Morphogen synergisms during antepartum synchronic evolution of placental hemochorial and pulmonary alveolar-blood biologic barriers in Homo Sapiens

Petru Razvan Melinte¹, Gheorghe S. Dragoi²,*, Ileana Dinca¹, Elena Patrascu¹

Abstract: During the ontogenesis of Homo Sapiens there are two systems for the gas exchanges that incites towards the study of the anatomic characteristics of ecoton areas, that is transition areas between adjacent biocenosis. The microanatomic analysis of placental hemochorial and pulmonary alveolar-blood biologic barriers was carried out on 9 fetus-placenta systems with gestational age between 16 and 26 weeks and on 3 embryos of 7 weeks of gestation. The comparative study of these two biological barriers involved in gas exchange, offered arguments for understanding the morphogen synergisms from the perspective of their structural synchronism. The authors consider that at the base of their morphogenesis, stand the reciprocal induction and interrelations between epithelium and mesenchyme that generate structures adapted to gas exchange between different environments: blood-blood antepartum and air-blood postpartum.

Key Words: morphogen synergism, respiratory biologic barrier, placental villi, pulmonary alveoli.

During the epigenesist of placental hemochorial and pulmonary alveolar-blood biologic barriers, one can define a certain convergence for morphogenesis of structures and a divergence for the different functions in time and space. The synchronism and significance of phenotype changes undergone by the structural elements of those biologic barriers have not been studied properly. The interest for knowing those barriers is determinant by the search for answers to numerous problems regarding their functional anatomy: What are the microanatomic characteristics of space distribution of blood capillaries networks inside the structures involved in gas exchanges such as antepartum placenta and postpartum lung? What are the consequences for tissue interactions and reciprocal induction between epithelium and mesenchyme during the antepartum genesis of placenta hemochorial and pulmonary alveolar-blood barriers? What is the dynamics of phenotype changes undergone by chorial villosities during placenta epigenesis? Why the advent placental and pulmonary vascular structures belong to the arterial system (umbilical artery and pulmonary artery) and the outgoing to the venous system (umbilical vein and pulmonary veins)? Could the gas exchange through hemochorial barrier be considered as a phylogenetic review of branchial respiratory system in fish? Are there any flow-regulating structures inside placenta vascular system? What is the contribution of cytotrophoblast in the genesis and remodeling of placenta villosities? What are the determining factors for the decrease of gas exchange surface in hemochorial and alveolar-blood barriers?

The purpose of this paper is to highlight the theoretical and practical problems of genesis and evolution in time and space of placental and pulmonary

1) University of Medicine and Pharmacy of Craiova, Department of Anatomy
2) Romanian Academy of Medical Sciences, Bucharest, Romania
* Corresponding author: Prof. MD, PhD, Email: dragoigs@gmail.com
respiratory systems from the structural synchronic and functional diachronic perspectives in Homo Sapiens.

We proposed ourselves to perform a micronanatomical analysis of space distribution and relations between the structural elements that take part to the formation of placenta hemochorial and pulmonary alveolar-blood barriers; they create transition areas between liquid biocenosis of placental blood and air and liquid biocenosis in lungs.

**MATERIALS AND METHODS**

The study was carried out on a set of 9 fetus-placenta systems with gestational age between 16 and 26 weeks and on 3 embryos of 7 weeks of gestation. The macroanatomical analysis of placenta was performed on 3 specimens injected through the umbilical vessels with a Tehnovit suspension and on 6 specimens fixed in 5% formaldehyde solution buffered at 7.2 pH. The macroanatomical analysis was performed on embryos and on fragments of placenta and lung harvested from fetuses. The fragments were included in paraffin using classical methods. The 5 microns sections were stained using Hematoxyline-Eosine, Mac Manus and reduced silver nitrate Gomori method. The sections were examined using Nikon Eclipse 80i microscope. The microanatomical imagery was captured by Nikon Digital Sight DS-Fi1 High Definition Color Camera Head. The macroanatomical pictures were captured by Digital Camera Eos 1ds Mark II equipped with Macro Ultrasonic Lens EF 100 mm f/2.8.

**RESULTS**

**A. Microanatomical analysis of space distribution and relations between the structural elements that take part to the formation of hemochorial barrier.**

Our attention was particularly drawn by: the shape and structures of the walls of spaces between villosities as location for arterial maternal blood; by the variable space distribution of trophoblast inside umbilical blood vessels that carry fetus venous blood; by the relations of argirofile collagen fibers with the inter-villi structures and last but not least by the location and relations of flow-regulating structures. When examining by 4x objective the serried sections through placenta fragments harvested from pregnancies at 24 weeks of gestation, we could identify the inter-villi space that is crossed by placenta villosities sectioned under variable angles. The stem villosities present crenelated margins due to the presence of mature intermediate villi and of villus terminalis (Fig. 1A).

