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PhD THESIS

FETAL MORTALITY – MORPHOLOGICAL IMPLICATIONS IN THE THANATOGENESIS OF SINGLETONS AND TWINS PREMATURELY BORN

PhD Thesis Summary

PhD supervisor: **Prof. Univ. Dr. Mariana Aşchie**

PhD Student: **Trofimov (Ghiţoi) Sînziana-Andra**

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The doctoral thesis includes:

• 221 pages, of which 40 pages are the General Part

• 106 figures

• 26 tables

• 533 bibliographic references

Note: in this summary, the content has been kept in the same form as in the doctoral thesis.

INTRODUCTION

The aim of this work is to identify new risk factors, potentially modifiable through currently available means of prevention, which are involved in the thanatogenesis of premature babies.

The latest research in the field emphasizes the impact of pulmonary surfactant in the pathology of the premature, not only neonatal, but also perinatal, with an essential role in fetal immunological defense, including intrauterine. However, the actual study of the fraction of surfactant proteins, as well as the correlations between their presence/absence and other gestational and fetal parameters, is currently just at the beginning of the road. Taking into account these aspects, the need to carry out additional research is highlighted, with the aim of ordering the already existing information and expanding it by obtaining new results that indicate the future directions of deepening the subject, the impact of surfactant being major on a casuistic segment booming.

As a result, we opted for the deepening of this direction of study and we characterized from an immunohistochemical point of view the presence of three protein fractions of pulmonary surfactant on lung tissue samples, originating from a batch of premature twins, who died perinatally.

1. Physiological pregnancy

1.1 Development of the physiological pregnancy

The first 8 weeks of development, during which the zygote develops from a unicellular organism to the existence of organ primordia, is called the period of organogenesis or embryogenesis. This is followed by the fetal period during which the differentiation processes take place in parallel with the weight gain of the future child.

The three germ layers - endoderm, mesoderm, ectoderm - represent the substrate of all the tissues of the future organism, as follows:

- The endoderm will give rise to the epithelia of the digestive and respiratory tracts, the liver and pancreas, the thyroid;
- The mesoderm gives rise to axial, paraaxial and lateral domains that contain precursor cells for a multitude of future organs and systems: muscle, cartilaginous and bone tissues, cardio-circulatory and urogenital system, including gonads;

The ectoderm will differentiate into neuroectoderm - the developmental basis, following the phenomenon of neurulation, for the neural tube and neural crests - and superficial ectoderm from which the epidermis, subcutaneous glands, hair and nails, the anterior pituitary and, respectively, the anterior ectodermal ridge will evolve. Also, the superficial ectoderm has hormonal regulation functions (through the pituitary gland), being involved in homeostasis processes. Thus, the idea can be supported that the structures derived from this embryonic sheet will make the connection between the newly formed organism and the external environment.

The fetal period extends from the beginning of the third month of gestation until the time of parturition. It is characterized by the maturation of the organs simultaneously with an accelerated rate of growth, initially more evident in length - approximately 5 cm each in the 3rd, 4th and 5th months, then more evident in weight, with a gain of about 750 g per month in the 8th and 9th months.

1.2. Singleton pregnancy and multiple pregnancy

While singleton pregnancies remain numerically dominant worldwide, recent decades have seen a significant increase in multifetal pregnancies in the United States alone, with the incidence of twin births increasing by 76% between 1908 and 2009. However, some decrease in incidence - of 4% in the period 2009-2018 - for the first time after thirty years of upward trend. During the same period, rates of triplet and higher-order multifetal gestations increased by more than 400% in the 1980s and 1990s.

In the long term, the upward changes in the incidence of pregnancies and multiple births are mainly correlated with two factors: the progressively increased age of the mothers - the advanced age of the mother at the time of conception correlating with a higher probability high rate of natural occurrence of a multiple pregnancy - and, respectively, the increasingly widespread use of assisted reproductive techniques (ART) following the increase in infertility rates. Thus, if in Europe the transfer rate of a single embryo during IVF procedures was around 10% in the 1990s, this percentage reached over 40% in 2017, while the number of transfers with two embryos remained constant around the 55% figure.

In fact, the research so far seems to objectify the presence of some differences - not just incidental - between the vital prognosis (neonatal, but also late), morbidity and long-term neuro-cognitive development between children from single pregnancies and children from multiple pregnancies , at the same gestational age. Even though the observations are

limited to the average prematurity, 32-36 weeks of gestation - boys from multiple pregnancies are at greater risk of morbidity and mortality. However, the conclusions so far take into account twin pregnancies more frequently, and the actual study of the causality of these differences is lacking.

Among all multiple pregnancies, the most common are twin pregnancies, with a total of 97-98% of them. A correct assessment of gestational age and chorionicity in the first trimester are essential for subsequent monitoring, considering the existence of some peculiarities. Thus, monochorionic twin pregnancies are much rarer, representing 20% of all twin pregnancies, but are associated with a rate of intrauterine fetal death before 24 weeks and a perinatal mortality rate approximately 10 times higher than dichorionic pregnancies, due to placentation characterized by multiple anastomoses between the two fetuses. Thus, in the case of monochorionic twin pregnancies, pathologies such as intrauterine growth restriction (IUGR) or transfusion-transfused syndrome are more common, with the consequent increase in the risk of premature birth and the negative impact on vital prognosis.

2. Fetal pulmonary apparatus

2.1 Lung embryological development

Three major periods of the developmental process, partially overlapping, have been described, two of which occur during intrauterine life - the embryonic period (starts on day 22 and lasts from weeks 4-7, with the formation of the main airways and pleura) and the fetal period - and the third being the period of postnatal development. The fetal period is, in turn, subdivided into three stages: pseudoglandular (between weeks 5 and 17, with the development of the bronchial tree and lung parenchyma), canalicular (between weeks 16-26, includes the development of the distal airways, with the formation of acini, the membrane alveolo-capillary and surfactant) and saccular (continuation of the acinar expansion process). Also, the postpartum period includes two substages: classic alveolarization and, respectively, continuous. Basically, the period of alveolarization begins around week 36, so peripartum and continues throughout childhood.

