

Placental Calcification Score: a new semiquantitative method to assess pattern and grading of placental calcifications

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Abstract

The relationship between placental calcifications and pregnancy outcome is still controversial. In this study, we examined the occurrence of placental calcifications, and we proposed a histopathological score system, Placental Calcification Score (PCS). We assigned a score (from 1 to 3) to calcifications according to their pattern (dusty = 1; single = 2; cluster = 3) and grading (low = 1; moderate = 2; high = 3). Multiplying the pattern score with that of grading, we obtained a score. After that, summing the score of each one of the three calcification patterns, we achieved the PCS. We examined 47 consecutive monochorionic placentas, searching calcifications in placental parenchyma (PP) (in which we distinguished four subsites: intervillous, intravillous, sub-amniotic fetal floor and decidua), extraplacental membranes and Wharton jelly of the umbilical cord. We collected clinical data relative to 47 mothers (age, gestational age at delivery, kind of gestation and hypertension) and 51 products of conception (kind of products of conception, gender, preterm birth, and intrauterine growth restriction [IUGR]), corresponding to the 47 placentas. We found calcifications in all placentas examined (47/47 = 100%), and all placentas showed calcifications in PP (47/47 = 100%). Calcifications were more frequent, respectively, in intravillous (36/47 = 77%) and intervillous (47/47 = 100%) subsite of PP. Besides, our preliminary data showed a mean PCS higher in mothers ≥ 35 years, with gestational age ≥ 37 W + 0 D and suffering from hypertension,

than in mothers < 35 years, with gestational age < 37 W + 0 D and without hypertension. Not preterm newborns, male gender, and presence of IUGR were associated with a mean PCS higher than preterm newborns, female gender, and absence of IUGR.

PCS is a new histopathological tool that might be useful to clarify the correlation between placental calcifications and clinical data of mothers and products of conception. Further investigations are needed, with a large number of placentas, to confirm the trend shown by our data.

Keywords

Placental calcifications, calcification pattern, calcification grading, Placental Calcification Score, intervillous calcifications, intravillous calcifications.

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Introduction

In everyday practice, it is widespread to find calcifications in the histologic section of placental specimens, especially from the placental parenchyma (PP).

What is the meaning of these calcifications? Is there a correlation between placental calcifications and adverse pregnancy outcome, including both maternal and fetal/neonatal outcomes?

Data from literature, regarding the relationship between placental calcifications and pregnancy outcome, are discordant. Some authors claim that placental calcifications might not have any clinical significance, representing a physiological aging process of the placenta [1-4]. Other researchers argue that placental calcifications occurring before 36 weeks of gestations (called preterm placental calcifications [PPCs]) might be

associated with fetal and maternal complications, such as intrauterine growth restriction (IUGR) [5-9], low birth weight [5, 6, 8-11], low Apgar score [10], fetal distress [6] and pregnancy-induced hypertension [5, 7, 9, 12].

Based on Grannum classification for ultrasound placental grading [13], placental calcifications, characterized by indentation or ring-like structures, have been defined as Grade III. Two studies [14, 15] have revealed that Grade III PPCs might represent a risk factor for the adverse maternal outcome (post-partum hemorrhage, placental abruption and maternal transfer to intensive care unit) and neonatal outcome (preterm birth, low birth weight, low Apgar score, and neonatal death) in both low-risk and high-risk pregnant women.

A third study [16] reported that Grade III PPCs occurring at 28 weeks of gestation are correlated with a higher incidence of intrauterine fetal death (IUFD), representing an independent risk factor for IUFD.

In this study, we examine the occurrence of placental calcifications at different gestational ages, and we propose a histopathological score system, based on pattern and grading of calcifications. Our aim is to provide a new tool that might be useful to clarify the relationship between placental calcifications and clinical data of mothers and products of conception.

Materials and methods

We selected 47 consecutive monochorionic placentas received by our Department between July 2016 and January 2017. All 47 placentas were fixed in 10% buffered formalin. Each placenta was sampled according to the Amsterdam Placental Workshop Group Consensus Statement [17]: 1 block for a roll of the extraplacental membranes (EM) from the placental margin (including part of the marginal parenchyma) to the rupture edge; 1 block for two cross-sections of the umbilical cord: one at 5 cm from the placental insertion end and another from the fetal end; 3 blocks, each containing a full-thickness section of normal PP: one sample adjacent to the EM insertion site; two samples from the central two-thirds of the PP, of which one close to the umbilical cord insertion site. Only samples from normal-appearing placental tissue were included in this study.

Blocks were processed with Tissue Processor TPC 15 Medite Medizintechnik and paraffin-embedded. A 5 μ m-thick section from each

paraffin block was stained with hematoxylin and eosin (H&E) by Leika Autostainer XL CV 5030.