The examination with the 63x objective of the walls bordering the inter-villi spaces allowed us to visualize the relations between conjunctive-vascular stroma and vascular-syncytial membrane. This membrane is made by the trophoblast around villi, the walls of villi capillaries and by a thin layer of villosity stroma interposed between the two structures (Fig.2A). At the level of villus terminalis we visualized a network of sinusoid capillaries that expand to the periphery of villi; thus, a vascular complex is achieved, resembling “a bunch of grapes” (Fig. 3A). At the base of villus terminalis, we could identify fascicles of muscle cells stained by Hematoxyline-Eosine (Fig. 3 A). When examining the sectioned processed by reduced silver nitrate Gomori method, we could easily visualize the reticular lamina of subtrophoblast subendothelial basal membrane and the presence of paired argirofile collagen fibers with spiral trajectories towards the base of villus terminalis (Fig. 3B).

Neutral mucopolysaccharides visualized by Mac Mannus method are present in subendothelial basal membrane area of capillaries within terminal villosities structure (Fig. no. 3C). Significant changes were observed in the spatial distribution of perivillous trophoblast, by formation of syncytial knots which are prominent outside the villous surface; syncytial sprouts present during the development of lateral villosities; syncytial and interfibrous-villous bridges; the partial absence of syncytiotrophoblast nuclei which lead to the development of “vascular-syncytial membrane”. Within this structural unity, argirofile collagen fibers are present as “reticular blades” of subtrophoblastic and subendothelial basal membranes (Fig. 5B). Similarly, we noticed the presence of perivillous fibrous substance deposits between syncytium and cytrophoblast (Fig. 3 C-E). Rupture of placental membrane was frequently observed. There was also noticed the crossing of nucleate fetal red blood cells into the inter-villi space of the mother. When examining by 100x objective, terminal villosities are well visualized: sinusoid capillaries, vascular-syncytial membrane, subendothelial and subtrophoblastic basal membranes, trophoblast islands within pericapillar stroma (Fig. 5 A, B).

**B. Microanatomical analysis of spatial distribution and relations of structural elements that participate to the formation of hemochorial barrier.**

Seriate sections of 7 weeks embryos and of harvested fragments from fetuses lungs aged 16, 20, 24 and 38 gestation weeks, revealed a great variability of air-ducts shape and structure and also a heterogeneity of epithelial-mesenchymal interrelations and differentiation of periductal and perialveolar capillary networks. When examining by 4x objective, sections through 7 weeks embryos, it is easily noticeable the presence of bronchial ducts system, which forms a pseudoglandular structure (Fig. 3 E). Examination of seriate sections of pulmonary fragments from 16 and 20 gestation weeks fetuses, allowed a microanatomical visualization of bronchi and bronchioles lumen and also the presence of per bronchial capillaries (Fig. 3G). In 24 gestational weeks fetuses we concluded that each terminal bronchiol branches off into two or more alveolar sacks (primitive alveoli) (Fig. 3 H). When examining with the x63 objective the alveolar sacks walls, we noticed the presence of a flat epithelium consisting...
Figure 2. A. Shape and limits of intervillous space in placenta at 24 weeks of gestation. B. Shape, location and communication of alveolar ducts and sacks within fetal lung at 24 weeks antepartum. 1. Vascular-syncytial membrane; 2. Spatium intervillosum; 3. Alveolar duct; 4. Alveolar sack; 5. Type I pneumocyte; 6. Type II pneumocyte. Paraffin sections. Hematoxyline- Eosin stain. Macrophotographs taken with Nikon Digital Sight DS-Fi1 High Definition Color Camera Head x 420 (A, B).
of alveolar cells type 1 and from point to point, clear cytoplasm cells, such as alveolar cells type 2. Capillary network is well visible around alveolar sacks. This network was visualized per alveolar, after injecting China ink to a 3 weeks newborn (Fig. 3 I).

C. Mesoscopic anatomic analysis of branchial respiratory system in fish.

Branchial structures in fish were visualized after sectioning the operculum at the level of implantation basis (Fig. 4 A). Underneath each operculum, we visualized four branchial arches. Each branchial arch consists of a bone which inserts through “branchiospines”, branchial blades dispose in fan-shape and contain highly vascularized filaments (Fig. 4 B).

DISCUSSION

During the ontogenesis of human bio system the respiratory gas exchanges are achieved by two independent organs – placenta and lungs – that are specially adapted from anatomic and functional point of view to transfer oxygen and carbon dioxide between the two identical different biocenosis. Placenta presents the respiratory hemochorial barrier that plays an important role as the embryo-fetus lung and is known as an “eco-ton area” between the two liquid blood biocenosis, maternal and fetal. It separates the maternal blood from the inter-villi spaces, from the fetus blood inside the blood capillaries of chorial villotissies, and does not allow direct contact between them. Lungs present the alveolar-blood barrier and they are equally an “eco-ton area” between two different biocenosis: air from pulmonary alveoli and blood inside pulmonary vessels. Morphogenesis of lung takes place in two successive stages: antepartum for the system of alveolar sacs and postpartum for the alveolar system that triggers the respiratory function. The antepartum stage is contemporary with the morphogenesis and plain function of placental structures. Their identification and evaluation was difficult and controversial in the history of knowledge regarding the functional anatomy of fetus-placenta system.

