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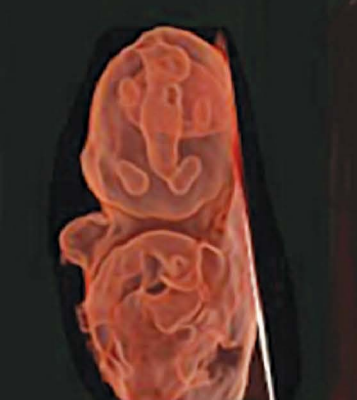



Donald School
EMBRYO
AS A PERSON AND AS A PATIENT

Asim Kurjak
Frank A Chervenak

Foreword
Milan Stanojevic

JAYPEE

Contents

1. Neurogenetic Processes in the Lateral Telencephalon during Intrauterine Development of the Human Embryo **1**

Ivica Kostović, Iris Žunić Išasegi, Željka Krsnik

- Development between 22 days to 7 Postconceptual Weeks *3*

2. Morphogenetic and Differentiation Powers of the Human Embryo **12**

Srećko Gajović

- From the Fertilization to the Activation of the New Genome *12*

3. Sonoembryology **19**

Ritsuko K Pooh

- Modern Embryology *19*
- Abnormalities at Embryonal Stage *22*

4. Controversies on the Beginning of Human Life **28**

Asim Kurjak, Lara Spalldi Barišić

- Science and Religion: Models of the Interaction *29*
- The Scientific Study of the Science and Religion *29*
- Academia and Religious Beliefs *30*
- The Definition of "Life" *30*
- The Facts of Human Embryogenesis *32*
- Influence of the Genetics and Epigenetics *34*
- Personality *34*
- Embryo as a Patient *35*
- Legal Status of the Embryo *38*
- Arguments for Beginning of Human Life and Human Person at Fertilization *39*
- Arguments against the Beginning of Human Life at Fertilization *40*
- Different Religious Teachings and Historical Aspects *41*
- Clinical Controversies *42*
- Visualization of Early Human Development *43*
- New Possibilities for Studying Embryonic Movements and Behavior *45*

5. Embryonic and Early Fetal Abnormalities Diagnosed with Three-dimensional Ultrasound in the 1st Trimester **51**

Eberhard Merz, Sonila Pashaj

- Embryonic Period *51*
- Normal Sonographic Development of the Embryo *51*
- Fetal Period *52*
- Embryonic/Fetal Malformations *52*
- Yolk Sac Abnormality *52*
- Umbilical Cord Cyst *52*
- Acrania, Anencephaly and Exencephaly *52*
- Encephalocele *53*
- Holoprosencephaly *53*

- Dandy–Walker Malformation *54*
- Cleft Lip/Palate *54*
- Retro-/Micrognathia *55*
- Low Set Ears *55*
- Enlarged Nuchal Translucency *55*
- Cystic Hygroma *56*
- Fetal Hydrops *56*
- Spina Bifida *56*
- Heart Defects *56*
- Abdominal Wall Defects *57*
- Urinary Tract Anomalies *59*
- Limb Anomalies *60*
- Conjoined Twins *60*
- Single Umbilical Artery *60*

6. Behavior of the Embryo **65**

Toshiyuki Hata, Uiko Hanaoka, Mohamed Ahmed Mostafa AboEllail, Nobuhiro Mori, Kenji Kanenishi, Megumi Ito

- Neurological Development of the Embryo *65*
- Two-dimensional Sonographic Study on Embryonic Behavior *65*
- Four-dimensional Ultrasound Study on Embryonic Behavior *66*

7. Pre-embryo: Medical, Moral and Legal Aspects **75**

Joseph G Schenker

- Events of Fertilization *75*
- Pre-embryo Development in vitro *75*
- Pre-embryo Culture Conditions *75*
- Composition of the Embryo Culture Medium *76*
- Pre-embryo Cryopreservation *76*
- Pre-embryo Assessment—Noninvasive Methods *77*
- Genetic Testing of Pre-embryo *77*
- Legal Status of the Preimplanted Pre-embryo *78*
- Moral Status of Pre-embryo *79*
- Human Genome Editing *79*
- Mitochondria Manipulation: A Three-parent Babies *80*

8. The Moral Status of the Embryo in Professional Obstetric Ethics **82**

Frank A Chervenak, Laurence B McCullough

- Ethics *82*

9. Invasive Diagnostic Procedures in Embryonic Period **89**

Giovanni Monni, Ambra Iuculano, Cristina Peddes, Maria Carla Monni, Valentina Corda

