Placental angiogenesis biomarkers (VEGF) and potential pathways involved (COX-2 and CASPASE 3) in pregnancies complicated by hyperglycemia

Simone Angelica Leite de Carvalho Silva¹, Iracema Mattos Paranhos Calderon², Renee Laufer Amorim³

¹Medical Preceptor of Medical Residence at University of Pernambuco and the Integral Institute Professor Fernando Figueira (IMIP); PhD student at the Postgraduate Program in Gynecology, Obstetrics and Mastology / Botucatu Medical School, (UNESP)
²Associate Professor of Obstetrics, Postgraduate Programme in Gynecology, Obstetrics and Mastology / Botucatu Medical School, (UNESP)
³Professor of Veterinary Pathology / Department of Clinical Veterinary / School of Veterinary Medicine and Animal Science of Botucatu, (UNESP)

E-mail: simoneangelicacarvalho@gmail.com

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ABSTRACT

Diabetes associated with pregnancy progresses with adverse perinatal outcomes (RPNA), directly dependent on the quality of maternal metabolic control and integrity of the placental function. The hypoxic metabolic model, described to explain the pathophysiology of RPNA in these pregnancies may be related to different ways of acting in the trophoblast. The objective of this review was to explore the relationship between maternal hyperglycemia and markers of placental angiogenesis, as well as markers of possible pathways involved in this process. Interestingly, the pathways related to inflammation and / or proliferation and cell apoptosis, potentially related to the processes of intrauterine hypoxia, a feature of pregnancies complicated by uncontrolled hyperglycemia. To this end, we selected markers of activity in the trophoblast, among them the proliferation of endothelial factor (VEGF), the enzyme cyclooxygenase (COX-2), a marker of inflammation and also of regeneration and cell proliferation, and proteolytic enzyme family of caspases (CASPASE-3), a marker of cells undergoing apoptosis. As a strategy used to search, defined as keywords of interest in databases with public access (Lilacs, SciELO, PubMed / Medline, Virtual Health Library / BIREME, Cochrane, among others), and the manual search books on specific texts.

KEYWORDS

Placenta; Diabetes; Angiogenesis; Inflammation; Apoptosis.

INTRODUCTION

The placenta is highly vascularized organ that shows rapid growth in a short time and should meet the increased demand of metabolism necessary for fetal growth and development. To perform their functions,
however, depends largely on the proper development of fetal and uterine capillary network, with such a plasticity, which allows the necessary changes along the pregnancy\(^8\).

Diabetes Mellitus is a syndrome characterized by chronic elevation of fasting glucose and/or postprandial, in absolute or relative defect in insulin production or decreasing its effect on target organs. Gestational diabetes is defined as any degree of glucose intolerance first identified in pregnancy\(^2\). In pregnancies complicated by diabetes for the mother’s exposure to chronic hyperglycemia, which results in different metabolic disorders, leading to fetal and placental injury.

In the placenta, in particular, are related to changes due to endothelial proliferation, exaggerated vascular growth and adhesion of pericytes\(^3\). However, much of these informations and the real role of markers involved in these processes and, consequently, in the perinatal outcomes are not well established in the literature.

### VASCULOGENESIS AND ANGIOGENESIS

Vasculogenesis and angiogenesis are the mechanisms that induce the formation of new blood vessels. Vasculogenesis occurs during embryonic development, from the mobilization of pluripotent mesodermal precursors present in the yolk sac of embryos of birds and mammals and in specific organs in the adult\(^4\). These precursors, identified as angioblastic, form endothelial cells, yet do not exhibit the characteristic markers of these cells nor the ability to form tubules. In the yolk sac, hematopoietic precursor cells and angioblastic differentiate in close association with each other, forming the so-called blood islands. Subsequent processes of fusion of blood islands and the formation of vascular lumen lead to primary vascular network\(^5\).

