I. Project Title: Evaluation of the Biomedical Significance and Prognostic Value of Calcification in the Placenta

II. Grant Information: American Heart Association AHA19CDA34660038

III. Project Description: This project investigated whether placental vascular calcification is an early marker of placental dysfunction. Mineral homeostasis and ectopic deposition are regulated by complex pathways, both active and passive. In order to investigate ectopic mineralization pathways during pregnancy in normal and diseased conditions, our team first work towards determining biological fluid concentrations of essential minerals which are consumed through dietary consumption, including calcium, magnesium, and phosphate (phosphorus). The enclosed datasets include experimentally determined biological fluid phosphate concentrations. Conditions, procedures, and characteristics are as follows:

Maternal urine and placental tissue section samples from 9 normotensive pregnant women and 7 preeclamptic women were purchased from the Global Alliance for the Prevention of Prematurity and Stillbirth (GAPPS). Preeclampsia was defined by standard clinical criteria. Gestational ages were between 28.7 and 33 weeks. Samples were coded by GAPPS without any patient identifiers and analyzed without knowledge of the disease status. Specimens from participants with a history of smoking were excluded from this study. Participants were characterized with preexisting pregnancies and divided in two groups as follows: first pregnancy and second pregnancy. Participants were characterized with preexisting diabetes mellitus, which included Type I, Type II and Gestational Diabetes Mellitus, and divided in two groups as follows: normal glucose tolerance (NGT) and diabetes mellitus. Aliquots were stored at −80 °C until analysis.

De-identified amniotic fluid samples were obtained from OHSU (MTA-OUT16-095). A total of 11 s trimester and 9 third trimester samples were obtained. Gestation age of delivery (GAD) ranged between 25 and 39 weeks on average. Gestation age of sample collection (GASC) ranged between 17 and 35 weeks on average. We tested whether amniotic fluid phosphate levels were an indicator for preterm or early pre-term birth for second and third trimester of pregnancy. Aliquots were stored at −80 °C until analysis.

IV. How was the project carried out: In this study we assessed phosphate levels during pregnancy. Two cohorts were studied. The first cohort provides information on maternal

urinary phosphate levels. The second cohort provides information on amniotic phosphate levels.

Pi Quantification: Maternal urine Pi levels were determined with the Phosphate Assay Kit (Sigma Aldrich, St. Louis, MO, USA; MAK308) and amniotic fluid Pi levels were determined with the QuantiChrom Phosphate Assay Kit (Bioassays Systems, Hayward, CA, USA; DIPI500) according to the manufacturer's instructions.

Methods for Von Kossa Staining and Imaging of Placenta Tissue: The von Kossa staining was performed as previously described in Speer et al. 2009 [31] with the inclusion of an optimally reduced 22 min von Kossa treatment. Placental tissue section from 8 normotensive pregnant women and 8 preeclamptic women were stained, and images of mounted sections were acquired on a Keyence BZ-X800 microscope using the Keyence BZ-X software (Keyence, Ozaka, Japan) at 4× magnification. File names were coded. Individual calcified lesions were imaged on a Nikon E800 Upright Microscope (Nikon Corp., Tokyo, Japan) at 2.5 and 10× magnification and file names were coded. Semi-quantitative analysis of calcified lesions was performed on all tissue sections using ImageJ/Fiji (NIH, Bethesda, MD, USA) histomorphometry software.

Statistical Analysis of Experimental Data: The following statistical tests were used to analyze quantitative data in GraphPad Prism 6 software for Windows (GraphPad Software Inc., La Jolla, CA, USA). For comparison of two groups, a p-value was determined by a two-tailed Student's T-test with unequal variance. For comparison of three or more groups, a p-value was determined by a Welch's T-test with unequal variance. Slope calculations were obtained by Deming (model II) linear regression.

V. How to cite:

MDPI and ACS Style: Correia-Branco, A.; Rincon, M.P.; Pereira, L.M.; Wallingford, M.C. Inorganic Phosphate in the Pathogenesis of Pregnancy-Related Complications. *Int. J. Mol. Sci.* 2020, 21, 5283. https://doi.org/10.3390/ijms21155283

OR

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VI. Licenses: N/A