- Genetic-obstetric Counseling *89*
- Ultrasound and Prenatal Invasive Techniques *89*
- Embryonic Invasive Diagnostic Procedures Chorionic Villus Sampling–Preimplantation Genetic Diagnosis *89*

10. The First Four Weeks: Ultrasound and Doppler Assessment of Normal and Abnormal Implantation **96**

Sanja Kupesic Plavsic

- Ultrasound and Doppler Studies of the Endometrium *96*

11. Ultrasonographic Evaluation of Embryonic Cardiac Development **105**
Gwang Jun Kim

- Carnegie Stage 9 (19–21 Days of Conception, 5 Gestational Weeks), CRL 2 mm *106*
- Carnegie Stage 10 (22–23 Days of Conception, 5 Weeks 1–3 Days of Gestation), CRL 2.5 mm *106*
- Carnegie Stage 11–12 (23–26 Days of Conception, 5 Weeks 2–5 Days of Gestation), CRL 3–3.5 mm *106*
- Carnegie Stage 13 (28 Days of Conception, 6 Weeks of Gestation), (9.5 Days of Rat Embryo), CRL 4.5 mm *106*
- Carnegie Stage 14 (32 Days of Conception, 6 Weeks 4 Days of Gestation), 10 Days of Rat Embryo, CRL 6 mm *107*
- Carnegie Stage 15 (36 Days of Conception, 7 Weeks 1 Day of Gestation), 11.5 Days of Rat Embryo, CRL 8 mm *107*
- Carnegie Stage 16 (40 Days of Conception, 7 Weeks 6 Days of Gestation), CRL 10 mm *108*
- Carnegie Stage 17 (42 Days of Conception, 8 Weeks of Gestation) 12.5 Days in the Rat Embryo, CRL 11 mm *109*
- Carnegie Stage 18 (44 Days of Conception, 8 Weeks 2 Days of Gestation), 13 Days in the Rat Embryo, CRL 13 mm *110*
- Carnegie Stage 19–21 (48–52 Days of Conception, 8 Weeks 2–5 Days of Gestation) 13.5 Ed in the Rat Embryo, CRL 16–23 mm *110*
- Carnegie Stage 22 (54 Days of Conception, 9 Weeks 1 Day of Gestation), 14.5 Ed in the Rat Embryo, CRL 26 mm *113*

12. Preimplantation Genetic Diagnosis **116**
Elitza Markova-Car, Krešimir Pavelić

- Biopsy Techniques *116*
- Use of Polymerase Chain Reaction *116*
- Microarray Platforms *118*
- Next Generation Sequencing *118*

13. Vanishing Twin Syndrome **121**
Maria Carla Monni, Ambra Iuculano, Cristina Peddes, Valentina Corda, Giovanni Monni

- Vanishing Twin Syndrome in ART Pregnancies *121*
- Effects of Vanishing Twin Syndrome on Surviving Twin *123*
- Influence of Vanishing Twin Syndrome on Maternal Serum Markers *124*
- Influence of Vanishing Twin Syndrome on Nips *125*
- Influence of Vanishing Twin Syndrome on Invasive Prenatal Diagnosis *126*
- How Early can the Suspicion of Vanishing Twin Syndrome be Posed? *127*

14. Genomic Editing, Human Enhancement and Transhumanism: A Brief Overview **131**
Kresimir Pavelic, Sandra Kraljevic Pavelic

- Genomic Editing *131*
- Human Enhancement *134*
- Designing a Baby with GE *135*
- Transhumanism and "The Culture of Life" *137*
- Living Indefinitely Long *139*

Index *145*

CHAPTER

2 Morphogenetic and Differentiation Powers of the Human Embryo

Srećko Gajović

INTRODUCTION

The current advancements of biomedical technologies resulted with a surprising outcome, that nowadays the embryo can be considered not only as a patient, but as well as a potential cure. Together with taking care and monitoring the normal sequence of developmental events, the medical interventions are possible and some of them are already in the everyday medical practice. Furthermore, new emerging technologies are envisaged to generate the embryo-derived cells and organs, aimed for innovative medical treatments of the adult patients, in particular in the regenerative medicine.[1]

There is no doubt that the whole process of arising of the adult human from a single cell of the fertilized egg is extraordinary. The embryo period is when these transformations are the most prominent and fastest. The goal to be accomplished for the human embryo to reach the stage of the fetus is rather complex and demanding even in the sense of normal development. Although embryo is mostly hidden during this period and the changes occur very fast, still the new technologies provide very detailed insight in the events going on.

