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Unraveling the Complexities of Fetal Growth Restriction: Insights into Management and Placental Pathology Mechanisms

ABSTRACT

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ABSTRACT

Fetal Growth Restriction (FGR) is a complex condition with profound implications for both maternal and fetal health.

This doctoral thesis aims to unravel the intricacies of FGR by investigating various aspects related to its management, placental pathology, and the role of ultrasonography. Through a comprehensive review of the existing literature, this thesis provides an in-depth understanding of FGR's definition, classification, prevalence, etiology, pathophysiology, clinical manifestations, diagnosis, and management.

The scientific objectives of this doctoral research are multifaceted and encompass several key areas of investigation. Firstly, we strive to examine the influence of appropriate delivery timing on neonatal complications in fetuses with early-onset FGR. This objective aims to shed light on the optimal timing for delivery, considering its impact on neonatal outcomes. Secondly, we aim to delve into the placental pathology associated with early-onset FGR, with specific emphasis on its correlation with preeclampsia. This investigation seeks to unravel the intricate relationship between placental dysfunction and the development of early-onset FGR, contributing to our understanding of its underlying mechanisms. Lastly, we endeavor to establish correlations between placental histopathological and immunohistochemical changes and the occurrence of preterm birth in cases of FGR. This objective holds paramount significance in comprehending the links between placental alterations and adverse pregnancy outcomes.

The Special Part of this thesis comprises three independent personal studies, each dedicated to distinct aspects of FGR.

Study 1 investigates the impact of appropriate delivery timing in early-onset FGR on neonatal complications, guided by Doppler parameters.

This study aims to assess the probability of neonatal complications contingent upon the timing of birth in fetuses with early-onset FGR, utilizing Doppler parameters as a foundation. Notably, the time of delivery for early-onset FGR cases is primarily determined based on Doppler parameters, meticulously considering the potential risks and benefits for the infant.

In light of this objective, a case-control study was carried out at the Obstetrics Clinic of the Municipal Emergency Hospital of Timisoara during the period spanning 2018 to 2022, encompassing 205 consecutive pregnant women diagnosed with early-onset FGR. Subsequently, participants were categorized into two distinct groups, namely the "cases" and "control" groups, based on the management (time of delivery) of FGR.

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- Case group: 1) Infants diagnosed with early onset FGR; 2) Infants delivered at onset of UA—reversed end-diastolic flow (REDF) (in fetuses under 30 weeks of gestation) or onset of UA—absent end-diastolic flow (AEDF) (in fetuses between 30 and 34 weeks of gestation).
- Control group: 1) Infants diagnosed with early onset FGR; 2) Infants delivered at onset of absent or reversed DV a-wave (in fetuses under 30 weeks of gestation) or onset of UA—REDF (in fetuses between 30 and 34 weeks of gestation).

The results demonstrate that neonates delivered at the occurrence of umbilical artery absent or reversed end-diastolic flow exhibit a reduced likelihood of developing grades III/IV intraventricular hemorrhage and bronchopulmonary dysplasia. This underscores the critical significance of optimal delivery timing in mitigating neonatal complications in cases of early-onset FGR. The most prevalent Doppler abnormality observed in fetuses with early-onset FGR was absent end-diastolic flow (AEDF) in the umbilical artery, and it was notably more frequent in the case group. Moreover, fetuses included in the control group exhibited a significantly higher incidence of abnormal cardiotocography.

The overall neonatal mortality rate was found to be 2.0%, and there was no statistically significant difference between the two study groups ($p = 0.19$). Additionally, no significant distinction was observed in the rates of grades I–II intraventricular hemorrhages (IVH) between the two groups ($p = 0.41$). However, participants included in group two (control) displayed a higher occurrence of bronchopulmonary dysplasia and grades III–IV intraventricular hemorrhages (IVH).

Gestational age emerged as a significant factor influencing the occurrence of neonatal complications in infants with FGR, with the majority of complications being reported in those born before 30 weeks of gestation.

Among infants delivered up to 30 weeks of gestation (WG), grades III/IV intraventricular hemorrhages and bronchopulmonary dysplasia were found to be statistically significantly more frequent in the control group. Conversely, grades I/II intraventricular hemorrhages were more frequently

observed in fetuses whose delivery time was at the onset of umbilical artery (UA) AEDF/REDF.

The results of the univariate binomial logistic regression analysis on fetuses born under 30 weeks of gestation (WG) indicate that those included in the control group were 30 times more likely to develop bronchopulmonary dysplasia ($p = 0.002$) and 14 times more likely to develop IVH grades III/IV ($p < 0.001$).