The first important observations on placenta have been recorded in antique Greece. Diogenes from Apollonia (around 480 BC) was the first to consider this structure as a nutrition organ for the fetus. Aristotet (384-322 BC) [1] signaled the presence of fetal membranes and used the terms such as chorion and amnion. Galenus (180-201) [2] believed there is a direct communication between the maternal and fetus blood vessels. Anyway the term of placenta was introduced later by Matteo Renaldo Colombo (Columbus) (1559) [3]. Remarkable progress in the knowledge of the functional structure and relations regarding uterus-placenta and fetus-placenta systems was achieved after the diversification of the research instruments and methods: dissection of pregnant uterus (Leonardo da Vinci – 1492-1519) [4]; Andreas Vesalius (1543;1555) [5]; GiulioCezarAranzi (1584)[7]; experimental methods for the understanding of blood circulation (William Harvey (1628) [8]) and the gas exchanges during respiration (John Mayow (1674) [9]; Joseph Priestly 1772) [10]; Antoine Lavoisier (1778) [11]); the injection of blood vessels with marked substances ( William Hunter (1774) [12]; John Hunter (1786) [13]; Fillipo Civinni (1839) [14]); the invention of photonic microscopy (Antony von Leewenhoeck ( 1632-1732) [15]; Robert Hooke (1665) [16]); the visualization of blood capillaries (Marcello Malpighi (1661) [17] – established the anatomic basis regional arterial and venous blood circulation).

Two problems have been on top of the list for researchers: the relations between fetus-placenta and mother-placenta blood vessels on one hand, and on the other hand, the structure of placental villosities and the functional evaluation of inter-villi spaces. The hypothesis stated by Galenus that described a direct communication between fetus and maternal blood vessels was resumed and repelled by many scientists. Based on anatomic studies on pregnant uterus, Leonardo da Vinci[4] sustained the idea of no such thing as direct communication. While dissecting, Alexander Mauro-Primus (1734) [18] observed that there is no continuity regarding uterus and placenta vessels. He noticed that the extremity of fetus blood vessels expand beyond the base of placenta and pass inside depressions of uterine decidua where they just touch maternal blood vessels. In 1674, John Mayow [9] raised the problem of gas exchanges inside placenta that he named as “uterine lung”. After examining the anatomical specimens injected with marked substances (red or blue wax), William Hunter [12], John Hunter [13] and Fillippo Civinni [14] reached to the same conclusion. William Turner (1872) [19] proved that maternal blood circulates inside inter-villi spaces. William Campbell (1833) [20] noticed that placenta must be included in the fetus circulatory system; he makes a parallel between cardiopulmonary circulation and Cadillac-fetus-placenta one.

The evaluation of placenta structural anatomy was possible after the invention of photonic microscopy (Antony van Leewenhoeck [15]; Robert Hooke [16]. Ernst Heinrich Weber (1832) [21] was the first to describe the microanatomic structure of placental villosities and to notice that they contain branches of fetus blood vessels. Mathias Duval (1892) [22] demonstrated the invasion of uterus by placental tissue and its erosion by the trophoblast of uterine spiral arterioles. Otto Grossen in 1909 and 1927 [23, 24], proposed the structural classification of placenta after number and type of cellular layer which separates maternal blood from fetuses one into: epitheliochorial, syndesmochorial, endotheliochorial and hemochorial. In 1870 and 1882 Langhans [25,26] demonstrated the presence of two layers at the periphery of chorial villosities: a peripheric one represented by syncytiotrophoblast and an internal one represented by cytotrophoblast (“Langhans
layer”). Harland Mossman [27, 28] in 1926 and 1987 demonstrated, from anatomic and functional point of view, the existence of a reverse flow of maternal and fetal circulations. March and Simon in 2011 [29] elaborated the “turn-over” concept of trophoblastic cells and noticed the role of apoptosis in placental morphogenesis.

Langhans [26], Wolska [30], Rohr [31] and Nitabuch [32] draw attention over the fact that fibrinoid substance is an important structural component of placenta by its role in the phenotypic transformations during placental epigenesis. Fose and Sebire [33] in 2007 proposed a placental lesions classification into four classes: 1. lesions determined by disturbances of blood flow towards placenta via fetal-placental circulatory system; 2. lesions caused by disturbances of maternal blood flow towards inter-villi spaces; 3. retroplacental hematoma and non-vascular lesions.