In this chapter, I am introducing the concept of embryo powers, which is distinct from the embryo potentials and their realization, but complementary to them. When we consider the embryo potential at the time of fertilization, it is indeed the whole new being, but in sense of realization, it is still a single-cell zygote. Together with following how the stepwise realization of the potential in the developing structures leads to the healthy baby, I would consider as well the powers of the embryo. The embryo powers describe what embryo is capable at each stage. These powers determine quite directly the possibilities for medical intervention in case of "embryo as a patient", and as well enable the controversial enhancement of the embryo status in the "embryo as a cure".

The embryo in the current society is much more than a target of medical intervention. Through the powers exercised during this profound transformation, there are as well the societal powers of the embryo. The technologization of the embryo in the current society raises a wide array of controversies, not limited to the question when the embryo would start to be a little human.[2] The possibility to create an embryo through in vitro fertilization, to keep it frozen and use it not only for creation of new being, but as well for the embryonic stem cells, and the recent possibility of creation of human-animal chimeras, all these are some of the examples leading to the previously unimaginable situations with the human embryo in the focus. Conceiving a new life is central for the human species and for the human society. Therefore, the embryo had already a special place in society long before recent technological advances. Still, these technological advances bring new discourses to the old topics in consideration, which are frequently not possible to be solved and would remain opened for the future. These controversies, although important, would not be the topic of this chapter, as I would like to concentrate on the biological sequence of developmental events and the biological powers embryo has during this process. Nevertheless, the new individual experiences of the pregnancies, which would be generated by the emerging technologies, would influence the public debates and create new discourses, which eventually could solve some current, but in the same time generate new controversies in relation to the human embryo.[3]

FROM THE FERTILIZATION TO THE ACTIVATION OF THE NEW GENOME

At the moment of fertilization, the half of the genome of the new human being is contributed by the sperm cell and half by the egg cell. This completes new set of chromosomes having all genes necessary for the new being. In sense of the potential, it is at its maximum and the abilities written in the genome of the new human are assembled and defined. However, in sense of the realization, the embryo is a single

JAYPEE BROTHERS

cell, zygote, which would immediately start its first mitotic division. If we would like to discuss the powers of the embryo and of its new genome, contrary to its potential, at this moment it is completely powerless. The new set of the chromosomes is located in the egg cell provided completely by the mother. The new embryo-specific set of genes would need several days after fertilization to be activated, transcribed to mRNA, and create new proteins generated by the new genome. During this period, the cell components apart from the set of chromosomes are those of the egg cell, generated by the mother through the process of oogenesis. There is as well a substantial amount of the stored mRNA from the mother, which serves as a template for the new proteins needed for the development to start. The activation of the egg cell is achieved by the fertilization, when at the moment of fusion of the plasma membranes of the sperm and egg cells, calcium ions enter from the extracellular space in the oocyte and activate the sequence of developmental events. Subsequently, after calcium ions start the developmental sequence, the first period is not dependent on the embryo genome, but it is completely executed by the maternal genome, and RNA and proteins provided by the mother during oogenesis.[4]

The proteins of the mother will take care in the next days of the embryo genome. This actually implies that the mother will power-up the initially powerless embryo genome and help it to achieve the functioning state. This switch between functional proteins provided by the mother and new ones being generated by the embryo itself occurs gradually, and it is estimated that 3 days after fertilization the embryo has more of its own proteins than those of the mother, implying that the embryo starts to take over its own destiny.[5]

Together with taking care of the new genome, the mother-provided proteins start the first developmental phase, cleavage. The cleavage involves a series of mitotic divisions and creates new daughter cells, blastomeres, assembled in the morula. This first developmental phase is focused on the embryo genome, with the aim to generate, in a short time, new cells—each one with the additional genome copy. There is neither growth nor other noticeable activities during this period, to give maximum priority to the cell divisions, but as well due to the lack of space, embryo being encapsulated in the zona pellucida.

As the embryo genome is powerless at this stage, and in the caring hands of the mother, it is easy to understand that the outcome of this period is not at all dependent on the embryo quality, nor it is predictor of the embryo quality. Subsequently, neither chromosomal nor genetic anomaly of the embryo will be influencing the morula formation, and no prediction about embryo genome is possible by morulas obtained during in vitro fertilization.

