First trimester screening, forecasting adverse pregnancy outcomes

PhD thesis

Dóra Gerszi MD

Doctoral School of Basic and Translational Medicine
Semmelweis University

Supervisors: Szabolcs Várbíró, MD, DSc
Mária Eszter Horváth, MD, PhD

Official reviewers: Attila Keszthelyi, MD, PhD
László Tarnai, MD, PhD

Head of the Complex Examination Committee:
György Losonczy, MD, DSc
Members of the Complex Examination Committee:
Andrea Székely, MD, DSc
Ákos Jobbágy, MSc, DSc

Budapest
2023

TABLE OF CONTENTS

LIST OF ABBREVIATIONS 4

1. INTRODUCTION 6

1.1. Placental insufficiency - related pregnancy complications/pathologies 6

1.1.1. Intrauterine growth restriction (IUGR) 6

1.1.2. Preeclampsia 7

1.1.3. Gestational diabetes mellitus (GDM) 8

1.2. Pregnancy complications and pathologies – possibilities of early screening 9

1.2.1. Screening for intrauterine growth restriction (IUGR) 9

1.2.2. Screening for preeclampsia 10

1.2.3. Possible markers for early detection of GDM 10

1.3. The role of oxidative-nitrative stress 12

1.4. The role of steroids 14

1.4.1. Steroid metabolism and the placenta 14

1.4.2. Glucose and steroids metabolism 15

2. OBJECTIVES 17

3. METHODS 18

3.1. Measuring oxidative stress and pulsatility of the uterine artery in early phase pregnancies 18

3.1.1. Patient inclusion criteria 18

3.1.2. Study protocol 18

3.1.3. Measuring oxidative-nitrative stress markers 19

3.1.4. Statistical analysis 20

3.2. Prediction of gestational diabetes mellitus in the first trimester 20

3.2.1. Patient history, sampling 20

3.2.2. Current study 21

3.2.3. Groups 21

3.2.4. Determination of oxidative-nitrative stress related parameters 21

3.2.5. Measuring steroid levels 22

3.2.6. Statistical analysis 23

4. RESULTS 24

4.1. Oxidative stress and uterine artery pulsatility index (UtAPI) in early pregnancy 24

4.1.1. Clinical parameters and anthropometric data in low and high UtAPI groups 24

4.1.2. Oxidative-nitrative stress in low and high UtAPI groups 26

4.1.3. Pregnancy outcomes and anthropometric data of newborns in low and high UtAPI groups 27

4.1.4. Correlations between oxidative-nitrative stress, UtAPI and clinical data in the total study cohort 28

4.2. Gestational diabetes mellitus – prediction in the first trimester 30

4.2.1. Patient characteristics in early pregnancy - at the end of the first trimester 30

4.2.2. Glucose, CRP, fructosamine, liver function and creatin kinase 31

4.2.3. Oxidative – nitrative stress parameters and SuPAR 32

4.2.4. Steroid metabolites 33

4.2.5. Correlation analysis of „classical" GDM risk factors and potential new candidates for marking risk 35

4.2.6. Classic risk factors vs proposed new candidates for assesment of risk – findings regarding potential additional predictive value 37

