

Diploma Thesis

Gestational diabetes effects on placental development and
fetal outcome

submitted by

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Abbreviations

ADIPOQ	Angiopoietin
ATP	Adenosine Triphosphate
BMI	Body Mass Index
CO ₂	Carbon Dioxide
DHEAS	Dehydroepiandrosterone Sulfate
DNA	Deoxyribonucleic Acid
eNOS	Nitric Oxide synthase
EPO	Erythropoietin
EPOR	Erythropoietin Receptor
FGF	Fibroblast Growth Factor
FGR	Fetal Growth Restriction
FHF	Fibroblast Growth Factor Homologous
FLT1	Vascular Endothelial Growth Factor Receptor 1
GDM	Gestational Diabetes Mellitus
GLUT	Glucose Transporter
H ₂ O	Water
HAPO-Study	Hyperglycaemia and Adverse Pregnancy Outcome
HbA1c	Glycated Haemoglobin
HCG	Human Gonadotropin
HCT	Human chorionic thyrotropin
hENT	Human Equilibrative Nucleoside Transporter
HIF	Hypoxia Inducible Factor
HPL	Human Chorionic Thyrotropin
hPMEC	Human Placental Microvascular Endothelial Cell
HRE	Hormone-Responsive Element
HUVEC	Human Umbilical Vein Endothelial Cells
IADPSG	The International Association of the Diabetes and Pregnancy
IGF	Insulin-like Growth Factor
IgG	Immunoglobulin G
INS/IGF	Insulin/Insulin-like growth factor
IRCP	Immuno Reactive C-Peptide
IRES	Internal Ribosomal Entry Site

IUGR	Intrauterine Growth Retardation
KDR	Vascular Endothelial Growth Factor Receptor 2
LEP	Leptin
LGA	Large for Gestational Age
MBG	Average blood glucose level
mRNA	Messenger Ribonucleic Acid
NGT	Normal glucose tolerance
NICE	National Institute for Health and Clinical Excellence
NO	Nitric Oxide
NPH-Insulin	Neutral Protamine Hagedorn-Insulin
O ₂	Oxygen
OADs	Oral Antidiabetic Drugs
oGTT	Oral Glucose Tolerance Test
PAF	Platelet Activating Factor
PAPP-A	Pregnancy-Associated Plasma Protein A
PDM	Postpartum Type 2 Diabetes
PIGF	Placental Growth Factor
PPAR	Peroxisome Proliferator Activated Receptor
PROK	Prokineticin
ROS	Reactive Oxygen Species
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
TNF- α	Tumor Necrosis Factor- α
TNFR	Tumor Necrosis Factor Receptor
VE-Cadherin	Vascular Endothelial Cadherin
VEGF	Vascular Endothelial Growth Factor
VEGFR	Vascular Endothelial Growth Factor Receptor
ZO1	Zonula-Occludens-1

Abstract

Gestational diabetes mellitus (GDM) has become a hot topic over the last few decades regarding its influence on the maternal metabolism and fetal outcome. In this thesis, a substantial focus is put on its effects on the placental vascular development and correlations with fetal outcome. The diabetic metabolic situation causes hyperglycemia, hypoglycemia, hyperinsulinemia and oxidative stress in the mother and the fetus. These alterations trigger a sequence of changes in the development of the vessels in the placenta. Consequently, the fetus is affected by this lack of supply of necessary substances or overstimulated by pathological factors with significant risk for abnormal development. This thesis focuses on the GDM associated factors on the alteration of functional and structural placental development in terms of vascular development. Maternal and fetal adaptations are taken into account as causes for pathologies and diverse outcomes. This paper also addresses GDM effects on proangiogenic factors and endothelial function, which are crucial in understanding the changes in the placenta and fetus in this disease.

The research for this thesis was conducted using the international online database PubMed and the inventory of the Library of the Medical University of Graz. The information used originates from 300 sources, such as books, reviews and studies published in the years 1967-2018.

Both the fetus and placental circulation provide a range of growth factors necessary for angiogenesis and vascular growth. The major regulators of angiogenesis are the VEGF system, the FGF system, angiopoietin system, EG-VEGF/PROKR system, EPO, IL6 and TNFA, Insulin and IGFs, Leptin and adiponectin. All of these are sensitive to hypoxia, which is one of the key changes from hyperglycemia and hyperinsulinemia caused by GDM. Diabetic metabolic changes are associated with enlarged and distinct hypervascularization of the placenta. Oxidative stress, inflammation, changes in the transport of nutrients, and endothelial dysfunction under the influence of adenosine, transporters, and receptors in the GDM placenta are responsible for the adaptive responses in the mother, the fetus and the placental vasculature that correlate with GDM associated pathologies and fetal malformation.

Zusammenfassung

Gestations Diabetes Mellitus (GDM) und die damit verbundenen metabolischen Veränderungen und Konsequenzen für den Feten ist eine Erkrankung, die in den letzten Jahrzehnten zu einem wichtigen Thema wurde. In dieser Arbeit wird ein wesentlicher Fokus auf die plazentare Gefässentwicklung unter GDM und die Folgen für die Entwicklung Hyperglykämie, Hypoglykämie und oxidativer Stress betreffen Mutter und Fetus. Diese Veränderungen führen zu einer Reihe von abnormalen Entwicklungen der Gefässe in der Plazenta. Die Konsequenz ist die Unterversorgung des Feten oder eine erhöhte Zuführung unnötiger bzw. schädlicher Substanzen, was zu einem signifikant erhöhtem Risiko für die abnormale kindliche Entwicklung führen kann. Mütterliche und fetale Anpassungsmechanismen können der Grund für konsekutive Pathologien und krankhafte Ergebnisse sein. Die Arbeit befasst sich auch mit den Einflüssen, die der GDM auf die proangionetischen Faktoren und die endotheliale Funktion nimmt. Die Kenntnis der pathophysiologischen Zusammenhänge im Hinblick auf diese Faktoren ist wichtig um die komplexen Auswirkungen des GDM auf die Plazenta und den in dieser Erkrankung zu verstehen.

Für die Recherche dieser Arbeit wurde der Bestand der Bibliothek der Medizinischen Bibliothek Graz und die online Datenbank PubMed verwendet. Die Informationen stammen aus 300 Quellen, die aus Studien, Zusammenfassungen und Büchern bestehen und in den Jahren ...-2018 publiziert wurden.

Der Fetus und die plazentare Zirkulation sind die Quelle für eine Reihe an Wachstumsfaktoren, die verantwortlich für die Angiogenese und das Gefässwachstum in der Plazeta sind. Die wichtigsten Regulatoren sind das VEGF System, das FGF system, das Angiopoietin System, EG/VEGF/PROKR System, EPO, IL6 und TNFA, Insulin und IGFs, Leptin und Adinopoectin. Diese sprechen alle auf eine Stimulation durch Hypoxie an, welches eine der Ergebnisse von Hyperglykämie und Hyperinsulinämie ist. Diese diabetischen und metabolischen Veränderungen werden mit vergrösserten Plazenten und dessen Hypervaskularisierung assoziiert. Der unter GDM hervorgerufene oxidative Stress, die Inflammation, die veränderten Transportmechanismen und die endotheliale

Dysfunktion, die durch Adenosin und dessen Transportern und Rezeptoren beeinflusst werden, spielen eine grosse Rolle in den adaptiven Veränderungen in der Mutter, dem Feten und der placentaren Gefässentwicklung, die letztendlich zu Pathologien und fetalen Malformationen führen.

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I. Introduction

This thesis will discuss how placental angiogenesis is altered in maternal diabetes. An introduction is given on the placenta as an organ itself. Further discussion includes the disease gestational diabetes and the specific mechanism of placental vascular development physiologically and in GDM. Consequential influence on the maternal and fetal outcome will be elaborated in regard of the key factors. Finally, a concluding summary and an analysis of the limitations of the paper, as well as a proposition on future research on the topic is given.

A. The Placenta

The placenta is an organ that temporarily develops and functions during pregnancy. It plays a role in a wide range of functions, including endocrine and transport activities, and serves as barrier between the maternal and fetal blood circulation. (3) Its key role is the exchange of substances between the mother and the fetus. The placenta is made up of blood vessels, conjunctive tissues, and trophoblastic cells. It produces large quantities of hormones throughout the pregnancy until the time of birth. (4) During fetal development, the placenta functions as both a unique agent of human symbiosis and as gateway for fetal renal, respiratory, hepatic, gastrointestinal, endocrine, and immune systems while dependent on the mother. (5)

1. Formation and structure of the placenta

The origin of the placenta is the trophoblast from the outer cell layer of the morula. After implantation, it proliferates and differentiates into cytotrophoblasts and syncytiotrophoblasts. (6, 7) The fertilized egg cell moves through the fallopian tubes to the cavity of the uterus, then develops into a blastocyst. On the 7. day post conception it implants into the lining of the endometrium (Decidua). It already consists of an outer trophoblastic layer and an inner embryoblast, which will develop into an embryo. (8) Trophoblasts provide nutrients to the embryo and will develop into a large part of the placenta. Villous trophoblasts have two cell populations:

undifferentiated cytotrophoblasts and fully differentiated syncytiotrophoblasts. The syncytiotrophoblasts are a continuous, specialized layer of epithelial cells.(9)

In the fetal part of the placenta between the chorionic plate and basal membrane in the lacuna system of the syncytiotrophoblast, there are trabeculae. Starting on the 12. day after conception, the cytotrophoblast sprouts into trabeculae with an increased rate of proliferation from the chorionic plate and form primary villi. They run openly into the lacunae and have maternal blood flowing through them. On the 15. day, mesenchymal tissue reaches the villi and now forms the secondary villi. On the 19. day, the vascularization and the tertiary villi begin to develop, which continues until the end of pregnancy.(10) On approximately the 21. Day, embryonic blood circulation in the villi starts. The chorion, which encases the embryo, is covered with uniform villi until the 8. week p.c., at which point they degrade on the side of the decidua capsularis.(11)

During this period of adjustment where the placenta must meet the demands of the growing fetus, the maternofetal exchange surface is expanding and becoming more permeable, while the exchange distance is decreasing. More and more villi develop, but the individual villi are decreasing in surface area. The number and lumen size of fetal intervillous vessels grow while the villous stroma is displaced.(10) These villi trees fill up most of the intervillous space. They are confined by a layer of merged tissue consisting of fetal trophoblastic cells and the decidua on the basal side (facing the uterus).(8, 11) This is the place where the chorionic frondosum develops as the fetal part of the placenta. The maternal portion is made up of the decidua basalis.(11)

The stem villi branch out from the chorionic plate into the space between the chorionic frondosum and the decidua basalis, which is filled with maternal blood. From here, septums grow and form cotylons, which function as units of the placenta. Each cotylon is made up of two or more villi trees and the placenta has 10-38 of these lobules. The cotylons are connected to each other and do not touch the chorionic plate.(11)

Around the 14. week the placenta has finished developing into its final structure. The growth in width is practically complete around this time point and

moving forward only a growth in surface is detectable. In the 20. Week, the diameter is about 10cm, which doubles on the date of birth with a width of 2-4 cm. Its weight of about 500g correlates with the weight of the child.(11)

2. Placental circulation

The maternal blood flows through approximately 70 spiral arteries from the decidua basalis into the intervillous space of the placenta. It reaches the chorionic plate under high pressure, sprinkles back onto the basal plate, and shifts around the cotylons. Due to these conditions, intensive substance- and gas exchange with fetal blood is prohibited. It flows back to the maternal circulation through the decidua veins. The blood exchange, which has a volume of 150-200ml, occurs in the intervillous space and happens 3-4 times per minute. Two umbilical arteries transport blood, which is low in oxygen and high in degraded products, from the fetus to the placenta. Arteries branch out from the chorionic plate and reach arteries stemming from the cotyledons. In the wide capillary network, an indirect contact with the maternal blood occurs on the surface of the villi (total of 10-15 m²).⁽¹¹⁾ The placental vessels do not have an auto regulating system. A drop in blood pressure in the mother consequently leads to a decrease in the blood supply to the fetus.⁽⁸⁾

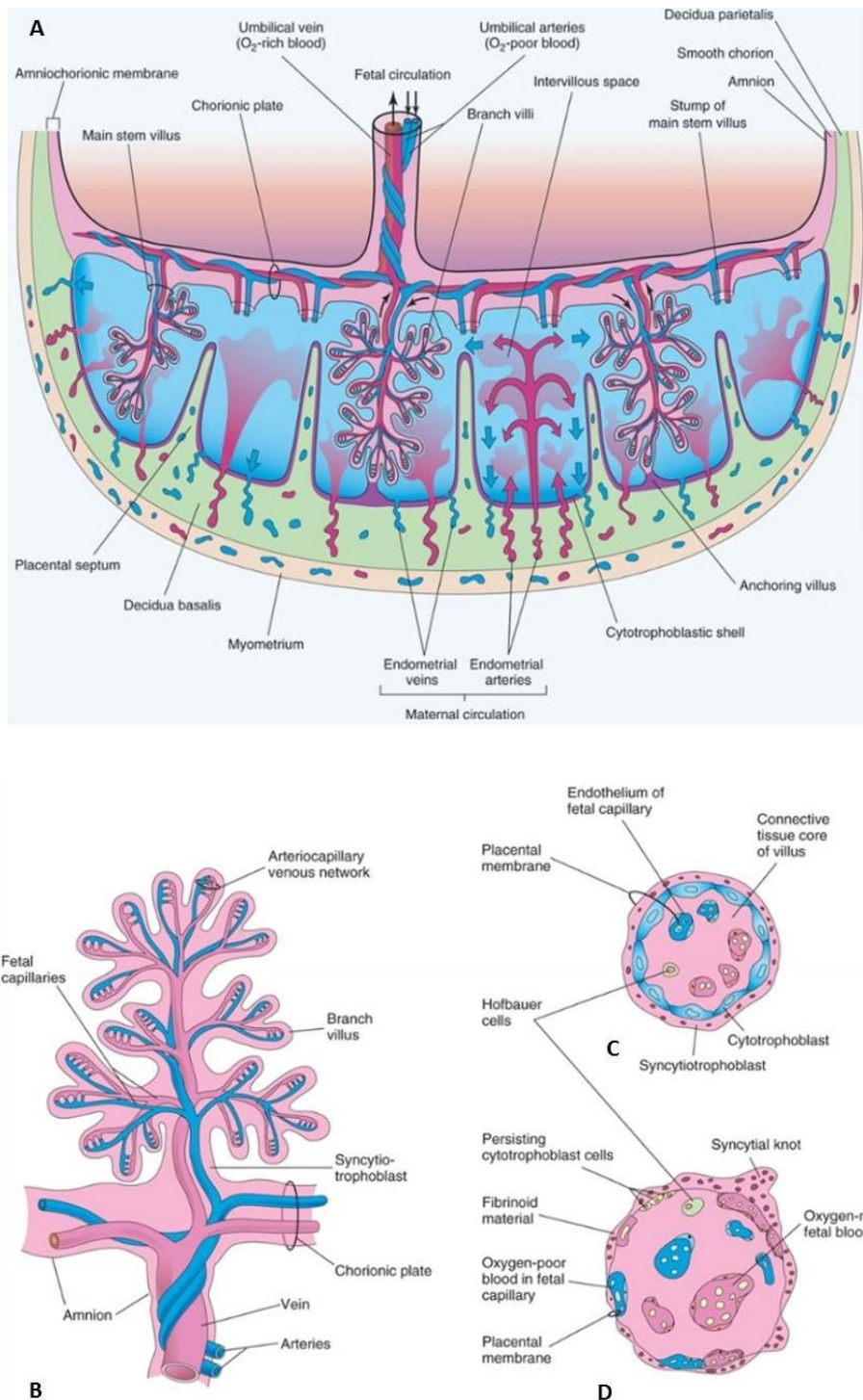


Figure 1: Drawing of the schematic placenta and chorionic villi

A) The relationship between the villous chorion and the basal decidua, the fetal placental circulation, and the maternal placental circulation in a transverse section of a full-term placenta. Projections of the basal decidua form the placental septa, which separates the cotyledons. B) Arterio-capillary-venous system. C) Section of 10 weeks-old term chorionic villi showing the placental membrane formed from syncytiotrophoblasts and cytotrophoblast. Villous mesenchyme with embedded fetal capillaries and Hofbauer cells. D) Section through full-term chorionic villi with vanishing cytotrophoblasts and increasing surface area in exchange of decrease in

the thickness of the barrier. Multiplication of the fetal vessel branches closer to the membrane of the placenta. Syncytial knots are formed from groups of syncytiotrophoblast nuclei. (12) (picture from Moore et al.(12)):

3. Exchange of substances

The syncytiocapillary membrane (2-4µm thick) makes up the surface of the villi and its main function is to separate the maternal and fetal blood.(10, 11) The exchange of substances across the placental barrier uses two basic mechanisms. 1) Passive transport: Diffusion of oxygen, water, carbon dioxide, and urea; Diffusion is dependent on molecule-carrier for glucose and lactate; Diapedesis for viruses and bacteria. 2) Active transport of amino acids and ions; Pinocytosis/ Endocytosis to transport proteins, maternal antibodies, and fats.(10, 13)

4. Passive exchange of substances

a) Diffusion

Respiratory gases (O₂, CO₂, H₂O), urea, liposoluble substances, vitamins (vitamin A, D, E, and K), as well as pharmaceuticals are exchanged by diffusion.(8) Diffusion is the main process the placenta uses to exchange substances and requires no energy. The partial pressure difference is the driving force to cross the border between arterial maternal blood and fetal blood (14).

b) Facilitated Diffusion

Facilitated diffusion uses a carrier for substance transport without energy consumption. Transporter proteins with a limited capacity are found in the cell membrane of the syncytium, which are specifically used for the exchange of substances. Glucose, which is the main energy source for the fetus, is mobilized into the placenta in large quantities using the insulin-dependent GLUT1-Uniportcarrier. Because of the large amount of glucose used, the glucose concentration in the blood of the umbilical cord is 1-2mmol/l less than the maternal blood. This enables a gradient direction to the fetus.(3, 8) To avoid an increase in the concentration of lactate in the fetus, it also uses a carrier enhanced transport from the mother to the fetus.(8)

c) *Diapedesis*

Cellular blood components such as erythrocytes and lymphocytes have restricted movement across the placental barrier. The substances cross through membrane pores or defects in trophoblasts. In addition, diapedesis is enabled when water is exchanged and moved along due to the hydrostatic or osmotic pressure difference (filtration) (8, 15). Pharmaceuticals with a molecular weight above 600, viruses, bacteria, and protozoans can all be transmitted across the placental barrier (11).