If initially the immature alveoli look like swellings invading the primary septa, as the alveolar sacs continue to grow these protrusions also increase in size. Newly formed, longer and less thick septa are called secondary septa. These are the structures responsible for the final part of the division of the respiratory tree from the alveolar sacs to the alveoli. This septation process occurs in areas of fibroblastic activity and collagen and elastin secretion in the interstitium and has an accelerated pace during the first approximately six

months of extrauterine life, then slows down but continues until the age of three, with alveolar numerical growth having direct consequence of the increase in lung size. Parallel to the growth process, structural maturation occurs with the thinning of the alveolar-capillary barrier, due to the fusion of the double capillary network into a single one, each of them in an intimate functional relationship with two alveoli.

The finalization of the lung growth and maturation process takes place around the age of 8 years.

2.2. Notions of pulmonary anatomy

The respiratory system is the complex structure that provides the necessary means for atmospheric oxygen, indispensable and necessary in continuous flow to living cells, to reach them. The respiratory system is made up of airways (conductive area, having the role of bringing air from outside the body into the system) and lung parenchyma (whose portion called "respiratory" ensures gas exchange between the atmosphere and the body).

The conducting portion or the airways starts from the nasal cavities and further includes the nasopharynx, larynx, trachea, which divides into the two main bronchi, which branch arborescently at the level of each of the lungs, up to the terminal bronchioles. From this level - of the respiratory bronchioles - the respiratory portion begins, including the alveolar ducts and sacs and ending at the level of the terminal alveoli where the actual gas exchange takes place (hematosis).

The lung is a paired anatomical structure - with an indispensable vital role and complex structure - located in the thoracic cavity, on either side of the mediastinum. It is the essential organ of the respiratory process, responsible for the supply of oxygen to the body through the gas exchange between atmospheric air and blood, through the alveolo-capillary membrane.

The pulmonary parenchyma includes bronchioles, alveolar ducts and alveoli, has a spongy structure, and an elastic consistency, and due to the post-partum air content, the fragments float when immersed, and crepitations are noted upon palpation.

From an anatomical point of view, each lung is lined by the pleura - a double-layered serous membrane with a protective role and in the dynamics of respiratory movements - it has two surfaces, three edges, a base (considered by some authors the third surface) and an apex, subdivides into lobes and segments, having, as a whole, the form of a half-cone in oblique section, from apex to base.

From a structural point of view, the lungs are made up of multiple components: the bronchial tree, the stromal component, the parenchymal component, the vasculo-

nervous component.

The right lung communicates - through this face - with the esophagus, with the right brachio-cephalic arterial trunk, with the superior vena cava, with the azygos vein and its arch, and with the pericardium, through the right branch of the phrenic nerve and the pericardial vessels frenzy In the right pulmonary hilum, the topography of the elements notes the bronchus in a postero-superior position, with the artery anterior and the veins located inferior to the artery.

The left lung comes in relation to the esophagus, the descending portion of the aorta artery and, respectively, its arch, the left subclavicular artery and the pericardium, from which it is separated by the left branch of the phrenic nerve and by the pericardio-phrenic vessels. The left pulmonary hilum has a different topography compared to the right one, the artery being in a superior position, with the bronchus below, and the veins being located: one anterior, the other inferior to the bronchus.

From an anatomical point of view, the bronchial tree arises through the emergence of the main (primary) bronchi - right and left pulmonary, respectively - from the trachea at the level of the sternal angle, corresponding to the plane that intersects the fourth thoracic vertebra (transverse thoracic plane).

The trachea is a cylindrical structure whose supporting skeleton consists of incomplete cartilaginous rings, in the shape of the letter "C", with the opening to the esophagus, which thus allows it to dilate during the swallowing process. The primary bronchi are structurally similar to the trachea, but its cartilaginous skeleton consists of complete rings.

Functional vascularization - brings deoxygenated blood from the heart to be oxygenated at the level of the pulmonary parenchyma - is achieved by means of the pulmonary arterial trunk, which originates at the level of the right ventricle. The two pulmonary arteries, right and left respectively, start from this trunk, one for each lung, the branching process accompanying the bronchial branching, so that lobar, then segmental branches and, finally, a dense perialveolar capillary network are described.

Nutritive vascularization is part of the systemic circulation, being provided by the bronchial vessels, originating from the thoracic descending aorta and accompanying the bronchial ramifications up to the level of the respiratory bronchioles. They will form capillary plexuses in the bronchial mucosal and muscular tunics, these being connected with pulmonary arterial branches and being drained by pulmonary veins. Also, some of the bronchial branches have ramifications at the level of the connective tissue and flow

into the bronchial veins, others, externally, give rise to a subpleural plexus.

2.3. Pulmonary histology

The pulmonary morphofunctional unit - seat of gas exchange - is the acinus, whose definition has varied over time, most frequently being considered to be the portion of lung parenchyma served by a single bronchiole, so that a lobe would be constituted by a variable number of acini , usually between 2 and 10.

The alveolar walls are very thin, made up of 2 types of epithelial cells with a specialized role, distinct from each other, called pneumocytes (alveolocytes). Type I pneumocytes have a squamous morphology, and hematosis is achieved through their cytoplasm, while type II pneumocytes, with a cuboidal morphology, are rarer, appear interspersed among the others whose progenitors they are and have the role of pulmonary surfactant synthesis, as well as post-traumatic epithelial regeneration.

Interstitial, connective-type tissue is richly represented throughout the lung, including - although invisible in optical microscopy - in the alveolar cell walls, where elastic and collagen fibers, mesenchymal cells and, rarely, inflammatory elements are present.

2.4. Pulmonary physiology

The respiratory system is designed to carry out the breathing process - gas exchange between the external environment (atmosphere) and the cells of the body. In order to fulfill this function, all the components of this system are said to physical processes of a mechanical and, respectively, chemical nature, preceding, but the central role belongs to the lungs, at the level of which hematosis is achieved. Ultimately, oxygen is used in the production of ATP, and carbon dioxide, along with other metabolic products, will be eliminated.

From a biomechanical point of view, there are four components involved in respiratory dynamics: lung and chest wall compliance, respectively (defined as volume change per unit of pressure change, or basically elastic resistance), airway resistance, and respiratory rate.

The actual gas exchange takes place at the alveolar level - a sine qua non condition being that the alveolus is ventilated and perfused - as a result of a diffusion process, by means of a functional structure composed of two elements, each of them belonging to a different system :pulmonary and, respectively, circulatory: alveolo-capillary membrane. Thus, the success of the process also depends on the morphofunctional integrity of the

cardio-circulatory system.