The formation of the first blood vessels occurs by differentiation in situ of the angioblastic cells and is characterized as vasculogenesis. From these pre-existing vessels, new capillaries are formed by budding, resulting in a vascular plexus elongated and highly branched, and this process is called angiogenesis\(^5\). These mechanisms are considered essential for the development and growth of the embryo, fetus and newborn\(^6\). In most adult tissues, however, the capillary endothelium represents a stable cell population, with low mitotic index.

Angiogenesis in adults is observed in pathological conditions, occurring persistently and unregulated, as in cases of cancer, and fibrosis, retinopathy and rheumatoid arthritis, or in strictly controlled situations, as in follicular development and corpus luteum formation, endometrial post menstruation and placentation\(^9\).

Angiogenesis is essential and dynamic process that occurs from venules or capillaries, and that, after transduction of differentiation signals, develops into six major steps: (1) vasodilatation and increased vascular permeability; (2) activation of proteases produced by endothelial cells, which break the basement membrane of the vascular wall and destroy the adjacent matrix; (3) proliferation of endothelial cells; (4) migration of these cells, through an extension toward the angiogenic stimulus, forming solid cords of cells aligned and referred to in literature as “sprouts” (buds); (5) formation of a lumen, by the coalescence of intra and intercellular vacuoles and, in sequence, (6) recruitment of pericytes to stabilize the vascular structure and form arterioles and venules\(^7\).

The possible pathways of activation of the angiogenic process may include, alone or in combination, the following events: i) increased production of growth factors and cytokines, through direct action on the activation of endothelial cells or indirectly on others cells that promote angiogenesis; ii) synthesis of enzymes that enable the binding of angiogenic factors to their receptors; iii) specific incentives for the production of enzymes by endothelial cells and stroma adjacent to the capillaries, capable of inducing the degradation of basement membrane and extracellular matrix perivascular and iv) interruption of the synthesis and expression of physiological inhibitors of angiogenesis, probably in the early stages of the angiogenic response, and stimulating the synthesis and/or activation of angiogenesis inhibitors, probably in late stages of the angiogenic response. In general, angiogenesis inhibitors and stimulators work together, controlling the growth of blood vessels. Disturbances in this process, however, can lead to numerous “angiogenic diseases” in different tissues\(^8\), such as pre-eclampsia\(^9\).

Among the factors regulating activation highlight 1 and angiopoietin 1 and 2 (Ang-1 and Ang-2) and its receptor Tie-2, the components of the family of vascular endothelial growth factor (VEGF-A, B, C and D).
and its receptors VEGFR-1 and VEGFR-2, basic fibroblast growth factor (FGF-b or FGF-2) and placental growth factor (PIGF).

MARKERS OF ANGIOGENESIS

The angiopoietin and VEGF are essential and multifunctional regulatory molecules that regulate growth and morphogenesis of the vascular system, acting as a specific endothelial mitogen regulated by different factors and also by hypoxia\cite{4}. Inhibiting the activity of their specific receptors inhibits angiogenesis and causes destabilization of the vascular wall and inhibit the migration and proliferation of endothelial cells and their precursors\cite{4}.

VEGF is part of a family consisting of six members: VEGF-A (or VEGF), PIGF, VEGF-B, VEGF-C, VEGF-D and VEGF (homologous to the VEGF gene encoded by orf virus-parapoxvirus). The loss of a single VEGF allele leads to embryonic lethality, which makes a single factor in the development of the vascular system\cite{8}. It is a factor expressed by different tissues of low-angiogenic activity as the brain, kidneys, liver and spleen, where it regulates vascular permeability. It is also essential to the process of angiogenesis during development, which features a variety of effects on endothelial cell (proliferation, migration and survival) and the formation of blood vessels in general organization in tube and lumen formation). This action is also observed at sites of embryo implantation and formation of the placental vessels\cite{8}.