The action of the embryo genome is present and visible in the following stage of development, compaction. The rounded blastomeres of the morula change their shape to polygonal and create intercellular junction characteristic for the epithelial tissue. Before compaction, whatever was present around blastomeres, (e.g. molecules of water, ions, and sugars) could not be assigned to be either inside or outside the embryo. After compaction, the cells create a superficial layer of tightly connected cells and this clearly separates the center of the embryo (now being "inside"), from the surface of the embryo (being "outside"). The process is dependent on the activity of the cytoskeleton changing the shapes of the cells and formation of the intracellular junction sealing the spaces between the cells to make the layer impermeable. The compaction is a sign of the activated embryo genome and the compacted embryos have higher probability to start pregnancy during in vitro fertilization.[6]

The embryo at compaction stage has some distinguished powers, as it is not powerless any more. It has already active genome, and it has generated its first form, the superficial layer of the epithelial cells. It is not any more a loose aggregation of blastomeres generated by the cleavage, but a compact mass of connected cells. Compaction represents a milestone between two stages. Before it, the loose blastomeres could be removed from the morula without damaging the embryo, which is used in preimplantation diagnostics. The isolated cells of morula serve to get the insight in the embryo genome, and diagnose gene alterations. Taking cells for preimplantation diagnostics after compaction is theoretically possible, but it would require the removal of the intracellular junctions and "uncompacting" the embryo in order to remove a cell or two. The biopsies are again possible at the blastocyst stage explained in the next chapter, where thin layer of trophoblast offers this possibility.[7]

In the same way as the cells can be taken out of the morula, they can be added to it, and the additional cells would be incorporated in the embryo by compaction process. This is widely used in animal experiments on mice and rats as "morula aggregation". This technique enables creating one animal from multiple embryos, or merging embryonic stem cells with the host embryo to

JAYPEE BROTHERS

generate animals derived from embryonic stem cells. Human embryos are not subject of these types of the experiments, but the human embryonic stem cells could be used to merge with animal embryos, (e.g. pigs or cows). These human-animal chimeras are not produced only due to scientific curiosity, but as well by intention to eventually generate human organs ready for transplantation, (e.g. human kidney growing in the body of the pig).[8]

How the Embryo Escapes the Imminent Dangers Threatening its Survival

The compaction is a prerequisite for the following stage of development, blastocyst stage. The fact that the embryo has the superficial epithelial sheet allows the liquid to accumulate in its center. This creates the rapidly expanding hollow structure, blastocyst, with liquid in its central cavity, blastocoel. The surface epithelium, trophoblast, differs from the small group of cells protruding in the blastocoel, inner cell mass.

As embryo with compaction acquires the power of compartmentalization using its newly formed epithelial sheet at the blastocyst stage it uses this power for its own undertakings. Embryo needs to escape two imminent dangers and this would be its first priority during the following days. Just to mention, the embryo does not consider anything close to "building a new human" at these early stages as any of its activities. This is because embryo has bigger concerns, which threaten him and it needs to use its powers to counteract them first.

The first danger is zona pellucida, which is an inert capsule, made by glycoproteins. The zona pellucida does not provide any space for the embryo growth and it isolates embryo completely from the eventual contacts with surrounding tissues. This could be beneficial, as eventual contacts between oviduct epithelium and the embryo can cause it to stay in the oviduct and eventually start extrauterine pregnancy. However, as the embryo approaches uterus it would need to free itself before implantation. During blastocyst formation, the liquid accumulates fast inside the embryo, and puts the pressure to break zona pellucida. This makes minute lacerations of the zona pellucida resulting with a tiny hole through which water filled blastocyst can easily exit out (what would not be possible if it would be a solid structure made by cells only). This "hatching" of the blastocyst makes it exit to the uterus and establish cell-to-cell contacts with uterine endometrium necessary for implantation.[9] The explained power of the embryo, to form epithelial sheets, accumulate water and easily expand, is used later for creating spaces for the unhindered baby development, i.e. amniotic and chorionic cavities.