2.5. Pulmonary surfactant: structure and physiology

Pulmonary surfactant is a complex substance with surface-active properties, composed of proteins and phospholipids with the function of reducing surface tension at the pulmonary air-liquid interface.In the early 1800s, two scientists - the Frenchman Pierre-Simon (marquis de) Laplace, and the British Thomas Young, independently recognized the relationship between surface tension (ɤ), radii of a sphere (r1, r2) and pressure (p), under the form to an equation that will bear their name - the Young-Laplace equation: $\Delta p = \gamma \left(\frac{1}{r_1} + \frac{1}{r_2}\right)$. Due to the properties of the surfactant, its surface tension has a high value and, as a result, it expands rapidly, covering the air-liquid interface, including at the level of larger airways. The surfactant can therefore be collected even from the level of the airways with a larger caliber, when the approach is necessary for the analysis of the biochemical structure.

It is noted that phospholipids are part of the structure of cell membranes and, together with other lipids or proteins, are part of the constituents of numerous fluids in the body: lung surfactant, but also bile, secretions from the pleura, peritoneum, the surface of the Eustachian tube or the gastrointestinal ones. There are also four specific proteins associated with surfactant: SP-A, SP-B, SP-C, SP-D.

Surfactant proteins B and C are hydrophobic peptides with low molecular weight - 14 and 16 kDa, respectively - participate in defining the biophysical properties of the surfactant, being also involved in its packaging and recycling/reuse.

SP-B is essential in maintaining the useful respiratory surface area and in the lipid packing process necessary for the generation of lamellar bodies. Its absence in newborn surfactant results in respiratory failure incompatible with survival.

A large number of respiratory diseases from the palette of respiratory distress syndromes, in children and adults - including perinatal deaths - are related to the absence or significant quantitative reduction of the SP-C protein, confirming its role in the homeostasis of the respiratory system, together with the more recently described and the insufficiently studied involvement of this protein in the modulation of the direct and indirect immune response to local pathogens.

SP-A is currently considered to be involved in three important physiological processes: the regulation of surfactant homeostasis, the decrease of pulmonary surface tension at the alveolar air-liquid interface, together with the other surfactant proteins, as

well as in non-specific innate immune activity at the lung level.

The functions of SP-D join the role of SP-A, having particular importance especially in the innate, non-specific immunological processes at the lung level, where it recognizes, opsonizes and is a promoter of phagocytosis and the elimination of microbial agents and, respectively, various micro or nano particles entered the system by inhalation.

3. Pathological pregnancy. Mortality and fetal thanatogenesis

3.1. Pathological pregnancy

Pathological pregnancies following the presence of nidation anomalies refer to ectopic implantation of the zygote and cases of placenta praevia, respectively. Although only about 1% of pregnancies are located outside the uterus, this fact leads to the arrest of the development of the embryo in the first weeks of pregnancy and can affect the reproductive prognosis or even maternal life.

Preterm birth is defined as occurring before the 37th week of amenorrhea, with some variability in the literature regarding the lower gestational age limit that differentiates it from miscarriage. The incidence of this phenomenon is currently increasing due to the increase in the frequency of use of assisted reproduction techniques. Although these techniques result in an increase in multiple pregnancies, including singleton pregnancies obtained by in vitro fertilization (IVF) have an increased risk of preterm birth compared to those obtained naturally. The impact of this pathological entity is major. , being responsible for over half of long-term postnatal morbidity and 75% of perinatal mortality.

Pathological pregnancies due to associated maternal pathology represent a category with a very high variability. Although it is a physiological phenomenon, pregnancy affects and changes the parameters of maternal homeostasis for a certain period, requiring specific adaptive biological efforts. Thus, in the situation of the existence of a maternal pathology pre-existing the pregnancy, this or the necessary treatment for it may present a risk for the fetus or for the physiological development of the pregnancy. There is also the possibility that the onset of pregnancy aggravates the maternal pathology.

The main maternal pathologies include hypertension (preexisting or triggered during pregnancy), diabetes (preexisting or installed during pregnancy), anemia, neuropsychiatric disorders, asthma (and respiratory pathology), but also infectious pathology.

The pathology of pregnancy also includes abnormalities related to the ovulatory elements. Thus, polyhydramnios is part of the pathological processes of the amniotic

fluid, being the excess volume of this fluid, and which predisposes to numerous perinatal, maternal and fetal complications. Only 20% of cases of pathological excess of amniotic fluid are related to congenital anomalies, up to 70% of cases are idiopathic. Oligohydramnios is the decrease in the volume of amniotic fluid and is easily monitored by ultrasound, as it has a specific curve during pregnancy, with an initial progressive increase, followed by a plateau period - about 400 ml from 36 weeks to term, starting to decrease after the 40-week threshold.

According to the 2016 Amsterdam Consensus, four placental lesion patterns are currently recognized: maternal vascular malperfusion, fetal vascular malperfusion, acute chorioamniotitis and villitis of unspecified etiology.

3.2. Fetal mortality. Perinatal mortality. Thanatogenetic mechanisms.

Fetal mortality - intrauterine death of a fetus, regardless of gestational age
- currently represents a major public health problem, globally. In the United States alone, in the year 2021, the fetal mortality rate was 5.73 deaths at a gestational age of more than 20 weeks of amenorrhea, compared to 1000 live births. In 2022, compared to the previous year, the figure decreases by a percentage of 5%, up to a value of 5.45%.

Neonatal mortality includes all deaths of newborns in the first 28 days of life. The period is recognized to be of maximum vital vulnerability, the first 7 days being known as the early neonatal period. In 2013, 36% of neonatal deaths globally occurred in the first 7 days of life, with 35% of these occurring in the first 24 hours postpartum compared to 27% of deaths occurring between the 7th and the 27th day of life. However, a significant decrease in the neonatal mortality rate is noted, with 19.5 deaths per thousand live newborns: from 36.8 in 1990 to 17.3 in 2022.

The main intrauterine thanatogenetic mechanism is the reduction of the amount of oxygen available to the fetus. The underlying causes of fetal asphyxia are varied, but the most frequently blamed are: maternal hypertensive disorders, premature detachment of the normally inserted placenta, accidents related to the umbilical cord (especially vascular ruptures in the case of velamentous insertion), prolonged labor and/or the presentation pelvic, frank (buttocks forward) or full (legs forward).

The main thanatogenetic mechanisms incriminated in the case of neonatal deaths are also infections and asphyxia, which are joined - without being able to name a mechanism - prematurity. During the last decades, neonatal mortality has decreased significantly, especially in developed countries, based on the decrease in the prevalence of some

conditions as well as the improvement of diagnosis and therapies available for other conditions.