4.2.7. Maternal and neonatal pregnancy outcomes 37

5. DISCUSSION 39

5.1. Uterine artery pulsatility index and oxidative - nitrative stress in early pregnancy 41

5.2. First trimester prediction of GDM 43

5.2.1. First trimester SuPAR and oxidative-nitrative stress markers in GDM 44

5.2.2. The role of glucocorticoids 45

5.2.3. Androgens 46

5.2.4. The role of mineralocorticoids 47

5.2.5. Prediction of later onset GDM 48

5.3. Limitations and strength of our study 48

6. CONCLUSIONS 50

7. SUMMARY 51

8. REFERENCES 52

9. BIBLIOGRAPHY OF PUBLICATIONS 65

10. ACKNOWLEDGEMENTS 67

LIST OF ABBREVIATIONS

AC: Abdominal circumference

ALP: Alkaline phosphatase

ALT: Alanine Transaminase

AST: Aspartate transaminase

BMI: Body mass index

BPD: biparietal diameter

CRP: C-reactive protein

DHEA: Dehydroepiandrosterone

DHEA-S: Dehydroepiandrosterone sulfate

DHT: Dihydrotestosterone

DM: Diabetes mellitus

EFW: Estimated fetal weight

FL: Femur length

GDM: Gestational diabetes mellitus

GGT: Gamma glutamyl transferase

HC: Head circumference

HDL: High density lipoprotein

HRP: Horseradish peroxidase

HSDs: 11beta-hydroxysteroid dehydrogenase

IADPSG: International Association of Diabetes and Pregnancy Study Groups

IUGR: Intrauterine growth restriction

LDH: Lactate dehydrogenase

LDL: Low density lipoprotein

LGA: Large for gestational age

3-NT: 3-nitrotyrosine

OI: Oxidative index

PAI-1: Plasminogen activator inhibitor-1

PAPP-A: Prengnancy-associated plasma protein A

PCOS: Polycystic ovary syndrome

PI: Pulsatility index

PIGF: Placental growth factor

PIPX: (UtAPI/PRX) * 100

PRX: Total plasma peroxide

RI: Resistance index

RNA: Ribonucleic acid

ROS: Reactive oxygen species

S/D ratio: Systolic/diastolic ratio

sFlt-1: Soluble fms-like tyrosine kinase-1

SGA: Small for gestational age

SHBG: Sexual hormon binding globulin

SuPAR: Soluble urokinase plasminogen activator receptors

T: Testosterone

TAC: Total antioxidant capacity

T2DM: Type 2 diabetes mellitus

TNF: Tumor necrosis factor

UA: Uric acid

UtAPI: Uterine Artery Pulsatility Index

1. INTRODUCTION

1.1. Placental insufficiency - related pregnancy complications/pathologies

Placental insufficiency is a defect in the formation or function of the placenta and many diseases can be the result of this condition.

1.1.1. Intrauterine growth restriction (IUGR)

Low birth weight carries importance as it may increase the risk of both neonatal morbidity and mortality (1). Intrauterine growth restriction (IUGR) is classically defined by the weight of the newborn being under the 10th percentile. Abdominal circumference below the 10th percentile threshold for the given gestational week may also be used to define IUGR (2). The smaller the fetus, the greater the risk for morbidity rises from 13 to 39% from the 5 to10 percentile (3).

IUGR may be traced back to fetal, placental and maternal factors (4). The following genetic abnormalities may be grouped as fetal contributing factors: aneuploidy, single gene mutations, partial deletions, or duplications, trisomies. These may be held responsible for 5-20% of all IUGR cases (5). 5-10% of IUGR may be contributed to infections, most commonly cytomegalovirus and toxoplasmosis (5). Congenital and genetic anomalies are often concurrent. Multiparity is also more commonly associated with pregnancy complications that may also play a role in the development of IUGR: preeclampsia, twin-to-twin transfusion syndrome, abnormalities of the placenta and the umbilical cord (4).

Chromosomal mosaicism is a relevant placental factor; in these cases, the chromosomal abnormality is limited to the placenta, and it does not affect the fetus. It is often accompanied by trisomy and demonstrated a strong correlation to IUGR (6). In 10% of idiopathic IUGR chromosomal mosaicism was diagnosed post-partum.

Clinical manifestations of ischemic placental insufficiency are preeclampsia, abruption of the placenta, stillbirth, or a combination of these. Unfortunately, they are likely to recur in following pregnancies.

Arteria umbilicalis singularis, anomalous umbilical cord insertion, marginal cord insertion, bilobe placenta, circumvallate placenta, and placental hemangioma all fall

under the category of umbilical contributing factors. These show a weak correlation with IUGR (5).

Further placental abnormalities include mesenchymal dysplasia of the placenta, which is a significant factor regarding the development of IUGR and perinatal death (4). Maternal disease may play a role in IUGR through their adverse effect on the placenta. (5).

1.1.2. Preeclampsia

Preeclampsia is specific to human pregnancies, and it occurs in around 2-8% of all pregnancies. Worldwide it may be one of the most relevant causes underlying maternal mortality and morbidity. Preeclampsia presents following 20th gestational week (in some cases of trophoblastic abnormalities, or disease, e.g. in hydatiform mole, it may even manifest earlier (7)) and it is resolved at six weeks post-partum (8). Leading clinical symptoms include hypertension (systolic pressure values >140 mmHg and/or diastolic pressure values >90 mmHg in normotensive pregnant women – measurements are to be taken before the 20th week of pregnancy) and proteinuria (values over ≥0.3 g within a 24-hour timeframe).