5. Active transport

Energy dependent active transport is used for the transmission of macromolecular substances, as well as the enrichment of substances such as lipid soluble vitamins. It can work against the concentration gradient but conditions an intact trophoblast function. A countless number of active transporters use ATP as their source of energy or an electrochemical gradient to function. They are localized on either the maternal or fetal side of the membrane and allow for the exchange of nutrients such as amino acids, vitamins, and glucose as well as pharmaceuticals that have chemical structures similar to the body's own substrates, in both directions (14). Glucose is transported into the fetal circulation via the glucose transporter (GLUT) and is depended on the maternal-fetal concentration gradient (3).

a) *Pinocytosis/ Endocytosis*

Several different proteins, iron, and fats are “eaten up” for transfer. For example, iron is bound to transferrin in the maternal blood. This complex sticks to the multivillous membrane of the syncytium and undergoes endocytosis. It is separated from the protein and transferred into the fetal blood through the basal membrane.(4)

Endocytosis of larger protein molecules is selective. Particularly relevant are the maternal antibodies (IgG-antibodies), which are passed across the placental barrier to supply the fetus with immune protection. Because of this, the fetus has a similar immune profile to the mother against infections.(8) Endocytosis is very slow and has no role in the exchange of pharmaceuticals.(16)

6. Synthesis function of the placenta

A key role of the placenta is to act as an endocrine organ. It produces several different hormones that circulate in the maternal blood, where they can be traced and evaluated throughout the pregnancy (8). The proteohormones produced exclusively by the trophoblast are especially important (17): human chorionic gonadotropin (HCG), human placental lactogen (HPL), and human chorionic thyrotropin (HCT) as well as steroid hormones including progesterone and estrogen (11). The fetal liver and adrenal gland need to provide the precursor DHEAS specifically for estrogen. In addition to the hormones listed above, the placenta also synthesizes messenger substances such as tachykinin, acetylcholine, platelet activation factor (PAF), and prostaglandin (18). The placenta also produces proteins that can be developed not only from trophoblastic tissue, such as PAPP-A, but also proteins that the mother produces outside of a pregnancy in much lower quantities, such as the α_2 -pregnancy associated glycoprotein (17).

II. Methods and Materials

This paper is a review of the summary from literature found on the placental vascular development in gestational diabetes. Research was conducted from December 2017 until April 2018 using all sources available at the Library of the Medical University of Graz, as well as the online database PubMed. Books and sources published between the years 1967 - 2018 were used and cited.

The beginning of research consisted of obtaining basic knowledge on the topic. The placental physiological development and purpose as well the pathology and background of gestational diabetes were studied. Information was found by searching “placenta”, “function”, “structure” “circulation”, “vasculogenesis”, “angiogenesis”, and “gestational diabetes”, “epidemiology”, “classification”, “pathophysiology”, “diagnostics”, “management”, “therapy” alone or in combination with one another in the source index of the Grazer library. The same terms were also searched on Pubmed to increase the number of available sources.

Once there a strong foundation of background knowledge was established, the next step was to search for more specific information relevant to the topic. This exceeded the literature available at the Grazer library and primarily the result of using PubMed and searching terms such as “placental”, “gestational diabetes”, “vascularization”, “trophoblast”, “syncytiotrophoblasts”, “maternal”, “fetal”, “adaptation”, “hyperglycemia”, “hypoglycemia”, “hyperinsulinemia”, “embryopathy”, “macrosomia”, “vasculogenesis”, “angiogenesis”, “endothelial function”, “regulation”, “proangiogenic factors” alone or in combination. The literature found was filtered based off relevancy of the abstracts and narrowed down to the best matches specific to this topic. The search concentrated on alterations in the placenta only for gestational diabetes and eliminated literature focusing on T1D and T2D, although it was imperative that some parallels were included. Of all the sources found, their respective bibliographies were then used as a resource for a secondary literature.

A deeper look into the topic was conducted by searching Pubmed for specific hormones, proteins and influencing factors in GDM on the placenta, maternal metabolism, fetal outcome, and endothelial dysfunction. The terms “placental”,

“hypoxia”, “VEGF”, “PIGF”, “angiopoietin”, “FGF system”, “insulin”, “IGFs”, “leptin”, “adiponectin”, “EG-VEGF/PRKR system”, “EPO”, “IL6”, “TNF- α ”, “macroscopic”, “microscopic”, “endothelial dysfunction”, “fetoplacental”, “oxidative stress”, “adenosine”, “NO”, “NO synthase” were used alone, in combination with each other or in combinations listed previously from prior searches. Once again, the abstracts of the search results were analyzed for finding the relevant information and their bibliographies served as a source for secondary literature. The third stage of research was the most extensive and the literature had to be filtered in order to reduce the amount of information to only sources that were crucial to the topic. Consideration of the effects on the fetus were especially taken into consideration, since it is valuable to the essence of the thesis as to what all of these changes in the maternal metabolism and corresponding changes in the placenta lead to. The works of the authors Wang, Bernischke, Desoye, were closely investigated upon suggestion by the supervising professor, Dr. Desoye.

III. Results

A. Gestational Diabetes

The definition of gestational diabetes mellitus (GMD) is the first-time diagnosis of a carbohydrate intolerance resulting in hyperglycemia in women during pregnancy in weeks 24-28 (19, 20). In Europe, GMD is one of the most common complications during pregnancy and has a prevalence of 2-6% (20).

1. Pathophysiology

The insulin resistance begins near the second half of the pregnancy and progresses through the third trimester. In late pregnancy the insulin sensitivity falls by about 50%. Among the effects of insulin resistance are the insulin desensitizing effect of the placental hormones (21), as well as alterations in growth hormone and cortisol secretion, which acts as an insulin antagonist. The HPL secretion produced by the placenta in the second half of the pregnancy affects fatty acids and glucose metabolism as well as promoting lipolysis and decreasing glucose uptake (21, 22). It not only stimulates the production of insulin but also inhibits the peripheral uptake of glucose in the mother (21). The placenta also produces insulin and its secretion facilitates metabolism of insulin. Additionally, the disruption of the glucose insulin balance is promoted by estrogen and progesterone. Furthermore, an increase in the mothers weight, a decrease in exercise, and increase in calorie uptake contribute to a relative glucose intolerance (22).

2. Diagnostics

In most cases of GDM, the typical clinical symptoms of thirst, polyuria, and weight loss are not distinct (23). To diagnose GDM, a standardized 2-hour 75g-oral glucose tolerance test (oGTT) is recommended. All pregnant women should undergo an OGTT to exclude GDM during weeks 24-28 (24). The guidelines by the IADPSG (The International Association of the Diabetes and Pregnancy Study Groups), which are based on the outcome of the HAPO-study (25, 26), state that the data should be collected from venous plasma directly or data collected from

venous blood should be converted to a plasma glucose measurement using a factor 1,11 (+ 11 %) (27).

Pregnant women with unknown blood glucose levels should undergo the glucose tolerance test in the morning, with the first blood sample being drawn after a fasting period of at least 8 hours and ingestion of a diet high in carbohydrates. The woman should drink a glucose solution (75g glucose in 300ml of water) in a 5 minute period while remaining seated and not smoking (27). Plasma glucose is evaluated at two time points, 1 hour and 2 hours after drinking the solution. The diagnostic thresholds for plasma glucose levels are shown in Table 1. When one score is pathological the mother is diagnosed with GMD (21).

Table 1: Gestational diabetes mellitus diagnostic thresholds for 75-g oral glucose tolerance tests(21)

Time	[mg/dl]	[mmol/l]
Fasting	>92	5,1
1h	>180	10,0
2h	>153	8,5

Women with an increased risk should undergo the test as soon as the pregnancy is confirmed.

Risk factors for gestational diabetes mellitus include (28):

- Advanced maternal age (≥ 35 a)
- Maternal obesity
- High parity
- Previous delivery of macrosomia infant
- Family history of type 2 diabetes mellitus
- Maternal short stature
- Polycystic ovary disease
- High levels of saturated fat in the diet
- Prior gestational diabetes

- Prior neonatal death
- Prior cesarean delivery
- Previous stillbirth or congenital malformations
- High blood pressure during pregnancy
- Multiple pregnancies

3. GDM Classification

Classification of gestational diabetes is done using a modified version of the original scheme developed by Priscilla White (1949). The Clinic of University of Graz adapted and widened this initial concept. GDM is categorized into the subgroups White-A (GDM W/A) and White-A/B (GDM W/AB). Patients with T1D and T2D are graded in further subgroups depending on the onset of the disease. The GDM classifications are shown in Table 1(29).

Table 2 GDM White-Classifications as modified by the Clinic of University of Graz (adapted from (29)):

White A	Blood glucose level exceed oGTT threshold
White B	Fasting glucose level is >95 mg/dl
White A/B	2 or more pathological oGTT scores
White B, C, D	Pre-existing insulin-dependent diabetes

4. Therapy

When a pregnant woman is diagnosed with GDM, she must be informed of the consequences for both herself and the child. A plan of action with an outlined schedule should be determined and include: ambulatory therapy, self-testing of blood glucose levels, medical nutrition therapy, weight gain goals, physical activity routine, and if necessary a pharmacological therapy (24).

a) *Dialectological Care*

Medical Nutrition Therapy

Diatological support as treatment for GDM with individually adapted nutritional plan should be incorporated or discussed as a treatment option. The goal of medical nutrition therapy is to achieve normoglycemia, control weight gain, avoid ketosis, and ensure the wellbeing of the fetus. The daily caloric intake is determined by the patient's body weight (21).

Table 3:: *Calorie allotment and weight gain in pregnancy. (Adapted from Nihal et. al (21))*

Current weight (as % of ideal body weight)	Category	Recommended daily caloric intake (Kcal/kg)	Recommended total weight gain in all three trimesters (kg)
<80-90	Underweight	36-40	12.5-18.0
0-120	Ideal	30	11.5-16.0
120-150	Overweight	24	7.0-11.5
>150	Obese	12-18	At least 6

The proportion of carbohydrates of the total calories should be restricted to 40-45%. The remaining caloric intake should be divided between proteins and fats. Three meals and three snacks should be consumed per day, and one meal/snack should be eaten at bedtime to prevent ketosis while sleeping (21, 27).

b) *Physical activity*

For all patients capable of exercising, 30min of physical activity/day is recommended. It can be divided into 10-minute periods of walking or arm exercises while sitting after each meal. The focus should be on exercises that minimize mechanical stress on the trunk and instead use upper body muscles (21).

c) **Pharmacological therapy**

If the thresholds listed in Table 3 are exceeded or a single measurement of the blood glucose levels are too high regularly or the average blood glucose level (MBG) is ≥ 110 mg/dl during a 6-point day profile, as well as the abdominal circumference of the fetus above the 75. percentile for the gestational age, the patient will need to start an insulin therapy (24, 27).

Table 4: Capillary blood threshold for glucose levels (adapted from Kautzky-Willer (27))

Recruitment targets	Capillary blood (mg/dl)
Fasting (pre-prandial)	65-95
1h post-prandial	<140
2h post-prandial	<120

The maternal blood-glucose-levels should be in the target-range, especially during birth to avoid neonatal hypoglycemia and failure during adaptation. The HbA1c is unsuitable for diagnosing GDM, however it can be used for monitoring the progress of the metabolism. It should stay in the physiologic reference range for a healthy individual (27).

HbA1c is a common marker used to diagnose TD2. It has not yet been established as an adequate indicator in diagnosing GDM. However, studies comparing HbA1c thresholds of non-diabetic pregnant women with GDM patients show that HbA1c possibly has clinical usefulness in diagnosing GDM as well as predicting the risk for future TD2 development (30). The study from Kwon et al. found that HbA1c was significantly higher in the GDM group than in the normal control group. For GDM diagnosis, it showed high sensitivity with relatively low specificity at a cut-off value at 5.05% (32mmol/mol). Patients with postpartum TD2 (PDM) had higher levels of HbA1c during pregnancy. HbA1c above 5.55% (37mmol/mol) is a possible predictor of postpartum diabetes (30).

The insulin therapy should be designed to control blood glucose in fasting periods and post-prandial. Aggressive medical management can achieve target blood glucose measurements

Insulin: Short-acting types of insulin such as Aspart and Lispro are equivalent to human insulin and can be used during pregnancy. They can be used to achieve postprandial control (21). So far there has been no verification that analogs were advantageous during pregnancy in terms of outcome, maternal hypoglycemic rates or the maternal metabolic adjustment compared to normal insulin (31, 32). Glulisin has contraindications due to lack of evidence in use during pregnancy. As there are no study outcomes showing a benefit in the use of long-term type insulin analogs (such as Glargine, Detemir(21)) in comparison to NPH-insulin, their application is not recommended (27).

Oral antidiabetic agents (OADs): Specific oral antidiabetic agents such as the sulfonylurea Glibenclamid biguanid or Metformin are sometimes recommended as an alternative to insulin (e.g. NICE) during pregnancy (27). One third of women will still need insulin to meet glycemic targets. Metformin is placenta current and Glibenclamid is only in small amounts (33). Other OADs like Toldbutamide, which diffuses across the placenta most freely, followed by Chlorpropamide and Glipizide, are not used during pregnancy. They cause fetal hyperinsulinemia and prolonged neonatal hypoglycemia. The use of Metformin alone or in combination with supplemental insulin does not show an increase in perinatal complications in comparison to insulin (21) if these OADs could achieve assailable control in maternal metabolic control (33). In comparison to women treated with only insulin, those receiving a combined treatment of Metformin and insulin required a lower dose of insulin and gained less weight. Metformin is currently the preferred OAD over Glibenclamid during pregnancy (21). Based off of current knowledge, treatment with Glibenclamid may be preferred (e.g. when an insulin-therapy is refused), especially in overweight women. For insulin-resistant patients, Metformin treatment alone or in combination with insulin might be preferred. In this case, the patient should be informed of the advantages and disadvantages when determining which therapy to use (27). The patient should be monitored 1-3 times per week to outline a profile for blood glucose, blood pressure, weight gain, urinary sample, and evaluate if any modifications to the therapy are needed (insulin dose) (27).

d) *Obstetrical Care*

After GDM diagnosis has been confirmed, the patient should have 1-2 weekly appointments. In early pregnancy hyperglycemia an ultrasounds-screening is recommended to exclude malformation of the fetus (especially in heart and kidneys.) Check-ups should include evaluating biometry, amniotic fluid, and heartbeat via Doppler. The growth curve should be watched closely to monitor asymmetric growth or polyhydramnios during abdominal growth. It is extremely important to monitor for hypertension, preeclampsia, and infection throughout the pregnancy. It is crucial to figure out a birth plan, including delivery date and method of delivery (27).

5. Maternal adaptations

A range of physiologic changes occur following implantation. Glucose metabolism adapts to meet the nutritional demands of the fetus and mother (34). Estrogen and progesterone can initiate hyperplasia and hypertrophy in the β cells located in the islets of Langerhans. Glucose crosses the placenta via facilitated diffusion during early pregnancy to supply the fetus with its glucose requirements. This enhanced glucose transport may cause maternal fasting hypoglycemia. The increase in synthesis and secretion leads to elevated postprandial insulin levels in contrast to seemingly normal basal insulin secretion (28).