The second imminent danger for the embryo is menstrual bleeding, which would shed not only uterine endometrium but as well the embryo itself. To cancel the menstrual bleeding the embryo would need to interfere with the hormonal regulation of the mother, which implies it needs somehow to get access to the maternal blood. This is achieved through the process of implantation, which in addition allows the embryo to use the maternal blood as well to get nourishment and to get rid of the waste products. Although combining this with release of embryo-derived hormones to the blood is very convenient, the embryo at this stage gets all necessary nourishment by simple diffusion through the endometrial epithelium and from the secretions of the uterine glands. Therefore, at the implantation stage, the first priority for the embryo is cancelling menstrual bleeding, and all other handy advantages of ingredients' exchange with maternal blood would become important later, during the period of the fetal growth.

To achieve the task of the implantation, the embryo will develop a new power, power of differentiation. By the use of its activated genome among its cells organized as the epithelial layer, it will create first type of cells with dedicated function. The final product of differentiation process is fully functional cells, which are difficult to reverse in another type of the cells. Therefore, every differentiation comes on the cost of losing potential. The reason for this is accumulation of many long-lasting proteins specific for the given function, and eventual dedifferentiation and redifferentiation would be costly process of getting rid of these high quality proteins and in the meantime synthesizing new proteins for some other function. Subsequently, although dedifferentiation is possible, it is an exception achieved mostly in experimental conditions, and not something a living being would consider as an appropriate option. Therefore, the embryo at this stage needs to dedicate a group of cells to be differentiated, but as well to keep another group of cells in undifferentiated state in order to use them later on for all other differentiation purposes. As the danger of menstrual bleeding is indeed very serious, the embryo will dedicate actually around 90% of its cells for the task of differentiation, and keep only around 10% in the undifferentiated state. The differentiation takes place in the trophoblast layer

JAYPEE BROTHERS

generating highly specialized cells, syncytiotrophoblast. The undifferentiated cells stay loosely attached to the trophoblast as a bulge of cells toward blastocoel, inner cell mass.

Syncytiotrophoblast is a very potent and highly active cell type. It disrupts the epithelium of the uterine endometrium, dissolves the extracellular matrix underneath, and aggressively erodes the closest capillaries. Upon reaching the blood, it releases a hormone, chorionic gonadotropin, which instead of luteinizing hormone (LH) of the mother, supports corpus luteum in the ovary to produce progesterone, and evade menstrual bleeding by keeping endometrium healthy and active. As syncytiotrophoblast is so complex and versatile cell type, differentiating well-functioning syncytiotrophoblast requires activity of many genes dispersed across almost all chromosomes. Therefore, achieving high quality syncytiotrophoblast, which would generate enough of chorionic gonadotropin, represents a major checkpoint of the embryo quality.[10] In case of chromosomal anomalies or some major genetic anomaly, the embryo would not be able to secrete necessary amounts of chorionic gonadotropin to the maternal blood and subsequently the necessary levels of progesterone would not be maintained leading to the spontaneous abortion. This is an "all or nothing" principle, the embryo will either escape the danger of endometrial shedding and survive, or it would not be able to cope with the task and perish. There are no malformations at this stage as there is no body plan yet.

In case of in vitro fertilization, mothers are treated frequently by progesterone to provide some advantages for the embryo during the implantation. Still, in case of major chromosomal anomalies the external progesterone would not help to maintain the pregnancy.[11] The levels of chorionic gonadotropins increase and can be measured in the maternal blood to assess the embryo vitality, and the slow increase can indicate chromosomal anomalies, e.g. Down syndrome.

Providing Extraembryonic Membranes as a Necessary Infrastructure for the Development

The inner cell mass of the blastocyst is a small group of undifferentiated cells within the blastocyst. Although epithelial, these cells lack polarity due to their arrangement expanding toward the blastocoel. Their characteristics are very similar to blastomeres, but those centrally located, which did not turn into the trophoblast. The undifferentiated state is reflected in their gene expression being protected not to activate the syncytiotrophoblast program. Human blastocysts are possible to be obtained by in vitro fertilization, and they are the last stage of human embryo available for manipulation as after implantation the embryo would be hidden somewhere in the uterine endometrium. Therefore the inner cell mass was taken as an ideal source for embryonic stem cells. Compared to the morula stage embryo, their potential to make trophoblast is significantly reduced, they are undifferentiated, and due to the lack of epithelial polarity, still easy to be removed from the blastocyst and grown further in the culture.

Just opposite to that, the blastocyst is as well the last stage when something can be added to the embryo, which is widely used in animal experiments to add embryonic stem cells and generate chimeric animals. For human embryo, this could represent an entry level for genetically modified stem cells with corrected genome to cure the embryo, but these experiments were not considered yet. This is as well, the stage where human cells can be added to the animal embryo creating human-animal chimeras.