3.3. The thanatogenetic impact of prematurity. Implications of surfactant pathology in fetal thanatogenesis.

The main causes of neonatal death in premature patients include: respiratory distress syndrome (RDS) due to surfactant deficiency, intraventricular cerebral hemorrhage, necrotizing enterocolitis and various infections, but also hypothermia or jaundice.

Various studies conducted have established the existence of predictive factors of the risk of death, among which are: gestational age, APGAR score, neonatal respiratory distress syndrome, perinatal asphyxia and malformative pathology.

Pulmonary surfactant deficiency is a determinant factor, clearly documented in the literature, for neonatal respiratory distress syndrome, which is a leading cause of fetal morbidity and mortality. An insufficient amount of surfactant will directly translate into reduced lung compliance and increased surface tension, with the risk of post-expiratory alveolar collapse. Thus, the hematoma surface and the alveolo-capillary diffusion capacity are suddenly and significantly reduced, with a hypoxic effect and with hypercapnia. Due to the development calendar of the fetal lung apparatus, the risk of developing post-partum respiratory distress is greater the lower the gestational age, so that this syndrome represents one of the main etiologies of mortality in premature infants, even after the introduction of large-scale introduction of exogenous surfactant administration.

Thus, both the deficiency of pulmonary surfactant and the deficiency of its fractions are directly involved in fetal and neonatal thanatogenesis, dependent but also independent of gestational age.

PERSONAL CONTRIBUTION

4. The motivation, purpose and objectives of the study

The motivation of the present research derives from the fact that the thorough study of the causality and of the different thanatogenerative pathogenic sequences is of fundamental importance in optimizing both the management of these patients and in optimizing the available medical resources.

4.1. Purpose and objectives of the study

In the present paper, I aim to identify new anatomic-clinical and morphological risk factors, potentially modifiable through currently available prevention means, and which are involved in the thanatogenesis of premature girls.

In order to achieve the stated goal, we have established the following general objectives:

Determination of anatomical-clinical and morphological risk factors involved in the thanatogenesis of single premature girls, necropsied between January 1, 2018 and December 31, 2022, within the Pathological Anatomy Clinical Service of the Emergency County Clinical Hospital "St. Apostle Andrew"

Determination of anatomic-clinical and morphological risk factors involved in the thanatogenesis of girls born from twin pregnancies and who died before or after birth

3) Determination of the risk potential for fetal mortality of lung surfactant fractions SP-A, SP-B and pro-SP-C.

5. Material and method

This work was carried out within the Pathological Anatomy Clinical Service of the "St. Apostle Andrei" Constanța. In two retrospective studies, the cases of single girls born prematurely and who died before or after birth and, respectively, the cases of girls from twin pregnancies and who died before or after birth in the Obstetrics-Gynecology and Neonatology departments of the same hospital, necropsied during January 1, were analyzed 2018 – December 31, 2022 within the Pathological Anatomy Clinical Service, in order to determine the causes of death, according to the actual legislation.

The autopsy procedure has three execution times: the external examination of the corpse, the examination after its opening and sewing. The external examination is carried out in a general manner and then by regions, it involves the observation of cadaveric changes and the constitutional type. After opening the corpse, the autopsy of the following cavities is performed: cranial and spinal canal, mouth and neck region, thoraco-abdominal, pelvic, upper and lower limbs.

Tissue sections for histopathological examination under routine hematoxylin-eosin (HE) staining were harvested from meninges, brain, lungs, heart, stomach, mesentery, intestines, liver, spleen, kidneys, and adrenal glands.

The parts were macroscopically described and processed, to obtain the final result: the slide stained with Hematoxylin-Eosin.

The aspects evaluated macroscopically were: color, dimensions, consistency, presence or absence of lesions, homogeneous or non-homogeneous appearance of parechymatous organs, permeability of cavity organs, presence or absence of areas of hemorrhage and necrosis. In case of macroscopic detection of lesions, each lesion has color, dimensions, consistency, delimitation, homogeneous or non-homogeneous appearance have been described.

The slide prepared in this way is examined microscopically to evaluate the organ structure and, respectively, the morphological class of the lesional processes, as well as their histological localization, dimensions, histological type of the lesion, architecture, possible presence and severity of cyto-nuclear atypia, presence or absence hemorrhage, presence or absence of necrosis.

Following the evaluation of the microscopic preparations, we selected the most representative paraffin blocks for immunohistochemical tests. Paraffin blocks were sectioned on a microtome at 4 μm thickness. Markers were used: SP-A, SP-B and pro-SP-C from NovusBio (Bio-Techne, USA). Each set performed was accompanied by positive control slide. The working protocols used in making the immunohistochemical slides are as follows:

Statistical data analysis was performed using SPSS version 26 (IBM Corporation, USA) and Microsoft Office Excel 2019 (Microsoft Corporation, USA). Indicators of central tendency (arithmetic mean, median) and dispersion indicators (dispersion and standard deviation) were used.

6. Study 1 – Risk factors involved in premature deaths through the lens of thanatogenic processes

6.1. Introduction

Preterm births include all pregnancies that end before 37 weeks' gestational age. In 2020, about 1 in 10 births was premature, resulting in more than 13 million babies born prematurely, and about 900,000 babies died from complications of prematurity.

Available data show that about 90% of preterm births occur in countries with low and middle living standards, mainly in Africa and South Asia. However, it is worth noting that with advances in assisted reproduction techniques, even well-developed countries are experiencing an increase in the occurrence of multi-fetal pregnancies. This type of pregnancy comes with an increased risk of prematurity and, consequently, associated

neonatal mortality.

Premature babies can have both short-term and long-term complications. These include breathing and feeding difficulties, but also the risk of cerebral palsy or impaired brain development throughout life. In addition, visual and hearing impairments can affect their overall prognosis. Premature infants, as a patient category, have a significant economic effect on individuals, families, and society. The survival rate of these children in developed countries is higher compared to developing countries. The difference may result from superior neonatal care infrastructure and lower levels of psychosocial inequality in higher-income countries compared to developing countries.

6.4. Results

In the period 2018-2022, a total number of 18,379 births were registered in the "Sf Apostol Andrei" County Emergency Clinical Hospital in Constanța. Of these, 17,609 were term births and 770 premature births (under 37 weeks). Out of the total number of newborns, 303 deaths were detected, of which 140 were premature girls. Of the cases of premature deaths, 85 children were born alive, and the death of these occurred before the age of one year. The remaining 55 premature babies died intrauterine, being born either by caesarean section or naturally.