The transcription of VEGF mRNA is induced by different growth factors and cytokines, among them, PDGF, EGF, TNF-\(\alpha\), TGF-\(\beta\) and IL-\(\gamma\), acting as indirect mediators of angiogenesis. The levels of VEGF can also be regulated by oxygen tension. Hypoxia induces a rapid and reversible expression of VEGF, increasing transcription and mRNA stabilization. On the other hand, normoxia regulates the production of VEGF, promoting the regression of newly formed vessels\cite{8}.

The action of this growth factor occurs through specific receptors present on endothelial cells or their precursors. Two binding sites of high affinity for VEGF are present in these cells: VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1 / KDR), which expression is regulated by hypoxia\cite{8}. Another member of this family is the VEGFR-3 (Flt-4), which binds to VEGF-C and VEGF-D\cite{8}.

The receptors VEGFR-1 and 2 show different properties of signal transduction. The interaction of VEGF with VEGFR-2 is critical for angiogenesis, however, the consequences of the pathway of VEGF mediated by VEGFR-1 are not yet fully established. During embryogenesis, the expression of VEGFR-1 and VEGFR-2 begins at the time of formation of the vascular islets. Thus, the presence of homozygous mutants, which inactivate these receptors is lethal because it prevents the development of embryonic vasculature\cite{8}.

ANGIOGENESIS IN HUMAN PLACENTA

Factors related to the formation of blood vessels play a prominent role in the process of placentation and fetal survival. Many of these factors, pro and anti-angiogenic, have been described in human placenta and other animals. However, their mechanisms of interaction have not been established, their possible consequences and their molecular changes involved in many pathologies of pregnancy.

In human placenta, VEGF plays a role of importance for the development of villous vascularization during the first trimester of pregnancy, and placenta at term, to maintain the integrity and vascular permeability. In the first trimester of pregnancy, the vascular growth factor is produced by the trophoblast, showing higher expression in villous cytotrophoblast than the syncytiotrophoblast. Its presence in the extravillous trophoblast is still questionable\cite{10}. In contrast, VEGFR-1 and VEGFR-2 receptors are expressed by endothelial cells of placental villi, whereas VEGFR-1 is also expressed in extravillous trophoblast\cite{10}. VEGF derived from the trophoblast cells seems to be paracrine function, acting not only in remodeling and permeability of the uterine microcirculation, but also in the formation of placental villi in fetal vessels, contributing to the effective increase in metabolic exchanges between the maternal and fetal compartments\cite{11}.

MATERNAL HYPERGLYCEMIA AND ALTERATIONS OF PLACENTAL VASCULARIZATION

Angiogenesis is an essential component for the re-
productive processes and depend on the ovular implantation of the blastocyst, organogenesis and plental development and embryogenesis\[11\]. Faults in any component of this process determine vasoproliferative disorders and inappropriate angiogenesis, commonly observed in many complications of the reproductive cycle, including hypoxia, inflammatory diseases and other syndromes. In many of these situations, angiogenesis does not cause signs and symptoms, but has direct effects on pathogenesis and prognosis of pregnancy\[6\].

In pregnancies complicated by diabetes, there is exposure of the maternal organism to chronic hyperglycemia, resulting in different metabolic disorders, including with regard to endothelial proliferation, vascular growth and adhesion of pericytes\[3\]. Hence the importance of diagnosis and correct and early treatment of hyperglycemic disorders in pregnancy.

The oral test glucose tolerance test (OGTT) is recommended to confirm the diagnosis of diabetes in pregnancy\[2\]. Nevertheless, the literature has been considering other forms of diagnosis of hyperglycemia during pregnancy, considering that not only diabetes but also the mild hyperglycemia or glucose intolerance should be diagnosed and treated during pregnancy\[2, 12\]. The association of OGTT and glucose profile (GP), applied in parallel during pregnancy, shows a group of pregnant women, by the normal response to OGTT are not diabetic, but which, since they change in PG, fit the current definition of mild hyperglycemia (TABLE 1).