Placental hemochorial and respiratory alveolar-blood barriers impress by their structural simplicity, as well as by time and space variability of structural elements relations. Anatomic antepartum formation and evolution of these barriers depends on interrelations and reciprocal inductions between epithelium and mesenchyme. Comparative microanatomic study of these two barriers offers the argument in understanding the morphogen synergisms, from the structural synchronal perspective. In the morphogenesis of the two respiratory models in human, there are stages during which epithelial-mesenchymal interactions occur, which represent the basis of ramification process: in lungs - ramification of airways system by the participation of endodermic epithelium and of pulmonary mesenchyme which assures morphologic differentiation of collagen fibers fascicles and the formation of perialveolar capillaries networks; in placenta - ramification of chorionic villous system due to the contribution of trophoblastic epithelium in implantation process inside uterine tissue and delimitation of inter-villi space, and due to the extraembryonary mesenchyme implication in genesis and differentiation of extracellular matrix and of vascular capillary network inside villi. These interactions assure the antepartum formation of respiratory system structures: placenta, adapted to gas exchange between blood-liquid biocenosis and pulmonary, adapted to the gas exchange between two different biocenosis - air and blood - liquid that becomes functional in postpartum.

Saunders et al. [34] in 1957 observed that mesenchyme is responsible for induction specificity into competent ectoderm. Competence represents the capacity to respond to an inductive signal (Waddington, 1940) [35]. Experimental embryogenesis researches, performed by Alescio and Casini (1962) [36] allowed the assessment of mesenchyme major role in the formation of pulmonary sprouts. During respirator pulmonary system, regional specificity of mesenchyme induction has an important role (Dragoi et al. 2010) [37]. Signal molecules involved in these dynamic and reciprocal interactions between epithelium and mesenchyme are poorly known. It appears that FGF10 is a signal molecule correlated to the formation of bronchus branches; it is expressed inside mesenchyme located above the developing buds (Bellusci et al., 1997) [38].

The respiratory biologic barriers from the structure of placenta and lungs are the consequence of reciprocal induction and interrelations epithelium-mesenchyme during the antepartum part of morphogenesis (Dragoi et al. 2007) [39].

The pulmonary alveolar-blood barrier is part of the structure of pulmonary alveoli. The structures derived from mesenchyme forming the alveolar wall – blood capillaries and type IV argirofile collagen fibers – can be found inside this barrier. Collagen fibers participate to the formation of capillary subendothelial and sub-pneumocyte basal membrane and they are part of common septal space. It continues to the conjunctive sheath of bronchi and blood vessels. Blood capillaries are incorporated inside the alveolar wall after the fusion of their basal membrane to the subendothelial basal membrane. Thus, the alveolar-capillary membrane or alveolar-blood barrier is formed; inside its structure one can easily identify epithelial structures (pneumocytes), endothelial structures (capillary endothelium) and the two basal membranes subendothelial and subepithelial, reunited as a double basal membrane.

The placental hemochorial barrier is part of the structure of terminal placental villi; similar to the pulmonary alveolar wall, one can find trophoblast epithelial cells, endothelial cells inside the walls of villi sinusoid capillaries and type IV collagen fibers that can be observed using reduced silver nitrate Gömörı method; collagen fibers appear as sub-trophoblast and sub-endothelial basal membranes that fuse in the sub-trophoblastic area (Fig. 5).

Comparative analysis of placental respiratory system in human to the branchial respiratory system in fish draws the attention to a functional similarity between the human placental villosities structure and filaments of branchial lamellas in fish (Fig. 4).

CONCLUSIONS

1. The comparative study of the epigenesis of hemochorial and alveolar-blood respiratory barriers brings arguments for understanding the synergisms during its morphogenesis.

2. The convergence of genesis and antepartum evolution of these biological barriers is synchronous to their functional divergence.

3. The placental hemochorial barrier represent an adaptation for surviving in a blood liquid environment and in Homo Sapiens, it represents the equivalent of branchial respiratory system in fish.
The pulmonary alveolar-blood barrier represent an adaptation for surviving in air environment and consequently it does not function antepartum.

5. The structural elements of respiratory barriers take part in achievement of their morphologic and functional homeostasis.

6. Blood capillaries networks have different locations inside the two barriers: inside villi in placenta and around alveoli in lungs.

7. The pattern for conjunctive-vascular relations is identical in the two types of barriers and it is determined by the reciprocal induction between epithelium and mesenchyme.

References

9. Mayow J. Tractatus quinque medico – physici; Quorum primus agit de salinitro et spiritu nitro – aereo: Secundus De respiratio; Tertius de respirazione foetu in utero, et ovo; Quartus de motu musculari et spiritibus animalibus; Septimus De rhachitide. E Teatro Shledoniano, Oxoni 1674.