Is inflammation inducing hypoxic tissue modifications in placenta? A retrospective appraisal

Abstract

Infectious causes of morbidity and mortality in the newborn are well documented, but the relationship of this to placental infection is not well known yet. Having the Ethical Comitee consent for the study, from the medical archives of University Emergency Hospital in Bucharest, Department of Pathology, and the Department of Obstretics and Gynaecology, fifty-seven placentas were selected over a period of 22 months. In this study there have been 15 (26.31%) cases of acute chorioamnionitis (COA) and 31 (73.68%) cases of chronic chorioamnionitis (COC), with an overall mean gestational age of 27.6 weeks. The cases with COA were having mean gestational age of 26.5 weeks (6-38 weeks), only five living new-born with a mean gestational age of 35.3 weeks. In the study, there were documented 10 abortions (17,54%) with a mean gestational age of 12 weeks. Most pathologic cases proved histologic abnormalities such as fibrinoid necrosis of villi, villitis, villous edema, few Hofbauer cells, villous or fetal changes, vasculosyncytial knots, that differentiated the cases from those with normal histology by a increased gestational age with a mean of 32.25 weeks. We conclude that a moderate degree of placental inflammatory status will not influence significantly the degree of hypoxia.

Keywords: *placental pathology, gestational inflammation, chorioamnionitis, hypoxia, vasculosyncitial knots, villous edema*

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Introduction

The placental inflamatory pathology still remains one of the most important obstetrical problems, mainly, when it has as a result the increase of new-born morbidity and mortality. Globally, newborn mortality finds itself in a increasing way, from 37% in 1990, at aproximately 44% in 2012[(1)]. The incriminated factors are: economic and social status of the mother, congenital anomalies, ante-partum haemorhage and prenatal infections[(2)]. This study search the relation between the histologic placental inflammation degree and other coexistive lesions, specially, secondary to hypoxia.

Methods

From a total 224 placentas that were collected from all gestations admittedin the Obstaetrics and Gynaecology departament between january 2012 and octomber 2013, there were selected a number of 57 cases, that were also diagnosed by the pathologist, at the Bucharest University Emergency Hospital. The study admission criterion was the histopathology diagnosis of chorioamnionitis (COA). Placental tissue, embedded in paraffin blocks, was sampled from those areas with obvious macroscopic lesions: calcification, infarction areas, significant mucus deposition, haemorrhage; the elective areas for sampling were preffered to be from the immediate vicinity of the ombilical cord insertion in the placenta. The purpose for this is to avoid the organ margins, this being a frequent source for misinterpretation and artifacts, as a result of surgery. After formalin fixation and parafin embedding, 3µm thick sections, that were subsequently with haematoxilin and eosin stained and entirely examined. High resolution photographs (14 megapixels) were performed from all sample fields, with all microscopic objectives. The microscopic lesions absence or presence was quantified, as well as their width (Table 1). Therefore, the resulting data was centralised in a database, toegether with the gestation age (weeks) and mother age (years), medical record and clinical diagnosis. Only those tissue samples with acute or chronic inflammatory lesions, were included. Image capture was performed with the Nikon E200 Eclipse microscope (40x10), one image per slide, always from the fifth field from left to right, at the upper left corner of sample that is positioned in the same way for each case studied. Eventually, with the aid of „ImageJ" software, still cited in similar studies[(3,4,5,6)], these images were scaled in micrometers. From the initial set of cases, there were selected a number of 17 placentas that were splited in two other sets: those with hypoxic and ischemic lesions (IP), 10 case, and those that did not have these histologic features (NIP), 7 cases, in order to become a subject for assisted computational morphometric analysis (ACMA). Included patients did not have any erithrocyteanomalies, allowing fast scaling using the normal diameter of a red blood cell, that in an adult healthy human, measures about 7,81 ± 0,63 µm[(7)]. A micrometric grid with a square area of 3000 µm^2, was placed over the photographs, and the

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Table 1 Morphometric differences between ischemic placentas (10 cases) and non-ischemic (7 cases) placentas

