Chapter 7

The Role of Placenta in the Fetal Programming Associated to Gestational Diabetes

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Additional information is available at the end of the chapter

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1. Introduction

Gestational diabetes mellitus (GDM) is a human pregnancy disease characterized by elevation of glucose levels (i.e., hyperglycemia) responsible for a several adverse perinatal outcomes included macrosomia, fetal hypoglycemia, requirement of neonatal intensive care and neonatal mortality, among others. Estimation of the epidemiological impact of GDM has indicated that at least 1 out of 10 pregnant woman is being affected by GDM worldwide. In addition, GDM causes not only short-term complication in both mother and fetus, but also is associated with elevated risk for long-term complication such as cardiovascular disease, obesity and diabetes. Even though it is not feasible to exclude the genetic component in the elevated risk for metabolic/cardiovascular disease later in life, the general agreement is that hyperglycemia generates an adaptive response in the fetus addressing to control the glucose level, characterized by hyperinsulinemia. Part of this adaptive response, might also include the elevation in the placental consumption of glucose and enhancement of the feto-placental blood flow, especially in fetus large-for-gestational age (LGA). On the other hand, due to lack of innervation in the placenta, the vascular tone is controlled by the regulation of the synthesis and release of vasoactive substances from the endothelium like vasoactive molecules, nitric oxide, adenosine, prostaglandin, among others. Interestingly, vasoactive molecules may also regulate endothelial proliferation and migration, suggesting that they also affect the vessel formation (i.e., angiogenesis). In this regard, several studies have shown that placenta from GDM is characterized by hypervascularization and elevation in the pro-angiogenic signals including the secretion and activity of the vascular endothelial growth factor (VEGF). In addition, hyperglycemia also generates a status of oxidative stress, where free radicals derived from oxygen (ROS) induces changes in the endothelial cell membranes producing an elevation

in the cell permeability. Therefore, it is feasible that the adaptive response -useful for surviving in a hostile medium (i.e, hyperglycemia) - may be imprinting in the fetus and once he/she is exposed to other different conditions after delivery, this response might constitute a risk factor for developing metabolic diseases. In this chapter, we will review the available literature focus on the role of feto-placental endothelial dysfunction as the possible main factor in the generation of short-term complication during GDM and speculate how it may program the response of the sibling exposed to GDM.

2. Gestational diabetes: Definition and epidemiology

Pregnancy is a physiological state where occurs a series of complex anatomical and functional adaptation in the mother to facilitate the development of fetus. For instance, during the normal pregnancy a "physiological" insulin resistance is necessary to provide glucose to the growing fetus [1]. However, this normal adaptation is no longer occurring in some conditions and generates a clearly pathological state of insulin resistance, which is called Gestational Diabetes Mellitus (GDM). Therefore, GDM has been defined as any degree of glucose intolerance with onset or first recognition during pregnancy [2]. Specifically, the World Health Organization (WHO) has stated that GDM encompasses impaired glucose tolerance and diabetes identified as fasting glucose level ≥ 7 mmol/L or ≥ 126 mg/dL; or 2 hours plasma glucose after oral glucose (75 g) tolerance test (OGTT) ≥ 7.8 mmol/L or ≥ 140 mg/dL [3]. Despite this recommendation, it has been worldwide accepted recently a new diagnosis criteria, which it has been given by the Diabetes in Pregnancy Study Group (IADPSG) based on the OGTT (fasting glucose ≥ 5.1 mmol/L or ≥ 92 mg/dl, or a one hour result of ≥ 10.0 mmol/L or ≥ 180 mg/dl, or a two hours result of ≥ 8.5 mmol/L or ≥ 153 mg/dL), which is still controversial based on the analysis of the risk for perinatal adverse outcomes [2]. This discrepancy has been extensively discussed in the literature but the general agreement is that adverse perinatal outcomes occur in lesser degrees of hyperglycemia than the recommended as diagnostic criteria by the WHO [4].

Prevalence of diabetes for all ages is increasing worldwide, including women in fertile age. Therefore, it is not surprising that diabetes diagnosis before or during gestation has been defined as a public health problem [5]. Epidemiologically speaking, it has been estimated that near to 90% of the diagnosis of diabetes in pregnancy is actually GDM [5]. More precisely, GDM affects from 1.4% to 25.5% of pregnancies, however, its incidence will depend on the population, which it has been tested and the diagnostic criteria used [6,7]. Thus, taken into account the origin of the population, it has been described that women from Asian, African American, and Hispanic background exhibit twice the risk for being diagnosed of GDM compared to those of non-Hispanic White origin, a phenomenon observed also in women in the lowest socio-economical quartiles compared to women in the highest quartiles [8,9].

The underling mechanisms responsible for GDM are under investigation; however, likewise to other causes of type 2 diabetes, GDM is characterized by a dysfunction in the pancreatic β cell, which does not produce enough insulin to meet the increased requirements of late

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pregnancy. Mechanistic studies reveal at least three possibilities: 1) The presence of anti-islet cell antibodies (<10% cases); 2) Genetic variants of monogenic forms of diabetes (1-5% cases), and 3) Presence of obesity and chronic insulin resistance (>80% cases) [10]. In addition, it has been described that the large majority of the insulin secretory defects present in the third trimester of gestation, are actually manifesting before and soon after pregnancy [10,11]. In this way, considering that a) obesity, is a condition of insulin resistance and a common risk factor to GDM, and b) insulin secretion during pregnancy increases according to gestational age in women with and without GDM [10]; it has been reinforced the concept that chronic deficiency rather than gestational-acquired deficiency of insulin secretion is the underling cause for GDM. Consequently, these evidences have broken the traditional vision of GDM pathogenesis, where the imbalance in glucose level at the third trimester of gestation has been consider exclusively as a defect in the "physiological" insulin resistance present in pregnant women.

With regard to insulin, it is well known that it reduces the elevated level of blood glucose; however, insulin is also regulating the metabolism of amino acids and lipids. Indeed, selective damage of β-cell in animal models generates a severe lipid defects that induce animal death [12,13]. This idea reinforces the general agreement of hyperglycemia is not the unique feature that may be taken into account during GDM management. In addition, it has been reported that in general, hyperglycemia is resolved after birth; however, there are epidemiological evidences showing that GDM constitutes a risk factor for development of diabetes mellitus type 2 (DMT2), as well as it constitutes a risk factor for hypertension in both mother and offspring. Thus, it has been estimated that about 10% of women with GDM have diabetes mellitus soon after delivery; whereas the rest will develop diabetes mellitus at rates of 20-60% within 5-10 years after the manifestation of GDM in the absence of specific interventions to reduce their risk [10]. Therefore these evidences have suggested that metabolic defects in GDM, characterized by hyperglycemia, and fundamentally, insulin deficiency (relative in GDM) are maintained after birth being a risk factor for metabolic and cardiovascular diseases in the mother and her sibling.