Three main different placental sites were analyzed: PP, EM and Wharton jelly of the umbilical cord (WJ).

In the PP we recognized four different subsites: intervillous, intravillous, sub-amniotic fetal floor and decidua.

Calcifications appeared as basophilic bodies at H&E. We evaluated their presence distinguishing three different patterns: dusty – aggregates of fine, particulate calcifications – (**Fig. 1**), single – more significant than the previous ones, solitary, round or oval and well-defined calcifications – (**Fig. 2**), and clusters – voluminous aggregates of at least two extensive calcifications – (**Fig. 3**). Each one of the three patterns was assessed on 10 random fields at 10 magnifications, according to the following grading: low (< 5 calcifications); moderate (5-10 calcifications); high (> 10 calcifications).

The tissue surface occupied by calcifications, depending on pattern and grading, is not the

same. Hence, we attributed a different weight to calcifications by assigning a score, from 1 to 3, based on pattern (dusty = 1; single = 2; cluster = 3) and grading (low = 1; moderate = 2; high = 3). Multiplying the pattern score with that of grading, we obtained a minimum score of 1 and a maximum score of 9 (**Tab. 1**).

After that, summing the score of each one of the three calcification patterns, we achieved Placental Calcification Score (PCS) that ranged from 1, if only low-grade dusty calcifications were present ($1 + 0 + 0 = 1$), to 18, in the presence of high-grade calcifications in all three patterns ($3 + 6 + 9 = 18$).

In **Fig. 4** and **Tab. 2**, an example of assessment of pattern and grading on 10 fields at 10 magnifications is shown.

Afterward, we collected clinical data relative to 47 mothers and 51 products of conception, corresponding to the 47 placentas examined.

The maternal clinical data that we have gathered included: age, gestational age at delivery, kind of

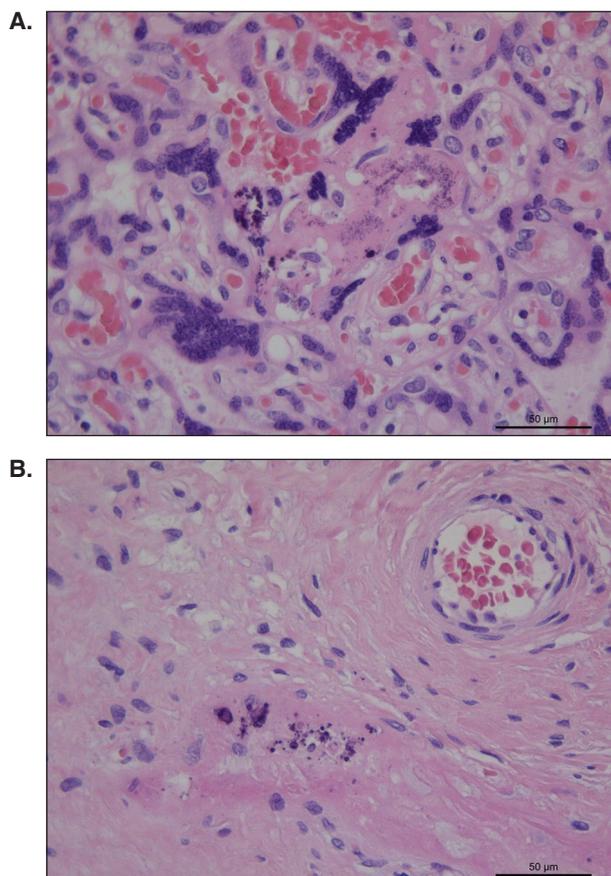


Figure 1. The dusty calcification appears as an aggregate of fine, particulate calcifications (H&E, x40 magnifications). **A.** Dusty calcification in intervillous subsite of placental parenchyma (PP). **B.** Dusty calcification in intravillous subsite of PP.