Following the implantation, the inner cell mass goes through the process similar to compaction and its cells acquire polarity. It results in accumulation of liquid inside the inner cell mass in the same way it has accumulated during formation of the blastocysts.[12] The new lumen generating by this process is amniotic cavity and the epithelium divides in the two domains. The outside domain consists of two epithelia, the epithelium of the amnion consisting of the amnioblasts, and the epithelium of the hypoblast destined to make yolk sac. The inside-facing domain is epiblast, which is present only near the hypoblast, creating bilaminar embryonic disk. The epiblast keeps the undifferentiated state, while the outside epithelia are involved in generating extraembryonic membranes.

The implantation marks the end of the first week of development after fertilization, and the formation of the extraembryonic membranes will take the complete second week. This is a long period still without any body plan, but the extraembryonic membranes are necessary infrastructure to be provided for the embryo development. Together with the power of differentiation, the embryo would add the ability to combine different cell types in the specific forms, morphogenesis. In addition to the epithelial tissue, the primitive connective tissue or mesenchyme would appear as extraembryonic mesoderm. This as well

JAYPEE BROTHERS

allows the different types of cells to influence each other through so-called epithelial-mesenchymal interactions. The new structures evolving include amnion, chorion, yolk sac, and allantois. The amnion would supply the isolated, but ample space for the embryo body to develop. Chorion would create the interface with the uterine endometrium, now referred as decidua, which would contribute to the placenta and the amniochorionic membrane. The yolk sac in human embryo represents the first sight for vasculogenesis and hematopoiesis, and allantois contributes with its blood vessels to the formation of the umbilical cord. Through this process, the extraembryonic membranes provide roomy compartments within the endometrium, while uterine cavity obliterates. The connection between mother and embryo stabilizes in a form of placenta and umbilical cord. Still, parallel to this extensive extraembryonic morphogenesis, epiblast would stay in the undifferentiated form until the 3rd week of development.

The Formation of the Body Plan and Organogenesis

At the beginning of the 3rd week, the embryo has collected and exerted already significant powers. It has activated its genome and tested its quality through the implantation process, it is able to form two types of tissues, epithelium and mesenchyme, and it can differentiate fully functional cells, which can be combined in the definite forms through the process of the morphogenesis. All this helped embryo to create a comprehensive setup to activate finally its ultimate power—the power of creating the body plan. In case of molar pregnancy, most of the powers described previously were achieved, but there is no body plan, highlighting the distinguished property of this crucial power. Moreover, not only the embryo without body can be made like in molar pregnancy, but, opposite to that, the embryo with two or multiple bodies could be created in form of the monozygotic twins. The monozygotic twins arise through the division of the undifferentiated group of cells either at the stage of morula, inner cell mass or epiblast, highlighting the undifferentiated state of these structures.[13]

The body plan appears in the epiblast by formation of the primitive streak and the process of gastrulation. The primitive streak defines the major axes of the embryo, cranial-to-caudal and lateral-to-lateral.[14] As primitive streak is formed the epiblast cells start to migrate through the primitive streak and change their positions in relation to each other.

The human, similar to other vertebrate embryos, does not follow determined development, where the sequences of events are predetermined and follow in hierarchical order. At the first sight, the determinate (mosaic) development seems as the best way to control the intricate process. Although it serves well for simple organisms (like nematode *Caenorhabditis elegans*), it is not appropriate in case of the complex organisms. Therefore, at some point of evolution it was replaced in higher organisms by regulative development, where the developmental plan is executed by interactions among cells, in particular different types of cells. The regulative development offers necessary flexibility to achieve increased complexity. By adapting to different situations, it reduces the developmental mistakes and speeds-up the evolution.[15]

Gastrulation process uses the principle of regulative development at its best. It provides the axial orientation for the cells by establishing the primitive streak, and subsequently lets cells to pass through it and end at the different locations across newly formed trilaminar embryonic disk. This achieves two goals, first to bring different types of cells to interact with each other at the different parts of the newly formed structure, and second it primes them with their future tasks by the process of invagination through the primitive streak. The nature of cell priming by the primitive streak is still speculative, but the information they get is the location along the primitive streak and the timing.[16] The cells entering first would end more cranially and those that follow would take positions more caudally. The migration through the primitive streak would result, instead of epiblast, in three new germ layers, ectoderm, mesoderm, and endoderm. The hypoblast layer would be pushed away to be part of the yolk sac. The three germ layers are the representations of the established body plan and the interactions between germ layers and different cell types within the layer will lead to establishing the organ primordia during next step of development, organogenesis.[17]