3. Fetal and neonatal outcomes in GDM

Gestational diabetes is associated with multiple adverse perinatal outcomes which include in the mother, haemorrhage, hypertensive disorders, obstructed labor, infection/sepsis, and maternal mortality [14]. Thus, in the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study [4], which included a large number of participants (23.316) in nine countries, who were divided into 7 groups according with the fasting and glucose plasma level observed during OGTT; and importantly considering a level of glycaemia lower than the WHO criteria, showed a linear relationship between glucose level (both fasting and after OGTT) with the occurrence of adverse perinatal outcomes such as birth weight and cord-blood serum C-peptide level above the 90th percentile, cesarean section, neonatal hypoglycemia, premature delivery, shoulder dystosia or birth injury, intensive neonatal care, hyperbilirubinemia, and maternal pre-eclampsia. On the other hand, at the fetal side, this study describes that the higher level of glucose, the higher risk (between 1.37 and 5.01) of elevated birth weight. Thus,

considering data on the difference in the birth weight between the lowest and the highest glucose categories was about 300g. Therefore, this study suggests that maternal hyperglycemia, even in the "normal" range according with the WHO criteria, is related to clinically important perinatal disorders.

Considering this report, a recent meta-analysis [2] which included a large number of patient (44.829), containing who were included in the HAPO study and using the criteria recommended by the WHO, showed that diagnosis of diabetes was associated with high risk (RR=1.37 to 1.88) for presenting macrosomia, large for gestational age, perinatal mortality, preeclampsia and cesarean delivery. When the authors excluded the HAPO study from their meta-analysis, the relative risks for the analyzed perinatal outcomes were minimally altered. Specifically, and considering the highest risk described in this meta-analysis, women with GDM exhibited a high risk for macrosomia (RR=1.81) and large for gestational age (RR=1.73). This association between GDM and macrosomia is particularly important for our discussion, since it has been described that fetal growth defects are associated with long-term complication, including obesity and diabetes [15,16]. Nevertheless, another highlight of this meta-analysis is that reduction in the criteria for "hyperglycemia" recommended by the WHO, should be considered for the next generation.

Although discrepancies in cut off value of glucose level for diagnosis of GDM, most of the alterations observed in GDM have been related with "hyperglycemia". For instance, it has been shown that intraperitoneal injections of high glucose in early pregnancy were associated with a modest but significantly increased placental weight and fetal weight [17]. Therefore, authors suggest that increased fetal growth may be explained by a large placenta and delivery of more nutrients to be transferred to the fetus. Since macrosomia is also present in "normo-glycemic" pregnant women, it has been suggested that other factors rather than high glucose by itself may take part in the pathophysiology of maternal and fetal-neonatal complication present in GDM [18]. In this way, other clinical components in GDM, included metabolic alteration such as insulin resistance, as well as high levels of cholesterol, triglycerides, adenosine, nitric oxide, and several other factors may disrupt normal function of maternal, placental and fetal tissues. Specifically, it is well accepted that hyperglycemia in the fetus exposed to GDM, generates a compensatory elevation of insulin; which in turn, is not only affecting glucose level, but also is acting as a growth factor. In addition, insulin is also regulating the transport of other nutrients such as amino acids or other regulatory elements such as adenosine [19,20,21]. In particular, it has been described that insulin increases the L-arginine uptake in human umbilical vein endothelial cells (HUVEC), a phenomenon associated with generation of vein relaxation and increasing Sp1-activated *SLC7A1* (for human cationic amino acid transport type 1, hCAT-1) expression [22]. In addition, it has been described that insulin increases the activity of neutral amino acid through the system A [23]. On the other hand, insulin recovers the reduced adenosine transport mediated by the *E*quilibrative *N*ucleside *T*ransport type 1 (ENT-1) in HUVEC, an effect that was associated with increased relaxation of the umbilical vein [24]. Therefore, the general consensus is that the fetus's tissues (and in particular the placenta) are able to generate a compensatory response characterized by hyperinsulinemia, and aimed to revert the deleterious effect of GDM (i.e., hyperglycemia) and ultimately improve

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fetal survive. In this context, it has been suggested that this adaptive response (i.e., fetal programming) may not match with the extrauterine environment, in both early and later life, and it would be responsible for either neonatal complications after birth or long-term diseases [15]. In the next section, it will be reviewed some of these evidences and the mechanisms linked with fetal programming in GDM.

4. Programming and GDM

Programming is defined as "the phenomenon whereby a stimulus occurring during a critical window of development, namely the prenatal and early postnatal periods, which can cause lifelong changes in the structure and function of the body" [25]. In this regard, the concept that the intrauterine environment might affect health later life became evident with the surprising observation that low birth weight was associated with increased cardiovascular disease 40 years later [15,16,26]. Numerous epidemiological studies extended these observations to suggest a role for the intrauterine environment as a leading cause of schizophrenia, depression, cardiovascular diseases, stroke, diabetes, cancer, pulmonary hypertension, osteoporosis, polycystic ovarian syndrome, among others in adult life [27,28,29,30]. These observational relationships are supported by animal experiments, which fetal growth manipulation by changing maternal nutrition or reducing blood flow to the placenta resulted in obesity, increased blood pressure and other cardiovascular abnormalities in the offspring later life [31]. As indicated, most of this observation included newborns with restricted growth but the contrary phenomenon (i.e., macrosomia) is observed in GDM.

In addition, a clear association between maternal diseases (including GDM) and future implication in health in the offspring has been affected by several confounding variables such as genetic factors (a particular phenotype may be genetically transmitted to the offspring), paternal implication (the father genotype may affect the phenotype), gender (hormonal differences may induce a particular gender-linked phenotype), diagnosis criteria used for maternal disease (in the particular case of GDM, the level of glycaemia), retrospective evidences (most of the epidemiological analysis coming from retrospective rather than prospective studies), among others. Despite those confounding factors, most of the available data in the case of GDM supports a predominant role for intrauterine exposition to hyperglycemia as one of the underling mechanisms for future chronic disease in the offspring exposed to this disease [25]. Among the evidences that support this assumption, it has been described that children born after a diabetic pregnancy in Indian Pima women exhibited a high (6-fold) prevalence of type 2 diabetes than those who were born from a non-diabetic pregnancy. Interestingly, this high prevalence persists after a multivariable analysis, taken into account paternal diabetes, age of onset of parental diabetes in father and mother and obesity in the offspring [32]. Besides, another study showed that the risk of diabetes was significantly higher (≈ 4 fold) in siblings born after GDM than those who were born before the mother has been diagnosed with diabetes [33]. In the next section we will highlight some of those evidences.

Offspring "exposed" to GDM shows a high risk for developing obesity, impaired glucose tolerance, type 2 diabetes, malignant neoplasm and hypertension in adulthood

[34,35,36,37,38,39]. For instance, initially, it has been reported that offspring (10-16 years) "exposed" to maternal diabetes showed a higher prevalence (6-fold) of impaired glucose tolerance and body mass index than controls non-exposed [40]. Furthermore, this finding was confirmed in another study including children (1-9 years) who their mothers presented pregestational insulin-dependent diabetes (IDDM) or GDM [41]. Following to this study, prospective data from the Framingham Offspring Study [42], which included a large sample (2.527 subjects), found that offspring (26-82 years) of women with diabetes showed a high risk (≈3-fold) to impaired glucose tolerance and type 2 diabetes compared to individuals without parental diabetes. This risk was almost three times higher in children belong to diabetic mothers <50 years. Moreover, another study also confirms these findings, where offspring "exposed" to GDM exhibited ≈ 7 folds increase in the prevalence of type 2 diabetes or impaired glucose tolerance compared to offspring from non-diabetic pregnancy [39]. Interestingly, this risk was even higher than offspring of women with type 1 diabetes who presented ≈ 4 fold risk for being diabetic [39], reinforcing the idea that maternal intrauterine environment generates a particular phenotype which is not explained only by heritage. Nevertheless, Clausen et al (2009) have reported a high risk (≈ 2 fold) for developing overweight or metabolic syndrome in offspring of women with GDM or type 1 diabetes compared to offspring from non-diabetic pregnancies. It has been also reported that the higher hyperglycemia in the mother [36] or the weight for gestational age in children exposed to GDM [43], the higher risk for metabolic syndrome in the offspring in future life.