	Mean gestational age (weeks)	Mean mother age (years)	Mean number of villi in selected ROI	Mean villi diameter in ROI (µm)	Mean vessel number in ROI	Mean vessels diameter in selected ROI (µm)	Mean minimum endotheloum-intervillous space distance in ROI (µm)
Ischemic placentas	29.6	32.8	3.5	70.1	9.8	21.2	5.1
Non-ischemic placentas	30.1	31.1	3	86.2	9.1	15.2	11.08

first 9 complete squares identified in the upper corner of the image, was designated as the region of interest (ROI) for ACMA. The measurements were: villi diameter, the diameter of the blood vessels within the villi mesenchyma, the distance between the endothelial pole of most external blood vessels within the villi mesenchyma and the intervillous space, the number of villi per ROI.

Results

From 224 placentas, there were identified 109 new born live births (48.45%), 94 abortions (41.78%) and 22 cases (9.78%) with different pathologies, in which the most frequent diagnosis is that of hidatiforme mole. In the selected set of cases for the study, the mean gestational age was of 27,6 weeks, as premature births, while the mean mother age was 31.4 years old, within the range of 15 to 43 years old. There have been found 25 (26.31%) cases of COA and 31 (73.68%) cases of chronic chorioamnionitis (COC) with a mean gestational age of 27.6 weeks (Figures 1 and 2). A

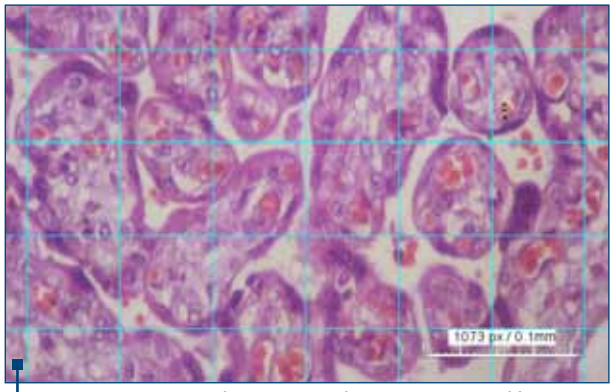

Figure 1. Late trimester placenta (28 weeks gestation). It is visible a 3000 µm² grid positioning with a random offset (personal collection)

percent of 17,54% of the selected set was the result of spontaneous abortion, gestation interruption and stillbirths, both in-utero and postpartum (Figure 3). The most important feature is the presence of discrete chronic inflamamtion represented by limphocytes and