The elevation of several hormones such as HPL, glucocorticoids and progesterone, as well as free fatty acids and tumor necrosis factor- α , leads to the development of insulin resistance during the second half of pregnancy. In late pregnancy, in comparison to non-pregnant women, ingestion of glucose results in higher and more sustained levels of glucose and insulin. GDM develops when hyperinsulinemia cannot further compensate for exaggerated postprandial hyperglycemia. Patients are at risk for numerous gestational diabetes-associated morbidities (28):

GDM maternal risks(28, 29, 35):

- Preeclampsia, Eclampsia
- Macrosomia
- Caesarian section
- Traumatic Labor
- Instrumental delivery
- Birth injury and shoulder dystocia
- Postnatal uterus atony
- Postpartum loss of blood a require for transfusion
- Early delivery
- Perineal tear
- Diabetes mellitus Type 2 development post-pregnancy
- GDM in following pregnancies
- Urinary and genital tract infection
- Soor colpititis
- Retinopathy
-

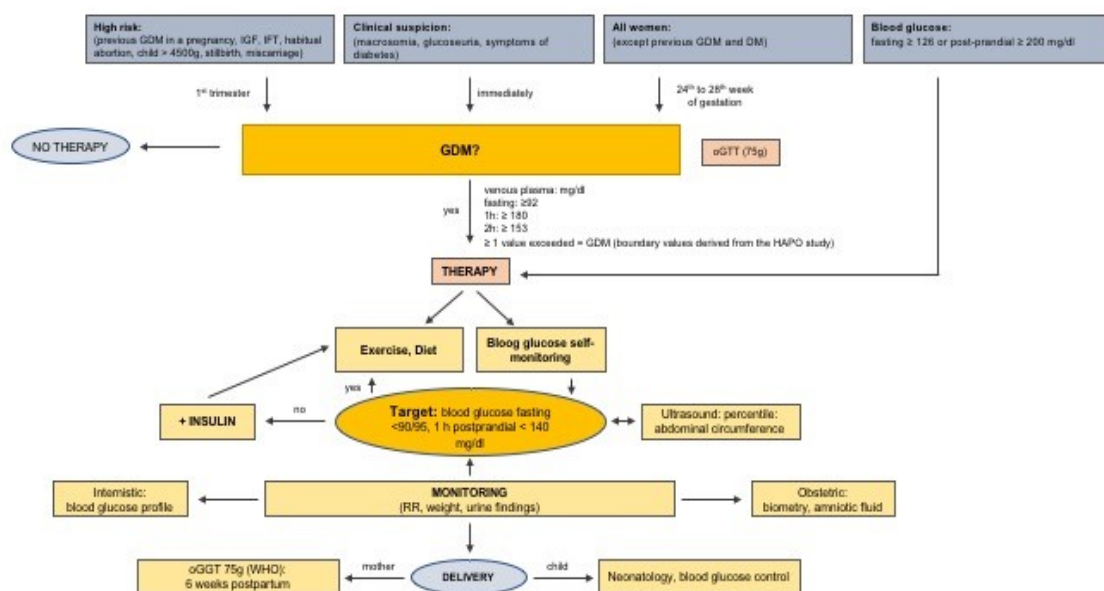


Figure 2: Risk evaluation and diagnosis of Diabetes Mellitus (adapted from Kautzky et. al (27)):

6. Obstetric Complications for mother and child

The perinatal mortality of children of mothers with GDM is no higher than those of metabolically healthy mothers. The perinatal morbidity is seen in elevated incidents of respiratory distress syndrome (36) (surfactant production is reduced due to fetal hyperinsulinemia(37)), hyperbilirubinemia, and hypoglycemia in the early perinatal period (36). GDM per se is not necessarily an indication for a caesarean section, whereas a large fetus is. An estimated birth weight of >4000g (for women <155cm: 4250g) elevates the risk for shoulder dystocia with the consequence of clavicular fracture (37). Inducing labor with either oxytocin or prostaglandin at weeks 38-40 is reasonable depending on the mothers cervical state (38).

Perinatal blood glucose levels should be targeted to be within a range of 70-120 mg/dl (3,9-6,1mmol/l). During the beginning of the birth, it is crucial to use a short-acting type of insulin and avoid long-acting insulin in order to maintain tight control.(37) During birth, the maternal insulin requirements are reduced and then continue to decrease even more during the first few hours postpartum (38). An insulin pump can then be used at a lowered basal rate of 50% until delivery of the placenta and then be reduced to 30%. In many cases of GDM, the delivery can take place without any therapeutic insulin. A few hours postpartum, insulin may not be needed. Daily blood glucose monitoring for 2 days postpartum can determine whether any further pharmaceutical therapy is necessary. The newborn of a GDM mother should be evaluated by neonatology within the first 24h after birth or immediately following delivery if any complications arise (37).

7. The fetus in gestational diabetes

In the beginning of pregnancy, the fetus is dependent on having adequate nourishment, normal glycolysis, and sufficient glucose levels. Physiologically, after the second trimester and the placenta is finished developing, it is supplied with glucose from the maternal blood via facilitated transport using GLUT 1-transporters. The transport capacity is saturated at a blood glucose level of 360 mg/dl (20mmol/l). The glucose concentration reaching the fetus is therefore directly dependent on the acute maternofetal glucose gradient (37).

During maternal hyperglycemia, an increased amount of glucose is fed into the fetal metabolism. The fetus compensates by having an increase in the production of insulin. Unlike glucose, insulin is not able to pass the placental barrier and causes the fetus to develop hyperinsulinemia. Postpartum this can lead to a manifest hypoglycemia (23).

The risk for a hyperglycemic pregnancy to develop an embryopathy is high. In many cases, GDM is diagnosed without any knowledge of possible earlier hyperglycemic metabolic situations (fasting blood glucose level >70 mg%, postprandial >120 mg%) in the earlier stages of pregnancies (before the oGTT during weeks 24-28) or even preconception (36). The longer glucose levels are uncontrolled, the higher the risk for abnormal embryonic development (23). The pathogenic factor of adverse outcomes can be caused by glucose and possibly other metabolic substrates. The fetus is exposed to an altered environment when the metabolism is impaired, with changes occurring in gene expression, free oxygen radicals, cellular damage, and increased teratogenesis. Glycemic control at all times of development as well as during antenatal are crucial for minimizing complications (39). Fetal malformations are not only caused by hyperglycemia, but also ketoacidosis, hypoglycemia as a consequence, and hypoxia. Embryopathy is characterized by deformations, particularly in closing of the neural tube, cardiovascular malformations, and caudal regression syndrome (23).

If an embryopathy is excluded, fetal development must be monitored biometrically to detect an incipient macrosomal or hypertrophic growth development. If the mother's metabolism is poorly adjusted (therapeutically), hypotrophy may develop or polyhydramnios can occur. In case intrauterine growth retardation (IUGR) is observed, placental insufficiency or a diabetic angiopathy must be considered (36). Presenting secondary to hyperglycemia is fetal polyuria, which is one of the causes for polyhydramnios (21). Polyhydramnios can initiate premature contractions, rupture of the membrane, and preterm delivery (23). Macrosomia is the result of the fetal hyperglycemia which induces the β -cells of the fetal pancreas to synthesize insulin. When glucose from the maternal blood reaches the fetus transplacentally, it is incorporated into the fetal cell with help of the insulin (36). This is accompanied with subcutaneous fat deposits and postpartum hypoglycemia (38).

Hyperglycemia during pregnancy not only places the mother at risk for metabolic complications but also puts the fetus at risk for an adverse outcome (21, 29).

GDM fetal risks include (28, 29, 35):

- Polyhydramnios
- Malformation
- Early delivery
- Abortion
- Stillbirth
- Myocardial hypertrophy
- Macrosomia
- Intrauterine growth retardation
- Intrauterine death
- Postnatal polyglobuly
- Postnatal hypoglycemia
- Postnatal respiratory dysfunction
- Postnatal transitory hypocalcemia
- Postnatal transitory hypomagnesemia
- Hyperbilirubinemia
- Postnatal polyglobuly
- Hypocalcemia
- Metabolic syndrome in later life
- Obesity
- Gestational diabetes (if female)

8. Fetal sex differences in GDM risks and delivery mode

Generally, it is known that having a fetus of the male sex are a risk factor for adverse perinatal outcomes. In GDM, findings suggest a significantly higher risk for delivery via caesarean section (40, 41). This could be due to increased fetal weight (42), higher rates of fetal distress, and the lack of fetal hormone contribution during labor in the male fetus (43). There are no published sex differences in the normal range of blood glucose in newborns of GDM mothers, however it is known that

females are more resistant to insulin than males (44, 45). This could indicate that males are more vulnerable to neonatal hypoglycemia. Persson et. al (40) suggest an increased risk for neonatal hypoglycemia in the male newborn according to their findings in a retrospective review (40). A case-control study by Depuy et al. (46) documents such a tendency in newborns from healthy mothers, which could bolster this hypothesis (46). In addition, there have been studies reporting higher risks for respiratory disorders and higher risk of major malformation in male offspring. Interestingly, no significant differences were found in offspring of mothers with DM Type 2 (40).

B. Vascular Development of the Placenta

1. Placental Vasculogenesis and Angiogenesis in Normal Pregnancy

The process of vessel development in the placenta is generally accepted as including vasculogenesis and angiogenesis, with one following the other. Vasculogenesis forms the initial blood vessels, while during angiogenesis vessels are further developed from the already existing ones (47). The two mechanisms differ regarding their control and mechanism (48) and can be broken down into three steps. In Step 1, the vasculogenesis begins with the differentiation of the cytotrophoblast, which is induced by haemangiogenic stem-cells and is regulated by VEGF in a paracrine manner. Step 2 is Angiogenesis 1, during which a pre-vascular network forms. Hofbauer cells and growth factors produced by the cytotrophoblast cells play a key role. In step 3 a process of remodeling and differentiation of perivascular cells happens during Angiogenesis 2. Contractile vessels are formed during this step (7, 47).

The process of vasculogenesis begins in angioblasts, which are endothelial cells. The mechanism can be divided into three steps as well:

1. Fibroblast growth factor induce haemangioblast and angioblast.

2. Vascular endothelial growth factor mediates the assembly of primordial vessels.
3. The transition from vasculogenesis to angiogenesis is activated by the receptors FGF and VEGF correspondingly (9).

The de novo formation of blood vessel begins with precursor cells derived from the mesoderm in the placenta (48). It initially occurs in the mesenchymal layer of the secondary yolk sac in the conceptus. Shortly after this, the earliest developments of villous vessels are seen on approximately day 18 to 35 p.c. while the mesenchymal villi mature from intermediate villi. The earliest primitive capillaries are formed when haemangiogenic stem cells, which are derived from the pluripotent mesenchymal cells, undergo differentiation. They then undergo further differentiation to other hemangioblastic stem cells that persuade angioblast cells, which are the progenitors of endothelial cells (49, 50). During day 21 to 32 p.c., the earliest endothelial tubes are formed (48). The result of local de novo formation of capillaries from the mesenchymal precursor cells in the placental villi is responsible for fetal vascularization, rather than embryonic vessels that grow into the placenta (7).

When the preexisting vessel bed is expanded and new vessel branches are created from preexisting ones, the process of angiogenesis commences. It also involves the longitudinal growth of vessels (48). The early stages of this mechanism start on day 21 p.c. and continue throughout gestation (9). All of the developing vascular supply of immature intermediate villi, stem villi, mature intermediate villi, and terminal villi in the placenta rely on the principle of this mechanism. Angiogenesis can be further subdivided into branching angiogenesis and nonbranching angiogenesis depending on the mechanism and resulting geometry. The term branching angiogenesis describes capillary sprouting or intussusception (endothelial pillar divides the lumen of a vessel), resulting in a complex and multi branched capillary network. The simple elongation of branches is mechanism for the process of nonbranching angiogenesis (48).

The cytotrophoblast core is covered with a thick layer of syncytiotrophoblasts during the formation of the primary villi. It disperses irregularly with the development of the secondary villi(9). A basal lamina can be recognized around the villous

vessels at 6 weeks of gestation. Up until weeks 10-12, the formation of villous capillaries from hemangioblastic cells can be recorded. Hemangioblastic cells develop into villous capillaries until approximately weeks 10-12. After moving into the trophoblastic layer, they twist, bulge, and form sinusoids. In the second half of the pregnancy, some capillary sprouts can be seen even though reliable signals for vessel formation are missing (9).

The first cells are most likely seen in the trophoblastic basement membrane due to the richness in angiogenic growth factor of the cytotrophoblasts. In the cytotrophoblast layer, homogenous connective tissue cells can be seen. Primitive tight junctions and desmosomes unite the cells in aggregates of polygonal cells (hemangiogenic cords) in a string-like formation (48). Around day 23 p.c., the first lumen formation can be seen. Apoptosis has been said to be involved in this process due to morphological and immunohistochemically evidence (51). Long, clearly defined, polygonal capillary in most villi are seen around day 28 p.c. surrounded by endothelial cells, which flatten. Mesenchymal cells set in around the endothelial tubes. These cells are typically thought to develop into pericytes and are characterized by richly developed rough endoplasmic reticulum. These cells may extend focally and be located between endothelial cells (52).

The first hematopoietic stem cells excorticate from the primitive vessel walls and differentiate even more when the first capillary lumens have been formed. They do not circulate at this point because all of the endothelial tubes are independent, unconnected parts, and lack an anatomic connection to the embryonic circulation (the cord). The final connection between the placental vascular beds and intraembryonic is made when allantois vessels rise within the allantois and spread in the direction of the placenta and embryo (53).

In the past it has been claimed that after a phase of branching angiogenesis from day 32 p.c. to week 24, there was a switch to nonbranching angiogenesis (54, 55). This hypothesis assumes that terminal villi form mostly in the second half of gestation and is brought about completely by capillary elongation and looping. However, more recent findings suggest that terminal villi are formed toward the end of pregnancy almost equal amounts by capillary looping and sprouting (56, 57). The

hypothesis that a divisional shift in patterns occurs no longer seems justifiable regarding the lack of confirmation by any longitudinal quantitative data (48).

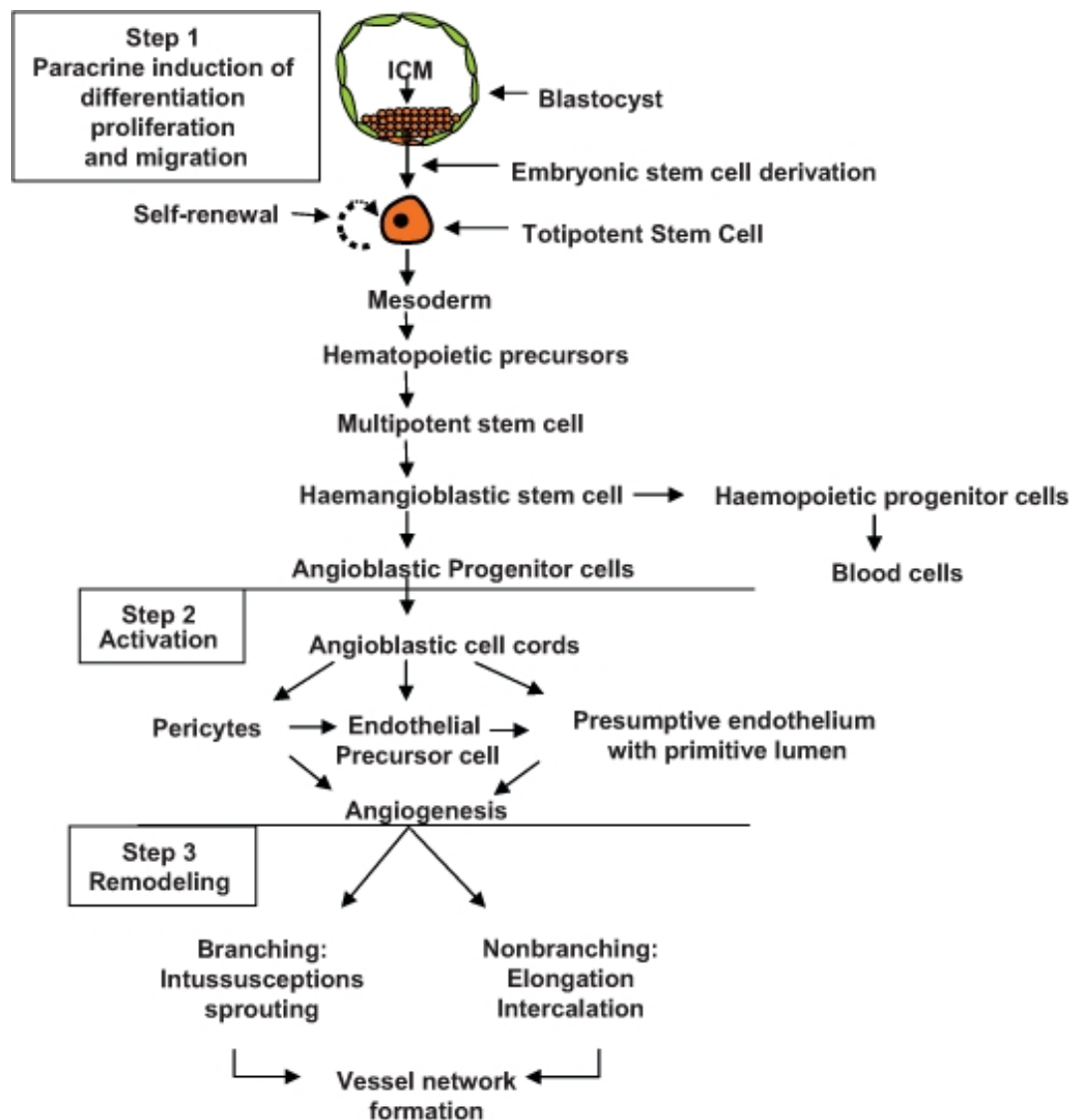


Figure 3: The placental development of villi. Process in three steps of vasculogenesis and angiogenesis (picture from Wang et. al. (7, 9, 47))

The contact between endothelial tubes to each other and to the fetal allantoic vessels is established around day 32 p.c. in the promotive umbilical cord. The first of a presumptive fetoplacental circulation is established. From here until reaching term, vasculogenesis with de novo formation of capillaries and the growth of the villous vascular system by angiogenesis establishes the placental vascularization. There are three periods in this process of angiogenesis which overlap in time (58).