Moreover, GDM is also associated with high risk for cardiovascular diseases in the offspring. Thus, in a large cohort study, it has been reported that children exposed to GDM had higher systolic blood pressure (≈3 mm Hg) than non-exposed children [44]. Moreover, other study [38], which also included children (5-9 years) "exposed" to maternal diabetes, reported a higher level of insulin resistance (i.e., HOMA index) than control subjects. On the other hand, another recent report including more than 1.7 million singleton born in Denmark found that sibling (followed for up to 30 years) "exposed" to GDM exhibited a high risk for developing malignant neoplasm (2.2-fold) and for diseases of the circulatory system (1.3-fold) [45]. Interestingly, a significantly higher risk for those groups of diseases were also observed in children whose mother had type 1 diabetes or pre-gestational type 2 diabetes. Therefore, a hyperglycemic intrauterine environment seems to be part of the pathogenesis of chronic metabolic and cardiovascular disease in the offspring of GDM [36,37,39].

The mechanisms linked with fetal programming during GDM have been associated with hyperglycemia, through the hypothesis of fuel-mediated toxicity (Freinkel's hypothesis) [46], which indicates that fetus experiences a "tissue culture" environment, in such circumstances, where high availability of nutrients may induce a "fuel-mediated teratogenicity". In this scenario, high metabolism of glucose in the fetus may generate an excessive consumption of oxygen (i.e., relative hypoxia) and consequently, it may generate an oxidative stress condition. This last phenomenon, would affect the normal development of organs or systems that are completing its development during late gestation and/or perinatal period, such as placenta, kidney and vasculature [25]. Particularly, we will focus on the alterations observed in the placental vasculature, early in life, that may support the association between elevated risk for

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cardiovascular disease during adulthood and GDM. In this regard, two branches of the knowledge may be discuss: 1) Anatomical and functional alterations that are involved in the deregulation of placental vascular tone control [47]; and 2) Alterations in the formation of new blood vessels (i.e., angiogenesis) [48]. Since placental circulatory system form a continuous network with the fetal circulation, it is feasible to propose that changes in the function and regulation of all these vessels early after birth may give clues of the abnormalities that will occur later in life.

5. GDM and placental anatomy/histology

The placenta- endocrine organ and an immune barrier - is the functional unit where occurs the exchange of oxygen and carbon dioxide, the absorption of nutrient and the elimination of metabolic waste. The placenta prevents the passage of macromolecules over 700 Daltons, whereas the smallest particles can cross (for instance melatonin, catecholamines and other hormones) [49,50]; therefore, this tissue exhibits a selective permeability that is known as the placental barrier. In the formation of human placenta, the maternal vessels are invaded by trophoblastic cells, which in turn are in direct contact with maternal blood. This type of structure is named hemochorial placenta [51]. Two layers coexist in this structure, the maternal and the fetal one. In the maternal side, a laminar degenerative process in the junctional zone forms the maternal layer or uterine surface, which in general are formed by maternal vessels where the endothelium has been replaced by placental cells (invasive cytothrophoblast), remnants of endometrial glands and connective tissue. Moreover, grooves is shown in this structure, which subdivide the surface of placenta in about 10-40 elevated areas similar to lobules named maternal cotyledons, which are in perfect correlation with fetal cotyledon [51]. The fetal component, cotyledon, is formed by several villous trees (1-3 villous trees per fetal cotyledon), which in fact are formed by chorionic villus. This anatomic and functional structure are formed by syncytiotrophoblasts/cytotrophoblasts, stromal core villi and fetal vascular endothelium [52].

Cytotrophoblast and differentiated syncytiotrophoblast are derived from trophoblastic cells. The syncytiotrophoblast is a multinucleated and continuous layer of epithelial cells, which is formed by the fusion of cytotrophoblasts. In the other hand, syncytiotrophoblast is covering the villous trees and it is in direct contact with maternal blood, therefore, it is the area where direct exchange of oxygen, nutrient and removal of waste products occurs [53]. Moreover, syncytiotrophoblast have an endocrine function characterized by production of human chorionic gonadotrophin (hCG) regulated by progesterone [50]. Besides, those cells also secrete a variant of growth hormone (GH), human placental lactogen (hPL), insulin-like growth factor I (IGF-I) and endothelial growth factor [50,53]. On the other hand, cytotrophoblasts (or Langhans´cells) are continually differentiating into syncytiotrophoblast. In addition, this layer also may synthesize hCG [54]. There is a trophoblastic basement membrane supporting these two layers, cytotrophoblast and syncythiotrophoblast. This membrane forms the physical separation of those layers with the stromal core villi, a structure formed by connective tissue where the fetal vessels are immersed.

In the placenta, the blood vessels constitute the largest component among the structures creating the cotyledons. These vessels are an intricate network coming from and going to the fetus. In fact, placental vessels constitute a continuous circulatory system with the fetal cardiovascular system. In the placenta, the veins are conducting oxygenated blood toward the fetus, whereas the arteries contain deoxygenated blood toward the placenta. Anatomically, from the umbilical cord to the deep in the placental cotyledons, the umbilical arteries and veins branch themselves to form chorionic arteries and vein, respectively, over fetal surface of the term placenta, and those branches subdivide themselves before entering into the villi. The chorionic arteries generally cross over the chorionic veins [53]. Likewise other vascular beds, in the placenta the veins are more elastic, exhibit high capacity and a miniscule layer of both smooth muscle cells and adventitia compared to arteries; which in turn are vessels that offer a high resistance. These characteristics, especially those observed in the umbilical cord, have been used for functional non invasive studies, like Doppler, in order to analyze the status of the feto-placental circulation. Finally, and similar to any other tissue, the placenta blood vessels are lined by the endothelium. In fact, these cells are obligatory constituent of blood microvessels [55]. The endothelial cells are supported by a basal membrane and pericytes, both of them involved in vessel permeability and integrity, and importantly in the endothelium differentiation [56].