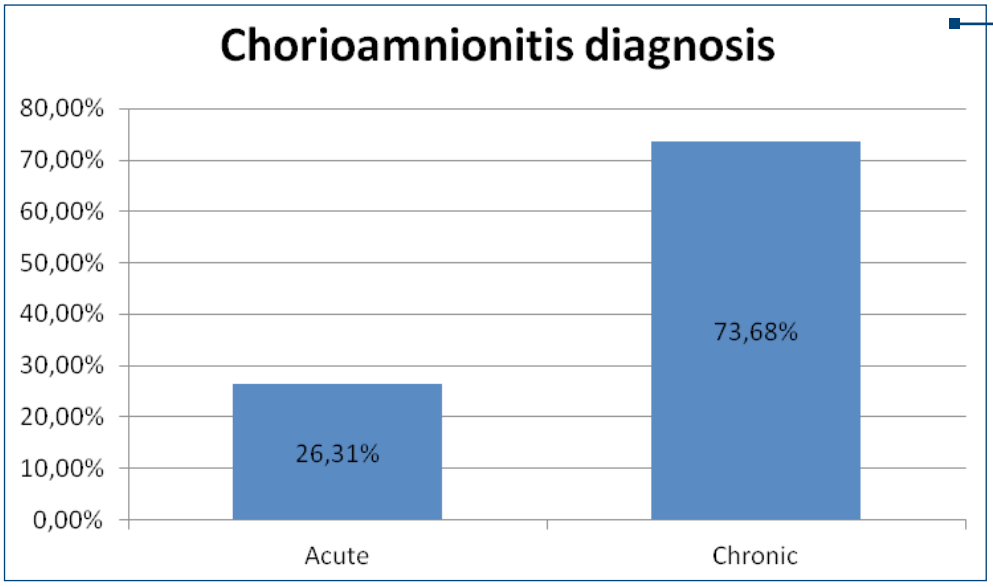

Figure 2. Chorioamnionitis diagnosis in our study

plasmocytes (75.47%) along with a moderate degree of fibrin deposition (57.69%) and placental villi necrosis (49.06%). Discrete, ubiquitous, presence of Hofbauer cells has been identified in 83.02% of cases, mainly in COC. Umbilical cord inflammation is visible in 17.81% cases from which all proved arterial involvement, while the venous one is existent in only two cases. Neither anatomical, nor embriological abnormalities were detected. Amnionic membranes shows similar lesions those discovered at the placental mesenchyma, but in lesser amplitutide and the acute inflammation was less obvious (20%). From the selected cases for ACMA, the IP come from gestations with a mean age of 29.6 weeks, the female patients having the mean age of 32.8 years old, while NIP have a mean gestational age of 30,14 weeks with a mean mother age of 31.14 years old.

From the histologic lesions observed in IP and in NIP, the most obvious ones were the chronic inflammation (70%), in a moderate degree (57.14%) in IP, and in discrete, focal, degree (42.86%) in NIP (Figures 3, 4, 5, 6 and 7). Villi necrosis was easily visible in IP (90%) than in NIP (57.14%). Hofbauer cells were more frequent in IP. Villi diameter proved a smaller variation, in IP (70.1 μm) with lesser extent than NIP (86,2 μm), while the diameter of the blood vessel within the placenta villi was significantly larger in NIP than IP, while the distance from these vessel to the intervillous space was significantly smaller in IP than NIP (Figures 8, 9, 10, 11, Table 2). Therefore, the more the gestational age becomes older, the smaller becomes the distance between the villi blood vessels and the intervillous space.