1. Preponderance of branching angiogenesis to form capillary networks from day 32 to week 25 p.c.
2. In weeks 15 to 32 p.c., reverting of peripheral capillary webs and forming of central stem vessels.
3. Preponderance of non-branching angiogenesis to form terminal capillary loops (9).

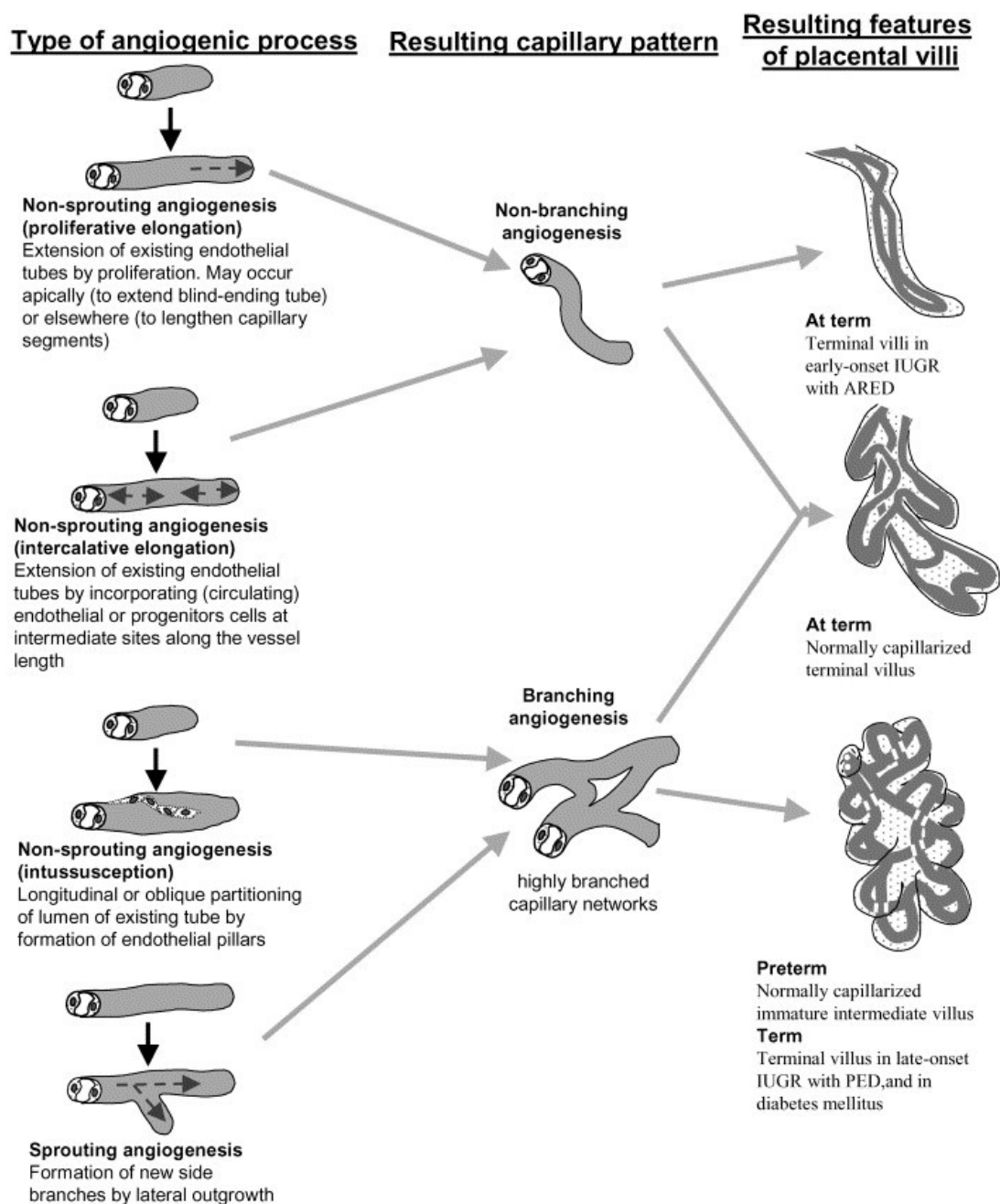


Figure 4: Process of angiogenesis (picture from Charnock-Jones et. al (59)):

Table 5: Measures of the mean cross-sectional sizes and shapes (areas, μm^2 ; perimeters, μm ; shape coefficients, $\mu\text{m}^2/\mu\text{m}^2$) of villi and capillaries in the healthy placenta (adapted from Mayhew et. al (60)).

Variable	Measurement
Villous area	3870 (436)
Villous perimeter	194 (15)
Villous shape	10.0 (0.74)
Capillary area	283 (20)
Capillary perimeter	46.5 (2.51)
Capillary shape	7.8 (0.56)

2. Regulation of Placental Angiogenesis

Placental vascularization is established all throughout gestation by vasculogenic and angiogenic processes. The fetoplacental endothelium is targeted by neighboring cells such as trophoblasts, Hofbauer cells, pericytes, endothelial cells (placental cells), and smooth muscle cells all play a key role in producing a series of angiogenic factors. Some factors may also be present in the fetal circulation and originate from the placenta or fetus itself (1, 9).

A broad range of angiogenic factors are responsible for the regulation of placental angiogenesis. The key modulators include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)-2, placental growth factor (PIGF), and peroxisome proliferator-activated receptor (PPAR)- γ (61-63). Further promoters of the placental angiogenesis are erythropoietin (EPA), leptin (LEP), angiopoietin (ADIPOQ), and the insulin/insulin-like growth factor (INS/IGF). Mediator cell surface receptors specifically regulate their effect in the endothelium (1). In the following passage the findings of Cvitic et. al (1) from a gene expression analysis of levels of the receptors for these hormones respectively, are summarized in order to layout the basis of molecular regulation of angiogenesis. The highest levels of receptors expressed were for VEGF, angiopoietin, and adiponectin (1).

a) Hypoxia

Local oxygen regulates capillary and vascular structure and is self-evidently crucial for adult and fetal life. Alterations in supply or demand leads to a change in oxygen concentration and can cause cellular and organismal responses that are acute or chronic (59). When the metabolic demands exceed the supply of oxygen the metabolism becomes hypoxic. This hypoxia is a major regulator of angiogenesis. The process of placental and embryonic development is driven by a state of low oxygen levels, which is the physiological state during early pregnancy. After the first trimester, levels of fetoplacental oxygen levels increase quickly (64). Hypoxia has a major influence on the regulation of transcription in angiogenic factors and alters mRNA transcript levels. It can alter the mRNA stability by mediating transcriptional activation or alterations (59). Findings that their mechanisms have been upregulated under hypoxic conditions only explain the parallel of high levels of hypoxia sensitive proangiogenic factors and low oxygen environment in the early placenta. The hypoxia-inducible factor (HIF) transcriptionally regulates many genes regulated by hypoxia. It plays a central role when it comes to the variety in development, as well as physiological and pathological responses to hypoxia (59, 65). One of the subunits of this heterodimer HIF-1 complex is the HIF1A subunit. Its transcription and level of activity are closely regulated by the cellular oxygen concentration. The HIF1A unit is stabilized at low oxygen levels due to an internal ribosome binding site (IRES) in their mRNA. Another example of a preganglionic factor which includes mRNA containing IRES is VEGF. HIFs bind to hypoxia response elements (HRE) in their promoters, introns or enhancers and trans activate pro-angiogenic genes (1, 59, 66).

b) VEGF System

The vascular endothelial growth factor (VEGF) system is a considerable regulator in the formation of vascular development in the placenta. The five members of the VEGF family are referred to as VEGF-A to VEGF-E.(67) Considerably the most important factor is VEGF-A, which is the key promoter in differentiating mesenchymal cells into hemangioblastic stem cells. They play a role in placental vascular development, especially in early pregnancy, where they are associated with the formation of the progenitor cells and hemangiogenic cords. Villous trophoblasts mainly secrete VEGF during the first trimester (1, 68). As the pregnancy advances, more of the other angiogenic factors as well as macrophages,

other stromal cells, and smooth muscle cells take over control of blood vessel formation (1, 7, 69-71).

The two main tyrosine kinase receptors VEGFR1 or FLT1 or VEGFR2 or KDR are associated with VEGF (48). VEGFR1 is localized in the syncytiotrophoblast, cytotrophoblast, and macrophages. It has a key role in the regulation of VEGFR2 (72, 73). VEGFR2 is detected within the endothelium cells, but not within the trophoblast and is highly expressed only during the first trimester of pregnancy (72, 74). A lesser extent of expression of VEGF immunoreactivity is seen during the second and third trimester of pregnancy in the syncytiotrophoblasts and not at all in the macrophages. VEGFR1 can be detected in very low levels in the villous trophoblast and endothelial cells, whereas VEGFR2 is no longer detectable (72).

For the other members of the VEGF family (VEGF-B-E), there is no sufficient data on their role in the vascular development of the placenta. However, it has been shown that they have an effect on endothelial cells and other systems (48).

c) *Placental Growth Factor*

One other member of the VEGF family is the placental growth factor (PlGF). It is expressed in the syncytiotrophoblasts (71), as well as in smooth muscle cells, which are located around the fetoplacental vessels (63). During early embryogenesis, giant trophoblast cells secreting PlGF may very well be the initiator and coordinator of the vascularization process in the decidua and placenta (75). It stimulates the formation of the highly branched capillary networks (76, 77).

Even though PlGF can bind to VEGFR1 but does not bind to VEGFR2, which suggests it may have more influence on angiogenesis than vasculogenesis, it is assumed that it might contribute to the mobilization of mesenchymal precursor cells, which do contribute to vasculogenesis (78). In vivo data gives an indication that PlGF might be as important and potent in stimulating growth of new vessels in the placenta as VEGF-A (79). Like the soluble form of VEGFR1, VEGF-1 expression increases as the pregnancy progresses (48, 80). The general pattern shows a gradual rise of VEGF-A throughout the pregnancy and a rather sudden increase of free PlGF in weeks 28-32 p.c. (48).

Expression of VEGF and PlGF are both regulated by physiological oxygen tension. Hypoxia is responsible for the downregulation of PlGF and VEGFR1 autophosphorylation, whereas an increase in oxygen tension upregulates PlGF protein in terms of placental villous explants (81).

d) *Angiopoietin System*

The angiopoietin signaling system is an important controller in the maturation of vessels, as well as in the regulation of vascular smooth muscle cell recruitment. It is also involved in quiescence necessary for maintaining blood vessels (82). The system includes four kinds of ligands, which are angiopoietin-1 (ANGPT1), angiopoietin-2 (ANGPT2), and angiopoietin-3/4. They interact correspondingly with the angiopoietin tyrosine kinase receptors Tie-1 and Tie-2 (50, 83). The balance of interaction between ANGPT1 and ANGPT2 with Tie-1 and Tie-2 is said to be responsible for maintaining the outer layer of the vessel walls. ANGPT1 and ANGPT2 mRNA and protein are found where larger arterioles/arteries and venules/veins develop in the perivascular cells of immature intermediate villi (1, 84). Tie-1 and Tie-2 mRNA are found in the vascular endothelium and placental trophoblast (85, 86). Studies have found a direct correlation of the functional consequences of the angiopoietin-Tie-system to VEGF-A presence. Cell migration and proliferation under the control of ANGPT2 and therefore angiogenesis only function in the presence of VEGF-A. Consequently VEGF-A inhibition leads to vessel regression and endothelial cell death (87, 88). HRE mediates transcriptional induction of ANGPT2, which is sensitive to hypoxia and also regulated by increasing mRNA stability (1, 89, 90).

e) *FGF System*

Both acidic and basic forms of the fibroblast growth factor can be seen in the cytotrophoblast immunoreactivity in the first trimester, which suggests that the early stages of vasculogenesis and angiogenesis are influenced by these factors (48, 91). They might also be involved in remodeling due to the fact that the mRNA encoding basic fibroblast growth factor localizes to the smooth muscle cells, which encircle the vessels with small and large stem villi (48).

So far 22 members of the FGF-family and 4 members of the FGF receptors have been identified in humans. The molecules each of have a molecular weight of about 14-16kDa (92, 93). They can be divided into three groups based on the functional, structural, and characteristic differences. FGF1-1- binds to FGF receptors. FGF1 is the acidic form whereas FGF2 is the basic form. FGF 11-14 are the FGF homologous factors 1-4 (FHF1-4), which do not bind to the FGF receptors but rather to the islet-brain-2 (IB2) (9, 94). Humans do not have FGF15, and FGF16-23 have yet to be well characterized. FGF1 and FGF2 have been studied the most from the FGF group and one of the identified functions is their potent angiogenic potential. They are promoters of endothelial cell proliferation and vasculogenesis (95).

FGFs are heparin-binding proteins which interact with the cell-surface associated heparin sulfate proteoglycans. They play an essential role in FGF signal transduction and are responsible for the proliferation and differentiation of many diverse cells. Of the many biological effects that FGFs have, pluripotency and angiogenesis are essential in the vascular development of the placenta.(96) Their functions on endothelial cells are exerted by binding to the tyrosine kinase receptors FGFR1 and FGFR2. Among proliferation and differentiation, they have a key role in ECM degradation, migration, and modulation of cell-cell interactions (97). Expression of FGF2 is highest during the early development of the placenta, similar to VEGF. It is thought to be involved in the recruitment of haemangiogenic progenitor cells. Towards the end of pregnancy its expression is seen more so in the syntiotrophoblast, villous stroma, and fetal vessels (98-100). The fetoplacental endothelium is target to FGF2 action due to parallel expression of FGFR1. Also, like VEGF, FGF2 involves HREs and IRES, making it sensitive to regulation by hypoxia at both the translational and posttranslational levels (101).

f) *INSULIN and IGFs*

As reviewed by Cvitic et al. (1) the insulin/insulin-like growth factor (INS/IGF) system regulates fetal and placental growth and development (102). It consists of insulin, insulin growth like factors 1 and 2 (IGF1 and IGF2). Additionally, three mediator cell-surface receptors for biological effects of INS and IGFs, insulin (IR) and the receptors IGF1R and IGF2R for IGF1 and IGF2 respectively, as well as a group of IGF-binding proteins (IGFBPs) are part of the system (1, 103).

There is a significant involvement of Insulin and IGFs in the development and growth of the placenta and fetus. Continuous synthesis of IGF1 and IGF2 in macrophages and endothelial cells, which are placental mesenchymal cells, is seen. IGF1 is present throughout gestation, mainly in the trophoblast compartment. The expression of IGF2 is only seen during the first trimester in villous and extra villous cytotrophoblasts and not observed in the syncytiotrophoblasts at all and not at term of gestation (102, 104-108).