Premature live births had a downward trend from 2018 (22.35%) to 2022 (16.47%).

By comparison, premature stillbirths had a steady upward trend from 2018 (12.73%) to 2022 (32.73%).

Most live infants were very preterm (VPT) neonates (64.71%), and the mean gestational age was 27.45 weeks (22–35 weeks, ± 0.37).

Most intrauterine dead boys fall into the category of very premature newborns (VPT) (41.82%), the mean gestational age being 30.73 weeks (22-35 weeks, ±0.49). For premature infants who died intrauterine, the morphometric aspects noted: mean length of 40.45 cm (24-54 cm, ±0.83), mean cranial perimeter of 28.30 cm (16-39 cm, ±0.53), perimeter mean chest girth 25.85 cm (14-39 cm, ±0.63) and mean abdominal girth 22.34 cm (11-31 cm, ±0.53).

We observed a difference between the degree of prematurity by gestational age and viability at birth. Stillborn girls had an increased gestational age (moderate prematurity and late prematurity), while liveborns had a low gestational age (extreme prematurity and very preterm neonates) ($p<0.001$). We also found a similar difference between the degree of prematurity in relation to the weight of girls and viability ($p<0.001$). A result of these aspects affects some morphometric parameters. The cranial

perimeter and the thoracic perimeter showed a higher value, in accordance with the degree of maturation and the viability of the girls ($p<0.001$ and $p=0.001$, respectively).

The mean survival time was 301.76 hours, ±80.64 (1-4380 hours), in live-born preterm infants. The most frequently found APGAR score at birth was 1 (1-8). Increased gestational age was associated with an increased APGAR score ($p<0.001$). Also, the gestational age correlated directly proportionally with the weight, length, cranial perimeter, thoracic perimeter and abdominal circumference of the girls, an aspect that denotes their harmonious development ($p<0.001$). In addition, increased weight and abdominal circumference were associated with longer survival in girls ($p=0.023$ and $p=0.002$, respectively). Unfortunately, APGAR score was not associated with survival ($p=0.722$).

Univariate analysis did not identify risk factors among demographic aspects (sex, gestational age, APGAR score, degrees of prematurity). Instead, in the multivariate analysis of the data we noted as risk factors involved in mortality extremely low birth weight (HR=5.141, $p=0.009$) and very low birth weight (HR=4.177, $p=0.018$).

Univariate analysis of morphometric data identified protective factors: length (HR=0.957, $p=0.027$), chest circumference (HR=0.937, $p=0.008$) and abdominal circumference (HR=0.922, $p=0.003$). The multivariate analysis of the morphometric aspects (weight, length, cranial perimeter, thoracic perimeter and abdominal circumference) noted as risk factors on mortality the cranial perimeter - for each centimeter HR=1.134, $p=0.018$, while the abdominal circumference remains a protective factor (for each centimeter HR=0.901, $p=0.041$).

In 45.45% of cases, mothers with intrauterine deaths had no pathological history. At the other pole, the most common maternal conditions were pregnancy-induced hypertension (27.27%), scarred uterus (10.91%), urinary tract infections (3.64%) and cervico-isthmic incompetence (3.64%).

The presence of malformations correlated with increased parity ($p=0.005$). A special aspect that we have identified is represented by the predictability of morphometric data on fetal development. A birth weight ≥1505g has increased predictability with sensitivity of 87.50% and specificity of 80.50% (AUC=0.825, $p=0.003$).

It also adds a length ≥41.5 cm (87.50% sensitivity and 84.40% specificity, AUC=0.876, $p<0.001$), ≥28.5 cm cranial circumference (87.50% sensitivity and specificity of 77.90%, AUC=0.840, $p=0.002$), chest circumference ≥25.75 cm (sensitivity of 87.50% and specificity of 77.10%, AUC=0.793, $p=0.007$) and abdominal circumference ≥22.5 cm

(87.50% sensitivity and 70.1% specificity, AUC=0.770, p<0.012).

6.5. Discussions

Globally, 15% of premature babies are born before 32 weeks, 10.4% between 28 and 32 weeks and 4.2% before 28 weeks of pregnancy. In the study by Kwasawneh W et al., the rate of prematurity was 15.7%, with the majority of deaths occurring in babies born before 28 weeks' gestational age. In this study, most preterms were born between 28-32 weeks of gestation (55.71%), followed by late preterms (15.71%), extremely preterms (15%), and moderate preterms (13.57%).

In terms of live premature births, we observed a downward trend over the five years from 22.4% to 16.5%, while in the case of stillbirths, their evolution had an upward trend from 12.7% to 32.7%. The data from this research is consistent with the currently available literature on the topic, although this study does not cover the COVID-19 pandemic. There are many European studies on the link between this pathology and the pandemic years, and most of them indicated a decrease in prematurity rates during the pandemic, perhaps suggesting an impact of emergency measures.

Many variable factors associated with the mother may be related to the risk of preterm birth, including socioeconomic and demographic characteristics, behaviors and habits, health status, biological and reproductive characteristics, and pregnancy complications. Regarding health status, we observed statistically significant differences between maternal pathology and viability of preterm infants. Thus, the presence of gestational diabetes and uterine cerclage were correlated with premature live births, while the other pathologies were associated with premature stillbirths.

Also, over time, other maternal risk factors have been identified, the consequence of which is premature birth, these being: age, ethnicity, primiparity, dietary factors and lifestyle - smoking, chronic alcohol consumption, access limited to obstetric care, and low levels of education.

According to the study by Schindler T et al., the main causes of death identified, regardless of the gestational age studied, were intraventricular hemorrhage, acute respiratory pathology and sepsis. In their study, the most important factor predicting death was the number of weeks of pregnancy at which delivery occurred for all identified causes of death. Similarly, in the present study, we observed similar causes of death, the most common being hemorrhages of different location and acute respiratory pathology.

In the study by Honein MA et al., malformations were twice as common in preterm compared to fetuses born after 37 weeks of gestation, and in the case of very

preterm babies, the frequency of malformations was more than 5 times higher in the contrast of term births, the most important associations being with damage to the central nervous system and the cardiovascular system. Regarding the connection between this category of pathologies and moderate prematurity, the strongest associations that the authors identified were with defects of the central nervous system and the gastrointestinal tract (especially with esophageal atresia and small intestine). In this study, the brain (cerebellar hypoplasia, Dandy-Walker syndrome, total holoprosencephaly) and cardiovascular (atrial and ventricular septal defect) malformations identified contributed to the death of the infants through the determined hypoxic consequences. Moreover, we observed a pattern of anthropometric data in malformed children. Thus, the morphometric data that should raise suspicion for the presence of a malformation are low or normal birth weight, late and average prematurity (MPT), a cranial circumference ≥28.50 cm, a chest circumference ≥25.75 cm and a circumference abdominal ≥22.50 cm.