The differences in the likelihood of developing grades III/IV IVH and bronchopulmonary dysplasia continue to be significantly higher in the control group even after adjusting for gestational age at birth.

Moreover, a total of 120 newborns were delivered between 30 and 34 weeks of gestation (WG). Among these newborns, there were no reported neonatal deaths, bronchopulmonary dysplasia, or cases of necrotizing enterocolitis. However, 31 newborns did develop intraventricular hemorrhage, with 29 of them being classified as grades I/II and the remaining 2 as grades III/IV. Notably, grades I/IV intraventricular hemorrhage was found to be statistically significantly more common in newborns delivered at the occurrence of absent end-diastolic flow in the umbilical artery ($p < 0.0001$). Furthermore, grades III/IV intraventricular hemorrhage occurred exclusively among newborns included in the control group (those delivered after the occurrence of reversed end-diastolic flow in the umbilical artery).

The results of univariate logistic regression revealed that the likelihood of developing grades I/II intraventricular hemorrhage was 7.76 times higher among newborns whose delivery was delayed at the time of the occurrence of reversed end-diastolic flow (REDF) on the umbilical artery (UA) compared

to those born at the time of the onset of UA absent end-diastolic flow (AEDF). Moreover, the multivariate logistic regression analysis demonstrated a 14-fold higher odds of developing IVH grades I/II in infants included in the control group, even after adjusting for gestational age.

In conclusion, Newborns with gestational age (GA) less than 30 weeks, delivered at the presence of umbilical artery (UA) absent or reversed end-diastolic flow (AEDF/REDF), before the onset of absent or reversed ductus venosus (DV) a-wave, demonstrate a reduced likelihood of developing grades III/IV intraventricular hemorrhage and bronchopulmonary dysplasia. Given the significant advancements in neonatal care for preterm infants, our findings support the conclusion that the optimal time for delivery of infants with GA less than 30 weeks and early-onset FGR is at the occurrence of UA-AEDF/REDF. Additionally, for FGR infants with GA greater than 30 weeks, delivery at the occurrence of UA-AEDF is associated with a decreased risk of early neonatal complications compared to infants whose birth was delayed until the occurrence of UA-REDF.

Study 2 delves into placental pathology in early-onset FGR, with a specific focus on its association with preeclampsia.

The objective of this study was to conduct a histopathological (HP) examination of placental pathology in pregnancies affected by early-onset fetal growth restriction (FGR), with a specific focus on conducting a comparative analysis between cohorts with and without preeclampsia (PE).

To achieve this objective, a cross-sectional study was conducted on 85 consecutive women diagnosed with fetal growth restriction (FGR) who

delivered at the Municipal Emergency Hospital in Timisoara, Romania, between the years 2020 and 2021.

The study included 85 pregnant women diagnosed with early-onset fetal growth restriction (FGR). The participants had an average age of 29.72 years, and the median gestational age (GA) at the diagnosis of FGR was 31 weeks. Placental measurements revealed a median weight of 261 g, with a median maximum diameter of 15 cm, and a fetal-to-placenta weight ratio of 4.9. Significant differences were observed among the groups in various placental measurements. Specifically, pregnancies with preeclampsia (PE) and early-onset FGR exhibited diminished placental weight (242 g vs. 289 g; $p<0.001$), reduced maximum diameter (15 cm vs. 16 cm; $p<0.001$), and decreased minimum diameter (10 cm vs. 11 cm; $p<0.001$).

Furthermore, the volumetric analysis of the placenta indicated a notably lower placental volume in cases of PE and early-onset FGR (325 cm3 vs. 365 cm3; $p<0.001$). Notably, there were no statistically significant variations observed in the maximum and minimum placental plaque thickness, as well as the fetal/placental ratios, between the investigated groups.

Among the discerned placental lesions in pregnancies complicated by early-onset fetal growth restriction (FGR), as delineated by the Amsterdam Placental Workshop Group Consensus Statement, are retroplacental hemorrhage, accelerated villous maturation, placental infarctions, and fibrin deposits associated with calcification.

Furthermore, the immunohistochemical (IHC) analysis revealed the presence of para-villous B-lymphocytes, identified through staining with an

anti-CD20 antibody, as well as T-lymphocytes, detected via staining with an anti-CD3 antibody. Additionally, paravillous and intravillous macrophages, along with intravillous mast cells, exhibited positive immunoreactivity upon staining with an anti-tryptase antibody in normal placental villi.