In the early stages of pregnancy, the IGF1R is expressed on the syncytiotrophoblast, cytotrophoblasts, and extra villous cytotrophoblasts as well as on placental macrophages. In the third trimester it is also expressed on the fetoplacental endothelium (102). The predominant location of the IR is at the syncytiotrophoblast and during the third trimester of pregnancy, it can be found in low levels on the cytotrophoblast. The main location for the IR towards term are the fetoplacental vessels (109). This suggests that insulin and IGFs are responsible for the regulation of fetoplacental angiogenesis during the later stages of pregnancy. It can be assumed that they do not contribute to placental vasculogenesis due to the absence of receptor expression on the fetoplacental endothelium in the first trimester (1).

g) Leptin

The adipokine leptin (LEP) is an endovascular factor of proangiogenic factors and growth factors in the placenta. It is expressed in fetoplacental endothelial cells, cytotrophoblasts, syncytiotrophoblasts, and the amnion. Most of its secretion is directed into the maternal blood circulation. This may cause elevated levels of leptin in the mother (110-112). On the other hand, almost none of the leptin is secreted into the fetal circulation (5%) (113). During the third trimester, the syncytiotrophoblast is the primary location for the leptin receptor (114). Similar to VEGF and FGF2, the expression of LEP mRNA is regulated by hypoxia and hypoxia-inducible transcription factor HIF1A, but only on the transcriptional level (1, 115).

h) Adiponectin

Adiponectin (ADIPOQ) is an assumed endovascular factor of proangiogenic factors as well as having insulin sensitizing properties. It was assumed that it is primarily expressed in the syncytiotrophoblast reportedly in the term placenta (116), until the findings from

a recent study proved it is absent (117). Adiponectin most likely has a physiological effect on the development of the placenta due to the presence of its receptor, ADIPOR2 (not ADIPOR1), in the cytoplasm of placental syncytiotrophoblasts and cytotrophoblasts (1, 118).

i) EG-VEGF/PROKR system

lfaidy et al. reviewed a new group of angiogenic mitogens listing EG-VEGF/PROK1 and PROK 2 and their receptors PROKR1 and PROKR. They are characterized as having high tissue specificity, especially for the reproductive tract and placenta for EG-VEGF (119-121). There are low expression levels in the cytotrophoblasts and it is mainly expressed in syncytiotrophoblasts (122). It increases vascular proliferation, migration, and the tube-like formation as well as the permeability of the placental microvascular endothelial cells due to its strong specificity for the vascular bed. It is not involved in Human umbilical vein endothelial cells (HUVEC) angiogenesis (123). The receptor PROKR1 cannot be found at all in cytotrophoblasts, Hofbauer cells, or the placental microvascular endothelial cells. Expression of PROKR2 can be seen in Hofbauer cells, extravillous trophoblasts, and syncytiotrophoblasts (122, 124, 125). Both EG-VEGF and the receptor PROKR1 are transcriptionally stimulated through oxygen tension (122).

j) Erythropoietin (EPO)

Erythropoietin is a major hormone regulator of erythropoiesis and is produced by the kidney in the adult, can be stimulated through hypoxia (126) at the transcriptional level through the binding of HIF to HRE elements in the EPO gene, as well as regulated without engaging IRES elements at the translational level (127, 128). The fetal liver produces EPO in the perisinusoidal cells during gestation as well as expression in trophoblast subpopulations in the placenta (129, 130). Furthermore, the corresponding receptor EPOR was found in villous core cells such as the fetoplacental endothelial cells and in the villous and extra villous cytotrophoblasts and syncytiotrophoblasts (131). In addition to the hematopoietic role of EPO, it functions as a stimulator of angiogenesis in the embryo, circulating endothelial progenitor cells, and mitogenesis. It also hinders apoptosis of cardiac myocytes in the ischemic heart due to its cardioprotective nature (132, 133).

k) *IL6 and TNFA*

The human placenta also produces cytokines, which are cells of the immune system and adipose tissue (3). IL6 and TNF- α (tumor necrosis factor α) have a key role in the inflammatory response. TNF- α is only produced by activated macrophages. Macrophages, muscle cells, epithelial cells, and fibroblasts are responsible for the expression of IL6 (134-136). IL6 has been indicated as being partially responsible for remodeling during vascular development and angiogenesis (137). In early pregnancy, its expression in trophoblasts and fetal vessels is relatively low but rises as full term is reached (138, 139). TNF- α is also represented more dominantly in the syncytiotrophoblasts than villous stromal cells and cytotrophoblasts in the first trimester, whereas this reverses towards the end of the pregnancy (140). The corresponding receptors IL6R, TNFR1, and TNFR2 are expressed in fetoplacental endothelial cells as well as placental trophoblast for the binding site of IL6 (141, 142). Hypoxia is able to stimulate TNF- α without HRE containing TNF- α mRNA, whereas IL6 gene release, transcription, and translation can be enhanced from the smooth muscle cells and endothelial cells (143).

Table 6: Levels and expression of proangiogenic factors in the placenta and cord blood in GDM pregnancies (adapted and expanded from Cvitic et. al (1))

Factor	Placenta	Cord Blood
VEGF	↓ protein (144)	↓ (145)
PlGF	↓ protein (144)	↓ (145)
FGF2	↑ protein ↑ mRNA (146, 147)	↑ (107, 146)
Angiopoietin	↑ mRNA (148)	
Erythropoietin		↑ (145, 149)
IL6	↑ mRNA (150)	↓ (151)
TNFA	↑ protein (152) ↑ mRNA (148) = mRNA (150)	= (153) ↓ (151)
Insulin		↑ (40, 154-156)
PPAR γ	↓ protein ↓ mRNA (157)	
Leptin	↑ protein ↑ mRNA (147)	

↑ indicates elevated levels of each factor in GDM compared to normal pregnancy ↓ indicates reduced levels of each factor in GDM compared to normal pregnancy (1)

C. The Placenta in gestational diabetes

The placental changes have been studied and reviewed closely by Gauster et. al and summarized as follows (3). To study the effects of gestational diabetes on the placenta, the question of its actual onset proposes an undefinable variable for many cases studied. From what point can placental changes be correlated to diabetes(158)? It is unclear as to how many women, who are diagnosed with GDM

in their antenatal exam (oGTT in weeks 24-28), suffered from a diabetic condition at an earlier onset. Around 3-40% of all pregnancies complicated with GDM were diagnosed with the disorder before week 20 p.c. (159, 160). It has been suggested that early onset pathophysiological conditions with no clinically recognized symptoms cause altered placental development in regard to structure, especially in terms of vasculature development as it is still incomplete at this point. The most effected part of development includes villous immaturity and irregular villous branching, which are completed in the first half of pregnancy (145, 161-163). Long-term effects of early pathophysiologic derangements, which result in the manifestation of GDM with clinical conditions, are changes in placental morphology. Short-term effects of an abnormal glycemic status in later pregnancy mainly affect the placenta functionality (3, 158).

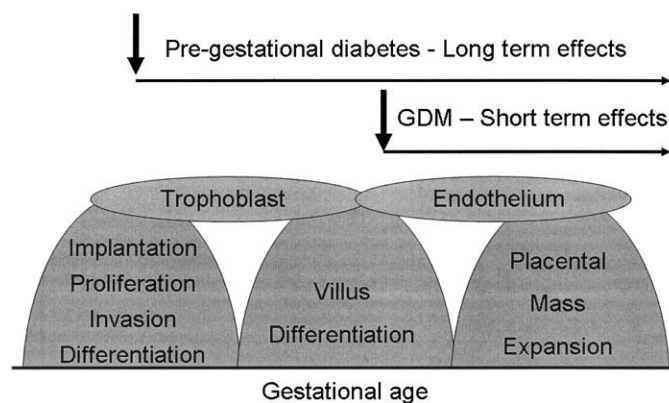


Figure 5: Three phases of placental growth and development. Placental growth and development are shown in three phases. Alteration of the development shown to set when exposure to an diabetic environment occurs. GDM has a short-term effect due to its later onset. (164) (Picture from (164))

To recall, GDM is responsible for many complications in the mother and fetus, both short-term and long-term, and alters the development of placental structure and function. Many causes can be traced back to factors found in a diabetic metabolic milieu in the mother. One of the key complications of late onset metabolic complications for the fetus are endothelial and vascular dysfunction (158).

GDM treatment and glycemic control reduces many risk factors for metabolic or obstetric complications for the mother and fetus. However, in general it is unclear if there is a direct accord to the degree of glycemic control and placental changes. According to some studies, the concept of a proportional correlation between hyperglycemia and damage is supported. However, other studies have found that having a tight control on glycemic levels alters the histology of the diabetic placenta in comparison to a nondiabetic placenta (145, 165, 166).

The macroscopic changes seen in the placenta are proportional to the degree of hyperglycemia and reflected in its increase in size, weight, thickness, and it is plethoric. This correlates with a decrease in placental and fetal weight ratio. This suggests that the placenta grows initially and then contributes to a higher glucose and nutrient transfer to the fetus, hastening its growth (3, 161, 162, 167-169). The placenta exceeding its normal weight may be a result of the increment in parenchymal tissue cellularity. This correlates with a higher DNA content of the placentae in diabetic pregnancies (170). Macroscopically, in regards to the shape or cord centrality index and the cord coiling index, there have been no anatomical differences seen in diabetic placentas compared to placentas from healthy pregnancies, other than enlargement (3).

However, differences have been found on a microscopic level. Diabetic placentas have several lesions, which are precursors for further complications due to altered maternofetal nutrient exchange or oxygen delivery, and abnormal developments can be found. This can be traced back to the villous fibrinous necrosis, where there is reduced formation of terminal villi and the villous stroma is replaced by fibrinous material (171, 172). An increase in the maturation of branching villi and elevated numbers of fetal nucleated red blood cells are evidently the result of a chronic ischemic condition of the fetus. This leads to a higher prevalence of placental infarction. An increase in vascular recruitment due to the enlargement of the placenta leads to chorangiosis and vascular hyperplasia of the chorionic villi (158). Madazli et al. (145) and Daskalakis et al. (162) found an increase in ischemic changes in the placenta and a rise in villous immaturity as well as chorangiosis, villous fibroid necrosis and nucleated red blood cells with no relation to the glycemic status of the mother (145, 158, 162). Further histological analysis found changes in syncytiotrophoblasts with fibrin deposits, villous edema, and marked

hyperplastic cytotrophoblasts (3, 173). In comparison to non-diabetic placentas, which showed no such changes, placentas from overt diabetic patients had fewer distinct alterations (3). For the fetus, a sudden shortage or excessive exchange of gas and nutrients may be dangerous due to a perfusion disparity caused by the growing distance of intervillous diffusion of the immature villi paired with the enlarged placenta (158).

1. Placental Angiogenesis in Gestational Diabetes Mellitus(1)

Regarding the onset of the diabetic metabolic condition, GDM sets the window for “damage” to the second half the pregnancy. This means it affects processes that occur in the later stages of pregnancies and most likely does not concern vasculogenesis. Most vascular developmental changes affected by GDM can be seen in angiogenesis and microvascular remodeling (1). This suggests that the effects of pre-gestational diabetes will have a different effect on the vascular development than GDM. It changes the placental development in the early pregnancy and leads to a reduced placental vascularization. However, the results are adverse. Signs for both an increase, as well as a decrease in placental vascular development were seen. Pre-gestational DM is responsible for changes of the placenta in the early pregnancy showing significantly inclined placental vascularization and lower PAPP-A values (174, 175). The GDM placenta has been less investigated compared to the Diabetes Mellitus Type-1 placenta, however similar abnormalities in vascular development have been found, although less observed. An enhancement of branching in villi is seen, as well as an increase in the capillary surface area (1, 57, 176). Another aspect that contributes to abnormal findings in GDM placenta is the growth of longer umbilical cords. This is an additional risk for the fetus to develop hyper- or hypo coiled cords (177, 178).

In the GDM placenta, the stem villi and free villi both show an increase in capillary structures of various sizes. In the syncytiotrophoblast cell layer, there are many vacuolar formations and a reduction of collagen fibers in the villus stroma can be found. The entire villus is covered with vascular formations (179). In GDM placentas, a tendency for hypervascularization was found, as well as changes in endothelial

resistance. A cutback of vascular-endothelial cadherin (VE-cadherin), beta-catenin (β -catenin), and adherent junctional molecules expressed on the surface were recorded. A downregulation of tight junction molecules, occluding and zonula-occludens-1 (ZO1) may cause commotion in the placental barrier function as well as angiogenesis (1, 180).

It is imperative to note that these changes in placental vascular development have an impact not only on the mother, but also on the fetus. It has a higher risk for cardiovascular complications and retinopathy due to changes in the vessels. The outcome of these complications are lower in children born to mothers with GDM compared to mothers with T1D and an increased BMI.(181) Nevertheless, Eriksson et al. have shown a parallel between exposure to hyperglycemia perinatal and inveterate anomalies (1, 182).

2. Effect of Maternal Diabetes on Proangiogenic Factors in Placenta and Fetus(1)

In the following chapter a summary of a review by Cvitic et. al on the effects of maternal diabetes on proangiogenic factors in the placenta and the fetus is given. The influence of several factors that contribute to the placental vascular changes are analyzed.(1) In Figure 7, an overview is shown.

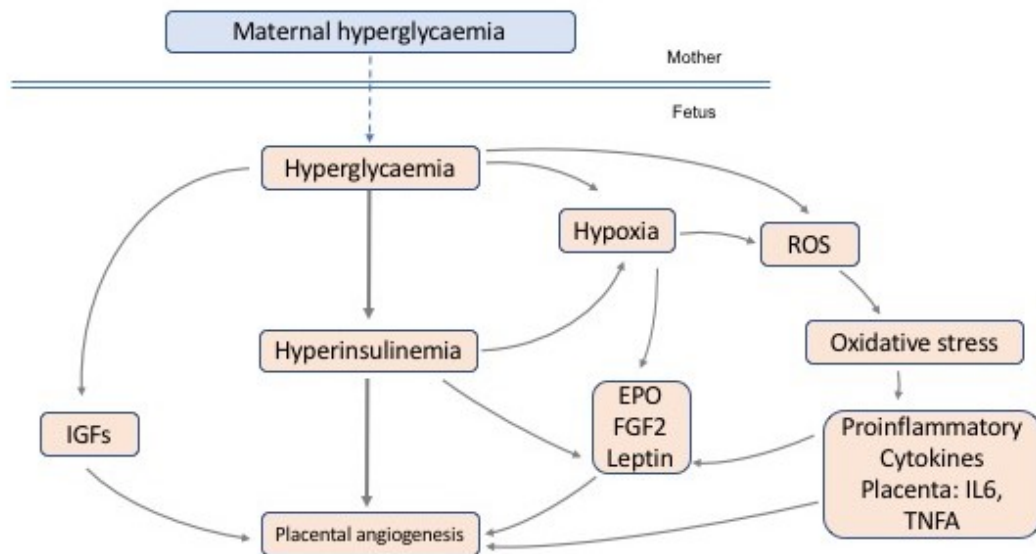


Figure 6: Hypothetical overview of how placental hypervascularization is generated. Consequential metabolic, hormonal and inflammatory changes due to fetal hypoxia, hyperinsulinemia, and hyperglycemia. (1) (adapted from Cvitic et al.(1))

Maternal hyperglycemia causes fetal hyperglycemia and hyperinsulinemia when the fetal production commences, both of which trigger further metabolic and hormonal disorders and lead to an increase in the demand for oxygen. Consequently, the fetus can often be challenged by chronic hypoxia (149, 161, 183). This triggers a chain reaction that stimulates placental vasculogenesis and angiogenesis because of the activating nature of hypoxia on proangiogenic factors and both fetal and placental expression of vascular growth factors (65). The factors VEGF, PlGF, FGF2, EPO, ANG2, and leptin are sensitive to regulation by hypoxia and may be elevated in maternal diabetes due to fetal chronic hypoxia. Consequently, there may be an increase in expression of regulators of IRES or HREs, which are sensitive to hypoxia (1). This sequence occupancy triggers an overstimulation in the expression and translation of FGF2 through the promoter. Its levels are elevated in the placenta and fetal cord blood compared to physiologic levels (98, 101, 146).

Fetal hypoxia due to maternal diabetes leads to an unchanged, or lower than what is physiologically normal, expression of VEGF towards the end of pregnancy (145, 184). Corporal increase of PIGF is inhibited (185). It is assumed that VEGF levels are preserved due to an increase in the expression of VEGFR1 (Flt1) and VEGFR2 (Kdr). This can be seen in placental tissues of diabetic pregnancies (1, 186).

Hypoxia further influences the expression of factors responsible for the vascular development of the placenta. It changes the main action of angiopoietin in its role to maintain and mature vessels. Hypoxia regulates ANG2 transcriptionally, which may be the reason for an increase in ANG2 mRNA levels in the placenta of a diabetic mother (1, 148, 187).

Regarding the hormone EPO, it is associated with an upregulation of the VEGF/VEGFR system which can be observed in experimentally in mice (131, 149). It is also affected by hypoxia and its level are increased in the diabetic fetal circulation towards the end of pregnancy. The receptor EPOR induces EPO action by expressing in the placental endothelial cells of the fetoplacental vessels (188). Hypothetically, it has been suggested that despite higher oxygen demands in the fetus, EPO can promote placental angiogenesis by upregulating the expression of VEGFR in the fetus and placenta, even though VEGF levels stay the same or are even lower in maternal diabetes. There has yet to be proof of this in humans (1, 144).

Hypervascularization in the placenta of diabetic mothers is initiated by hypoxia, which alters different proangiogenic factors at different levels. The standard genes sensitive to hypoxia regulation, such as VEGF, PIGF, and ANG2, stay the same in maternal diabetes while an increase of FGF2 and EPO are triggered. Accordingly, other factors that are associated with these alterations need to be taken into consideration. Two main factors that enhance or rework the effects of hypoxia are hyperglycemia and hyperinsulinemia (1). In order to isolate the effects of hypoxia on placental vascularization independent of hyperglycemia and hyperinsulinemia, chronic fetal hypoxia due to anemic conditions, high altitude, or a smoking mother have not shown similar results (189). Both maternal diabetes as well as other hypoxic conditions have been reported to have angiogenesis with increased

branching, but only in diabetic conditions has an increase in total vascular volume, surface area, and capillary length been described (1, 189).