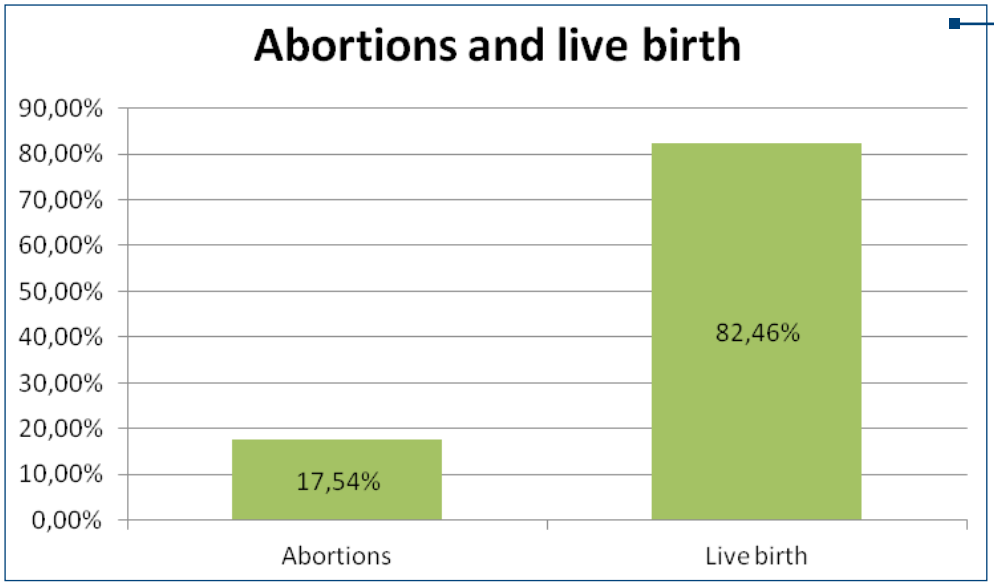

Figure 3. Descriptive statistics for survival in our study

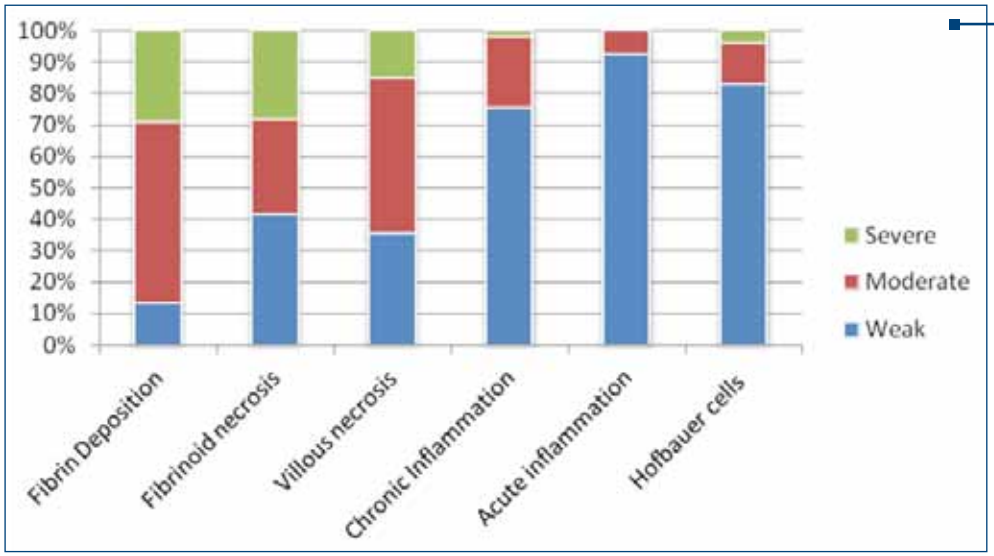

Figure 4. General histopathological features of the selected cases in our study (75 placentas)

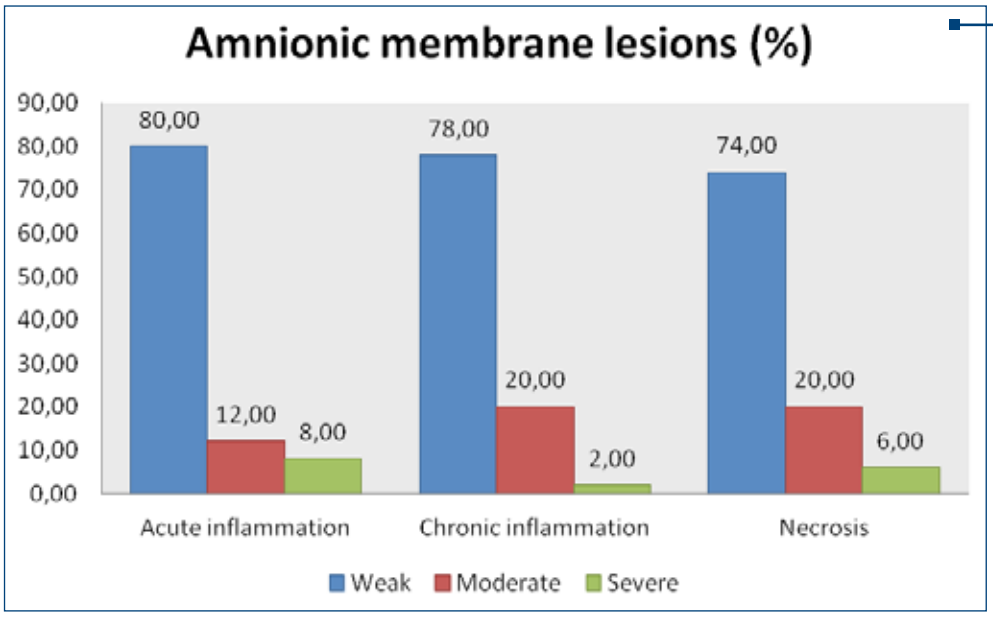

Figure 5. ***Descriptive statistics for lesions in amnionic membranes – in percentages***