**TABLE 1 : Diagnostic classification of Rudge\[13, 14\] in accordance with the responses to OGTT100g and GP**

<table>
<thead>
<tr>
<th>Groups of pregnant women</th>
<th>OGTT100g</th>
<th>GP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Diabetics / normoglicemics</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Mild Hyperglycemia</td>
<td>Normal</td>
<td>Altered</td>
</tr>
<tr>
<td>Gestational Diabetes</td>
<td>Altered</td>
<td>Normal</td>
</tr>
<tr>
<td>Gestational or Onset Diabetes</td>
<td>Altered</td>
<td>Altered</td>
</tr>
</tbody>
</table>

These women have been identified by Rudge *et al.* for over thirty years\[13\] and, since then, have been treated as suffering from gestational diabetes\[14\]. This discovery encouraged several specific searches and differentiated many perinatal and placental outcomes directly dependent on the intensity of maternal hyperglycemia\[15\]. The placentas of pregnant women with mild hyperglycemia have morphological changes similar to those with diabetes, but have a higher incidence of endarteritis that observed in placentas of diabetic pregnant\[16\].

In contrast, the placental vascular changes are different between groups of pregnant women with diabetes, or clinical or gestational (OGGT 100g altered and normal or altered GP), and mild hyperglycemia (OGTT 100g normal and altered GP)\[17]. In this study, the placentas of pregnant women with mild hyperglycemia were constituted by numerous and small terminal villous and also small and numerous vessels. Compared to the placentas of non-diabetic, this proliferation of vessels and villi resulted in increased villous vascular area and similarity in capillarization in the index, with significant increase of the surface of maternal-fetal exchanges. These results showed the vicariance of the placenta to ensure transport of oxygen and nutrients to the fetus. In onset and gestational diabetes, villous dimensions were comparable to those observed in placentas of nondiabetic pregnant women, but with less vascular area, depending on the type and intensity of maternal hyperglycemia. These changes marked developmental delay and failure of the placenta to ensure the maternal-fetal exchange\[18\].

The completion of this study related the effects of maternal hyperglycemia, from different backgrounds and intensity, with normal or altered the results of the pulsatility index (PI) in umbilical artery. Glycemic control was related to adequate maternal placental flow of low resistance, confirmed by normal results of umbilical artery Doppler. This result was associated with placental vascular proliferation and, according to the authors, should help ensure the macrossomia and fetal oxygenation. The poor maternal glycemic control was associated with lower vascularity and consequent increase in placental vascular resistance, confirmed by increased values of umbilical artery PI. This condition should favor the growth restriction and fetal hypoxia\[19\].

According to\[3\], the increase in fetal-placental angiogenesis and In villous capillarization appears to be independent of disease severity. According to other authors, the placenta in diabetes suffer a range of functional and structural changes dependent on a number of factors including the severity of maternal hyperglycemia during organogenesis and placental development. Apparently, these placentas have adaptations to main-
tained their functional capacity, facilitating the passage of glucose to the fetus and, consequently, fetal macrosomia\textsuperscript{[20]}. However, according to latest results, the more severe hyperglycemia can be a limiting factor for placental vascular proliferation, and in this condition, both macrosomia as fetal growth restriction may occur, associated with differential risk of hypoxia and perinatal death (PD)\textsuperscript{[19]}. Fetal macrosomia is responsible for about 0.49\% of the PD and 11.4\% of perinatal morbidity in pregnancies concurrent with hyperglycemic disorders\textsuperscript{[20]}. On the other hand, fetal growth restriction is an anticipated risk in pregnancies complicated with uncontrolled diabetes, usually of long duration and associated with vascular complications. The attributable risk (AR) of PD is 6.12\% in pregnancies complicated by gestational or onset diabetes (OGTT100g and GP altered) and 4.16\% in pregnant women with mild hyperglycemia (OGTT100g normal and GP altered). Hyperglycemia and consequent changes in placental vascularization. Thus, maternal glycemic control and the diagnosis of intrauterine hypoxia would be decisive in preventing the MPN in pregnancies associated with diabetes and mild hyperglycemia\textsuperscript{[16]}.