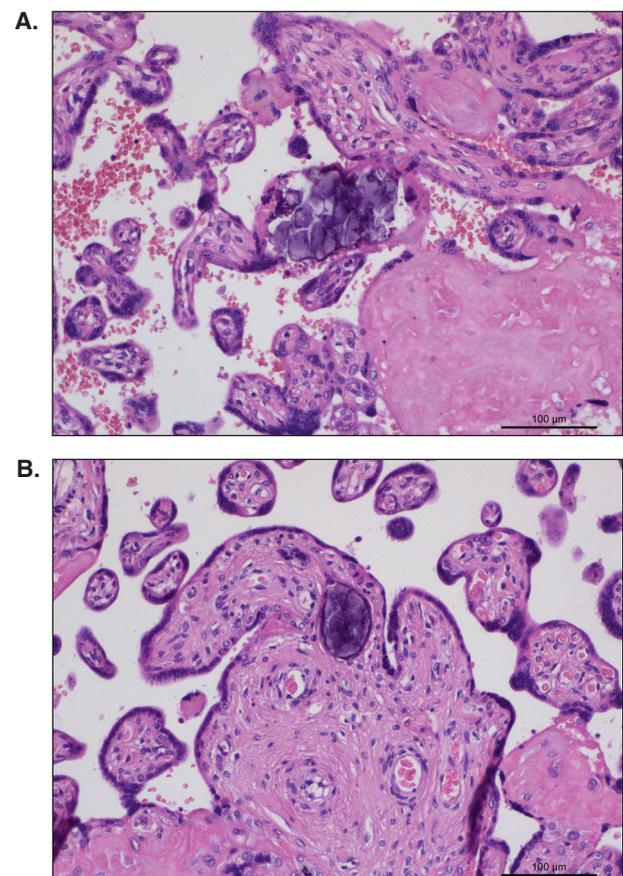


Figure 2. The single calcification appears as a solitary, round or oval, well-defined calcification (H&E, x20 magnifications). **A.** Single calcification in intervillous subsite of placental parenchyma (PP). **B.** Single calcification in intravillous subsite of PP.

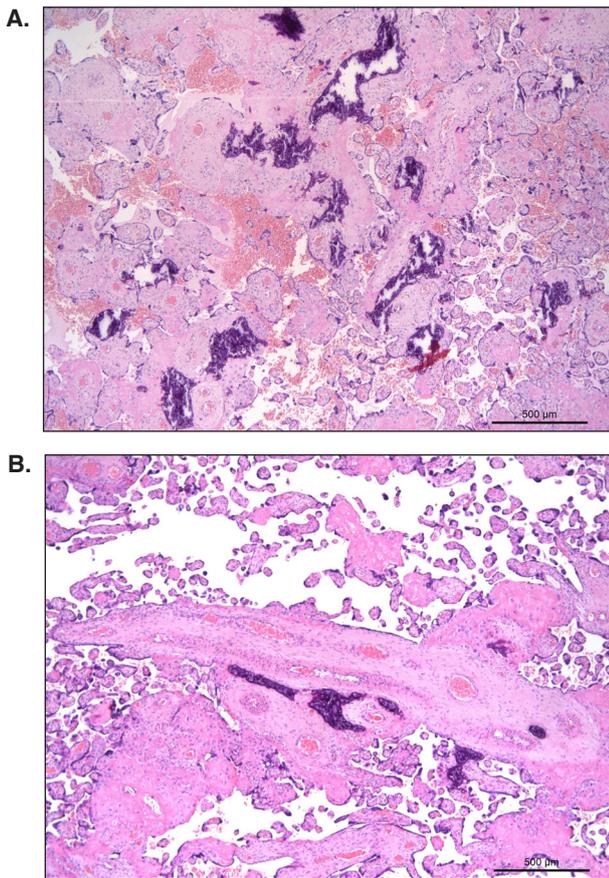


Figure 3. The cluster is a voluminous aggregate of at least two large calcifications (H&E, x4 magnifications). **A.** Cluster of calcifications in intervillous subsite of placental parenchyma (PP). **B.** Cluster of calcifications in intervillous subsite of PP.

Table 1. Multiplying (X) the pattern score with that of grading, we obtained: a minimum score of 1 and a maximum score of 3 for dusty calcifications; a minimum score of 2 and a maximum score of 6 for single calcifications; a minimum score of 3 and a maximum score of 9 for clusters.

		Pattern		
		Dusty (= 1)	Single (= 2)	Cluster (= 3)
Grading	X			
	Low (= 1)	1	2	3
	Moderate (= 2)	2	4	6
High (= 3)	3	6	9	

Low: < 5 calcifications/10 fields at 10 magnifications.
 Moderate: 5-10 calcifications/10 fields at 10 magnifications.
 High: > 10 calcifications/10 fields at 10 magnifications.
 X: multiplication.