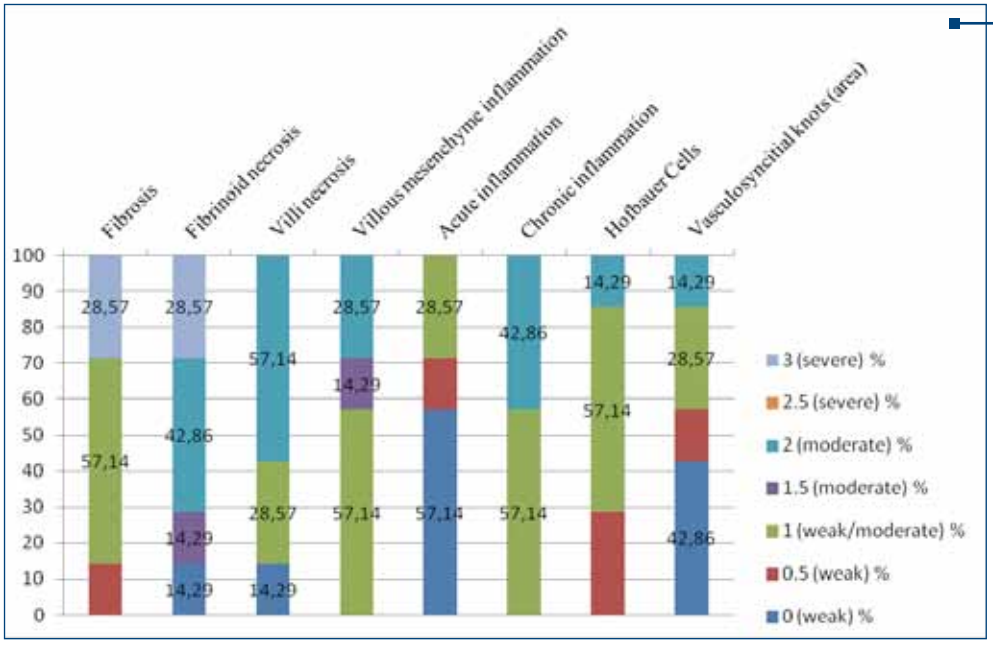

Figure 6. ***Histopathologic features in non-ischemic placentas (7 cases) - in percentages***

Discussion

Chorioamnionitis complicates almost 2% of all pregnancies, thus becoming a risk factor for fetal alterations, like periportal iron deposition, the increase of tissue neutrophils and of mielopoesis foci with leucocyte clusters[8]. The risk for inflammation secondary to infection in amnion and placenta becomes higher from the gestational age of 16 weeks old, fact visible in our study, by higher prevalence of inflammatory lesions at this age[9]. Infectious agents with ascendent tropism are a frequent cause for inflammatory systemic response on the fetal side. Clinical studies proves that antenatal exposure to inflammation becomes a high risk for the lung embriogenesis in premature babies, like lower respiratory rate associated with arcuate nuclei hipoplasia and pre-Bötzinger nuclei discovered after death[10]. However, the presence of chorioamnionitis is sometimes associated with a low mortality rate in small for age gestations[11]. Therefore, the observed mortality and morbidity observed in our study, maintain in a descendant trend, especially secondary to improved neonatal intensive care for mothers with poor social and economic status. Acute amnionitis with long term membrane rupture, with an incidence that rises fast in the first 24 hours from onset, has been associated, in some cases, with severe respiratory

distress that did not respond efficiently to reanimation measures. Between the lesions discovered in amnionic membranes, the most obvious was the necrosis, while the amnion apparantely prevents the spread of the inflammatory agent inside the amnionic sac and in the placenta. Therefore, the higher is the amplitude of inflammation in the amnios, the less obvious it becomes in the placenta tissue. In collagenase clostridium histolyticum (CCH), the limfocitic inflammatory response is usually discrete and does not involve vascular abnormalities, while it is associated with a small mortality rate[12]. This thing is observable in our study, too: the discrete, chronic inflammation does not influence significantly the vascular diameter, while the vasculosincityal knots have a larger diameter as an adaptation to higher oxigen needs and better gas transportation from intervillous space to fetal circulation. Also we observed that, for the same gestational ages and same mother age, the number of blood vessels does not modify significantly, while hipoxya is involved or not. The only measure detected with the aid of AMAC that varies significantly is the intervillous space volume, as it increases in hipoxic placentas. Therefore, the villi diameter and the distance between the maternal and fetal circulation lowers in IP, trying to minimise the placental tissue that is not vital for blood circulation. This modifications confirm the large, unicentric, studies for CCH as pathogenic factor in anti-fetal rejecting syndrome, increasingly incriminated in premature birth[13].