Placental leptin expression in GDM patients is increased compared to normal pregnant women (190). An inflammatory situation is established when leptin stimulates monocytes with the production of TNF- α and interleukin 6 (IL-6) to further increase secretion of CC-chemokine ligands. Leptin influences insulin secretion, glucose utilization, glycogen synthesis, and fatty acid metabolism by increasing insulin sensitivity. Therefore, there is a correlation between hyperinsulinemia in GDM and leptin production by adipocytes from fetal adipose tissue, which is the main source for leptin (190). This leads to a loop where elevation of fetal insulin leads to an elevation of fetal plasma leptin due to growth of adipose tissue as a result of fetal hyperinsulinemia (191).

3. Inflammation and Transport of Nutrients in the GDM Placenta

A proinflammatory environment and derangements of cytokines due to hyperglycemia further promotes changes in the placental vascular development. It triggers a chain reaction that promotes oxidative stress and enhances the proinflammatory environment in diabetic mothers (1, 192). Cells of the immune system and the adipose tissue are responsible for the production of cytokines. The two main cytokines contributing to an inflammatory environment are IL-1 β and TNF- α , which are sensitive to oxidative stress and may also affect and alter angiogenesis by adding to the proinflammatory situation (3, 193, 194).

a) *Abnormal transport of lipids*

Hyperglycemia contributes to the enhanced release of placental cytokines, which contribute to the lipid metabolism via expression of regulators. It is shown that levels of IL-6 are responsible for fatty acid inflation in primary term trophoblasts in vitro studies. In obese women with GDM, a significantly elevated concentration of endothelial lipase (a close relative of lipoprotein lipase (LPL)) was detected. This was not the case for slim GDM patients, thus suggesting a correlation of diabetic condition, metabolic inflammation, and obesity in contributing to an abnormal regulation of EL. It is commonly believed that the key regulators for placental EL

expression are TNF- α and IL-6 from cell culture experiments.(195, 196) In a microarray analysis of the placental tissue by Radaelli et al., the enhancement of genes participating in the lipid pathway in the diabetic placenta was shown. Characteristic accumulation of genes engaged in the lipid transport and metabolism, lipid activation, glucose metabolism, and hexosamine pathways were documented (197). Very interestingly, there was a sustainable difference between GDM and T1D gene expression. In the GDM placenta only selective pathways for triglycerides and cholesterol biosynthesis were seen, whereas in T1D placentae an increase in both the hexosamine pathways and glycosylation reaction were seen (3, 197).

b) *Abnormal transport of Amino acids in GDM*

Amino acids are not synthesized by the fetus or placenta. This calls for the necessity of their placental transport from the maternal circulation to the fetus in order to carry out their vital functions (198). Exchange and metabolism of amino acids in the GDM placenta is altered. This is documented in an analysis of amino acid concentration from the cord blood, where a significant enhancement of the presence of essential and nonessential amino acids had been detected in the arterial plasma and umbilical venous blood from GDM pregnancies that have been well managed. It is significant to note that these amino acid concentrations are not altered in the maternal circulation (199). Regulation of placental transport of amino acids has been associated with GDM conditions (200). Regarding system A, an elevation of leptin, insulin and glucose was observed to increase its activity (201-203). The data on transport capacity for systems A and L for neutral amino acids show an increase for system A transporter activity in pregnancies complicated by GDM and either an increase or no change for system L. Some studies show a decrease of system A in GDM pregnancies with macrosomic babies, however this information should be interpreted with caution due to the possibility of amino acid transporters in the syncytiotrophoblasts compensating for one another, as they both have the same substrate (3, 204). An interesting report by Jansson et. al (205) showed an increase of system L activity in pregnancies with LGA (large for gestational age) fetuses. This leads to the assumption that placental transport of amino acids via system L contributes to the excessive growth of the fetus in GDM (205).

c) **Abnormal Glucose transport in the GDM placenta**

The fetus depends on the maternal glucose supply considering his own production is very low (206). The maternofetal concentration gradient allows for the transplacental glucose flow a three classic glucose carrier (GLUTs) family transporter isoforms, of which GLUT1 is the most responsive (207). In the GDM placenta, glucose transport is reportedly altered (208-210). Gaither et. al (211) have found an elevation of GLUT1 expression and its activity in the basemembrane of syncytiotrophoblasts in women with diet controlled or insulin-dependent GDM. Even in women under competent glycemic control there was an incident of macrosomic fetuses in one out of three. This observation proposes that enhanced placental glucose transport is associated with fetal overgrowth in GDM due to the fetal hyperglycemia and hyperinsulimemia and the decrease in maternal glucose levels. (211) Other GDM-associated conditions could contribute to the increase in placental glucose transport as seen in the effects of oxidative stress and long-term glucose exposure. These factors downregulate GLUT1 expression. Overall the tendency in GDM is an increase in placental glucose transport (207, 208, 210, 212, 213). In combination with higher placental weights this leads to an increase in fetal fat accumulation. GDM and obesity correlate with an increase in inflammatory cytokines which again alters the gene expression for lipid pathways, as seen in Figure 9.

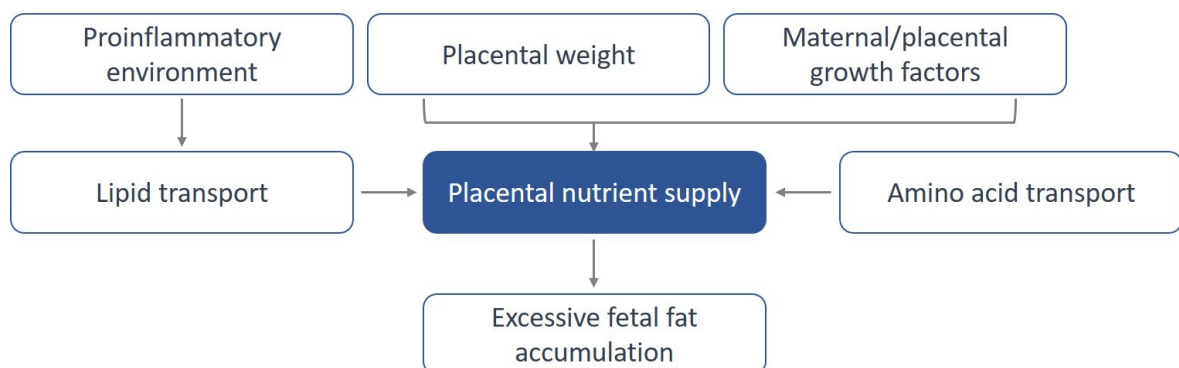


Figure 7: Augmented nutrient transport in the GDM. The fetal nutrient supply is increased due to several factors. An increased lipid transport molecules and the enhanced expression of amino acids. The higher placental weight acts substantially. (3) (picture is adapted from Gauster et al. (3))

D. Hba1c correlation to vascular changes in the GDM placenta

1. Elevated lactate levels correlation with HbA1c

The pathogenesis of GDM is associated with a range of regulatory hormones and growth factors of which one of the newest molecules considered is lactate. It has a transitional function in the carbohydrate metabolism (214). GDM mothers show significantly elevated lactate levels in correlation with HbA1c compared with normal pregnant women. Furthermore, a parallel to blood pressure and fetal birth is seen (215). A correlation between blood lactate and Immuno Reactive C-Peptide (IRCP) levels is seen (216). In the development of the insulin resistance in TD2 an increased blood lactate concentration and a change in substrate utilization are partly responsible of greater carbohydrate oxidation. (217). Fetuses born to GDM mother show significantly increased lactate concentrations and lower umbilical vein oxygen saturation and oxygen content. This is possibly the result of an enhanced fetal metabolism due to hyperglycemia and hyperinsulinemia (161). Studies have shown altered lactate levels in placental vessels in GDM women, even though the varied lactate level in the venous blood have not yet been explained (215). Lactate is responsible for relaxation in placental vessels in normal pregnancies. The mechanism involves hydrogen peroxide (H_2O_2) and stimulation of cGMP production while depending on oxygen. This vessel relaxing effect of lactate was seen less in GDM placental vessel and may be due to their faulty response to the catalase activity to H_2O_2 and lactate. This proposes impairments in the cGMP-mediated relaxation to exogenous and endogenous H_2O_2 in GDM placental vessels. Fetal hypoxia as found in GDM, might call for a compensating mechanism of relaxation to lactate in the placental circulation. The loss of this function and a higher titre of HbA1c in pregnant women with abnormal glucose metabolism could be ground for an increase in complications (218, 219).

In GDM pregnancies, the enlargement of the placenta, the reduced fetoplacental ratio, the altered placental morphology, the villous immaturity and increase in villous branching are seen. In addition, hyperplasia of the fetal-placental beta cell seems to influence pathogenesis of GDM (161).

2. Effect of HbA1c, C-peptide, insulin, IL-6 and maternal lipids on the placenta and the fetus in GDM

GDM has a significant effect on maternal lipid levels, which can contribute to adverse birth weight outcomes (220-224). GDM placentae show an altered lipid metabolism. The mechanism is still unknown, however it is hypothesized that high glucose levels reduce mitochondrial fatty acid oxidation and increase the accumulation of triglyceride in the placenta (225). A connection is seen to C-peptide levels in the cord blood as well as the relationship between maternal blood markers and association with LGA newborns (weight above 90th percentile for gestational age) in correspondence to maternal lipids, insulin and HbA1c. Compared to results from women who gave birth to AGA infants (weight above the 10th percentile, but below 90th percentile for gestational age) (226), women who delivered LGA newborns show higher levels of C-peptide, HbA1c and TG. Interestingly they had lower levels of HDL-C, LDL-C and TC than women who delivered AGA newborns (220, 227-229). High levels of TG are correlated with elevated maternal C-peptide levels and with birth weight (226). In GDM patients with elevated C-peptide levels an increase in maternal HbA1c concentration is seen (230).

The insulin-secretory activity of the pancreatic β -cells modulates fetal growth and is reflected by plasma c-peptide (230). Dube et al. (230) have found that women with GDM tended to have offspring with higher cord blood c-peptide (652 ± 324 vs. 511 ± 166 mmol/L, $p = 0.08$) and glycyemia (5.0 ± 1.0 mmol/L vs. 4.5 ± 0.9 , $p = 0.09$) compared to NGT (normal glucose tolerance) women (230). They show that cord-C-peptide levels correlate with maternal C-peptide, insulin and insulin sensitivity and give an indication for values after delivery. This proposes that fetal hyperinsulinemia stands in relation to maternal glucose-insulin homeostasis. It may also be an indicator during pregnancy for metabolic risk factors and to the outcome of glucose-insulin homeostasis shortly after delivery (230). Pallardo et al. a β -cell defect is responsible for an increased risk of postpartum diabetes, regardless of the degree of GDM or obesity (231). Maternal BMI is however a distinct predictor for GDM and weight of the newborn (232, 233). It is also an indicator for cord blood C-peptide level and maternal IL-6 at screening. These results lead to the assumption that GDM

and a high BMI are influential on the insulin-secretory activity of the fetal pancreatic β -cells of the offspring (230).

E. Sex specific differences in fetal sensitivity to the glycemic environment in GDM.

Maternal GDM has a potential influence on the growth and development of the offspring depending on its sex. A few studies have looked at cord blood changes in regulation of mitogenic hormones and adipokines, including leptin, adiponectin, insulin and the insulin-like growth factor (IGF) axis in correlation with fetal sex and found some differences (234). An association of the male sex in offspring from GDM mothers with growth factors and adipokines in their cord blood were found. This was not the case with girls to the same extent. These results propose sex differences regarding fetal overnutrition (234). Several studies have found that boys are more likely to turn out LGA (226). Boys, but not girls have elevated levels of several hormones, except cord blood levels of IGF-1 which exceeded in the female offspring of GDM mothers (234). This may explain the different adaptive responses of sexes to hyperglycemia and the increase of IGF-1 increase in females only. Females appear to be less sensitive to a glycemic environment in the uterus than male offspring. Increase in C-peptide, leptin, or insulin was only observed in male infants of GDM pregnancies (235, 236). Additionally, higher levels of IGF-2, while not directly correlated, might contribute to the higher fetal/placental weight ratio and the higher rates of perinatal morbidity as observed in male fetuses (237). The male fetus shows an increased sensitivity to hyperglycemia. This might result in an adaptive response of increased IGF-2 levels in the hyperglycemic uterine environment (235, 236). In summary Oken et al. (234) found that abnormal gestational glucose tolerance in mothers lead to different cord blood hormone profiles in infants compared with normoglycemia mothers and that these results varied by infant sex. Boys are more likely to show a dysmetabolism and are more sensitive to factors contributing to macrosomal development resulting in obesity in later life when born to GDM mothers (234).

F. Endothelial Function and Dysfunction in the GDM Placenta

A sequence of several mechanisms are responsible for abnormal development of the placental vascular system and promoting endothelial dysfunction, which will be discussed in the next chapter regarding the factors hyperglycemia, hyperinsulinemia, and further hypoxia (1, 194). Growth factors, oxygen, and cytokines control vasculogenesis, angiogenesis, and the development of the placenta as a whole. With the formation of a vessel network comes the development of the inner lining of the placental vascular systems, which is the endothelium composed of endothelial cells (238). Unconventionally the oxygenated and nutrient enriched blood are carried in the veins of the fetoplacental endothelium, and consequently the blood coming from the fetus which is low in oxygen runs through the arteries. Macro- and micronutrients are transport to the fetus and waste products from the fetus are carried out by the endothelium. The fetoplacental blood flow relies on the control of the vasotone by vasoregulators and synthesis of endothelial nitric oxide (NOs), which are expressed and regulated by the fetoplacental endothelium (239).

The fetoplacental endothelium is an extension of the fetal endothelium and therefore subject to the same hormonal and metabolic control. There are no direct impacts from the maternal hormones, but there are alterations to fetal metabolism, hormone levels, growth factors, and cytokines when the nourishment is commutated through hypo- and hyperglycemia, inflammatory mediators, and oxygen. In GMD pregnancies, these factors add to placental dysregulation in function and changes in vascular development, as well as influencing fetal growth (239).

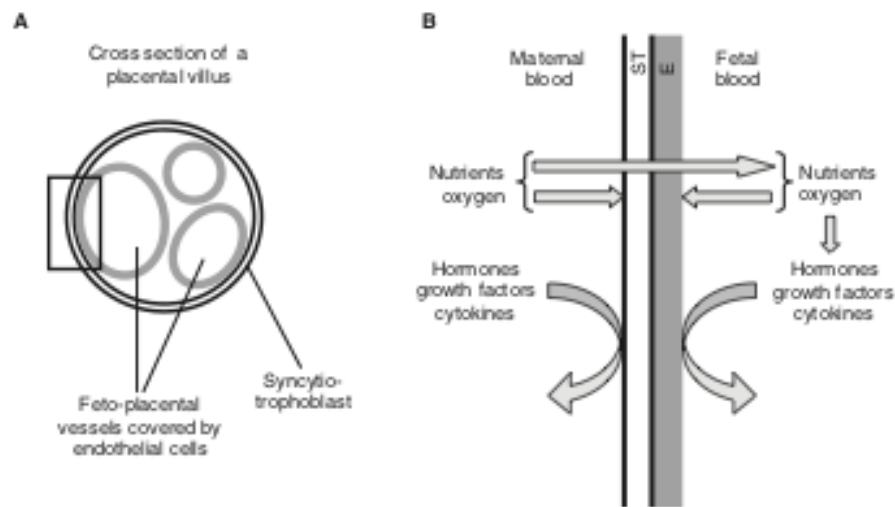


Figure 8: Placental villus cross section and fetal-maternal nutritional and gas exchange.

- A) *Placental villus cross section. The syncytiotrophoblast makes up the outer layer around the villus stroma in which the fetoplacental vessel is located. The site where direct contact between the fetoplacental endothelium and the base membrane syncytiotrophoblast is established is the vasculosyncytial membrane, shown in the rectangle.*
- B) *Presentation of fetal-maternal nutritional and gas exchange. The fetoplacental endothelium and levels of hormones, growth factors, and cytokines may be directly affected by the nutrients and oxygen that are transported from the maternal circulation into the fetal circulation. This possible alteration of the fetoplacental endothelium may be responsible for further placental changes such as proliferation and vascularization. Consequently, fetal growth is altered. (239) (picture from Wadsack et al. (239))*

The main functions of the endothelium are to control vascular tone, conserve the barrier function and play a role in angiogenesis (forming new blood vessels). It needs to enlarge exponentially to meet the demands of the growing fetus for sufficient nutrient and oxygen supply. Paracrine regulation of the placenta and endocrine control of the fetal circulation for growth and angiogenesis are the result of a number of growth factors (240). These include VEGFA, FGF-2, and ILGF2, but there are others that have not been as thoroughly studied. Little is known about how the placental vascular tone is regulated or about the permeability of the endothelium. Vascular dysfunction, as well as endothelial changes, are associated with long term effects of hyperglycemia and correlate with conditions such as inflammation, hypoxia, and hypertension (239). In recent studies, dysfunctions of the fetoplacental endothelium have been closely associated with alterations in the vascular function in GDM pregnancies and fetal growth restriction (FGR) (239).