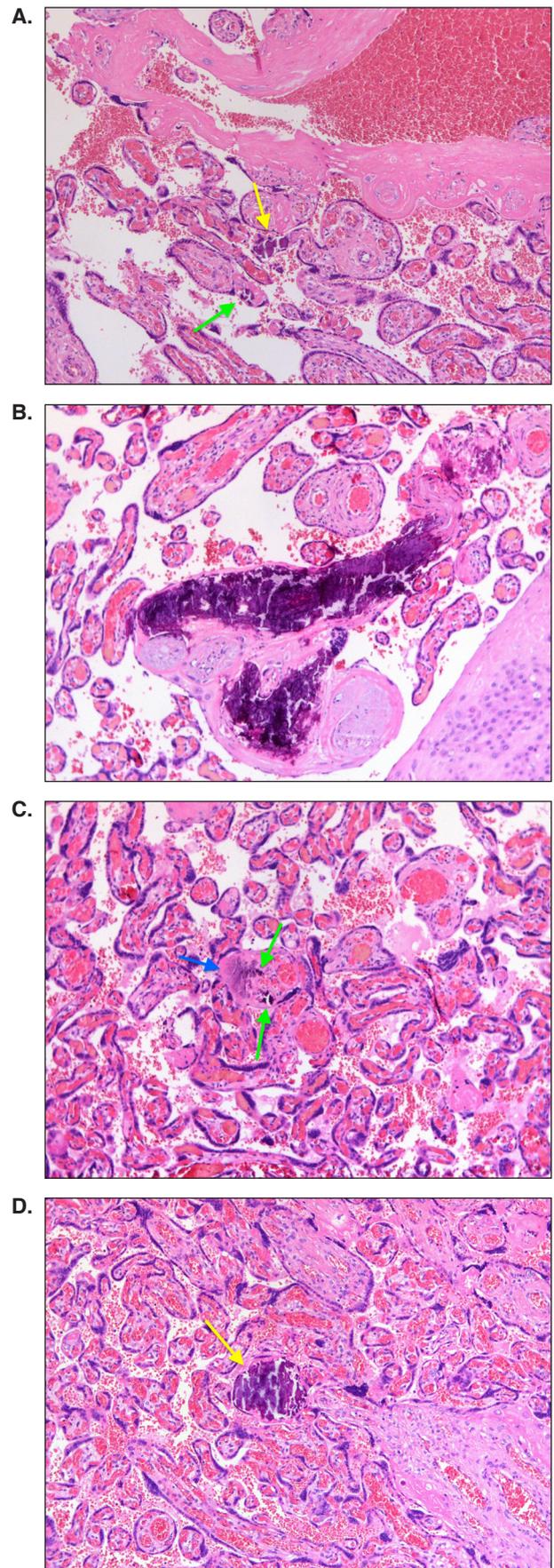


Figure 4 (continues on the next page). Assessment of pattern and grading of calcifications on 10 random fields (H&E, x10 magnifications).