Abdominal circumference is one of the most reliable predictive factors of fetal growth, being one of the evaluation parameters of intrauterine growth restriction (IUGR). Thus, to make this diagnosis, its value must be below the 10th percentile. Moreover, one of the risk factors for IUGR is maternal hypertension, a comorbidity also found in the present study. In addition, changes in the middle cerebral arteries with decreased oxygen pressure are noted during Doppler ultrasound evaluation. Also, hypoxia underlies the phenomena of vasodilatation, with the aim of increasing the blood supply at this level. On the other hand, the factors influencing the occurrence of intraventricular hemorrhage in premature infants are hypoxia and vasodilatation. This constellation of events may explain the correlation between low abdominal circumference and intraventricular hemorrhage identified in the present study. Also, in this research, we identified the association between low abdominal circumference and brain atrophy. A possible explanation lies in the development of hydrocephalus in patients with IUGR. On the other hand, according to the study by Lemmers PM et al., a prolonged suboptimal regional oxygen saturation was associated with a decrease in brain volume.

6.6. Conclusions

The number of premature babies born alive followed a downward slope during the 5 years of the study, from 22.35% to 16.47%, while the number of premature babies who died intrauterine was ascending during the same period, from 12.73% to 32.73%. 6.6.9. Univariate analysis of morphometric parameters identified as protective factors: length at

birth (HR=0.957, p=0.027), chest circumference (HR=0.937, p=0.008) and, respectively, abdominal circumference (HR=0.922, p=0.003) . Multivariate analysis of morphometric parameters identified cranial circumference as a risk factor for mortality, for each centimeter HR=0.901, p= 0.041.

7. Implications of surfactant proteins in the death of preterm twins

7.1. Introduction

From the point of view of incidence, singleton pregnancies are numerically dominant compared to multiple pregnancies, but the latter category is gradually becoming much better represented, mainly as a result of the increase in the use of assisted reproduction techniques (ART) , but also of maternal ages. Thus, the European average rate in 2010 was 16.8 twin births per 1000 births, with significant interstate variability.

Most of the studies carried out to date on multiple gestations have focused on twin pregnancies and have shown the joint involvement of genetic and environmental risk factors in the etiopathogenesis of these diseases, but the differences between singletons and twins born at the same gestational age and especially the differences between the evolution of the members of the same pair are currently not sufficiently studied and, respectively, characterized in terms of causality.

Lung immaturity, anatomical and functional, of premature newborns, translated by errors in surfactant production, is the primary etiology of respiratory distress syndrome (RDS). At the same time, the hypothesis that a quantitatively and/or qualitatively deficient surfactant has a significant impact also on pulmonary infectious susceptibility, with echoes on neonatal morbidity and mortality, is emerging more and more clearly.

7.4. Results

During the study period, from the total number of pediatric necropsies (n = 303), the autopsy files of the cases of premature newborns from twin pregnancies were analyzed (n= 12).

Mean maternal age at delivery was 27.25 ± 1.69 years (range, 21–38 years).

Fetal length at birth was shorter in the population of preterm twins born to multiparous multiparous mothers compared to those born to nulliparous primigravida.

Only one case of a premature twin fetus presented a congenital malformation, represented by giant encephalocele, associated with anal imperforation and facial dimorphism. Four of the six stillborn boys were born to multiparous women under the age

of 30.

The histopathological examination of the lung parenchyma taken from the patients included in the study revealed frequent lung changes: inflammatory, hemorrhagic and atelectatic. Meningeal and renal hemorrhages, circumscribed purulent collections (in live births), and congenital malformations have also been reported.

All cases presented pulmonary lesions, and 8 of the patients were associated with extrapulmonary lesions. The most frequently diagnosed were pulmonary hemorrhage in 58.33% of cases and meningeal and renal hemorrhage in 50% of cases.

Immunohistochemical examination to SP-A illustrated a negative response in 6 cases, a weakly positive response in 4 cases, and a strongly positive response in 2 cases.

Regarding SP-B immunoreactivity, 4 cases (33.33%) had a negative reaction, 3 cases (25%) were intensely positive in the areas of leukocytic alveolitis and pulmonary atelectasis, and the rest of the cases were weakly positive (41.67%).

Lung tissue immunolabeling with pro-SP-C was negative in 7 cases (58.33%) and weakly positive in 5 cases (41.67%), respectively. Intense focal positivity was not objectified.

Following the analysis of the obtained data, a positive correlation was found between SP-A and the diagnosis of bronchopneumonia ($\rho=0.695$; $p<0.05$) and, respectively, between SP-A and the APGAR score at 5" ($\rho=0.605$; $p<0 .05$). It was also found that SP-B immunomarking was positively correlated with pulmonary hemorrhage ($\rho=0.678$; $p<0.05$) in the studied group.

7.5. Discussions

SP-A has a role in tubular myelin formation, surface film formation, and contributes to the inflammatory response by binding to microbial pathogens invading the lungs, essentially in three different physiological processes of major importance: the decrease in pulmonary surface tension at the air-liquid interface alveolar, coordinated activity with that of other surfactant proteins, regulation of surfactant homeostasis and in innate, non-specific immunological processes at this level. SP-A is also found in small amounts in extrapulmonary sites such as the small and large intestine, mesentery, epithelium, salivary glands, prostate, thymus, amniotic fluid, and lacrimal apparatus.

SP-B, a member of the saposin-like family (SAPLIP), composed of low molecular weight proteins, having four or five amphipathic α-helices, stabilized by 3 intramolecular disulfide bridges involving 6 cysteines and, respectively, one more that will form the

bridge intermolecular disulfide of the covalent homodimer of 18 kDa is produced in Clara cells in the form of its precursor - pro-SP-B - and in type II alveolar cells in mature form.