In GDM, it has been reported macroscopical and histological alterations in the term placenta. For instance, placental size and placental weight [57] are elevated in GDM, which produce a reduced fetal/placental weight ratio compared to normal pregnancy [58,59], that means, the placenta growth is even higher than fetal growth. On the other hand, regarding studies, in syncytiotrophoblast from diabetes during pregnancy, have shown functional alteration in this cell type. Thus, it has been described an increase in the number of cytotrophoblast identified by number of nuclei [60], high fibrin deposit over syncytiotrophoblast and hyperplasia of cytotrophoblast [59,61,62], whose in turn may be related with the enhancement of the thickness of syncytial basement membranes in GDM compared to normal pregnancy [63]. Moreover, using functional studies of syncytiotrophoblast microvillous membrane vesicles, Jansson and collages [64] showed non-changes in the glucose transport in samples from GDM. Contrarily, other reports showed reduced glucose uptake and glucose utilization [65], as well as low expression of glucose transporter type 1 (GLUT1) and 3 (GLUT 3) in placentas from GDM compared with non-diabetic controls [66]. Other alterations in the throphoblastic cells from GDM were low expression of serotonin transporter (SERT) and receptors (5-HT2A) [67], as well as high activity of amino acid transporter system A [68]. Nevertheless, it has been reported a high expression of inducible nitric oxide synthase (iNOS) in the whole placenta but mainly in the trophoblastic cells using immunohistochemistry in GDM [69], a phenomena that may be correlated with high nitric oxide synthesis [24] and nitrative stress [70] observed in placentas from GDM.

In addition, it has been reported high level of degenerative lesions such as fibrinoid necrosis and vascular lesions like chorangiosis, as well as elevated signs of villous immaturity and presence of nucleated fetal erithrocytes in placentas from GDM compared to normal pregnancy [58]. In particular, the presence of microscopic signs of villous immaturity (i.e., hypervascu-

larization), as well as the presence of fetal erithrocytes and microscopic signs of ischemia [71] may suggest that placenta in GDM exhibits a high metabolic demand and oxygen consumption, which in turn, it is generating a "relative hypoxic" status in the fetus. Thus, it has been reported in GDM that the elevation of plasma glucose in the umbilical vein is associated with reduced oxygen saturation and oxygen content, as well as a significant increase of lactate concentration compared with normal pregnancy [59]. Interestingly, these changes were not observed in the umbilical artery, suggesting high placental oxygen consumption in GDM, which may generate a compensatory response in the placenta itself. In fact, as it will be described later in this chapter (see below), elevated vessel formation (i.e., angiogenesis) has been described in the placenta from GDM [18,72,73,74,75], which may explain the high placental "mass" observed in this disease. Therefore, placental alteration in GDM includes changes in the transport of nutrients (such as amino acid), enhanced blood formation and glucose consumption that may generate a "relative hypoxic" status. Unfortunately, all this findings are described in term placenta; therefore, non-invasive test such as Doppler will offer more clinically relevant information regarding fetal status and feto-placental circulation before delivery.

6. Placental blood flow and GDM

One of the non-invasive techniques used widely to estimate the blood flow in the feto-placental circulation is Ultrasound and Doppler. In this regard, the normal flow between 24 and 29 weeks of gestation in the umbilical vein is 443 ± 91.6 ml/min and normalized to fetal weight is 131.0 ± 19.8 (mL/kg/min) [76]. Moreover, the absence of end-diastolic blood flow before 36 weeks gestation is utilized clinically as indicator of fetal distress such hypoxia and acidosis [77] and this indicator is also associated to growth restriction [78].

Wharton's jelly area is surrounding the two arteries and the vein in the umbilical cord, and this jelly has a protective role for preventing interruption of flow by compression or twisting caused by fetal movement [79]. Wharton's jelly area can be determined by subtraction of umbilical cord area and total vessels area (arteries and vein), and interestingly it is significant correlated with gestational age and fetal anthropometric parameters [79,80], and also it has been described that alterations in this parameter are associated to hypertensive disorders, fetal distress, gestational diabetes and fetal growth restriction [80].

Doppler studies in umbilical vein from GDM have shown no changes neither in the pulsatile index value in the umbilical artery nor in the mean total umbilical venous flow in fetus exposed to GDM compared to normal pregnancy [81]. Interestingly, large for gestational-age fetus showed an increase in the total umbilical venous flow, suggesting that high placental flow toward the fetus may be associated with macrosomia. Moreover in macrosomic fetus without diabetes, it has shown an increase in the umbilical vein blood flow associated with high systolic velocity in the splenic, superior mesenteric, cerebral and umbilical arteries [82], suggesting an increased fetal perfusion especially in the liver. The underling mechanisms for this redistribution in the blood flow are unclear, but considering that GDM increases the synthesis of nitric

oxide in human umbilical vein endothelial cells [83], it is feasible to speculate that a overall vasodilatation in the pre-hepatic and hepatic circulation would be taken part in this process. Taken these evidences into account, it is feasible that elevated feto-placental blood flow and hyperglycemia would be responsible for macrosomia in GDM. However, the underling mechanisms of this relationship are part of ongoing investigations, and may include the synthesis and secretion of vasoactive substances from the fetal-placental endothelium, as it will be discussed in the next section.

a. Mechanism of vascular tone regulation in the placenta during GD: Role of endothelial cells

Endothelium in the feto-placental circulation is involved in a series of specific mechanism aimed to ensure the input of nutrients and oxygen to the fetus. These mechanisms include; maintenance of physiological barrier, regulation of vascular tone and angiogenesis. Importantly, feto-placental endothelium forms an uninterrupted tissue that will be extended until fetal circulation, where it is exposed to the same metabolic and hormonal medium than endothelium of the fetus itself [84]. Moreover, since the lack of innervation of the placenta, the regulation of vascular tone is mainly dependent on endothelial cells-mediated synthesis and release of several vasoactive substances including, nitric oxide (NO), prostacyclin, thromboxane, endothelial derived hyperpolarizing factor (EDHF), adenosine, mono or di or tri monophosphate of adenosine (AMP, ADP, ATP), among others [85,86,87]. These characteristics are summarized in the Figure 1, where it also described some functional alterations observed in GDM. On the other hand, there are emerging evidences showing that endothelial cells are able to dedifferentiate into mesenchymal cells, via a process called endothelial-to-mesenchymal transition (EndMT) [88,89], which in fact is related with the capacity of the endothelium to migrate away from the vessel-lining and colonize other tissues where dedifferentiation may occur in order to recover the particular capacity required by the invaded tissue. Additionally, endothelial cells exhibit a capacity to form new vessels (i.e., angiogenesis) via enhancement of its proliferation and migration toward the tissue where it would be required [90]. Both, dedifferentiation and vessel formation are mechanisms controlled by extracellular signals that are sensed by membrane receptors in the endothelium. Among others, transforming growth factor-β (TGF- β), VEGF, extracellular nucleosides (i.e., adenosine, ATP) have been related with endothelial function. Therefore, it is not surprising that endothelium exhibits a specialized function according with its cell localization and mainly according with the extracellular medium where they are seeded [91,92,93]. Taken these evidences into account, endothelium has been considered as a specialized endocrine organ which is able to control the vascular homeostasis in normal conditions; and its malfunctioning (i.e., endothelial dysfunction) has been related with several cardiovascular diseases, including hypertension and diabetes [86,87,94].