1. GDM effects on the fetoplacental endothelium

The result of endothelial dysfunction in GDM pregnancies is the alteration of the placental macro- and microvascular reactivity (2). Maternal and fetal hyperglycemia is one of the main causes of endothelial dysfunction by impairing its function. This results in macro- and microangiopathies that cause further pathologies (241). Nevertheless, the correlating consequences of hyperinsulinemia and hyperleptinemia cannot be disregarded in this matter (242, 243).

One of the effects that increased fetal insulin levels has during a GDM pregnancy is the stimulation of adipocyte growth and fat deposition due to the promotion of lipogenesis. As a final result, this leads to an increase in fetal fat (244). Adipocyte-derived leptin and insulin subsidize to endothelial dysfunction (245). Insulin promotes an increase in the oxidative metabolism of the fetus, which contributes to fetal hypoxia along with altered maternal and fetal blood flow. In GDM, the ratio of the oxygen affinity glycosylate hemoglobin is elevated and contributing to lower oxygen supply and hypoxia (145). Consequently, the demand and supply for oxygen are out of balance and lead to a diminished capacity of villous diffusion enhanced by the thickening of the fetoplacental basement membrane (145, 246). In GDM pregnancies this lack of exchange applies particularly to oxygen (246, 247).

Even though GDM effects the maternal and fetal metabolism for only a short period of time, it has a large impact on the fetoplacental vasculature and endothelial function, as seen in the morphological changes (248). Most changes in endothelial function have been detected in the umbilical vein endothelium and placental microvascular endothelium, rather than the macrovascular endothelium due to the higher expression of homeobox genes such as HLX1, TLX1, and TLX2. (2, 3) An increase in the branching of the vascular development of the placenta imply an enhancement in angiogenesis is mainly seen.(57) The proangiogenic force of hyperinsulinemia, hyperglycemia or hyperleptinemia as well as the hypoxia inducing growth factors such as VEGFA and FGF2 all result in an increase in capillary volume(247, 249) as the fetoplacental vascular tree is expanded. (110, 250)

2. Endothelial dysfunction and oxidative stress in the GDM placenta and endothelium

The placenta lacks innervation(251) and is therefore dependent on the release of locally released vasoactive molecules. Two of the many vasoactive molecules are the endogenous nucleoside adenosine and free radical nitric oxygen (NO).(252) When the adenosine metabolism is altered, it leads to an increase in NO synthesis. Elevation of NO leads to a downregulation of gene expression coding for nucleoside membrane transporters such as isoforms 1 (hENT1) and 2 (hENT2). This system promotes extracellular adenosine and activation of its receptors, which leads back to an increase in the synthesis of NO.(2)

NO is a free radical and an elementary diatomic gas with several biological effects, including mediation of vasodilation which is key in the development of the placental vascular development (192, 253). In normal pregnancies, the substrate for NO synthesis is the cationic amino acid L-arginine, which is catalyzed to L-citrulline via NO synthases. In GDM pregnancies this function of the endothelium is disrupted because the transport of L-arginine and NO synthase (eNOS) is altered (254-256). A significant increase in eNOS expression and activity in the HUVEC has been detected in isolated GDM placentas (257). Furthermore, no physiological inducible NO synthase has been recorded in GDM placentas (258). The proposed idea is that NO synthesis is increased in the placental veins, arteries, and HUVEC in GMD pregnancies due to higher NO expression and enhanced activity (218, 259-261). Studies show that fetal vascular dysfunction in GDM pregnancies is associated with dysfunctional NO-synthesis, uptake of L-arginine (257, 261), and decreased NO bioavailability to the endothelium and vascular smooth muscle in the placental circulation (252, 255). The increase of NO synthesis seems to lead to a reduction in endothelium dependent vasodilation (262, 263). This is due to the lack of NO bioavailability as a consequence of inactivation by reactive oxygen species (ROS) (264, 265).

Oxidative stress may be the result of a disturbed balance between the placental ROS production and antioxidant defense (192). The markers 8-isoprostame are measured and account for oxidative stress along with the activities

of the antioxidant enzymes superoxide dismutase and glutathione peroxidase (266). 8-isoprostane has been observed inducing vasoconstriction in the placenta. It derives from free-radical-catalyzed peroxidation of arachidonic acid. It can therefore be assumed that increases in ROS production and lipid peroxidation contribute to the changes in physiology of the placental vasculature (267). The placenta's response to oxidative stress may be caused by an increase in the expression of antioxidant enzymes as a way of adapting. The upregulation of glutathione reductase and catalase is seen in GDM placentas in comparison to healthy placentae (268, 269). If the changes are not sufficient, inflammatory conditions can arise (269).

3. The role of adenosine and adenosine receptors and nucleoside transporters in the placental endothelium in GDM.

a) *Adenosine*

Adenosine is an endogenous purine nucleoside and plays a key role in stimulating angiogenesis (270) as well as having an anti-inflammatory role (270). Synthesis of adenosine takes place both intracellularly and extracellularly at the endothelial cells plasma membrane and HUVEV by dephosphorylating AMP (271-273). Activation of adenosine receptors in different tissues and cell types contributes to these physical responses. They are members of the G-protein-coupled-receptors superfamily (271, 274, 275). Other biological actions of adenosine include homeostasis of the ATP metabolism (modulation of energy), triggering cell signal transduction mechanisms mediated by cAMP (271, 274), and activating the endothelial l-arginine/NO signaling pathway (2, 254, 255, 274, 276). Four subtypes of adenosine receptors have been identified. They are part of the P1 family and all isoform receptors, which are A2 adenosine receptor (A1AR), A2AAR, A2BAR and A3AR, are found in the placental endothelium (277, 278) and trophoblast cells (279, 280). A1AR, A2AAR, and A3AR are only found in the placental fibroblasts which demonstrates different biological effects depending on the expression location (271, 274). Adenosine also contributes to the control of vascular tone and blood flow in vascular tissue (270, 274, 275, 281). So far it has been found that low partial

pressure of oxygen activates A1AR and increases the intracellular calcium (Ca^{2+}) concentration, which enhances NO synthase (NOS) activity and NO synthesis. The activation of A2AAR is assumed to induce vasodilation NO-dependently based off studies using rat aortic endothelial cells (282). The variety of human placental macro- and micro-vascular endothelium function are in relation with the differential expression of adenosine receptor subtypes (252).

b) *Placental endothelium and nucleoside transporters*

Nucleoside transporters can be divided into two families of mammalian cells. The Na^{+} -dependent equilibrative nucleoside-transporters (ENTs) and the Na^{+} -dependent concentrative nucleoside transporters (CNTs) (2, 281, 283). The most important members of the ENT family solute carriers act as membrane transporters in the placental vasculature, which are hENT1 and hENT2. The uptake of nucleobases hypoxanthine and anticancer nucleosides, such as zidovudine by HENT2 (283-285), is crucial for the metabolic maintenance of normal extracellular adenosine levels through HUVEC and hPMEC. This modulates its broad biological effects. (270, 274, 275). The main mediator for adenosine transport in HUVEC is hENT1, whereas the rest are transported via HENT2. They are also expressed in hPMEC and have a similar contribution to the transport of adenosine (277, 286).

c) *Placental endothelium in GDM adenosine receptor (2)*

NO-dependent vasodilation is caused by adenosine and the activation of adenosine receptors (252, 257, 275, 282, 287, 288). This may be the reason for increased NO synthesis (289), more specifically the expression auf A2AAR in HUVEV from GMD (261, 287). In the placental vasculature, the correlation between adenosine and arginine/NO signaling pathway has been introduced as the ALANO pathway (i.e. Adenosine/l-arginine/nitric oxide) (257, 261). It is suggested that extracellular adenosine activates its receptors in GDM (290).

New discoveries in characteristics of ALANO pathway activation are considered necessary for a better understanding of hyperglycemia in GDM contribution to endothelial dysfunction. The human cationic amino acid transporter 1 (hCAT-1) expression is increased by the activation of ALANO-pathway. In addition this triggers NO-synthesis in HUVEC from GDM via eNOS and enhances transport

of L-arginine (276). Recently studies have shown different demands for molecules such as modulating L-arginine/ signaling pathway from the responding adenosine receptors (291). Changes seen in HUVEC and hPMEC cannot be assumed to be corresponding to changes in the human umbilical artery or chorionic vessels endothelium since various placental endothelial cells react differently to different molecules (164, 252).

The adenosine concentration in the umbilical vein blood in GMD pregnancies is elevated when compared to normal pregnancies. The reduction in adenosine uptake by the endothelial cell causes it to accumulate extracellularly and the ALANO signaling pathway is stimulated (257, 261, 286, 287, 292). A possible hypothesis can be stated from observing similar findings in the HUVEC from GDM pregnancies, which suggests that the blood flux will be altered in the placenta. This is a result of adapting to meet the demands of the growing fetus. A small number of studies using the Doppler technique found that the blood flux velocity is unchanged in the umbilical vessels in GMD (293-295). Follow up research implicates that the reactivity of the human umbilical vessels are unchanged. Nevertheless, modification of the A2AAR activation by extracellular adenosine is suggested to be the reason for the lowered basal tone in the human umbilical vein rings examined in GDM pregnancies (287). Furthermore, the effect of other vasodilators such as insulin are dependent on the on these subtypes of adenosine receptors in the normal placental vascular function (296).

d) *Effects of adenosine receptor changes on the fetus*

Strikingly, children born from GDM pregnancies are more likely to suffer from brain damage. Adenosine is correlated to modulation of the human brain function. (297) A fetus in GDM has higher chances of prospering brain damage in the first half of the pregnancy and developing long-term cognitive ramifications (298). The accumulation of adenosine in the fetal brain circulation is considered a crucial factor that causes functional change in this organ in GDM (2). This is due to the increase of adenosine supply for the fetus (287), because of the downregulation of hENT1 and hENT2 expression, caused by the activation of A1AAR and A2AAR receptors leading to a dominant presence of adenosine receptors (286).

e) Nucleoside transporters and placental endothelium in GDM (2)

In addition to elevations of adenosine concentrations in the human umbilical plasma, (254, 286) a correlation between GDM and the increase of extracellular adenosine concentration is seen in HUVEC and mPMEC due to diminished transport of this nucleoside (261). Lower hENT1 transport activity capacity and expression but unchanged stability of hENT1 regarding the protein and mRNA, results in GDM-associated adenosine transport reduction (261). Downregulation of hENT1 and hENT2 transport activity is reduced in hPMEC, however only hENT1 is downregulated in HUVEC in GDM. This clarifies why in vitro hindrance of hENT1 and hENT2 via nitrobenzylthioinosine (NBTI) mediation induces accumulation of extracellular adenosine in normal cell cultures as seen in GDM, whereas there are no additional adjustments in cells from GDM (261, 283, 287). The relocation of the membrane transporter hENT1 to the intracellular perinuclear regions reduces its presence in the plasma membrane of HUVEC (299). This mechanism causes a higher recycling of this protein and also contributes to a decrease in transport of adenosine in the fetal endothelium shown in Figure 8.

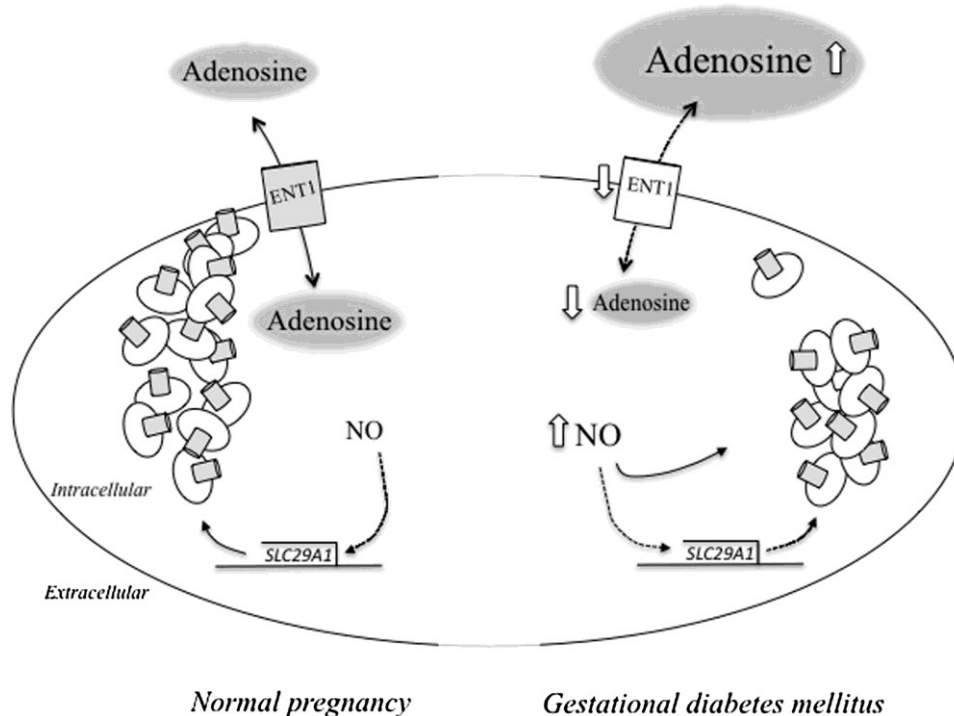


Figure 9: Altered adenosine transport in GDM

In the endothelial cell in GDM: the recycling of hENT1 contributes to a decrease in the transport of adenosine to the membrane, resulting in a decreased uptake of adenosine. Consequently, there is a lower concentration of adenosine intracellularly and increased extracellular concentration. Elevated NO synthesis

reduces SLC29A1 gene promoter for hENT1. In a healthy placenta the expression of the SLC29A1 gene codes for ENT1 and establishes its synthesis and normal subcellular distribution targeting the plasma membrane. The basal level of NO has a restorative influence on adjusting ENT1 activity synthesis downwards.(299) (Picture from Pardo et. al(2):

In the healthy placenta, the expression of the SLC29A1 gene codes for ENT1 and establishes its synthesis and normal subcellular distribution targeting the plasma membrane. It has been shown in studies that NO inhibits SLC29A1, which is crucial for understanding why extracellular adenosine concentration found in culture medium of HUVEC from GDM is elevated (299), as well as how the umbilical plasma adenosine concentration in the fetuses is increased (286, 287). It is possible that the adenosine metabolism and uptake has no correlation with the fetuses' body but rather takes place in the placental circulation (164, 256). This is supported by studies that have shown an increase in adenosine plasma concentration in the umbilical vein but not the arteries of GDM and findings that show a shortage of hENT1 and hENT2 expression and activity in hPMEC, even though no evidence has been found so far on other possible factors involved in this mechanism (164, 256, 257, 286).

Insulin has been reported to reduce hENT1 and hENT2 expression and activity in HUVEC of normal pregnancies (289). In the GDM placenta, insulin can reverse the reduction of hENT1 expression as well as restore SLC29A1 promotor activity (287). The effectiveness of insulin is only seen in hENT2 and SLC29A2, but not in terms of protein abundance and activity of hENT1 or promotor activity in hPMEC for SLC29A1 (286). There is also a proposal for insulin isoforms A and B in HUVEC (287) and hPMEC (286) being expressed differently in GDM. In fetal endothelial cells a similar mitogenic phenotype seems to be preferred when compared to the same cells in normal pregnancy. The insulin effect on hENT1 and hENT2 as seen in normal pregnancies is crucial for understanding its effect and role in these same cells in GDM. Macro and microvascular placental endothelial cell function may be influenced by the activation of adenosine receptors due to the increase in extracellular concentration of this nucleoside, which alters the role of insulin (254, 256, 296).

IV. Discussion

The purpose of this thesis is to review how placental angiogenesis is altered in gestational diabetes. It addresses the most influential factors on the outcomes, as well as defining and categorizing which changes in the maternal metabolism alter the placenta. It explains the different outcomes of studies on the topic and summarizes the key alterations in consideration to the mother, placenta, and outcome of the fetus. Although a wide range of papers have been written on this topic, most only focus on a few specific factors that cause pathologies in GDM. A thorough background of the vascular development of the placenta and the influence of GDM is summarized in this thesis and should give a strong overview of the topic.

The summary of the findings of this thesis on how gestational diabetes alters placental vascularization includes a wide range of influential factors. The placenta, the mother, and the fetus in GDM compensate and adapt to this new metabolic situation of hyperglycemia, hyperinsulinemia, hyperlipidaemia, and endothelial dysfunction in different ways with various consequences (2). Maternal changes, metabolically and clinically, are the result of pathological alterations in a series of mechanistic alternations in GDM. The outcome of the fetus and its development, perinatal and postnatal, are to be considered substantially.

The placenta is a very unique organ that is temporarily developed and only functions during pregnancy. It serves as a barrier between the mother and fetus (3), while also acting as a connector. The exchange of substances in the placenta between mother and child is dependent on diffusion, facilitated diffusion, diapedesis, active transport, and pinocytosis/endocytosis, which are all processes dependent on partial pressure differences, transporter proteins, functional membrane pores and trophoblasts, osmotic pressure, and ATP-dependent active transport, respectively (3, 4, 8, 14-16). Another key role of the placenta is its endocrine function and its ability to synthesize hormones and proteins (5). It supports the growing fetus by establishing its nutritional supply and shielding it from harmful quantities of substances. Any imbalance or malfunction will trigger a series of alterations in the angiogenic development, endothelial function, and substance balance in the placenta, consequently leading to pathological changes in the fetus and mother.