We differentiate between early (presentation of symptoms before the 34th week of pregnancy) and late (presentation of symptoms after the 34th week of pregnancy) onset preeclampsia – they may differ in terms of underlying cause, however the exact pathomechanism of preeclampsia has not been established yet (9). It is a placental disorder – this has been demonstrated – and it is therefore characterized by an invasion of defective cytotrophoblasts in the spiral arteries. Vessel resistance is also increased, which leads to chronic ischemia and increased oxidative stress levels in the placenta. There is also evidence to support that immunological and genetic factors may be contributing factors to the development of this disease. In this case the immune system of the mother produces an excess number of immunogenic mediators (i.e. tumor necrosis factor-α ((TNF-α)) as it does not recognize the foetoplacental unit as self but rather as foreign. These mediators induce apoptosis of the cytotrophoblast cells (10).

1.1.3. Gestational diabetes mellitus (GDM)

Genetic mutations, hormonal dysregulation of the placenta, more advanced maternal age and damage to the beta cells may all contribute to the development of gestational diabetes mellitus (GDM) (11). The effects of these and other risk factors have been found to be additive. The following contributing factors have been identified in high-risk GDM patients: body mass index (BMI)>30, positive family history regarding the occurrence of diabetes mellitus, higher values for fasting glucose, impaired glucose tolerance, GDM during previous pregnancies (rate of recurrence has been shown to be as high as 40%), polycystic ovary syndrome (PCOS), high maternal age (especially >40 years), high birthweight of previous newborns. Results from research conducted in the United States demonstrated that members of the following populations tend to have higher risk regarding GDM: African American, Native American, Hispanic American, Pacific Islander, South or East Asian (12-19).

Placental and growth hormones such as cortisol, progesterone, TNF-α and lactogen increase insulin resistance in physiological pregnancies: the development and the growth of the fetus is assured by the increased availability of maternal glucose. GDM occurs when increased insulin demand caused by the contra-insular hormones are not able to be balanced out by the rate of insulin production. (20). Usually, GDM develops between gestational weeks 24 and 28, while insulin resistance is usually increased on the 24th week of pregnancy – if the capacity for insulin secretion is not enough to maintain physiological blood glucose levels (21). GDM has been shown to have long term adverse effects regarding the health of both the mother and the fetus. GDM is also associated with an increased risk regarding pregnancy-related hypertension, preeclampsia, high blood pressure during pregnancy, polyhydramnion, premature birth, the need for caesarean section, neonatal morbidity and large for gestational age (LGA) newborns (20). The manifestation of GDM is also linked to an increase regarding the long-term risk for maternal type 2 diabetes mellitus (T2DM) and becoming obese, overweight or developing T2DM for the offspring (20).

Based on worldwide data, the incidence of GDM has been measured to be 1-14% (22). As both patient characteristics (i.e. BMI, maternal age) and diagnostic criteria differ, exact values regarding prevalence of GDM is not known; based on the data and criterion from the International Association of Diabetes and Pregnancy Study Groups (IADPSG-

2010) GDM prevalence is estimated to be 17% worldwide (25% in Southwest-Asia, 10% in North-America) (23, 24). Both obesity and the increase in maternal age contribute to the unfortunate rise in the prevalence of GDM (25).

1.2. Pregnancy complications and pathologies – possibilities of early screening

1.2.1. Screening for intrauterine growth restriction (IUGR)

As both fetal morbidity and mortality increases in case of IUGR, early detection and clinical monitoring is important. Our goal is to identify the fetuses that are high-risk regarding perinatal death, as they may benefit from induction of birth/performing a caesarian section (IUGR in itself is not an absolute indication for performing a caesarean section): observational studies have revealed protocols to identify and treat IUGR decrease the risk of stillbirth (4, 26).

The techniques that allow early detection of IUGR may also be used for monitoring:

- estimation of fetal weight through biometric measurements performed by ultrasound: head circumference (HC), biparietal diameter (BPD), femur length (FL) and abdominal circumference (AC). AC has been found to be the most sensitive biometric value regarding IUGR (especially in cases of disproportionate IUGR) (5).
- Doppler velocimetry: color Doppler examination of the umbilical cord is an effective tool to evaluate the fetus in terms of IUGR, if it is associated with placental dysfunction (4). The most commonly used Doppler indexes are the following for the umbilical arteries (4, 27, 28): the resistance index (RI) RI=(peak systolic velocity – end-diastolic velocity)/peak systolic velocity, the pulsatility index (PI) - PI=(peak systolic velocity - end-diastolic velocity)/time-averaged maximum velocity; and the systolic/diastolic ratio (S/D) – S/D=systolic velocity/end-diastolic velocity. PI is the most effective as it estimates the waveform more accurately than RI or S/D ratios (4, 29).