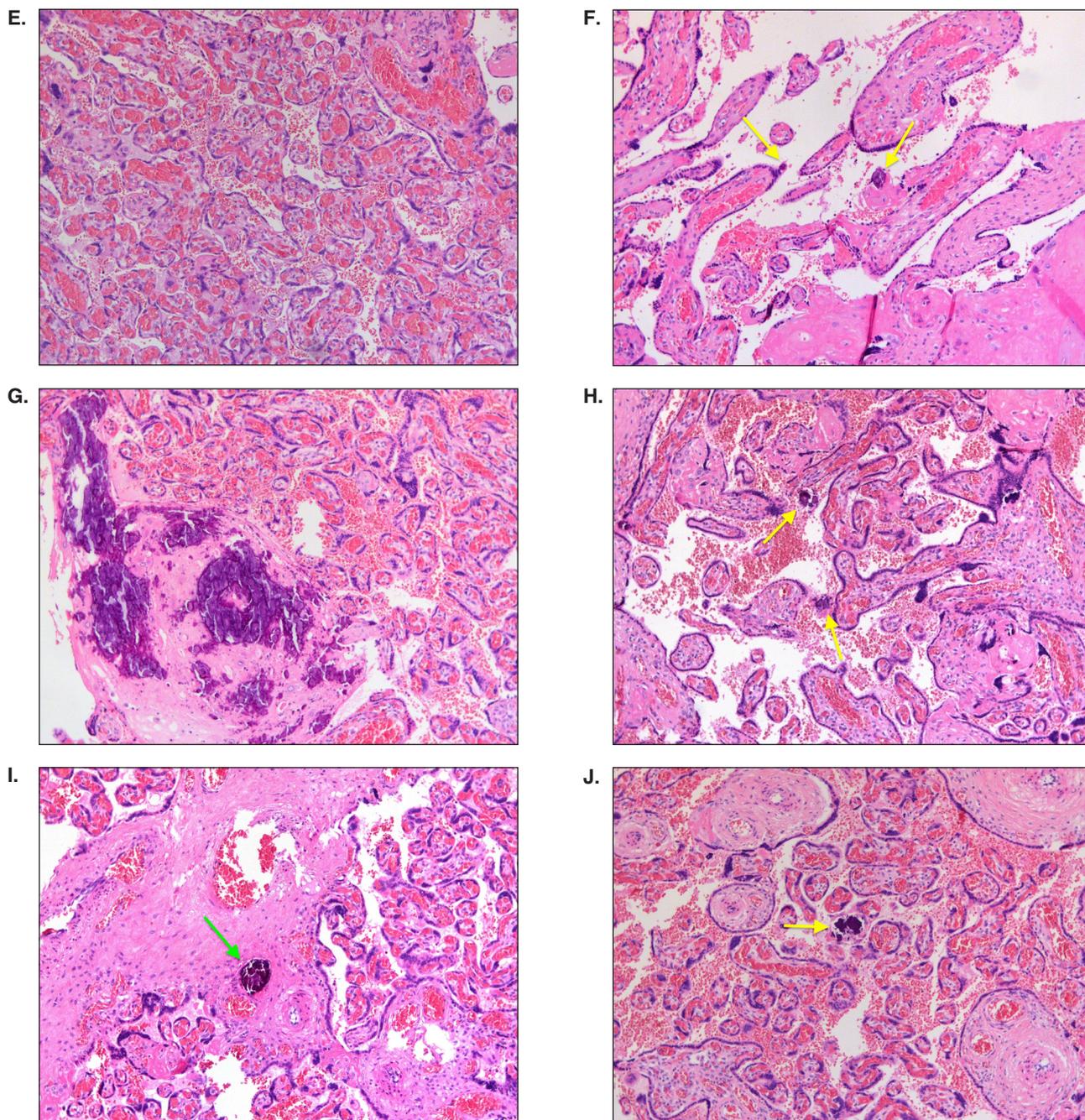


Figure 4 (continues from the previous page). Assessment of pattern and grading of calcifications on 10 random fields (H&E, x10 magnifications). **A.** One single calcification in intervillous subsite of placental parenchyma (PP) (yellow arrow) and one single calcification in intravillous subsite of PP (green arrow). **B.** One cluster of calcifications in intervillous subsite of PP. **C.** One dusty calcification in intravillous subsite of PP (blue arrow) and two single calcifications in intravillous subsite of PP (green arrows). **D.** One single calcification in intervillous subsite of PP (yellow arrow). **E.** Field without calcifications. **F.** Two single calcifications in intervillous subsite of PP (yellow arrows). **G.** One cluster of calcifications in intervillous subsite of PP. **H.** Two single calcifications in intervillous subsite of PP (yellow arrows). **I.** One single calcification in intravillous subsite of PP (green arrow). **J.** One single calcification in intervillous subsite of PP (yellow arrow).

gestation (single, twin or multiple), hypertension (chronic, pregnancy-induced or within pre-eclampsia). About kind of gestation, we had a twin pregnancy with the expulsion of one of two fetuses at 10 weeks of gestation and multiple pregnancies (three twins) with the expulsion of one of three fetuses at 11 weeks of gestation. In

the first case, we considered pregnancy as single, in the second case as a twin.

Clinical data regarding the products of conception included: kind of products of conception (liveborns, IUFD, voluntary interruption of pregnancy and therapeutic abortion), gender, preterm birth, and IUGR.

Table 2. The example in Fig. 4 shows, respectively, 0 dusty calcification, 7 single calcifications and 2 clusters in an intervillous subsite of placental parenchyma (PP), and 1 dusty calcification, 4 single calcifications and 0 clusters in an intravillous subsite of PP. Summing (Σ) the score of each one of three calcification patterns, the result is Placental Calcification Score (PCS) for, respectively, intervillous and intravillous subsite of PP.

		PP					
		Intervillous			Intravillous		
		Pattern			Pattern		
X	Dusty (= 1)	Single (= 2)	Cluster (= 3)	Dusty (= 1)	Single (= 2)	Cluster (= 3)	
Grading	Low (= 1)	-	-	3	1	2	-
	Moderate (= 2)	-	4	-	-	-	-
	High (= 3)	-	-	-	-	-	-
	Σ	0 + 4 + 3			1 + 2 + 0		
	PCS	7			3		

Low: < 5 calcifications/10 fields at 10 magnifications.

Moderate: 5-10 calcifications/10 fields at 10 magnifications.

High: > 10 calcifications/10 fields at 10 magnifications.

PP: placental parenchyma; PCS: Placental Calcification Score; X: multiplication; Σ : sum.

Results

We found calcifications in 47/47 (100%) placentas.

Calcifications were most represented in PP, where 47/47 (100%) placentas had calcifications, while 16/47 (34%) placentas showed calcifications in EM and only 1/47 (2%) in WJ.