Human placenta is an unique source of endothelial cells for studying functional differences considering vessel distribution. Thus, it has been estimated that >70% of the placental tissue is constituted by blood vessels and length of fetal capillaries would be covering an area of 223 miles [91]. In addition, since autonomic control of the vascular resistance will not be part of the mechanisms for controlling blood distribution, endothelial cells are responsible for

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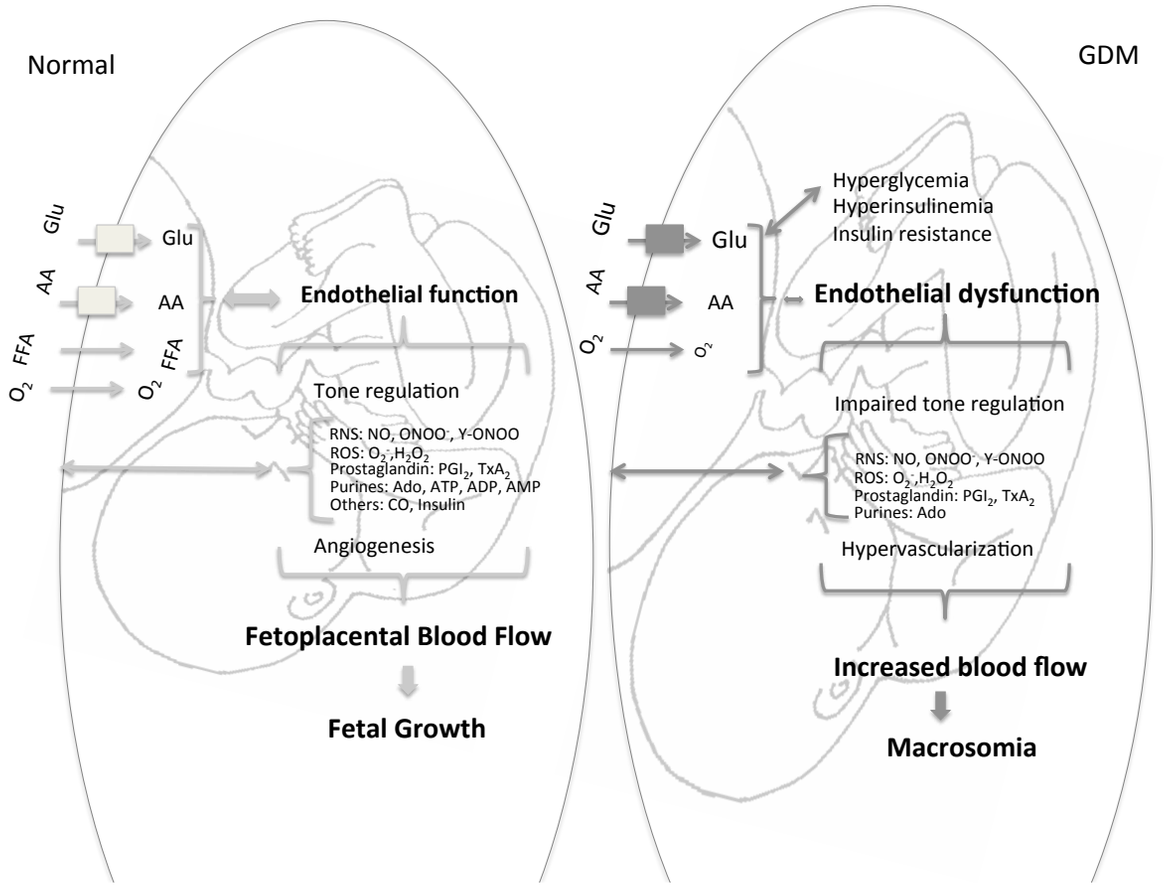

Figure 1. Role of feto-placental endothelial function in fetal growth during normal pregnancy and gestational diabetes. In normal pregnancy (Normal), the nutrients that includes glucose (Glu), amino acids (AA) are incorporated to the feto-placental circulation via specific transporters (boxes) whereas liposoluble molecules such as free fatty acids (FFA) and oxygen (O_2) pass through by simple diffusion (i.e., without transporters). These transport mechanisms are in a perfect equilibrium between demand and consumption, and they are highly dependent on the appropriated endothelial function in the placental vascular bed. In turn, endothelial function include: 1) the synthesis and release of vasoactive molecules including reactive nitrogen species (RNS) such as nitric oxide (NO), peroxinitrite (ONOO) and nitrotyrosine (Y-ONOO); reactive oxygen species (ROS), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2); prostaglandin such as prostacyclin (PGI_2) and thromboxane A_2; purines including adenosine (Ado), adenosine tri-di-or mono phosphate (ATP, ADP, AMP, respectively); and others factors such as carbon monoxide (CO), or insulin. 2) Capacity for vessel formation form pre-existing vessels (i.e., angiogenesis). Both, vasomotor and angiogenic properties are modulating the fetoplacental blood flow continually by a cross talking between placenta and fetus. On the other hand, there is an increase in the glucose level in the maternal circulation in gestational diabetes mellitus (GDM), which is transported to the feto-placental circulation generating and stated of hyperglycemia, hyperinsulinemia and insulin resistance. In turn this high glucose uptake may generate elevated oxygen consumption due to high metabolism. This elevation in the glucose metabolism in the placenta would generate an endothelial dysfunction characterized by elevation in RNS, ROS, prostaglandin and purine concentration in the feto-placental circulation, which consequently affects the tone regulation in the placenta. Moreover, relative hypoxic condition in GDM, may trigger an pro-angiogenic response generating a condition of hypervascularization in the placenta and therefore creating a vicious circle. Those alterations would be related with increased blood flow observed in Doppler studies. Finally, this high input of nutrients and elevated circulation will be responsible for macrosomia in GDM.

supplying this lack. Moreover, in human placenta, several studies have been shown that endothelium exhibits morphological and functional differences according to the vascular bed

where they are coming from [84,87,91,92]. Some of these differences have been described in recently published reviewers [84,87]. For instance and similarly to the pulmonary circulation in the adult, the feto-placental endothelium has a particular distribution. The veins transport oxygen and nutrients whereas the arteries contain de-oxygenated blood coming from the fetus. In terms of endothelial-derived vasomotor response, it has been described that acute hypoxia in the placental microvessels generates constriction [95], whereas this challenge generates augmentation of the umbilical blood flow [96]; this phenomenon is attributed to blood redistribution such occurs in the pulmonary circulation. Additionally, it has been also described in samples from umbilical endothelial cells exposed to hypoxia, that the one from the artery has a different response (i.e., high activation of endothelial nitric oxide synthase, without changes in arginase-2) than endothelium vein (i.e, low activation of eNOS, associated to high levels of arginase-2) [97]. In addition, HUVEC (i.e, macrovascular endothelium) showed a reduced synthesis of angiotensin II, thromboxane B_2, 6-keto-prostaglandin, and endothelin 1,2 compared to placental microvascular endothelial cells (hPMEC) [91]. Other functional differences occurred in the capacity for generating new vessels (i.e., angiogenesis). Thus, placental microvascular placental cells exposed to VEGF or placental growth factor (PlGF) showed a high mitogen response compared to HUVEC [91], a phenomena associated with high expression of VEGF receptor 1 (VEGFR-1) and 2 (VEGFR-2) [98] in this cell type. In addition, using feto-placental tissue it has been described that several genes related with angiogenic response are preferentially expressed in microvascular than macrovascular endothelium [86,87,91,92]. In this regards, studying functional differences between HUVEC and hPMEC, preliminary results (see Figure 2) showed that the tube formation in matrigel is faster in hPMEC than HUVEC, corroborating differences in the VEGF expression.

