Pathological Pregnancy, Placental Calcification, and Nannobacterial Infection:
Is there any Relationship between these Events?

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ABSTRACT
An important sign of pathological pregnancy is increasing placental calcification (PC). The nature and mechanisms of PC development remained undefined. Meanwhile, the ultra-small bacterial forms (nannobacteria) were found in other calcified sites—in kidney stones and in dental plaque. The nannobacteria were capable of inducing the calcification in vitro.11-14 Our article presents a new attempt to discover a cause of PC as a relationship between calcification of the placental tissues and the presence of nannobacteria there. Distribution of PC in the tissue samples was confirmed with standard ultrasonographic detection and microscopy; presence of the nannobacteria in the PC-containing samples was shown with electron microscopy. Numerous nannobacteria and grains of the mineral deposits were discovered in microcavities in the tissues; no nannobacteria were found in sites without calcification. The role of nannobacteria in placentas is discussed—they can play a pathological role in PC development through formation of mineral deposits in the placental microcavities where these bacteria form a supersaturated environment favorable for local calcification. Subsequently, the calcification process affects restricting the calcium delivery to the fetus and may cause pathological pregnancy.

Pregnancy is a physiological state characterized by increasing food intake and by important changes in carbohydrate, lipid, and protein metabolism. This adaptation is essential to sustain exponential fetal growth; it is coordinated through the placental state, namely, the maturation of placental tissues and their functional activity. The placenta is the temporary organ required for adequate delivery of oxygen, nutrients, and ions to a growing fetus and its protection against direct contact with infections of the mother. Chronic disorders of the vital functions of the placenta and its premature aging can disrupt normal development of fetal programming and lead to different diseases or fetal lethality.1, 2 The placental mineral deposits occur quite commonly among various placental pathologic findings; mainly they are presented in the form of calcification.3-5 Case reports in literature also present the same clinical observations about positive correlation between PC development and certain metabolic shifts in a woman’s homeostasis.6-8

The mechanisms of calcium biomineralization probably involve one of three known mechanisms of tissue calcification:4 physiological (like bone), pathological (ischaemia-related), and “metastatic” (mineralization in a supersaturated microenvironment), which may appear in soft tissues as placenta. According to the chemical composition, energy-dispersive X-ray analysis of PC, and its polymerase chain reaction analysis 3 have suggested that the PC development is progressing rapidly via a puzzling induction of a local supersaturated environment in the placenta. To the present time, a proposed placental site where the latter environmental conditions could appear is not yet at hand.

Considerable recent attention has been focused on ultra-small microorganisms, collectively referred to by geologists and biologists as "nannobacteria" or "nannobacteria" that are involved in biomineralization.9 Firstly, the "nannobacteria" have been found and isolated from human stones and from human dental plaque.10-14 In contrast, no nannobacteria were found during their search in a row of cases of tumoral calcinoses, subepidermal calcified nodule, and some others.15 By our point of view, the findings of nannobacteria raised the intriguing possibility of whether these microorganisms can act as promoting factors for
pathological calcification of placentas. Therefore, the goals of our study were to investigate the PC-containing tissues and to establish the presence of the nannobacteria there. The presented electron-microscopic data of the PC investigations presented below confirm validity of our suggestion.

The transmission electron microscopy discovered the presence of three types of objects of interest in the calcified sites of the placental tissues. They were: micravoids in the tissues, nannobacteria in the micravoids and around them and mineral microparticles (irregular grains) of calcium localized in the same sites. These objects were never found in normal placental tissues with the same methods of investigation. The micravoids were of 1 x 6 µm size, however, they served as a main place of localization or concentration of the nannobacteria and the mineral microparticles (Fig.1). Low electron density of the micravoid, in comparing with the surrounding tissues, testifies to the fact that the cavities were filled with some solution or gel favorable for accumulation of dissolved inorganic salts, i.e., for deposition of insoluble calcium compounds. The experimental finding confirms the earlier proposed mechanism of PC development, namely, that rapid formation of apatite mineral in placenta means the existence of some micravoids with the supersaturated environment. Thus, our publication presents evidence that the placental micravoids are the same micravoids where the proposed environment can be generated. The underlying cause for the micravoid formation as a fertile ground for calcium deposit is not yet known.

Our investigations on the contents of micravoids and the close surrounding tissues showed that presence of mineral microparticles can be confronted with distribution and concentration of nannobacteria (0.15-0.20 µm in diameter) within the sites. The revealed nannobacteria were coated with cellular membranes. It should be noted that some of the nannobacteria were found in direct contact with calcium microparticles, i.e., in localization similar with that described for nannobacteria in human kidney stones or in dental plaque. The cited authors explained localization of the nannobacteria on/in grain of mineral deposits as a cause and effect of the membrane-induced mechanism of calcification. Indeed, the bacterial membrane can accumulate inorganic ions. We found the situation during artificial formation of bacterial nannocells. However, it must be mentioned that localization of nannobacteria inside of mineral grains or coating of the whole bacterial cell with mineral envelope excludes bacterial growth and multiplication because it limits their outer space. Our observations on the PC formation in micravoids are more attributable to other mechanisms of calcification, namely, development of a local environment that is supersaturated with calcium and phosphate or oxalate as it occurs with urolith forma-

![Fig.1. Mineral deposits and nannobacteria inside of a microcavity in the calcified placental tissues (transmission electron microscopy, bar is 0.30 µm).](image-url)
tion. There are some known biochemical compounds or, as well, bacterial products identified as inductors and/or promoters of tissue calcification. Calcium is actively transported via the placenta throughout gestation, making the fetus relatively hypercalcemic. This action supports the cellular divisions in the uterus and placenta. According to clinical observations, enhanced PC is often seen in placenta associated with fetal growth restriction, probably via inadequate calcium delivery to growing fetal organs and/or disruption in Ca-dependent metabolic bonds. Moreover, a growth-restricted fetus often has abnormalities in the bone mineralization that may be observed in a delayed appearance of ossification sites. Complexes of these abnormalities can break down a fetus’s ability to survive or can give rise to development of different diseases.

The present study has provided the first demonstration of novel link between the PC development during a woman’s pregnancy and placental microcavities containing the nannobacteria. This paper reports our initial results on the investigation and discusses a possible participation of nannobacteria in the formation of calcium placental deposits and possibilities for development of fetus pathology. Mechanisms of the PC formation by nannobacteria are still under discussion and remain to be defined in the future.

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REFERENCES.

“In medicine one must pay attention not to plausible theorizing but to experience and reason together.”

– Hippocrates, (4th C. B.C.)