Calcifications were observed in all four subsites of PP: 47/47 (100%) placentas displayed intervillous calcifications, 36/47 (77%) intravillous calcifications, 5/47 (11%) decidual calcifications and 2/47 (4%) placentas revealed calcifications in sub-amniotic fetal floor subsite. Since calcifications were more frequent in intervillous and intravillous subsites of PP than in the other ones, we focused on intervillous and intravillous subsites (intervillous + intravillous = PP_{i+i}).

Single calcifications were present in 47/47 (100%) placentas, clusters of calcifications in 22/47 (47%) placentas, and dusty calcifications in 15/47 (32%) placentas. While, for the calcification grading, 45/47 (96%) placentas had low-grade calcifications, 39/47 (83%) moderate grade calcifications and 12/47 (26%) high-grade calcifications.

Regarding the clinical data of 47 mothers (Fig. 5), the mean age of our cohort was 35 years (range: 20 to 46 years) and, using this as cutoff value, we divided the cohort into two groups: Group A with 26 mothers ≥ 35 years and Group B with 21 mothers < 35 years. For each group we calculated

the mean PCS and the standard deviation in PP_{i+i} : Group A (≥ 35 years; $n = 26$) 8.46 ± 3.85 vs Group B (< 35 years; $n = 21$) 6.95 ± 4.70 .

The gestational age at delivery ranged from 14 weeks and 0 days (14 W + 0 D) to 41 weeks and 0 days (41 W + 0 D) with 44 (94%) single pregnancies, 2 (4%) monochorionic-diamniotic twin pregnancies and 1 (2%) monochorionic-triamniotic multiple (three twins) pregnancy. The cutoff value was 37 W + 0 D. We split up the cohort of 47 mothers in two groups: Group C including 18 mothers ≥ 37 W + 0 D and Group D including 29 mothers < 37 W + 0 D. The mean PCS and the standard deviation in PP_{i+i} , for each group, were: Group C (≥ 37 W + 0 D; $n = 18$) 8.61 ± 4.84 vs Group D (< 37 W + 0 D; $n = 29$) 7.28 ± 3.87 .

Hypertension was reported in anamnesis for 10 mothers (21%): 3 mothers had chronic hypertension, 4 pregnancy-induced hypertension, and 3 pre-eclampsia. 10 mothers with hypertension formed Group E, while 37 mothers without hypertension fell into Group F. The mean PCS and the standard deviation in PP_{i+i} have been calculated for each group: Group E (hypertension; $n = 10$) 9.40 ± 5.64 vs Group F (no hypertension; $n = 37$) 7.35 ± 3.79 .

Concerning the clinical data of 51 products of conception (Fig. 6), the cohort consisted of 41 (80%) newborns, 5 (10%) IUFD, 2 (4%) voluntary interruption of pregnancy and 3 (6%) therapeutic abortion. Referring to the WHO definition of pre-term birth [18], we examined the distribution of calcifications in 41 newborns. Therefore, the cohort