Several studies have reported dysfunction of feto-placental endothelium during GDM [18,74, 75,84,86,91,93,99]. In this regard, one of the most studied pathway in our group is the L-adenosine/L-arginine/NO (i.e., ALANO pathway) [100]. For instances, it has been described that L-arginine transport- mainly via the cationic aminoacid transport type 1 (CAT-1) - is increased in HUVEC from GDM [87,100,101,102]. Besides this alteration, it has been described high expression and activity of endothelial nitric oxide synthase (eNOS) [24,103] as well as iNOS [69] in both umbilical and placental endothelium from GDM. This enhancement would produce a high synthesis and release of NO [20,24], which in turn has been related with a nitrative status in the placenta and umbilical cord from this disease [104,105]. In addition, NO reduces the expression of adenosine transport via hENT-1 [83] and may generate augmentation in the extracellular level of adenosine in umbilical blood [106]. In turn, adenosine activates adenosine receptors (AR) spreading the vascular effects of NO in the feto-placental circulation in both vascular tone regulation [87,100,102] and promoting angiogenesis (see below). Therefore, it is feasible to speculate that the elevation in NO synthesis during GDM may explain the augmented umbilical flow observed in macrosomic fetuses [82].

b. GD and oxidative stress in the placenta

As detailed above, GDM has been associated with impaired placental development characterized by high placental weights and low ratios between fetal and placental weights [107]. Remarkably, one of the cellular mechanisms associated with the etiology of these

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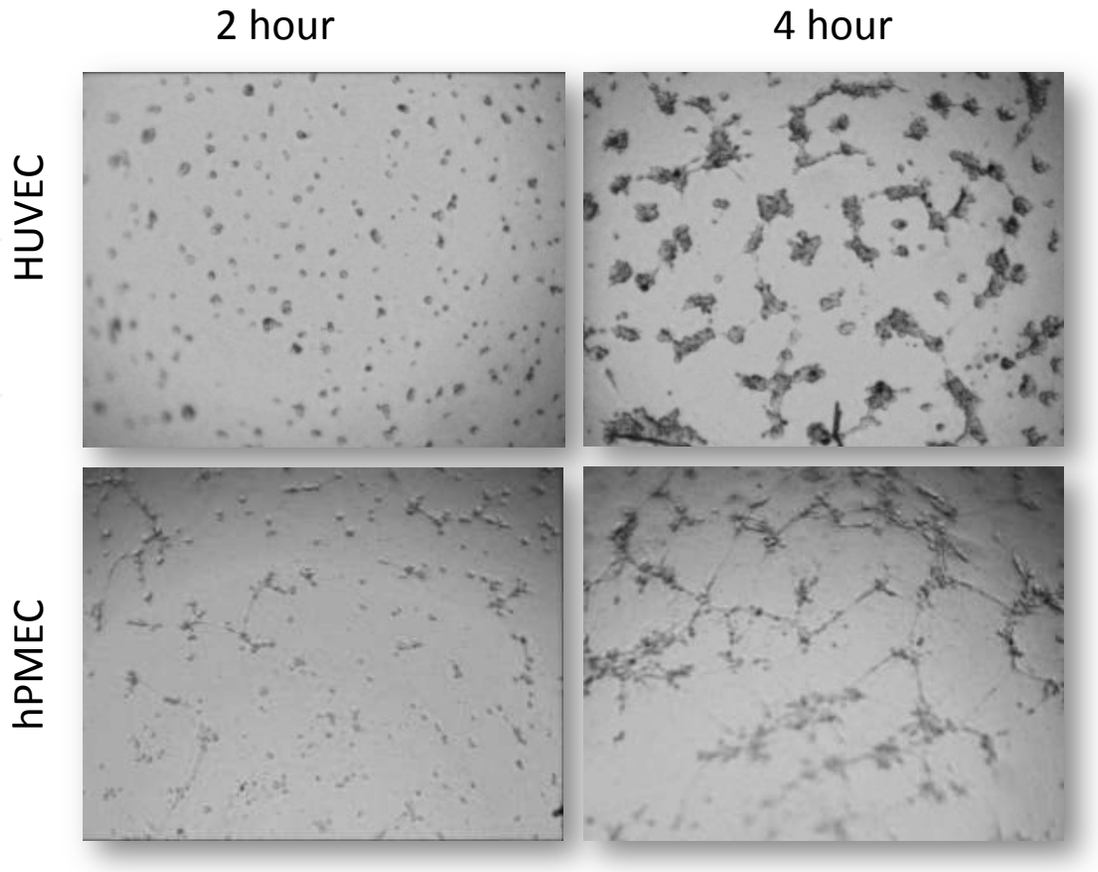

Figure 2. Differential capacity in the angiogenic ability of the fetal endothelium. Representative images of tube formation assay in matrigel using primary cultured human umbilical vein endothelial cells (HUVEC; i.e., macrovascular) and human placental microvascular endothelial cells (hPMEC; i.e., microvascular) isolated from normal pregnancy. After overnight serum deprivation, cells were seeded on the matrigel in a different number since 10 x 10^3 to 30 x 10^3 cells/ml prepared in culture medium (M199) without serum. In the images is presented only HUVEC that were seeded at 30 x 10^3 and hPMEC that were seeded at 10 x 10^3. Those cells were maintained in culture under standard conditions (5% CO_2, 37°C) during 0, 1, 2, 3 and 4 hours and cells were photographed. As indicated in the pictures, hPMEC exhibit a more rapid response for tube formation under our culture condition and require lesser quantity of cells than HUVEC, suggesting a differential physiological role of these cells in the fetal circulation.

changes is the oxidative stress, which is related with an imbalance between the synthesis of reactive oxygen and nitrogen species (ROS and RNS, respectively) and the activity of antioxidant enzymes. The most relevant free radicals are superoxide ($O_2^{\bullet-}$) in the ROS group; and nitric oxide (NO) and peroxinitrite ($ONOO^-$) in the RNS group. In addition, considering the diffusion distance, NO can diffuse from endothelial cells to smooth muscle cells, whereas $O_2^{\bullet-}$ and $ONOO^-$ would have actions within the cells where they were synthesized [70]. The main sources of $O_2^{\bullet-}$ in the placenta include the mitochondrial electron transport chain, xanthine oxidase, NADPH oxidase and uncoupled endothelial NO synthase (eNOS) [21,108], whereas the main source of NO are the endothelial and inducible NO synthases (eNOS and iNOS, respectively)[70,85].

The oxidative stress is an inherent condition of pregnancy related with the increasing metabolism of fetal and utero-placental tissues, which results in a continue delivery of

oxygen –and nutrients- toward the developing fetus. In fact, it has been described that the elevation in the normal metabolic rate of feto-placental tissues increases the oxidative stress in the placental [109]. Moreover, in placental tissue from early pregnancy has been determined a higher activity of NADPH oxidase; therefore, the synthesis of $O_2^{\bullet-}$ is more marked at the end of the first trimester than the activity in term placental tissue [110]. On the other hand, studies using samples obtained from patients with GDM showed that there is an increased activity of xanthine oxidase (XO) and a decreased activity of catalase in maternal plasma, umbilical cord plasma and placental tissue [111]. These findings showed that there is an impairment of antioxidant defenses in the placenta and blood from mother and newborn, which might be related with the high mortality and morbidity in both mother and newborn observed during GDM pregnancies. In addition, placental tissues from GDM exhibited a decreased response to oxidative stress induced by hypoxanthine plus XO, as was reflected by a reduced levels of catalase and glutathione peroxidase (GPx) after exposition to the pro-oxidative challenge, suggesting that placental tissues from GDM would be exposed to damage in an oxidative environment [112].