There is no data in the literature concerning the factors controlling the placental angiogenesis in pregnant women with mild hyperglycemia. Overwhelmingly, the studies focus exclusively on the gestational diabetes or pre-pregnancy, where the vascular adaptation is proportional to the hyperglycemia of intrauterine environment, a factor not always considered. Nevertheless, some results showed that placenta from women with adequate glycemic control had increased expression of fibroblast growth factor-2 (FGF-2) and lower expression of Ang-1, Ang-2, VEGF, VEGFR-1, VEGFR-2, Tie-2 and FGFR, findings opposite to those observed in placentas from non-diabetic\textsuperscript{[4]}. So far, the results of the expressions of biomarkers of placental angiogenesis in pregnancies complicated by diabetes or mild hyperglycemia are rare or nonexistent and, above all, extremely controversial. These findings justifies the search of some of these biomarkers in the presence of hyperglycemia with different severity and origin.

**MARKER OF INFLAMMATION AND PROLIFERATION AND CELL REGENERATION**

The cyclooxygenases (COX), also known as a synthetizer prostaglandin endoperoxidase H (PGH synthetase) is the enzyme required for the conversion of arachidonic acid in prostaglandin (PG), being first identified twenty years ago. For many years, was known only one type of enzyme cyclooxygenase, but a second enzyme was recently discovered, and then referred to as COX-1 (constitutive) and COX-2 (induced). COX-1 and COX-2 act in different physiological and pathological processes that extend from inflammation to neoplasia\textsuperscript{[22]}.

The products of arachidonic acid metabolism comprise a set of mediators to modulate inflammatory and immune response. These products result from oxidation of this acid under the action of phospholipase A2 on cell membrane phospholipids. The oxidation of arachidonic acid is carried out by two enzymatic pathways: the cyclooxygenase, also known as PGH synthase and lipoxygenase. The action of the cyclooxygenase enzyme system on the membrane phospholipids converts arachidonic acid to a stable PG endoperoxide, PGG2, which is subsequently reduced to PGH2. This, then serves as substrate for the synthesis of several other PGs such as PGE2, PGD2 and PGF2\textalpha. The PGH2 can be further converted into prostacyclin (PGI2) and thromboxane (TXA2)\textsuperscript{[22]}.

COX-1 (constitutive) is an essential enzyme for the organism and are expressed in many tissues. It is considered that is involved in the production of PGs that modulate physiological functions in different organs and cellular systems, including kidney, gastrointestinal tract and platelets. In contrast, COX-2 is undetectable in most tissues, but can be induced by proinflammatory signals. COX-2 is also related to the production of PGs that modulate physiologic events in development and cell growth and production of bacterial endotoxin (liposaccharides), of some growth factors such as transforming growth factor (TGF), factor epidermal growth (EGF) and fibroblast growth factor (FGF), and oncogenes\textsuperscript{[22]}.

Under physiological conditions, COX-2 is present in an unlimited fashion in the brain and spinal cord and
may be involved in nerve transmission, particularly in situations of pain and fever. PG produced via COX-2 play an important role in the rupture of the follicle at ovulation, probably mediated by direct generation or by the activation of proteolytic enzymes. During pregnancy, are key elements in ovum implantation and angiogenesis necessary for the formation of the placenta[23]. Regardless of gestational age, term or preterm, COX-2 is a marker of focus in the onset of uterine contractions of labor[23].

HYPERGLYCEMIC DISORDERS IN PREGNANCY AND CHRONIC INFLAMMATORY PLACENTAL PROCESS

Chronic inflammation of the placenta has complex diagnosis, which involves different types of immune cells in one or more sites of the placenta and decidua. More specifically, this entity is defined as chronic inflammation of the villi and the intervillous space, characterized by infiltration of mononuclear cells in the villi or intervillous space[24].