Gestational diabetes during pregnancy is one of the diseases that contributes to many different pathological changes in the mother, fetus, and placenta. The definition of GDM is the first-time development of a carbohydrate intolerance resulting in hyperglycemia in women during pregnancy in weeks 24-28 (19, 20). The insulin resistance develops near the second half of the pregnancy and shows clinical symptoms such as thirst, polyuria, and weight lost (21, 23). To diagnose GDM, a standardized 2-hour 75g-oral-glucose tolerance test (oGTT) is performed during weeks 24-28 of pregnancy (24). Risk factors for mothers to develop GDM include advanced age during the pregnancy, obesity, high parity, previous delivery of macrosomia infant, family history of type 2 DM, short stature, polycystic ovary disease, high level of saturated fat in the diet, prior gestational diabetes, prior neonatal death, prior cesarean delivery, previous stillbirth or congenital malformations, high blood pressure during pregnancy, and multiple pregnancies (28). Management of GDM is assessed with a medical nutritional restriction and physical activity as far as possible and complemented with pharmacological control in the form of insulin and oral antidiabetic agents. Special obstetrical care is called in for GDM deliveries (21, 24, 27).

Maternal adaptations in GDM lead to an elevation of several hormones such HPL, glucocorticoids and progesterone, as well as free fatty acids and tumor necrosis factor- α . This triggers the development of an insulin resistance in the second part of the pregnancy. Morbidities associated with GDM are risk of preeclampsia, eclampsia, macrosomia, caesarian section, traumatic labor, instrumental delivery, birth injury, shoulder dystocia, postnatal uterus atony, postpartum loss of blood, a require for transfusion, early delivery, perineal tear, DM type 2 development post pregnancy, GDM in following pregnancies, urinary and genital tract infection, soor colpitis, and rentinopathy (28, 29, 35).

The vascular development of placenta is generally accepted as vasculogenesis and angiogenesis, with one following the other. A series of mechanisms and control factors contribute to the establishment of the vessel system (47). The fetoplacental endothelium is a key source of angiogenic factors from the neighboring cells such as trophoblasts, Hofbauer cells, pericytes, endothelial cells and smooth muscle cells (1, 9). The key modulators are VEGF, FGF-2, PlGF, PPAR- γ (61-63), EPO, LEP, ADIPOQ, and INS/IGF regulated by the endothelium.

The highest levels of receptor expression were VEGF, angiopoietin and adiponectin (1). Physiologically, hypoxia is also a regulator of capillary and vascular structure. It influences the transcription via HIF (hypoxia-inducible factor) in angiogenic factors and alters mRNA transcript levels (59).

Needless to say, a diabetic milieu as such seen in GDM alters the physiological interplay of all factors contributing to the normal placental development and its vascularization and supply for the fetus as it grows. Glycemic control might have an effect on many risk factors for metabolic or obstetric complications for the mother and fetus, however histological changes are seen in diabetic placentas compared to nondiabetic placentas despite management of GDM (145, 165, 166).

The macroscopic changes seen in the placenta are proportional to the degree of hyperglycemia and reflected in its increase in size, weight, thickness, and plethoric appearance. A correlation between a decrease in the placental and fetal weight ratio suggests that the placenta grows initially and then contributes to a higher glucose transfer to the fetus, hastening its growth (3, 161, 162, 167-169).

On a microscopic level the GDM placenta shows several lesions, which are precursors for altered nutrient exchange or oxygen delivery with consequences for the fetus. Formation of the terminal villi are reduced, as well as villous stroma being replaced by fibrinous material resulting in villous fibrinous necrosis (171, 172). Chronic ischemic conditions in GDM lead to an increase in villous immaturity, maturation of branching villi, and elevated numbers of fetal nucleated red blood cells. Increased vascular recruitment in the enlarged placenta leads to chorangiosis and vascular hyperplasia of the chorionic villi. The risk for placental infarction is therefore elevated (145, 158, 162). Furthermore, histological analysis shows changes in syncytiotrophoblasts with fibrin deposits, villous edema and marked hyperplastic cytotrophoblasts (158).

In consideration of these many changes in the maternal and fetal metabolism and the functional, microscopic, and macroscopic changes in the placenta, we can take a closer look at the effects of GDM in the angiogenic development and proangiogenic factors in the placenta and the fetus. We know that the onset of GDM is in the second half of the pregnancy or it is most likely to be diagnosed during this time frame. It is hard to say if the disease has any precursor effects earlier, nevertheless focusing on later stages of pregnancies puts an emphasis on

angiogenesis and microvascular remodeling rather than vasculogenesis in the vascular development of the placenta (1).

It is debatable that pre-gestational diabetes will have a different effect on the vascular development than GDM, however the results are conflicting. Pre-gestational diabetes is known for changes occurring in the first trimester resulting in reduced placental vascularization. In the GDM placenta the same type of abnormalities as seen T1D patients can be observed. The branching of the villi is enhanced and the capillary surface area is increased. In addition, the GDM placentas have longer umbilical cords (1, 57, 176, 178). The stem villi and free villi both have an increase in capillary structure of various sizes. The syncytiotrophoblast cell layer shows many vacuolar formations, but reduced collagen fibers in the villus stroma while the entire villus is covered with vascular formations. Overall in the GMD placenta, there is a tendency for hypervascularization and changes in endothelial resistance are reported (1).

The proangiogenic factors in the placenta and fetus make a great contribution to the hypervascularization of the placenta. One crucial influence is hypoxia. The metabolic and hormonal disorder caused by fetal hyperglycemia and hyperinsulinemia increases the fetal demand for oxygen. In GDM the physiological effect and level of hypoxia are exceeded, and the fetus can often be challenged by chronic hypoxia (149, 161, 183). The activating nature of hypoxia on proangiogenic factors and both fetal and placental expression of vascular growth factors stimulate placental vasculogenesis and angiogenesis (65). It might be the cause for elevated levels of VEGF, PlGF, FGF2, EPO, ANG2 and leptin, which are all sensitive to hypoxia. Consequently, this means an increase in the expression of regulators of IRES or HREs. The overstimulation in the expression and translation of FGF2 though the promoter is seen in its elevated levels in the placental and fetal cord blood (98, 101, 146). VEGF expression in GDM is lowered towards the end of pregnancy resulting from an increase in the expression of VEGFR1 (Flt1) and VEGFR2 (Kdr). The angiopoietin actions are altered via hypoxia, which regulates ANG3 transcriptionally and increases the ANG2 mRNA levels effected by vessel maturation and maintenance (1, 148, 187). The hormone EPO shows elevated levels in the diabetic placenta towards term expressed in the placental endothelial cells and fetoplacental vessels. Hypothetically, EPO can promote placental angiogenesis by upregulating the expression of VEGFR in the fetus and the

placenta even though VEGF levels stay the same or are even lower in maternal diabetes. There has been no proof in humans, which would make further research on the effect of EPO in proangiogenic factors very interesting (1, 144, 188).

One of the major issues of GDM is creating an inflammatory situation in the placenta. Leptin expression is increased and stimulates monocytes, producing TNF- α and interleukin-6 (IL-6) to further increase the secretion of CC-chemokine ligands which all leads to an inflammatory environment. The presence of leptin from adipocytes in fetal adipose tissue promotes insulin secretion, glucose utilization, glycogen synthesis, and fatty acid metabolism by increasing insulin sensitivity (190, 191). The derangement of cytokines due to hyperglycemia is another factor contributing to the proinflammatory environment which further promotes changes in the placental vascular development and causes oxidative stress. This again correlates with TNF- α and IL-6 (1, 192, 194).

The elevated expression of placental cytokines in GMD contributes to an alteration of nutritional transport in the placenta (3). The enhancement of genes participating in the lipid pathway contribute to the increase in fetal fat accumulation. An increase in GLUT1 leads to increased placental glucose transport causing hyperglycemia (207-210, 212, 213). In combination with the higher placental weights, this contributes to the accumulation of fetal fat. The increased levels of leptin, insulin, and glucose are associated with a stimulation of system A transporters for amino acid activity (201-203). An increase of system L amino acid transporter correlates with pregnancies resulting in LGA fetuses, leading to the assumption that enhanced placental amino acid transport also results in overgrowth of the fetus (211).

The sequence of several mechanisms responsible for an abnormal development of the placental vascular system promotes endothelial dysfunction. The fetoplacental endothelium is an extension of the fetal endothelium and therefore subject to the same hormonal and metabolic conditions (239). Hyperglycemia impairs its function and hinders macro and microvascular reactivity resulting in pathologies. Adipocyte-derived leptin and insulin subsidize to endothelial dysfunction (244, 245). Hyperinsulinemia increases the oxidative metabolism of the fetus which leads to a diminished capacity of villous diffusion and results in the thickening of the fetoplacental basement membrane (145, 246). The most important

result of these mechanisms is that the placenta can no longer adequately meet its demands for exchanging substances, which particularly affects oxygen (246, 247).

In GDM pregnancies the function of the endothelium is disrupted because the transport of L-arginine and NO synthase is altered (NOs) (254-256). NO has several important biological effects for which vasodilation is key during the normal placental vascular development. It correlates with the vasoactive endogenous nucleoside adenosine. When adenosine metabolism is altered it leads to an increase of NO synthesis in the placental veins, arteries, and HUVECs in GDM pregnancies due to higher NO expression and enhanced activity. (2, 192, 252, 253) Reactive oxygen species (ROS) lower the NO bioavailability to the endothelium and vascular smooth muscle in the placental circulation. and contribute to a fetal vascular dysfunction due to reduction of endothelium dependent vasodilation (252, 255, 262, 263). An imbalance between the placental ROS production and the antioxidant response leads to oxidative stress. The placenta reacts with an increase in the expression of antioxidant enzymes as a way of adapting. This further contributes to the development of an inflammatory condition (192, 268, 269).

The summary appraisal of this thesis should be focused on the meaning of GDM regarding the consequences for the fetus. Maternal hyperglycemia leads to an increased amount of glucose supply to the fetal metabolism, which leads to its compensational reaction of raising the insulin production. Unlike glucose, insulin cannot pass the placental barrier and causes the fetus to develop hyperinsulinemia. This endangers the fetus for developing hypoglycemia post-delivery (23). The longer the fetus is exposed to an uncontrolled diabetic situation, the higher the risk for developing an embryopathy (23). Although the maternal hormones do not directly impact the fetus, its metabolism is altered. Hypo- and hyperglycemia, ketoacidosis, inflammatory mediators, and hypoxia change the hormone levels, gene expression, free oxygen radical, growth factors, cellular damage, increased teratogenesis and cytokines in the fetal metabolism and lead to abnormal embryonic development (10, 39, 239). Fetal macrosomia is one of the main pathological outcomes from GDM pregnancies. Among many complications, it endangers the mother and child during delivery. Fetal hyperglycemia induces β -cells from the fetal pancreas to synthesize insulin which allows glucose from the maternal blood to be incorporated into the fetal cell once it reaches the fetus. Another complication is postpartum hypoglycemia (36). Further asset to macrosomal growth is leptin. As we

know, the fetus suffers from hyperinsulinemia, which stimulates fetal plasma leptin due to the growth of adipose tissue. Fetal leptin correlates with fetal insulin. It is assumed that fetal insulin stimulates fetal adipocyte leptin production (191). This is a dangerous loop that contributes to excess fat deposits in the fetus (190, 191). Presenting secondary to hyperglycemia is fetal polyuria, which is a cause of polyhydramnions. This presents the danger of initiating premature contractions, rupture of the membrane, and preterm delivery (23).

It is still unknown if any vascular changes occur in the fetus in GDM like they do in the placenta (1). However, the fetus exposed to GDM is consequently impacted by the changes in vascular development in many ways. It is at higher risk for cardiovascular complications, caudal regression syndrome, and retinopathy due to changes in the vessels. Eriksson et al. have shown a parallel between the exposure to perinatal hyperglycemia and inveterate abnormalities (1, 182). For the fetus, these changes mean a sudden shortage or excessive exchange of gas and nutrients. The growing distance of intervillous diffusion of the immature villi paired with the enlarged placenta is potentially very dangerous due to perfusion disparity (158). The longer growth of umbilical cords places a risk for the fetus in danger of hyper- or hypocoiled cords (177, 178).

The elevated levels of adenosine supply to the fetus as seen in GDM is one of the potential risk factors for fetal brain damage (286, 287). Potentially adenosine accumulates in the circulation of the fetal brain. Extracellular hording of this nucleoside, paired with its relative expression and activation of its receptors, and hENT1 and hENT2 activity and expression, could alter the function and harmfully effect of this organ (2). There is a need to better understand the further effects of potentially elevated circulating adenosine plasma concentrations in umbilical blood on the effects on fetal growth and development or diseases associated (277, 300).

A very interesting aspect in the prediction of outcome and value of risk factors is the sex of the fetus (40, 41). In association with GDM, the male fetus has a much higher risk for delivery via caesarean section. Boys are more vulnerable to neonatal hypoglycemia and have a higher risk for respiratory disorders and major malformations (40, 44, 45). Further study on the reasons as to why there is such difference of outcome between the sexes could be very interesting to understand the effects of GDM and its pathologies. Why are girls less responsive to the the glycemic environment? Consideration of fetal adaptive capacities due to the access

of different hormone levels or other factors in male and female offspring could give further explanation.

This thesis was bound by some limitation. It was not possible to include all available literature. To focus on the information relevant to the topic of this thesis, some of the information from older literature was no longer applicable, although thoroughly researched. During the research for this thesis a problematic issue was the number of papers published from different authors quoting one another. It seemed that information was passed on and reused, which made it difficult to distinguish between actual facts and assumptions. This was obviated by trying to identify the original papers and to filter out any misinterpretation. Another problem was the difficulty of comparing results from studies from many different decades was the limited material and methods of testing for results. For one, the management of diabetes has improved considerably to the present day, which changes the outcomes of alterations seen in the placenta, mother, and fetus. Secondly, there are limitations for obtaining probes from GDM placentas with the problem of knowing its actual onset and difference to potentially pre-existing T1D or pre-gestational diabetes. Different outcomes from different studies may have been due to the method for investigation, different management availability, limitations of material, or knowledge at the time of study. Obviously the placental, fetal and maternal development evolved over the last three trimesters as well as being specific to each individual patient. Pregnancies are highly individual and researching metabolic changes due to GDM makes it hard to limit to one period of time or organ when examining the literature. This may have been the reason for controversial results from different studies.

In the future, the research field placenta and its development regarding GDM influence can be expanded greatly. It is predictable that the GDM incident will continuously rise in the future correlating with maternal obesity. In association with an increase of the BMI is an inflammatory situation resulting in elevated cytokine levels which trigger changes in the placenta, mother and the fetus. It should be interesting to investigate how obesity in combination with GDM and inflammation will take influence. One of the results are the altered amino acid and lipid transport due to hyperglycemia. Investigation of the transplacental transport of individual amino acids and further diverse lipids needs to be done in the normal placenta compared to the GDM placenta. The most interesting question is to what extend is

the intrauterine processes alter the outcome for the offspring and how gender differences contribute to different placental gene expression and function (1, 3).

How can HbA1c levels act as an indicator for fetal outcome and GDM development? Is there a difference between LGA groups and AGA groups? Does birth weight prediction correlate with the change of HbA1c between the first and second trimester? How does the alteration in placental lipid mechanism change the vascular development and outcome for the fetus? What is its mechanism? Is placental expression of vasoactive factors or the placental vasoactivity itself altered in addition to eNOS? Does this effect fetal outcome and could restoration of placental blood flow and vascularity decrease the risk for fetal malformations? Research should be conducted on drugs affecting maternal and fetal placental blood flow and circulation and further neonatal outcome.

The changes in placental vascular development in GDM have not yet been directly seen in fetus although assumed due to measurements of proangiogenic factors in the cord blood. The fetal compensation towards hyperglycemia resulting in hyperinsulinemia triggers further metabolic changes such as oxidative stress and alterations in the adenosine transport and increased NO synthesis. The potential influence of GDM and hypoxia on membrane transporters and endothelial dysfunction are substantial in the fetal development and still unknown to a great extent. Deepening in this complex subject matter of placental function altered in auto or paracrine regulatory mechanisms, could give insight as to how adenosine modulation and placental cell types alteration take influence in changing and placental vascular development and consequently endanger the fetus in GDM for adverse outcome (2).

In order to conduct better research on GDM, a disease that includes a highly individually developing and ever changing pregnancy, a standardized protocol of criteria is needed. The placenta is a temporary organ which makes the window of possible research short. Changes in GDM are influenced by various factors including management and control of harmful glucose levels, which need to be taken under consideration or masked out to put a focus on the entire range of metabolic changes in the mother and the fetus.

The subject of this thesis provides a better understanding of the potential risk factors of GDM for the mother and their offspring. It should give interest in understanding mechanism associated with alterations in the placental vascular

function and of all the placenta cell types concerning GDM for an improved therapeutic approach. This could help manage GDM for better outcomes in the fetus and mothers of GDM.

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