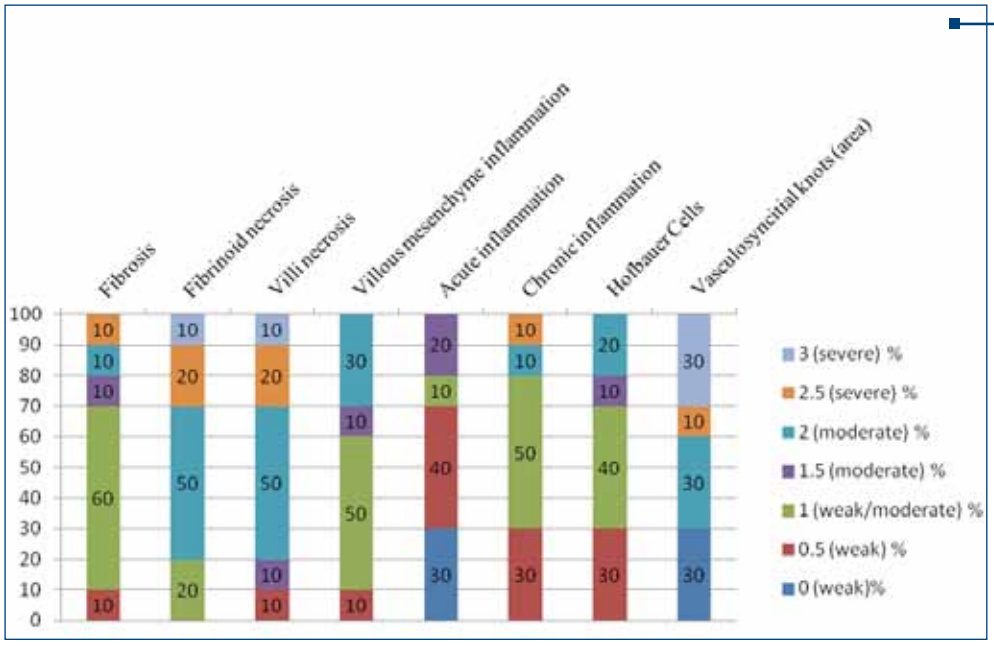

Figure 7. ***Histopathologic features in ischemic placentas (10 cases) - in percentages***

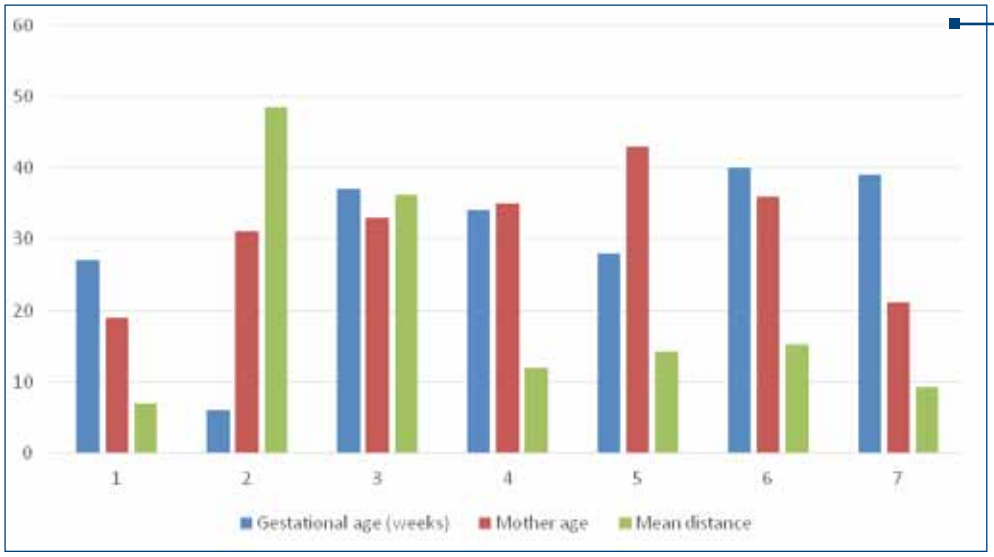

Figure 8. ***Gestational age, mother age and blood vessel-intervillous space distance (μm) in non-ischemic placentas***