Diabetes is known to be accompanied by chronic inflammation of the placenta, especially when related to fetal growth restriction, preeclampsia and extreme prematurity[24]. However, one wonders what would be the basis of this chronic inflammation. Although chronic placental inflammation may be related to a congenital viral infection, the predisposition to this type of infection is not confirmed in diabete. In contrast, the viral infection has been suggested as a causal factor of diabete due to immunological alterations present in both pre-diabetic condition such as diabete installed[25].

In literature no results were found on the in vivo expression of COX-2 in placental villi, which could reinforce this theory of chronic inflammation and regeneration, especially in pregnancies associated with diabete or mild hyperglycemia. This, perhaps, was more a causal factor of increased risk of fetal growth restriction in cases of difficult metabolic control. Only in experimental studies in diabetic rats, it was identified a lower activity of COX-2 in the placenta in malformed fetuses compared with normal fetuses of non-diabetic rats during organogenesis[26, 27]. Knowing that diabetes promotes an inflammatory environment during pregnancy, many studies have determined the occurrence of a higher expression of genes and receptors linked to an increased production of angiogenic and inflammatory mediators as a means of restructuring in hyperglycemic states[28, 29]. According Radell et al. (2006), interleukin-1 and TNF-α induce to a plethora response that profoundly affect the production of proteins in the matrix cell. This leads to fibrosis and a loss of integrity of placental endothelial cells. Thus, the authors believe that the expression of certain proteins in the placenta is responsible for adverse fetal reprogramming that occurs in pregnancies that occur with diabete[28]. Moreover, Peroxisome Activated Receptors (PPARs) act in lipid homeostasis and inflammation. As diabetes demonstrates high inflammatory environment, it is believed that this receptor plays a role in pathophysiology. In this study, the authors found that there is less expression of PPARs, but without affecting its fraction bound to DNA. This may facilitate the action of PPARs on pro-inflammatory cytokines, determining that environment in cases of diabete[29].

Thus, given the scarcity of in vivo studies involving specifically, the expression COX-2 in the placenta of pregnant women with diabete, specific searches are justified on the role of this biomarker in cases of fetal and placental development in both pregnancies low risk as those complicated by hyperglycemic disorders.

MARKERS OF APOPTOSIS

Apoptosis is cell death, induced by an intracellular program highly regulated, in which cells destined to die activate enzymes that degrade the nuclear DNA and cytoplasmic proteins. The plasma membrane of the cell remains intact, but its structure is changed and thus the apoptotic cell becomes primary target of phagocytosis. The dead cell is rapidly eliminated before its contents could spill and thus, this type of cell death does not trigger the host inflammatory reaction[30].

Apoptosis or programmed cell death is a process different from necrosis. In apoptosis, the cells go into self-destruction, for the body as a whole, survive. The individual requires that new cells are generated for the maintenance of vital processes, in contrast, the superfluous or altered cells, must be eliminated to safeguard the well being of the organism. In necrosis, the cell swells and cytoplasmic organelles, particularly, mitochondria are damaged and few changes occur in the nucleus.
These changes alter the internal balance, facilitating the free passage of water and ions into the cell, which ends up breaking. There is activation of the immune system, causing an intense inflammatory reaction by release of toxic cellular. Thus, some types of white blood cells, particularly neutrophils and macrophages, converge in the process of tissue necrosis and cause phagocytosis of dead cells. Despite causing cellular damage to surrounding regions, this process is important in cases of infections and also for the removal of cell debris[30].

Apoptosis can be initiated from external signals from specific receptors on the cell surface, or by internal stimuli of intracellular stress, such as ultraviolet radiation, chemotherapeutic agents, absence of growth factors, low amount of nutrients and increased levels of reactive oxygen species and corticosteroids. These different pathways and molecular stimuli culminate with the activation of proteases called caspases (“Cystine Aspartases”)[31].