SP-C is the only surfactant protein that is produced exclusively in

lungs by type II alveolocytes. Biochemically characterized as a monomer with a molecular weight of approximately 4.5 kDa and 35 residues forming a single α-helix, 37 Å long, whose central segment (23 Å) is intensely hydrophobic and spans thickness of a bilayer of dipalmitoylphosphatidylcholine with transmembrane orientation, SP-C has a role in the formation and stabilization of the surfactant film, decreasing the risk of alveolar collapse, as well as in the stabilization of intermembrane contacts. SP-C does not interact with SP-A and is not essential for the formation of myelin tubes, but acting synergistically with SP-B, the two surfactant proteins are responsible for the correct arrangement of functional membrane networks in surfactant complexes.

SP-D is a molecule with predominantly pulmonary localization, on the apical luminal surface of the respiratory epithelium, being secreted mostly by type II alveolocytes, but also, much less, by the so-called "club cells" - non- ciliated. In a significantly reduced amount compared to the level of pulmonary secretion, SP-D is also found extrapulmonary, at the integumentary level, in the parotid, lacrimal and sweat glands, at the level of the gallbladder and bile ducts, in the pancreas, esophagus, stomach and small intestine, kidneys and tract urinary, adrenal cortex and anterior pituitary, in the endocervical glands and in the seminal vesicles.

The results of the present study showed that the incidence of SDR is inversely proportional to GA, a fact confirmed by the data available in the specialized literature, where approximately 98% of newborns with a gestational age between 22-24 weeks develop this syndrome, but the percentage decreases to 25% for premature babies weighing between 1251g and 1500g, corresponding to higher gestational ages. Although it is a very common condition in this category of patients, not every respiratory distress in a premature baby less than 34 weeks is RDS, there are other conditions that can "mimic" this syndrome, such as meconium aspiration, intrapulmonary retention of fluid, pulmonary hypoplasia or persistent pulmonary hypertension. Early diagnosis is essential, as RDS worsens rapidly postpartum, threatening the vital prognosis through progressive respiratory failure.

The immunohistochemical examination showed a negative or weakly positive response to SP-A, SP-B and pro-SP-C in the majority of cases in the studied group (10 out of 12; 83.33%), in accordance with the data from the specialized literature. For example, Khoor et al., show in his study that, at the bronchiolar level, immunoreactivity for SP-A

appeared only in full-term newborns, while at the level of the epithelium of the terminal tracts, this protein was immunohistochemically detectable after 20 weeks of gestation. Studies in laboratory mice show that, although structural lung development is normal, mice die postnatally in the absence (or severe deficiency) of SP-B.[176] On the other hand, not only are SP-C levels decreased in preterm infants with respiratory distress syndrome, but ventilation with elevated oxygen concentrations further decreases SP-C expression.

In this study, 11 of 12 (91.66%) preterm twins were born with low birth weight, of which 7 were ELBW (<1000 g). Birth weight was not correlated with a specific surfactant protein deficiency, but was directly related to mortality. These results are consistent with previous studies showing that in infants with a birth weight <2500 g, the risk of death was approximately 200 times higher compared with infants with a normal birth weight.

Of the population of deceased preterm twins we analyzed, 8 of 12 (75%) were female. Studies have shown that male females have a higher - approximately double - risk of developing SDR, compared to female exponents of the same gestational age, a fact noted in the specialized literature under the name of"male disadvantage". This difference is not limited to small gestational ages or low neonatal weights, but can also be objectified in early prematurity or in full-term girls.

During the third trimester of pregnancy, the fetus is exposed to the mother's hormones, estrogen and progesterone. Estrogen has a positive effect on surfactant synthesis and SP-A and SP-B secretion by increasing the number of type II alveolar cells. But fetal exposure is interrupted in case of premature parturition. Correlating this fact with studies that have shown an increase in the concentration of surfactant phospholipids in the bronchoalveolar lavage of laboratory animals following maternal administration of estradiol, the greater natural exposure of the female fetus to estradiol may be a significant cause to explain,, the male disadvantage". In the present study, this disadvantage was not revealed. Regarding twin pregnancies, it was found that when one of the twins is female, the prognosis of the other twin and, respectively, of the pregnancy of the pregnancy are improved, an aspect also confirmed by the present research, by the fact that the average VG was lower in boy-boy pairs than in boy-girl and girl-girl pairs.

In this study, all live births had a 5-minute APGAR score <7, and a significant positive association was found between negative SP-A expression and a 5-minute APGAR score. These results were also previously observed in the study by Chambliss et al. which objectified the fact that an APGAR score at 5 minutes of ≤7 constitutes a risk factor for the development of SDR. These data have been confirmed by studies conducted

in different countries, such as Great Britain, USA, Cameroon, China and even Addis Ababa, premature newborns with APGAR greater than 7 at 5 minutes and having a significantly more favorable evolution. This was attributed to the fact that the APGAR score actually illustrates the degree of neonatal asphyxia, correlated with a reduced number of red blood cells, a fact that interferes with postpartum cardio-respiratory adaptation.

In the setting of in-utero fetal death, the role of surfactant in reducing the surface tension at the alveolar air-liquid interface does not have the necessary biological context to manifest itself. In this situation, a qualitative and/or quantitative deficiency of surfactant will have an impact on the immune protective functions, and apparently, on the induction of preterm labor. Inflammatory ligands (such as interleukin 1-IL1) are inducers of preterm labor, but surfactant modulates severe inflammatory responses in the fetal compartments (and not only), ameliorating them through the function of moderating inflammatory reactions. On the other hand, various transgenic experiments and other genetic evidence suggest that, in humans, surfactant proteins A, D or C (SP-A, SP-D, SP-C), expressed in fetal tissue, influence the time of onset of labor - at term or premature, so structural integrity and quantitative amount would have a significant role in the antenatal period as well. In this research, four stillborns were SP-A negative, 2 SP-B negative and 5 pro-SP-C negative.

Several other studies have shown that SP-A deficiency increases apoptosis and decreases cell viability, induces an inflammatory reaction, and causes acute lung injury. Pulmonary hemorrhage is frequently associated with RDS, possibly because of high capillary pressure caused by hypoxia, heart failure, and volume overload. Following endothelial injury, neutrophils are released, which will lead to increased levels of proteases, cytokines, and oxygen free radicals. These components will damage alveolar type II cells that produce SP, resulting in lower levels of surfactant proteins. On the other hand, exposure of preterm neonates with RDS to mechanical ventilation is another important risk factor for pulmonary hemorrhage. According to the study by Wiswell et al., pulmonary hemorrhage is a condition that aggravates the condition of 3-5% of preterm infants with severe RDS undergoing mechanical ventilation techniques. The pathophysiological justification implies that in pulmonary hemorrhage, the components of the exudate (hemoglobin, plasma proteins, membrane lipids) can lead to the inactivation of the endogenous surfactant, inopportunely pulmonary mechanics. Thus, the intake of exogenous surfactant would have a potential utility, and the aspect is equally valid for

other lung aspirates, such as, for example, meconium, describing in the literature the presumption that exogenous surfactant plays a therapeutic role in such situations . In the present study, we observed a statistically significant, positive correlation between SP-A expression and pulmonary hemorrhage. Of the seven cases with pulmonary hemorrhage studied, five of them showed negative expression of SP-A.