The hallmark of diabetes is hyperglycemia whose condition has been associated with increases of synthesis of ROS and RNS in tissues and cell cultures from umbilical cord and placenta. There is an increase of ROS levels in HUVEC exposed to high extracellular concentration of D-glucose mediated by activity of NADPH oxidase in a mechanism that involved a decrease of NO bioavailability and increases of vascular reactivity in umbilical veins [21,113]. In HUVEC, it has been described that the higher increase in the NADPH oxidase-mediated ROS induced by high concentration of D-glucose, the higher NO synthesis mediated by eNOS [21,113]. Considering the reaction rate between $O_2^{\bullet-}$ and NO, it is highly probable that the hyperglycemic condition induces the synthesis of $ONOO^-$; therefore, it contributes to the development of endothelial dysfunction in umbilical cord and placenta. Long-term incubation (7-14 days) of HUVEC with high concentration of high D-glucose increases the expression of regulatory subunits of NADPH oxidase $p67^{phox}$ and $p47^{phox}$ [114], whereas 24 hours incubation of the same cell type with high D-glucose increases the expression of the catalytic subunits NOX2 and NOX4 [113]. Thus, an increased expression and activity of NADPH oxidase would be a hallmark of HUVEC exposed to hyperglycemic, suggesting that the same phenomenon would be present in GDM.

On the other hand, recently data has been shown that in trophoblastic cells ACH-3P, incubated at 21 % oxygen and under normoglycemic condition, increases ROS levels after 3 days. Interestingly, ACH-3P cells treated (3 days) with high extracellular concentration of D-glucose increases the ROS levels only in cells exposed to lower percentage of oxygen (2.5 %). In fact, in ACH-3P cells, hyperglycemic conditions increase the mitochondrial $O_2^{\bullet-}$ levels. Therefore, this study is showing that ROS production in normoglycemia is oxygen-dependent but oxygen-independent in hyperglycemia. The mechanism involved in this phenomenon could involve the increase of expression and/or activity of oxidant enzymes present in placental tissue [115].

In summary, there is an imbalance in the control of redox cellular status in pathological conditions related with increases blood concentrations of molecules that induce oxidative stress, like GDM and hyperglycemia, probably due to a higher expression of oxidant enzymes like NADPH oxidase, XO and deregulation of metabolic pathways of NO.

c. Placental angiogenesis and GDM

Angiogenesis is a general term that involves the physiological process leading to growth of new blood vessels from a pre-formed one. This is a vital process involved in embryological growth, tissue development, would healing of damaged tissues and in the context of this chapter is a crucial process for placental development and fetal growth during normal and GDM. In this regard, as it has been remarked before, macrosomia, present in GDM, has been associated to increased nutrient delivery toward the fetus, a phenomenon that may be related with increased blood flow due to vasodilatation of placental vessels [100]. Moreover, angiogenesis in the placenta is also controlling blood flow toward the fetus, and in fact, the vessel formation proceed to any vascular function, this process (i.e., angiogenesis) has been studied as one of the underling mechanism for explaining macrosomia in GDM [116,117,118,119]. Thus, placentas from GDM exhibits elevated number of redundant capillary connections per villi, compared to normal pregnancy, suggesting a more intense capillary branching [120]. Moreover, there are increased placental capillary length, branching and surface area that have been reported in women with type 1 [121], pregestational and gestational diabetes [18,74], as well as elevated number of terminal villi and capillaries in women with hyperglycemia [73]. In addition, it has been reported that glycemic control was significant correlated with capillary surface area and capillary volume in women with pre-gestational diabetes [117]. Moreover, it is well known that diabetes is associated with increased angiogenic response in some specific tissues such as eye, where hyperglycemia can lead to retinopathy [122]. Nevertheless, it has been shown that GDM is associated to reduction in the circulating endothelial progenitor cells (EPC), in mother and fetus [123,124] a phenomenon that was linking with reduced capacity for recovering endothelial dysfunction in GDM.

Associated mechanism behind increased placental angiogenesis in GDM may be related to the pro-angiogenic effect of hyperglycemia [125], which in turn triggers an enhancement in the placental synthesis and release of VEGF, as well as the expression of VEGF receptors (VEGFR) and nitric oxide production [18]. Thus, it has been shown that the placentas from women with hyperglycemia exhibited high levels of VEGF and VEGF receptor 2 (VEGFR-2) but reduced expression of VEGF receptor 1 (VEGFR-1) [73]. Furthermore, it has been reported elevated placental levels of VEGFR-1 mainly in vascular and throphoblastic cells in women with GDM [73,126]. Also, alteration in VEGF-VEGFRs expression has been described in women with type 1 diabetes [18,61,75,127]. Thus, the increased secretion and activity of VEGF may explain hypervascularization observed in placentas from DGM [58,71]

On the other hand, increased placental angiogenic response may also be related with hyperinsulinemia present in GDM. In this regard, it has been described that insulin activates at least two types of insulin receptors (IR), type A (IR-A, associated with a mitogenic phenotype) and type B (IR-B, associated with a metabolic phenotype), which are elevated in both HUVEC [24] and hPMEC [106], respectively. This particular localization of insulin receptors in feto-placental endothelium, may be linked with the differential proliferative capacity of endothelial cells, since insulin increases the expression of several genes related with angiogenesis (i.e., EFNB2) and vascularization (i.e., EPAS1) mainly in placental derived endothelium [128],

therefore is feasible that functional clustering of insulin-regulated genes in this cell type may promote their mitotic potential.