Moreover, the presence of perilesional macrophages was observed in infarcted placental villi, as confirmed by staining with an anti-CD68 antibody. Furthermore, placental villi displaying positive immunoreactivity for anti-HIF and anti-VEGF antibodies were noted in placental infarcted villitis.

Maternal vascular malperfusion was observed in a significant proportion of cases, with an overall rate of 67.05%. The most common manifestations were increased syncytial knots (81.2%), followed by infarctions (67.05%). Among the infarctions, late infarctions were more prevalent, accounting for 44.6% of overall cases. Specifically, the incidence of infarctions was higher in the group with both preeclampsia (PE) and early-onset fetal growth restriction (FGR) compared to the normotensive group (83.7% vs. 44.4%; $p=0.015$). Furthermore, the group with both PE and early-onset FGR had a higher proportion of late infarctions compared to the normotensive group (58.1% vs. 27.8%; $p<0.001$). Regarding syncytial knots, both mildly increased (15–30%) and severely increased (≥30%) proportions were assessed. Mildly increased syncytial knots were observed in 40.0% of overall cases, with comparable rates between the group with both PE and early-onset FGR and the normotensive group (36.7% vs. 44.4%; $p=0.50$). However, severely increased syncytial knots were found in 41.2% of overall cases, with a higher prevalence in the group with both PE and early-onset FGR compared to the normotensive group (51.0% vs. 27.8%; $p=0.04$).

Regarding lesions associated with fetal malperfusion, the only identified lesion was thrombosis, detected in 11.7% of cases, with no significant difference between the group with both PE and early-onset FGR (8.2%) and the normotensive group (16.7%).

Furthermore, the logistic regression analysis revealed significant associations for certain placental findings. The presence of infarctions in the early-onset FGR placentas was significantly associated with PE, with an odds ratio (OR) of 6.40 [95% confidence interval (CI): 2.35–17.46, $p<0.001$] (Table 15). Similarly, late infarctions also showed a significant association, with an OR of 3.467 (95% CI: 1.37–8.72, $p=0.008$). These findings suggest that the occurrence of infarctions, particularly late infarctions, increases the likelihood of PE.

In conclusions, the consistent identification of maternal malperfusion-related lesions in these patients highlights their significant role in the pathogenesis of early-onset FGR. Moreover, our IHC analysis demonstrated a robust expression of VEGF in infarcted villi, suggesting its involvement in the vascular remodeling processes associated with placental dysfunction. The presence of infarctions, especially late infarctions, showed a strong association with the occurrence of PE, suggesting a potential link between these placental lesions and the development of PE in early-onset FGR cases. These findings contribute valuable insights into the underlying mechanisms and potential biomarkers involved in the pathogenesis of early-onset FGR.

The objective of the third study in this thesis is to establish a correlation between placental histological and immunohistochemical (IHC)

changes and preterm birth (PB) in the context of fetal growth restriction (FGR), comparing these placental changes with those observed in normal term pregnancy (TP). Microscopic structural and vascular placental changes found in singleton TP or prematurity have been associated with coexisting maternal and fetal clinical features.

The study included a group of 30 parturients with singleton gestation. Among them, 15 patients delivered at term, after 37 weeks of pregnancy, while the remaining 15 patients gave birth prematurely, between 32 and 35.6 weeks of gestation. The participants were hospitalized and gave birth in the Department of Obstetrics and Gynecology at the Emergency County Hospital, Craiova, and the Department of Obstetrics and Gynecology at the Filantropia Municipal Hospital, Craiova, Romania, during the period from January 2020 to January 2022.

The placental macroscopic aspects of both the term birth (TB) and preterm birth (PB) groups highlighted the presence of two placental surfaces (maternal and fetal side), the existence of amniotic membranes, and the umbilical cord on the placental fetal side. The microscopic examination of placental morphology in these two groups revealed various structural changes characteristic of TB and PB.

Classical hematoxylin and eosin (HE) staining technique allowed us to observe the normal structure of placental villi, with collagen fibers stained in pink, and the presence of syncytiotrophoblast at the periphery of the villi. However, we also identified some structural changes, including variable fibrinoid deposits stained in pink, massive calcifications stained in blue-violet, syncytial knots present at the villous periphery, intravascular thrombosis

stained in red, and infarcts represented as weakly stained areas without vascularization.

In the case of TB, small areas of perivillous amyloid deposition may be present, but in the case of PB, these areas are significantly larger, both intravillous and perivillous. Additionally, in PB cases, we frequently noticed the presence of massive intravillous calcifications, syncytial knots, as well as intravillous vascular thrombosis. Using the periodic acid-Schiff with hematoxylin (PAS-H) staining technique, we highlighted intra/extravillous vascular basement membranes, particularly the massive fibrin deposits rich in glycosaminoglycans (intense pink areas).