Hemorrhagic disease of the newborn is one of the main threats to the prognosis of premature girls, the occurrence and extent of these lesions being inversely proportional to gestational age and birth weight, respectively. The basis of the phenomenon is the cumulative effect between the immaturity of the capillary bed and that of the coagulation system, possibly adding a wide range of other risk factors, including respiratory distress syndrome, with associated cellular asphyxia, exposure to mechanical ventilation, sepsis. The most frequent extrapulmonary hemorrhagic lesions that we identified in this study are renal (41.66%) and adrenal (50%), followed by meningeal and plurivisceral hemorrhage. Of the 3 surfactant proteins we analyzed, SP-A was the only one that correlated with extrapulmonary hemorrhage.

7.6. **Conclusions**

Mean maternal age at delivery was 27.25 ± 1.69 years (21–38 years), with mean gestational age 8.17 ± 1.06 weeks (23–36 weeks). Birth weight ranged from 600 to 2900 g, with a mean of (1132.5 ± 189.03 g). 91.66% of cases were born with low birth weight, most being classified as ELBW. Histopathological examination revealed pulmonary lesions in all studied cases and extrapulmonary lesions in 8 of the 12 patients. At the pulmonary level, we identified inflammatory, hemorrhagic changes and pulmonary atelectasis. Extrapulmonary, the main diagnoses were represented by: meningeal hemorrhage, renal hemorrhage, circumscribed purulent collections - abscesses (in live births), congenital malformations. The expression of the SP-A surfactant protein was strongly positively correlated with the APGAR score at 5 minutes ($\rho = 0.605$; $p < 0.05$). The expression of surfactant protein SP-A was positively correlated with the presence of bronchopneumonia ($\rho = 0.695$; $p < 0.05$). The immunohistochemical expression of SP-B was positively correlated with the presence of pulmonary hemorrhage ($\rho = 0.678$; $p < 0.05$).

8. Conclusions

In the present research we identified a total number of 18379 births, of which 770 were premature, with a number of 140 deaths in singleton pregnancies and 12 deaths in twin pregnancies. We have observed that prematurity is a frequent phenomenon in singleton pregnancies and almost ubiquitous in multifetal pregnancies. Most cases of fetal-neonatal mortality are directly related to prematurity, as a consequence of structural and functional lung immaturity and, respectively, as a result of increased susceptibility to infections.

The main conclusions, which we have identified and transposed in the present work outline a complete morphological picture of premature babies who died perinatally. We identified pathophysiological correlates that can be used as prognostic factors, and ascertained and characterized the impact of the presence/absence of different surfactant protein fractions in fetal lung tissue.

These are the following:

1. For the study of single, premature, perinatally deceased girls:

-The progressive decrease in the frequency of deaths of premature babies born alive and, respectively, the increase in the frequency of stillbirths, highlights the current optimization of neonatal care and requires more frequent follow-up of cases with maternal pathology that threatens the vital prognosis of girls;

- The identification of a pattern of morphometric data in association with the presence of malformations leads to the establishment of some value thresholds of fetal development, when exceeding which the indication of genetic testing for the identification of some malformative syndromes becomes essential;

- Analysis of morphometric aspects identified cranial circumference as a predictive factor of mortality risk, by association with death diagnoses (such as intracranial hemorrhage), while abdominal circumference appears to be a protective factor.

2. Following the study of the immunohistochemical expression analysis of the surfactant protein fractions, we found that:

- The expression of the surfactant protein SP-A was positively correlated with the APGAR score at 5" and with the presence of bronchopneumonia, and the immunohistochemical expression of SP-B was positively correlated with the presence of pulmonary hemorrhage, both suggesting a possible functional deficiency of these fractions in the role of immunological modulation .

- 83.3% of stillbirths included in the study showed negative immunohistochemical expression for pro-SP-C, suggesting that the absence of this fraction is involved in other thanatogenerative mechanisms than SDR .
-We identified as risk factors for SDR caused by surfactant protein deficiency, the following parameters: low birth weight, birth weight and anthropometric data, GA, low APGAR score at 5 minutes, multiparity and female gender of the fetuses.

3. Respecting the aspects resulting from the research, I consider it essential to implement the following preventive and therapeutic measures, thus:
- design, organization and implementation of mass information programs for the general population regarding preterm birth and associated fetal risks.
- designing, organizing and implementing screening programs for risk factors for premature birth among pregnant women.
- - the implementation of monitoring protocols with increased frequency in the case of patients identified with risk factors for premature birth (multigravida-multiparous, over 30 years old, subject to assisted reproduction techniques, with pre-existing or pregnancy-related pathologies) that lead to administration of specific therapies as early as possible to prolong gestation and ensure fetal viability.

9. The originality and innovative contributions of the thesis

The originality of the present research derives from the way of approaching the studied topic, the detailed and complex statistical analysis - which includes gestational, fetal and maternal parameters - joining it with a highly specific analysis - the immunolabeling of surfactant protein fractions - on a batch characterized by a special feature: the origin of the patients from twin pregnancies.

After studying the specialized literature, I noticed that at the national level this pathology was not researched in such a way, without identifying multi-case studies, carried out post-mortem of twin patients or from multiple pregnancies. At the international literature level, the paucity of studies on this specific segment of patients is notable.

The results of the presented studies have great addressability both for multidisciplinary medical practice - obstetricians, neonatologists, pulmonologists, anatomopathologists, imagers - and for the scope of future research, highlighting the essential role of the complex approach and interdisciplinary collaboration both in clinical

and diagnostic approaches, as well as for the representativeness of future research.

The future research perspectives deriving from the present work are represented by: retrospective or prospective comparative studies on the fractions of surfactant proteins in premature infants from single and multiple pregnancies, on the fractions of surfactant proteins in women who died perinatally, of any gestational age, prospective studies that aim to establish possible correlations between surfactant protein deficiency and placental pathology.