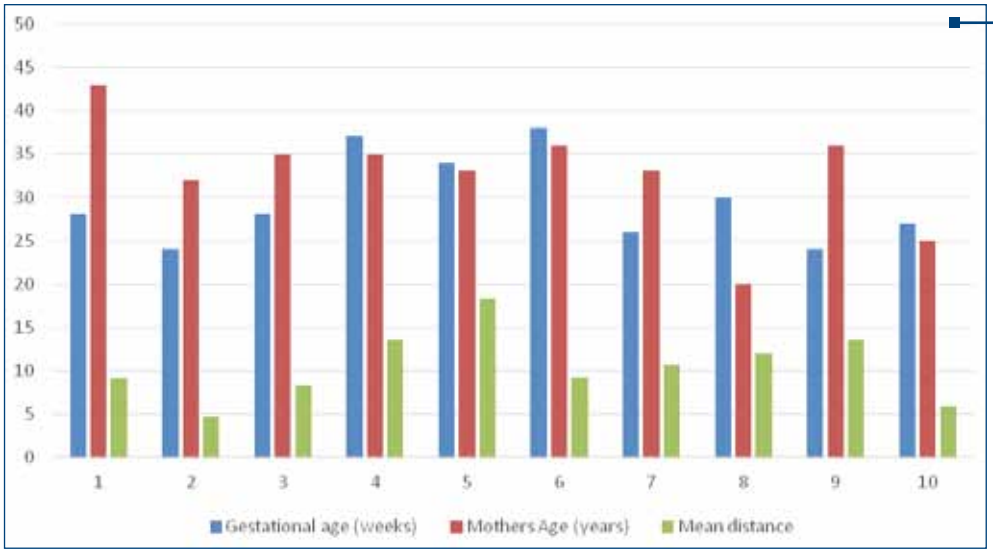

Figure 9. ***Gestational age, mother age and blood vessel-intervillous space distance (μm) in ischemic placentas***