Caspases are present in the cytosol in the form of inactive proenzymes and become active after proteolytic cleavage. Up to now it has been described, at least 39 members of this family. The caspases, when activated, have the ability to catalyze activation of other family members, resulting in amplification of proteolytic cascade. Some elements, such as caspase 8, occupy the proximal position in the cascade and act as initiators and regulators, others, such as caspase 3, are more distal and act as effectors of cellular fragmentation. Besides caspase 3, caspases 6 and 7 are considered executors. The other group is formed by the initiator caspases, which are part of caspases 8, 9 and 10 and possibly caspase 2, whose main role is to enable the executors[32].

**CELLULAR APOPTOSIS AND PREGNANCY**

In the development of normal pregnancies, the trophoblast undergoes changes in its structure to ensure the nutrition and oxygenation of the fetus. During pregnancy, placental apoptosis occurs especially in the third quarter, which may encourage obstetric complications[33]. Trophoblastic abnormalities seem to permeate all the events of pregnancy, promoting from inadequate placental implantation to different types of abnormalities that can result in serious complications, including, pre-eclampsia and preterm labor[33]. The placental apoptosis has also been studied in other obstetric complications such as post-term pregnancy[34], recurrent miscarriage[35] and fetal growth restriction[36].

**HYPERGLYCEMIC DISORDERS IN PREGNANCY AND PLACENTAL APOPTOSIS**

Sgarbosa et al. (2006), analyzed the balance between apoptosis and degree of expression of Bcl-2 (protein with antiapoptotic effect) in normoglycemic pregnant and women with diabetes and mild hyperglycemia. They found a high rate of apoptosis in women with mild hyperglycemia compared to women with diabetes[37]. Few other studies, most in vitro, analyzed the rate of placental apoptosis in pregnancies with hyperglycemia[38, 39, 40]. Moley (2001) [39] showed that hyperglycemia promotes best action of p53 and reduced activity of glucose transporters (GLUT 2 and 3), triggering the mechanism of mitochondrial death cascade. In vitro study, conducted in 2001 showed that cultures of trophoblast cells respond to hyperglycemia by increasing the rate of apoptosis[41]. Experimental study also determined that treatment of hyperglycemia in mice prevents the embryotoxic effects of glucose in vivo, including changes in apoptosis[42]. Review conducted in 2009[40] addresses the association between placental apoptosis and occurrence of fetal malformations. The maternal diabetes inhibits the expression of the Pax3 gene and as a result of this action, induces apoptosis of cardiac neural crest and neuroepithelial cells, explaining the association of defects in neural tube with diabetes.

These data are also controversial. Burleigh et al. (2004) found no difference in the rate of cell death in trophoblastic tissue, evaluating five diabetic and normoglycemic women[43]. However, none of these studies correlated the possible pathways involved in this process with the intensity of hyperglycemia. No one knows for sure how this hyperglycemia triggers apoptosis. In recent experimental study, Yang et al. (2008), found that high blood glucose significantly reduces VEGF expression in cultured primary endothelial cells of human umbilical vein. Moreover, the addition of VEGF in the culture of these cells, prevents...
apoptosis by suppressing the rate of Bax (pro-apoptotic factor) / Bcl-2 (anti-apoptotic factor) and activation of Caspase-3, and mitigate the generation of reactive oxygen species and calcium overproduction[44]. However, in vivo studies are needed to confirm this association.

**FINAL CONSIDERATIONS**

In this review there were few in vivo relevant studies addressing changes in vascular proliferation and the possible pathways involved (inflammatory and apoptotic) in the placenta of pregnant women with diabetes. So far, the placental vascular changes found in these women indicate an important role in the definition of perinatal outcome and, possibly, apoptosis and inflammation were associated. However, nothing is set in relation to these potential associations and quality of maternal metabolic control. This gap is also seen in relation to adverse perinatal outcomes, common in pregnancy with poorly controlled diabetes.

Undoubtedly, the literature is lacking in specific studies, including placental vascularization and potential pathways involved in pregnancies complicated by hyperglycemia with varying intensity. These studies will enrich the knowledge about the pathophysiology of this disease and especially to help prevent the risk of perinatal morbidity and mortality.

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