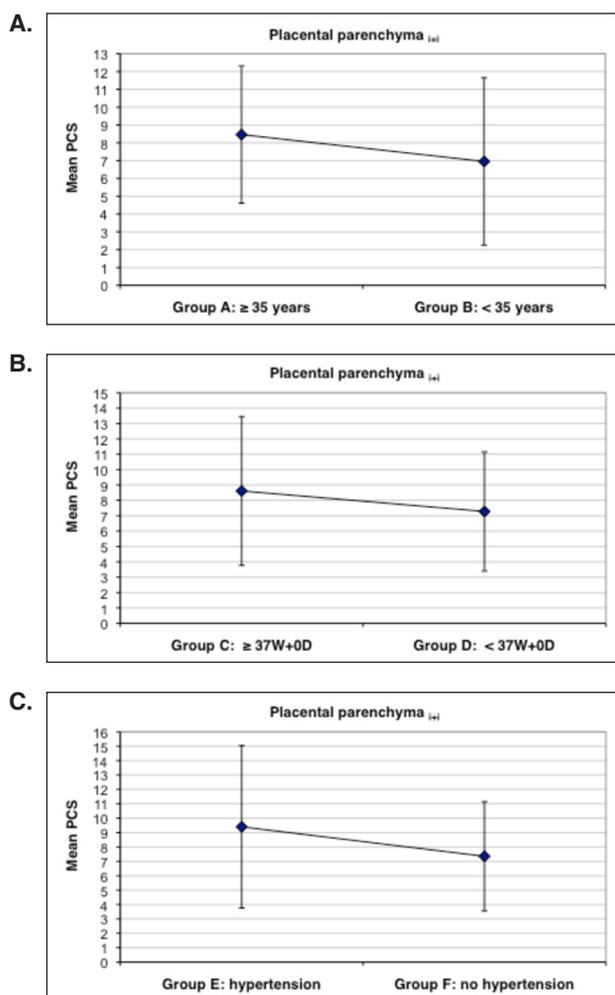


Figure 5. Maternal clinical data: the mean Placental Calcification Score (PCS) and the standard deviation in placental parenchyma_{i+i} (PP_{i+i}) (intervillous + intravillous = PP_{i+i}). **A.** Group A (≥ 35 years; n = 26) 8.46 ± 3.85 vs Group B (< 35 years; n = 21) 6.95 ± 4.70. **B.** Group C (≥ 37 W + 0 D; n = 18) 8.61 ± 4.84 vs Group D (< 37 W + 0 D; n = 29) 7.28 ± 3.87. **C.** Group E (hypertension; n = 10) 9.40 ± 5.64 vs Group F (no hypertension; n = 37) 7.35 ± 3.79.

of 39 placentas, corresponding to 41 newborns, has been divided into two groups: Group 1 including 18 placentas of 18 not preterm newborns (without distinguishing early-term, full-term, late-term and post-term) and Group 2 including 21 placentas of 23 preterm newborns. For each group we calculated the mean PCS and the standard deviation in PP_{i+i}: Group 1 (not preterm; n = 18) 8.61 ± 4.84 vs Group 2 (preterm; n = 21) 7.28 ± 4.69.

We looked at the different distribution of calcifications based on gender. The cohort of 51 products of conception was composed of 25 (49%) females and 26 (51%) males. We considered only 44 single pregnancies, excluding twin and multiple pregnancies. Consequently, the cohort

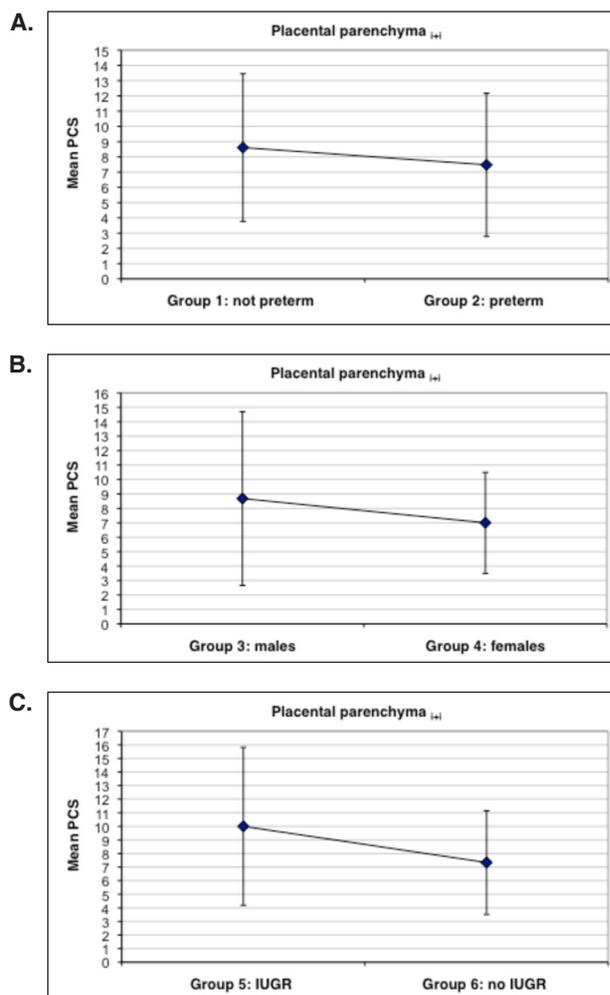


Figure 6. Clinical data regarding products of conception: the mean Placental Calcification Score (PCS) and the standard deviation in placental parenchyma_{i+i} (PP_{i+i}) (intervillous + intravillous = PP_{i+i}). **A.** Group 1 (not preterm; n = 18) 8.61 ± 4.84 vs Group 2 (preterm; n = 21) 7.28 ± 4.69. **B.** Group 3 (males; n = 25) 8.68 ± 6.02 vs Group 4 (females; n = 19) 7.00 ± 3.50. **C.** Group 5 (intrauterine growth restriction [IUGR]; n = 8) 10.00 ± 5.81 vs Group 6 (no IUGR; n = 39) 7.33 ± 3.82.

was reduced to 44 products of conception, split into two groups: Group 3 consisting of 25 males and Group 4 consisting of 19 females. For each group, the mean PCS and the standard deviation in PP_{i+i} were: Group 3 (males; n = 25) 8.68 ± 6.02 vs Group 4 (females; n = 19) 7.00 ± 3.50.