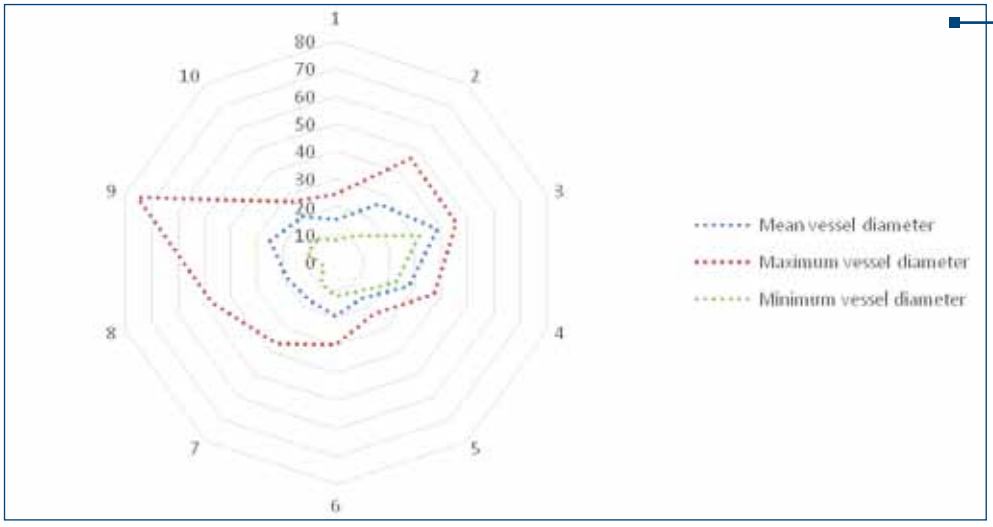

Figure 10. ***Blood vessel diameter (mm) measured in ischemic placentas.***

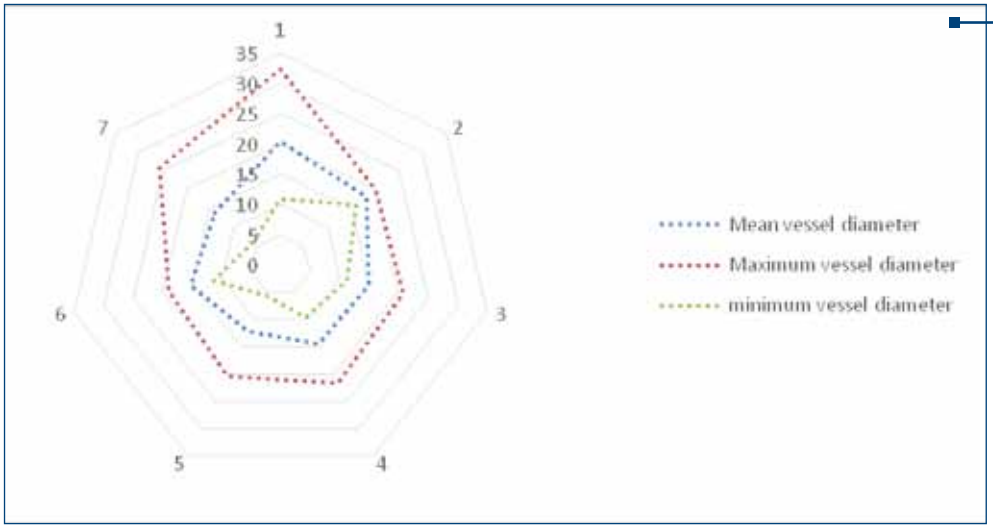

Figure 11. ***Blood vessel diameter (μm) measured in non-ischemic placentas.***

Table 2 Lesions quantification criteria for microscopic analysis applied in the study (*-10x10 PF, **-10x40 PF).

Lesion degree	Fibrosis	Fibrinoid necrosis	Villi necrosis	Villous mesenchyme inflammation	Acute / Chronic inflammation	Hofbauer Cells	Vasculosyncitial knots (area)
0 (mild)	<5%§	<5%§	0 vilus *	0-1 cells/HPF **	0-1 cells/HPF **	0 cells / HPF **	<5%§
0.5 (mild)	5-15%	5-15%	0-2 vilus/field	2-5 cells/HPF	2-5 cells/HPF	1-3 cells / HPF	5-15%
1 (mild/moderate)	15-25%	15-25%	2-4 vilus/field	5-8 cells/HPF	5-8 cells/HPF	3-4 cells / HPF	15-25%
1.5 (moderate)	25-35%	25-35%	4-6 vilus/field	8-10 cells/HPF	8-10 cells/HPF	4-5 cells / HPF	25-35%
2 (moderate)	35-45%	35-45%	6-8 villus/field	10-12 cells/HPF	10-12 cells/HPF	5-7 cells / HPF	35-45%
2.5 (severe)	45%-55%	45%-55%	8-10 vilus/field	12-14 cells/HPF	12-14 cells/HPF	7-9 cells / HPF	45%-55%
3 (severe)	>55%	>55%	>10 vilus/field	>15 cells /HPF	>15 cells /HPF	> 9 cells / HPF	>55%

Conclusions

Mild placental inflammation proves to be a less dangerous risk factor for stillbirths, even in small age gestations (25-28 weeks) and does not significantly influence the evolution of hipoxic lesions. The factor with higher impact seems to be the spreading of lesion compared with inflammation type - acute or chronic. Main lesions in examined placentas were: the fibrinoid deposition and villous necrosis, together with inflammation, mostly acute. As older the gestational and mother age becomes, the more obvious is the vasculo-syncitial knots wide spreading, especially in inflammatory context, rising the probabiliy for hypoxic and ischemic lesions in these placentas. ■

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