alteration in this interaction could be a pathological mechanism by which antiphospholipid antibodies cause pregnancy morbidity.
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PA.56.
OXIDATIVE STRESS AND NUCLEAR DNA DAMAGE IN HYPERGLYCEMIC PREGNANCIES

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Oxidative stress and DNA damage have been associated with a number of diseases, including endocrine, neurodegenerative and cancer.
Objective: This study was conducted to evaluate oxidative stress markers and nuclear DNA damage in blood from pregnant women exposed to different hyperglycemia levels as well as in umbilical cord blood.
Methods: This cross-sectional study included 80 pregnant women who underwent 75 g oral glucose tolerance test (GTT-75g) and glycemic profile (GP), which were applied in parallel between the 24th and 28th weeks of pregnancy. Pregnant women were classified into the following groups: Non-diabetic (ND; normal GTT-75g and GP; n=26), Mild gestational hyperglycemia (MGH; normal GTT-75g and abnormal GP; n=17), Gestational diabetes mellitus (GDM; abnormal GTT-75g in pregnancy; n=24), and DM2 (abnormal GTT-75g prior to pregnancy; n=13). Maternal glycemic control was evaluated by HbA1c levels (high-performance liquid chromatography) and the glycemia mean (GM) performed in GP. Maternal and umbilical cord blood was collected to evaluate nuclear DNA damage (by Gene-specific quantitative PCR) and oxidative stress markers (reduced thiol groups by DTNB assay and antioxidant capacity by Amplex →Red assay). The generalized linear model, LSMeans Test and Tukey test ($p < 0.05$) were used for statistical analysis.
Results: The glycemic control was less strict in pregnant women with the MGH, GDM and DM2 than ND. These groups showed higher HbA1c percentage and higher GM levels, confirming the presence of hyperglycemia. Pregnant women with GDM and DM2 presented higher levels of nuclear DNA damage, lower levels of reduced thiol group and no difference in antioxidant capacity when compared with MGH and ND groups. No differences were observed in umbilical cord blood.
Conclusions: Mothers with GDM or DM2 present increased oxidative stress, which may be associated with the higher levels of DNA damage observed in these pregnant women. *Supported by FAPESP (2011/18240-2; 2011/13562-1).

PA.57.
DIFFERENTIAL EXPRESSION OF CATALYTIC SUBUNITS OF NADPH OXIDASE IN HUMAN PLACENTA FROM GESTATIONAL DIABETES

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NADPH oxidase (NOX) catalyzes the synthesis of reactive oxygen species (ROS), mainly superoxide. In gestational diabetes mellitus (GDM), bioavailability of NO is reduced in response to the increase in the synthesis of superoxide induced by a state of sustained hyperglycemia, which finally leads to the development of endothelial dysfunction and cardiovascular complications.
Objectives: To determine the levels of expression of NOX2 and NOX4 in placental vessels from GDM and healthy patients.
Methods: Normal (n=27) and GDM (n=18) placentas were collected from Hospital Guillermo Grant Benavente, Concepción, Chile. Clinical data of the patients were collected to classify them in both groups and avoid disturbances in results due to other syndromes or pathologies (ethics committee approval and informed patient consent were obtained). Total RNA was obtained from chorionic plate artery (CPA), chorionic plate vein (CPV) and placental microvilli (PMV) and converted into cDNA by reverse transcription. mRNA levels for 28S, NOX2 and NOX4 were determined by PCR and amplification products were observed in agarose gel. Images were analyzed with ImageJ® and statistical significance ($p<0.05$) was calculated by GraphPism5®.
Results: NOX2 mRNA expression was increased ($p<0.05$) 12-fold in GDM-CPA as compared to controls, without significant change in CPV. In GDM-PMV, NOX2 mRNA diminished ($p<0.05$) 63% related to normal tissue. NOX4 mRNA levels decreased ($p<0.05$) 55% and 69% in CPA and PMV from GDM placentas, respectively.
Conclusions: There is a differential expression of NOX2 and NOX4 subunits in GDM placentas, with important increases in NOX2 in GDM-CPA, which counteract with lower expression of NOX4 in the same pathological vessel. In microvilli us tissue, both NOX2 and NOX4 are importantly diminished. These differences could be adaptations of the placenta, detected at the end of GDM gestation in response to maintained hyperglycemia and oxidative stress during pregnancy. Supported by FONDECYT 11100192 and VRID-Asociativo 213.A84.014-1.0.

PA.58.
INVOLVEMENT OF HYPOXIA AND INFLAMMATION IN EARLY PREGNANCY LOSS MEDIATED BY SHIGA TOXIN TYPE 2

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Symptomatic or asymptomatic Shiga toxin producing *Escherichia coli* (STEC) infections during early pregnancy may cause maternal or fetal damage mediated by Shiga toxin type 2 (Stx2). We have previously demonstrated that Stx2 produces early pregnancy loss in rats.
Objective: The aim of the present study was to elucidate the mechanisms responsible for early pregnancy loss in Sprague Dawley rats treated with Stx2.
Methods: Sprague Dawley pregnant rats were intraperitoneally injected on day 8 of gestation with a sublethal dose of purified Stx2 (0.5 ng of Stx2/g of total body weight, 250 μl). Control rats were injected with the same volume of vehicle. Experimental and control rats were sacrificed 48 h post-injection (day 10 of gestation) and blood and utero placental units were collected. Globotriaosylceramide (Gb3), Stx2 receptor, was detected by thin-layer chromatography and Stx2 was localized by immunohistochemistry. Hypoxia was evaluated using the Hypoxyprobe-1 kit. Local VEGF expression was studied by Western blot. Inflammation was evaluated by leukocyte infiltration and both systemic and utero-placental TNFα- expressions were evaluated by ELISA, 2 h post-injection.
Results: Our results demonstrate that Stx2 reaches the utero-placental unit where Gb3 is present. Stx2 injection triggers alterations in the utero-placental tissues and produces local hypoxia. However, no alterations in VEGF expression were detected in experimental rats compared to controls. Decidual TNFα- expression of Stx2-treated rats was increased.
Conclusions: These results suggest that hypoxia and inflammation contribute to the early fetal loss produced by Stx2 and that VEGF is not early involved in this process. We propose that an alteration in the utero-placental microvasculature due to a direct cytotoxic effect of Stx2 is responsible for the hypoxia in the utero placental unit. Poor oxygen supply accompanied with oxygen consumption and damage due to inflammation, could be responsible for early pregnancy loss.