The in vitro experiments using embryonic stem cells or the parts of the embryo, (e.g. epiblast and hypoblast) could recreate the powers of the embryo present before gastrulation including differentiation of functional cells and morphogenesis of the simple structure, but they did never recreate the power of generating the body plan, present specifically during gastrulation. Subsequently, although the gastrulation process seems quite free-formed

JAYPEE BROTHERS

and based on seemingly random migration, it is indeed a very complex and demanding process, which represents a key event during embryo development. This is the ultimate power of the embryo, to generate the body plan, the power lacking in all in vitro stem cells systems. The body plan appearance needs all prerequisites generated during two previous weeks of human development and only in this particular setting the epiblast is capable for gastrulation process.[18]

Although stem cells, in particular embryonic stem cells and induced pluripotent stem cells, lack the ability to generate body plan by themselves, when introduced in the embryo, they could obey the host embryo cells and together with them generate the body plan. The embryonic stem cells as well as induced pluripotent stem cells, corresponding to the inner cell mass of the blastocyst, by themselves lack the spatial relations of the epiblast. When introduced in the embryo, and reaching the epiblast stage, the body plan is generated and subsequently it makes stem cells capable for the normal organogenesis. All other types of the differentiating cells and partial organogenesis achieved in vitro from the stem cells, can supply by specific stem cells the eventual needs for therapy within the scope of regenerative medicine, but are (at least currently) not possible to recreate the full organogenesis, where organ is formed in accordance and integrated with all surrounding structures.[19]

This issue, how to create a fully functional organ suitable for transplantation, depends on the embryo power to create a body plan. Therefore, the evolving strategy to create the organs for transplantation is with the help of the embryos, but using animal embryos instead of human.[8] The strategy envisages the use of human-animal chimeras, where the host embryo is animal. The pig is chosen as the most suitable to be host according to the size and anatomy. If the human stem cells could be added at morula or blastocyst stage to the pig embryo, they would contribute to the body plan of the pig during the gastrulation process (using the power of the pig embryo to generate a body plan). As the organ with only human cells is needed for transplantation, the pig embryo should be genetically deficient, not to be able to produce the organ in question, (e.g. kidney). Consequently, the human cells will replace pig cells in this particular task and the pig will have all organs chimeric (pig and human origin), but the kidney will be human only. One can imagine the clean facility, where these specific pigs will be grown with human organs, generated by induced pluripotent cells to match exactly the patient in the need for transplantation.

Extreme ethical issues burden the technique described, and one of the most difficult to predict would be the levels of humanizing the pig by this procedure. As the contribution of the cells to the chimeric organs can vary, one can imagine the pig with mostly human-derived brain, which could then have the human-like mind. At the other hand, the humanized brain of such an embryo at the fetal stage can serve as a source of human fetal neural stem cells, which could provide an alternative source of the cells to be applied in the possible therapy of the neurodegenerative diseases.[20]

CONCLUSION

The morphogenetic and differentiation powers of the human embryo described here show how powerful and how flexible is the human embryo. The early development starts with maximum potential of the human embryo upon fertilization, which is realized gradually by developing powers of the embryo. They include the activation of the new genome, creation of the epithelial tissue and later on mesenchyme, functional differentiation of the specific cell types, and morphogenesis based on inductive interactions between different groups of cells. These powers are supplemented by the power of generating body plan, which appears in the epiblast at the gastrulation, and enables all other powers to start the concerted action of organogenesis.

Before generating body plan, the embryo is occupied by many important tasks to prepare the scene for the gastrulation. The developing powers are used to achieve these tasks and they are necessary proof that the new genetic set is capable to make a new living being. Through the "all or nothing" principle, most of the chromosomally and genetically anomalous embryos would not reach the body plan stage and would be spontaneously aborted due to the inadequate connection with the mother. As during these preparatory stages no body plan is present, the embryo appears to be flexible for various interventions, twin formation being the example of natural one. Adding or removing cells is possible throughout this time period, which could serve to treat the embryo as a patient, or use the embryo cells as a cure for other patients. Whatever intervention envisaged, it is highly ethically controversial. The embryo is one of the most controversial bio-objects, defying the existing classification and definitions.[21] As the

JAYPEE BROTHERS

evolving technologies would change the experience of the pregnancy, this would influence as well the societal debate on the embryo issues, and eventually redefine the current concepts. The unpredictable process of technical challenges and ethical controversies makes the developing powers of the embryo one of the most intriguing medical and social concerns.