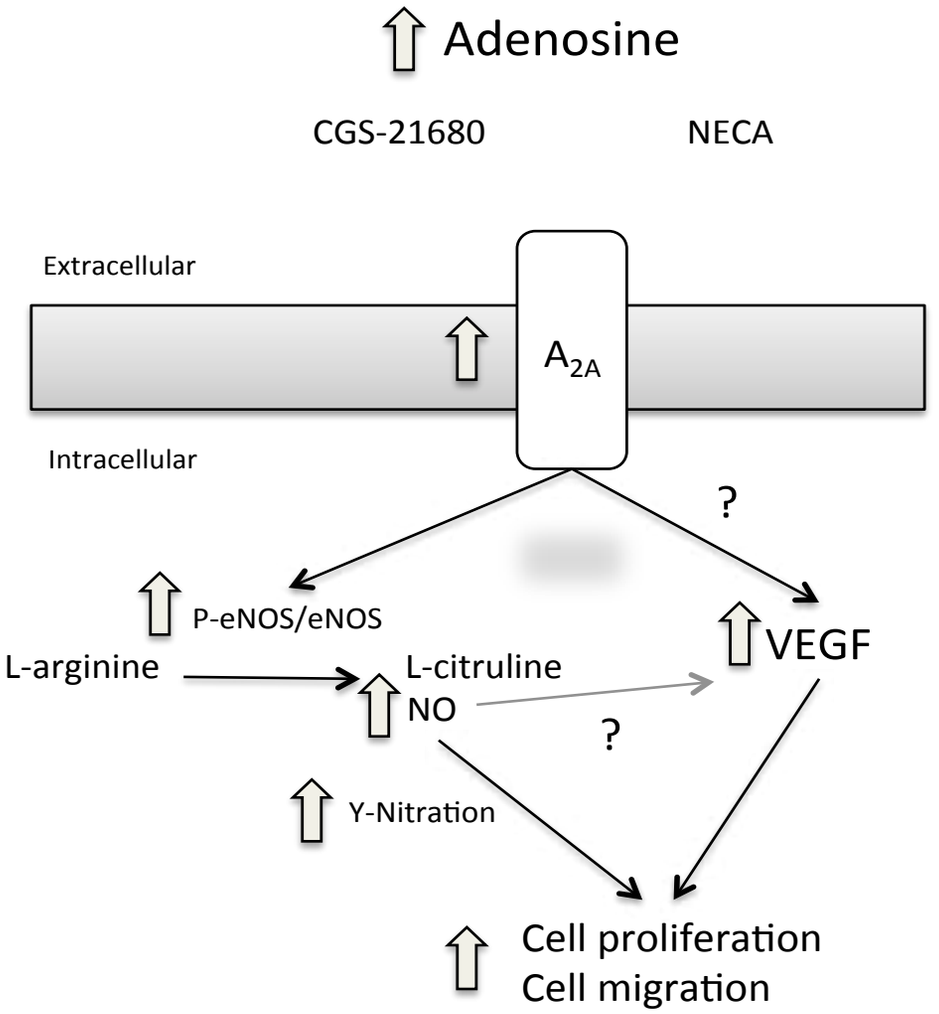

Figure 3. Potential role of A_{2A}AR/NO/VEGF signaling pathway in the cell migration and proliferation during gestational diabetes mellitus. In human umbilical vein endothelial cells (HUVEC), adenosine or adenosine receptor agonists (CGS-21680 or NECA) activate A_{2A} adenosine receptor (A_{2A}) and trigger the activation (i.e., phosphorylation in serine 1177, p-eNOS) of endothelial nitric oxide synthase (eNOS), without changes in total eNOS, which in turn is associated with elevation of nitric oxide synthesis (NO) and nitration of tyrosine residues (Y-nitration), enhancing the synthesis of vascular endothelial growth factor (VEGF) and then this activation generates cell migration and proliferation. It is unknown (?) the mechanism(s) associated with the regulation of expression of VEGF mediated by A_{2A}/NO signaling pathway. Preliminary results in our laboratory suggest that gestational diabetes mellitus (GDM) is associated with elevation of adenosine extracellular levels and high activation and expression of A_{2A} adenosine receptor characterized by high (⇧) eNOS activation, NO formation and Y-nitration whose are associated to enhancement of cell proliferation and migration and finally it would be occasioned elevated placental angiogenesis characteristic of this disease.

Another potential pathway, involved in the increase of placental angiogenesis during GDM, may be the ALANO pathway described before [100]. In this regard, we have previously proposed that a dysfunction in this pathway is taken part in the physiopathology of reduced placental angiogenesis in pre-eclampsia [129]. Therefore, it is feasible that the converse may

be occurring in GDM. Considering this idea, preliminary results have shown that HUVEC from gestational diabetes was associated with high expression of A_{2A} and A_{2B} adenosine receptors (AR) (~6 and ~2-fold, respectively). Moreover, CGS-21680 (A_{2A}AR selective agonist) and NECA (AR non selective agonist) increase (~2 and 2.5-fold) cell proliferation in both gestational diabetes and normal pregnancies; an effect that was blocked by ZM-241385 (A_{2A}AR selective antagonist) only in normal pregnancies. Interestingly, a shift in the curve of ZM-241385 by inhibiting the stimulatory effect of CGS-21680 was observed in diabetic pregnancies compared with normal pregnancies (calculated *Ki* 1.3 ± 0.2 and 12.8 ± 3.4 nM, respectively). Interestingly, co-incubation with L-NAME (NOS non-selective antagonist) blocked the CGS-21680-mediated proliferation in both normal and pathological pregnancies. Therefore, this results suggest that A_{2A}AR stimulation increases cell proliferation in gestational diabetes through an intracellular pathway dependent of NO synthesis. Remarkable, these results also suggested that a posttranslational modification in A_{2A}AR could be involved in the reduced affinity to ZM-241385 in GDM (Escudero A and Escudero C, unpublished results) (see Figure 3).

7. Concluding remarks

As detailed above, intrauterine exposure to diabetes has been associated with high risk of diabetes, obesity, as well as cardiovascular disease in the offspring. Although the genetic component is hard to discard, the general agreement is that intrauterine exposition allows the "transmission" of diabetes to the offspring. Several mechanisms have been proposed in order to understand the relationship between maternal GDM and the risk of metabolic and cardiovascular disease in the offspring. In this review, we have highlighted some information regarding the potential role of placental dysfunction and particularly placental endothelial dysfunction as one of the mechanisms linking with fetal programming in GDM. This relationship is not arbitrary because it may constitute the basis for explaining other pregnancy diseases such as growth restriction or pre-eclampsia, where the same alteration, might explain the predisposition that the children "exposed" to those diseases could develop metabolic or cardiovascular disease later in life. Therefore, if we consider this phenomenon (i.e., endothelial dysfunction) is a "normal" response in front of intrauterine stressful conditions, such as GDM or intrauterine growth restriction or pre-eclampsia; it would offer an opportunity to plan clinical strategies addressing to evaluate and control endothelial function as soon as the babies, exposed to those diseases, have been born. Finally, a general recommendation would be that it is necessary to establish a consensus for diagnosis of GDM. This is particularly important because hyperglycemia would be one of the most affecting factors involved in the endothelial dysfunction and fetal programming.

Abbreviations

Adenosine receptor (AR), Cationic aminoacid transport type 1 (CAT-1), Diabetes in Pregnancy Study Group (IADPSG), Diabetes mellitus type 2 (DMT2), Endothelial derived hyperpolariz-

ing factor (EDHF), Endothelial nitric oxide synthase (eNOS), Endothelial-to-mesenchymal transition (EndMT), Equilibrative Nucleside Transport type 1 (ENT-1), Gestational diabetes mellitus (GDM), Glucose transporter (GLUT), Glutathione peroxidase (GPX), Growth hormone (GH), Human chorionic gonadotrophin (hCG), Human placental lactogen (hPl), Human placental microvascular endothelial cells (hPMEC), Human umbilical vein endothelial cells (HUVEC), Hyperglycemia and Adverse Pregnancy Outcomes (HAPO), Inducible NOS (iNOS), nitric oxide (NO), Insulin-dependent diabetes (IDDM), Insulin-like growth factor I (IGF-I), Large for gestational age (LGA), Oral glucose tolerance test (OGTT), Reactive nitrogen species (RNS), Reactive oxygen species (ROS), Serotonin transporter (SERT), Transforming growth factor-β (TGF- β), Vascular endothelial growth factor (VEGF), Vascular endothelial growth factor (VEGF), VEGF receptor (VEGFR), Xanthine oxidase (XO).

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