Lastly, we focused on IUGR. It was reported in 8 products of conceptions (16%): 7 newborns and 1 IUFD. We divided the cohort of 47 placentas, concerning 51 products of conception, in two groups: Group 5 composed by 8 placentas corresponding to 8 products of conception with IUGR and Group 6 composed by 39 placentas corresponding to 43 products of conception without IUGR. The mean PCS and the standard

deviation in PP_{i+i} have been calculated for each group: Group 5 (IUGR; $n = 8$) 10.00 ± 5.81 vs Group 6 (no IUGR; $n = 39$) 7.33 ± 3.82 .

Discussion

Placental calcifications are depositions of calcium salts that may occur all over the placenta.

Three different mechanisms may lead to tissue calcification: physiological, dystrophic and metastatic. The physiological calcification occurs in bone and teeth. It is characterized by deposition of an osteoid matrix by osteoblasts, the necessary condition for hydroxyapatite formation on collagen fibers. The dystrophic calcification takes place in necrotic tissue. In this setting the integrity of cellular membranes is lost, allowing extracellular calcium to bind to intracellular phosphate. The metastatic calcification is exemplified by environmental supersaturation with calcium and phosphate as in urolithiasis [19].

The etiopathogenetic mechanisms of placental calcifications are still unknown. In 1998 Kajander and Ciftcioglu [20] proposed an alternative pathogenetic mechanism for calcification by introducing the role of nanobacteria. The exact classification of nanobacteria is still debated and beyond the scope of our study. Nevertheless, the intriguing aspect is that nanobacteria might trigger the calcification process in the placenta [21, 22]. In 2001 Poggi et al. [23] examined term and post-term placentas and suggested that the metastatic mechanism of calcification might be involved in placental calcification.

Calcium deposits appear as echogenic foci on ultrasound examination. The ultrasound grading system for the placenta, developed by Grannum, the Grannum classification, is based on the maturity of the placenta and the presence/extension of calcifications [13, 24]. Several studies [14-16, 25, 26] on the relationship between placental calcifications and pregnancy outcome have been conducted by performing ultrasonography referring to the Grannum classification for the degree of the calcifications in order to establish the diagnosis of placental calcification.

Calcium deposits may be seen at the macroscopic examination as small yellow-white granules and histologically appear basophilic with H&E staining [27].

To our best knowledge, there is a paucity of research evaluating the clinical significance of placental calcifications with histological

analysis of calcium deposits. In 2014 Nigam et al. [28] showed calcifications, on macroscopic and microscopic examination, in a significantly higher number of low birth weight babies placentas ($p < 0.01$) than in the control group (babies placentas with birth weight $> 2,500$ g). In this study, the authors evaluated the presence or absence of placental calcifications, without quantifying and defining the calcifications in detail. In 2017 Zeng et al. [29] examined the association between Intravillous and Intra-fibrinous Particulate MicroCalcification (IPMC) and adverse pregnancy outcomes. They found increased IPMC in cases of IUFD and with placental infarcts compared to placentas without adverse outcomes. The authors focused on IPMC, a specific type of placental calcification, visible only microscopically, and located at the basement membrane of chorionic villi.

We believe that a possible explanation for the prevalence of clinical-radiological rather than histopathological studies might be due to the lack of a histopathological grading system analogous to Grannum classification. Therefore, in this study, we propose a histopathological score system in order to evaluate the pattern and grading of calcifications in placental specimens.

In conclusion, our study focuses on histological evaluation of placental calcifications. Our data show that calcifications are commonly found in placentas, especially in an intervillous and intravillous subsite of PP.

The main strength of this study is PCS, a new histopathological tool that might be utilized by pathologists in the daily diagnostic activity. Although the high standard deviation indicates that data are spread out over a wide range of values, our preliminary data suggest that PCS, a histopathological semiquantitative scoring system, might allow a better evaluation of placental calcifications in order to help clarify the correlation between placental calcifications and pregnancy outcome. The mean PCS in the PP_{i+i} is higher in mothers ≥ 35 years old, with gestational age ≥ 37 W + 0 D and suffering from hypertension than in mothers < 35 years old, with gestational age < 37 W + 0 D and without hypertension. Regarding products of conception, not preterm newborns and males show the mean PCS in the PP_{i+i} higher than preterm liveborn and females. The presence of IUGR is associated with higher mean PCS in the PP_{i+i} than in products of conception without IUGR.

The main weakness of our study is the small cohort size. Further investigations are needed, with a large number of placentas, to confirm the trend shown by our data.

Finally, we believe it could be interesting to adopt a systemic approach in correlating histopathology with PCS, ultrasound placental grading by Grannum classification and fetal/neonatal and maternal outcomes.

Declaration of interest

The Authors declare that there is no conflict of interest.

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