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EMBRYO
AS A PERSON AND AS A PATIENT

The human embryo is of obvious importance in clinical practice and research in reproductive medicine and healthcare. Physicians and scientists continue to make striking advances, the implications of which are explored in the chapters of this book. These advances should be understood and appraised in the context of ethics and culture more generally. The unique feature of this book is that it complements chapters on the embryo in clinical practice and research with chapters on the embryo in ethics and culture. The result is a remarkable synergy of science and human values about the human embryo that can be found only in this volume. Scholars from many countries who are leaders in their fields contributed chapters in the book. Many of the chapters have been adapted from the author's own work, which is cited in the text and reference list.

Asim Kurjak MD PhD is a Professor, Department of Obstetrics and Gynecology, Medical School Universities of Zagreb and Sarajevo, and Professor (Emeritus), University Sarajevo School of Science and Technology, Bosnia and Herzegovina. He received his medical degree from Medical School University of Zagreb in 1966. From 1971 to 1972, as a British scholar, he was a research assistant at the Institute of Obstetrics and Gynecology, University of London. In 1974, he became a specialist in Obstetrics and Gynecology and in 1977 he got his PhD degree. In 1980, he was awarded the position of a Professor. From 1985 to 2002, Dr Kurjak served as Head of the Department of Obstetrics and Gynecology, Medical School University of Zagreb, Sveti Duh Hospital.

Dr Kurjak has published more than 500 papers in peer-review literature. His papers have been cited more than 11,000 times in Google Scholar bases. He has authored, co-authored or co-edited 117 books. He is a regular fellow of World Academy of Art and Science, European Academy of Sciences and Arts, International Academy for Human Reproduction, Italian Academy of Science, and Art of Reggio Puglia, Academy of Medical Sciences of Catalonia. He is the Honorary Member of American Institute of Ultrasound in Medicine and Biology, and foreign member of Russian Academy of Medical Sciences. He is the President of International Academy of Perinatal Medicine and Founder & Director of Ian Donald Inter-University School of Medical Ultrasound, Zagreb, Croatia.

He received a Doctor Honoris Causa from the Universities in Banja Luka, Budapest, Barcelona, Bucharest, Athens, Montevideo, Tomsk, Buenos Aires, St Petersburg, Moscow, Tirana, and Khartoum.

Frank A Chervenak MD currently serves as Chair of Obstetrics and Gynecology, Lenox Hill Hospital; Chair of Obstetrics and Gynecology and Associate Dean of International Medicine, Zucker School of Medicine at Hofstra/Northwell, New York, USA. Dr Chervenak was appointed Associate Professor of Obstetrics and Gynecology and Director of Obstetric Ultrasound and Ethics at New York Hospital-Cornell Medical Center in 1987. In 1991, he was named Director of Maternal Fetal Medicine and Director of Obstetrics. In 1992, he was made full Professor with tenure, in 1998, he was named Vice Chairman of the Department of Obstetrics and Gynecology and in 1999, and he was named Acting Chairman of that department. Dr Chervenak was named Chairman and Obstetrician and Gynecologist-in-Chief in 2000. He received his Master in Medical Management degree from Carnegie Mellon University in 2001 and earned fellowship status from the American College of Physician Executives in 2008.

Dr Chervenak has published over 300 papers in peer-review literature and has co-authored or co-edited 36 textbooks. Research interests include ultrasound and ethics in obstetrics and gynecology and physician leadership. He has been named a member of the National Academy of Medicine of the National Academies. Currently, Dr Chervenak serves as President of the International Society of the Fetus as a Patient, Vice president of the International Academy of Perinatal Medicine, and Co-director of the Ian Donald Inter-University School of Medicine and Ultrasound. He serves on the March of Dimes Bioethics Committee and is Chairman of the Ethics Committee of the International Federation of Gynecology and Obstetrics (FIGO).

He has been awarded Doctor Honoris Causa from Medical Universities throughout the world. He has been admitted as a fellow ad eundem of the Royal College of Obstetricians and Gynaecologists of Great Britain and as a foreign member of the Russian Academy of Sciences.

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