Through the special immunohistochemical (IHC) technique, we immunolabeled the intravillous capillary endothelial cells using an anti-CD34 antibody, which stained the vessels in brown. These small vessels of neoformation helped us obtain the numerical vascular density, which was found to be higher in the control group (TB).

Through the use of an anti-VEGF antibody, we observed the presence of signal proteins that promoted and stimulated the formation of neoformation vessels in the areas affected by the lack of vascularization, specifically at the level of placental infarction.

We conducted statistical analyses that revealed a higher number of blood vessels in the placental structure of TB compared to PB. The vascular density of the placenta associated with term female newborns (TNB-F) ranged from 125.5 to 150.75 vessels/×200, with an average value of 137.32 vessels/×200 (±8.34 vessels/×200). In the case of term male newborns

(TNB-M), the vascular density ranged from 125.5 to 150.75 vessels/×200, with an average value of 138.46 vessels/×200 (±8.37 vessels/×200).

For preterm female newborns (PNB-F), the vascular density varied between 100.25 and 125 vessels/×200, with an average value of 113.27 vessels/×200 (±8.94 vessels/×200). In the case of preterm male newborns (PNB-M), the vascular density ranged from 100.25 to 123.25 vessels/×200, with an average value of 114.83 vessels/×200 (±9.36 vessels/×200). Applying the ANOVA Single Factor test, we observed a highly significant difference between placental vascular densities in these groups, $F(3.29)=18.945$, $p<0.001$.

A directly proportional increase was also observed in the comparative study regarding fetal weight and placental weight. Additionally, by applying the Two-Sample t-test Assuming Equal Variances between categories, we identified statistically significant differences between the weight of the fetus by sex and the weight of its placenta.

In the comparative study between fetal weight and vascular density, a proportional increase between these categories was observed. Furthermore, by applying the Two-Sample t-test Assuming Equal Variances between categories, statistically significant differences were noted between the weight and sex of the newborn and vascular density.

At the end of these clinical-statistical comparisons, we noticed that the placental weight varies in direct proportion to the placental vascular density, and by applying the Two- Sample t-test Assuming Equal Variances between categories, we noticed that there are statistically significant differences between the placental weight of newborn’s sex and vascular density.

In conclusion, the presence of placental lesions throughout pregnancy, along with their potential interactions and stimulatory effects, may contribute to functional abnormalities in the placenta, leading to adverse pregnancy outcomes. However, investigating these consequences in vivo is challenging. As a result, establishing a direct and proportional link between maternal-fetal clinical factors and histological elements requires careful consideration. Nevertheless, identifying an antepartum risk group based on these factors could play a crucial role in preventing unfavorable pregnancy outcomes. Further research is warranted to fully understand the complex interplay between placental lesions and pregnancy outcomes, ultimately improving prenatal care and management strategies.

The primary objective of this thesis was to comprehensively investigate various aspects of fetal growth restriction (FGR) to gain a deeper understanding of its mechanisms, optimize delivery timing, explore placental pathology, and establish correlations with pregnancy outcomes. Through meticulous research and analysis, the thesis aimed to contribute valuable insights to the field of FGR.

The thesis has significantly advanced knowledge in understanding the underlying mechanisms of FGR, identifying key factors contributing to its development, such as placental dysfunction, maternal malperfusion, and vascular remodeling processes. Additionally, it has established evidence-based recommendations for optimal delivery timing based on Doppler parameters, potentially reducing neonatal complications and improving outcomes.

Moreover, the research has made substantial contributions to the understanding of placental pathology and its association with FGR, highlighting the roles of placental lesions and maternal malperfusion in FGR pathogenesis. The correlations between placental lesions and adverse pregnancy outcomes provide valuable insights for predicting and preventing complications, guiding clinical decisions.

These novel insights have potential clinical applications, informing personalized management approaches, early identification and intervention strategies, and improved neonatal outcomes. The findings underscore the importance of enhanced risk assessment and antepartum care to identify high-risk pregnancies and optimize outcomes.

The implications of this thesis for clinical practice include evidence-based strategies for managing pregnancies affected by FGR, individualized delivery timing, tailored interventions based on Doppler parameters, and early identification of high-risk pregnancies. The research also lays the groundwork for continued progress in the field of FGR, contributing to the overall body of knowledge and fostering opportunities for future investigations and collaborations.