The placenta is a highly vascularized organ that mediates the complex functional interaction between mother and fetus, being the endothelium, the main tissue involved in this interaction. Dysregulation of the endothelial function leads to pregnancy disorders such as gestational diabetes mellitus (GDM). It has been described that human placental microvascular endothelial cells (hPMECs) from pregnancies with GDM have high basal levels of nitric oxide (NO) and respond to insulin decreasing this synthesis to levels observed in untreated non-pathological cells. In contrast, in cells from normal pregnancies insulin increases NO levels. Thus, NO is synthesized by endothelial nitric oxide synthase (eNOS), whose expression is regulated by the transcription factors FoxO1/FoxO3a, whose expression is regulated by the transcription factors FoxO1/FoxO3a, among others. However, the role of insulin in this pathway is unknown. We propose that FoxO1/FoxO3a control the expression of eNOS in insulin response in these cells through of differential regulation of their receptors, Insulin Receptor IR-A and IR-B. We used cell line of skin microvascularutalure (HMEC1) in conjunction with hPMEC of healthy and DMG placentas. In HMEC1 (exposed to high or normal glucose levels) and hPMEC (from normal and DMG pregnancies), treated and untreated with insulin, analyzed the expression of FoxO1/FoxO3a by qPCR and nuclear/cytosolic localization by western blotting and immunofluorescence. Also, we evaluated eNOS expression in HMEC1 by these same methods. And we analyze intracellular NO levels by a fluorometric assay. Preliminary results suggests that FoxO1 cytosolic levels decrease in microvascular endothelial cells after insulin treatment. This could be related with FoxO1/FoxO3a nuclear activity increase and therefore eNOS transcription reduction.

Aim of study
To characterize a cellular model for the study of eNOS activity regulation by insulin in human microvascular endothelial cells, in the context of Gestational Diabetes Mellitus.

Materials and Methods

(A) hPMEC primary cultures

- Immunoselction (CD31+)
- mRNA expression (qPCR)
- Insulin Treatment
- Nucleus – Citosol Fractionation (WB)

(B) HMEC1 cell line cultures

- 5 mM Glucose
- 25 mM Glucose + 20 mM Manitol
- Insulin treatment

Results

Figure 1: mRNA expression of FoxO1 and eNOS in hPMEC primary cultures. qPCR assay for FoxO1 and eNOS in hPMEC primary cultures from normal (n=1; A-B) and GDM (n=2; C-D) pregnancies, after insulin treatment (1 nM; 0,2,4,6, 8 hours). eNOS mRNA expression shows a tendency to decrease after insulin treatment. Relative expression, normalized against β-actin.

Figure 2: Cytosplasmic and nuclear levels of FoxO1 in HMEC1 primary cultures. Western Blot analysis of citosolic and nuclear fractions of hPMEC primary cultures from normal (n=1, A) and GDM (n=2, B) pregnancies, after insulin treatment (1 nM; 0,2,4,6, 8 hours). FoxO1 citosolic levels show a tendency to decrease after insulin treatment.

Figure 3: Protein levels of eNOS, FoxO1 and FoxO3a in HMEC1 culture cells. Densitometric analysis of Western blot assays against eNOS (A), FoxO1 (C) and FoxO3a (D) in HMEC1 cultures after insulin treatment (1nM; 6 hours). FoxO1 and FoxO3a were detected only in citosolic fractions were shows a tendency to decrease. eNOS shows a tendency to increase in nuclear fraction (A).

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