Recently the number of publications has appeared in relation to early screening for IUGR. The goal of these studies is to be able to effectively predict IUGR risk sometime around the end of the first and the beginning of the second trimester, like screening procedures currently used to detect preeclampsia early in the pregnancy. Some studies appear to be

quite promising, but it is evident that implementing one type of measurement only i.e. Uterine Artery Pulsatility Index (UtAPI) does not provide nearly enough sensitivity and specificity. Neither average nor delta UtAPI values measured at gestational week 11-13 were found to correlate with birthweight in a prospective cohort of 415 specimens (30). A larger cohort (4610 pregnant women) found that first trimester UtAPI is only effective regarding prediction of preterm small for gestational age (SGA) condition (31). A recent retrospective study included 2746 pregnant women and using a multivariate model (combining test results from the first and second trimester) the research group was able to predict 51.6% of later onset IUGR with a false positive rate of 10% (32). The following factors were found to be significant predictors regarding later onset IUGR: maternal height, weight, medical history, average arterial blood pressure values in the first trimester, second trimester HC/AC ratio and estimated fetal weight (EFW).

1.2.2. Screening for preeclampsia

Regarding risk assessment for preeclampsia the following parameters are used:: (1) biomarkers - placental growth factor (PIGF), soluble fms-like tyrosine kinase-1 (sFlt-1) and prengnancy-associated plasma protein A (PAPP-A), (2) maternal characteristics: weight, preeclampsia during previous pregnancies, age, repeated blood pressure measurement on both arms; (3) Doppler ultrasound - UtAPI; (33). If risk is calculated to be high based on this model, the pregnancy should be monitored more closely, and urine protein and blood pressure values are measured on a more frequent basis; risk may also be decreased by oral administration of low dose acetyl-salicylate (150 mg) –this should be implemented before gestational week 16th (34). Early screening detection rates are 90% and 75% for the prediction of early and preterm preeclampsia, respectively, with a 10% false-positive rate (35).

1.2.3. Possible markers for early detection of GDM

Currently there we do not have a guideline or assessment tool that is available for early risk estimation of GDM. Possible contributing factors were evaluated for the purpose of establishing risk assessment: HgbA1c, fasting glucose levels. High HgbA1c levels alone correlate with the development of GDM only around gestational weeks 24-28. It may not

be considered an effective tool regarding early risk assessment; it may contribute to the prediction of increased risk (36).

Unfortunately, current GDM diagnostic criteria are not effective in predicting every case of GDM, and it also does not separate women at cardiovascular risk (11). When risk assessment is performed based on using risk factors is has also not been proven to be an effective/reliable clinical tool regarding GDM prediction - this is supported by the fact that 20% of GDM women do not have these known risk factors (37).

There is evidence of research in the literature regarding prediction GDM by measuring metabolite levels in maternal urine and blood. Uric acid (UA) levels in the serum were measured by Zhao et al. in pregnant women. The rationale behind this study was that T2DM risk has been linked with increased uric acid levels in non-pregnant women. A non-linear link was found between risk for GDM and levels of UA in a study including 85609 pregnant women; this association altered along the course of the pregnancy. Significant association was only found between increased GDM risk and elevated levels of UA at gestational weeks 13-18. Regarding GDM the quantile odds ratio at gestational weeks 13-18: first quantile and second quantile compared 1.11, the third is 1.27, the fourth is 1.37 and the fifth 1.7. Following the age of 35 the link between elevated UA and the development of GDM becomes significantly stronger (38).

Certain factors derived from the adipose tissue were studied between the first and second trimester by Lorenzo-Almorós et al: visfatin, adipopnectin, omentin-1, retinol binding-protein-4, fatty-acid-binding protein-4. In their study these metabolites demonstared an association with GDM risk. Placental metabolites such as afamin, sex hormone-binding globulin, fetuin-A, ficolin-3, fibroblast growth factors-21 and -23, specific microRNAs and follistatin have also been studied in regard ro assessment of GDM risk, and results were promising (11). Results from their research indicated that GDM may be predicted by measuring the following metabolites in the first trimester: sex hormone-binding globulin, retinol binding-protein-4, afamin, hs-C-reactive protein (CRP) (between gestational weeks 5 and 12), fatty-acid-binding protein-4, adiponectin (between gestational weeks 6 and 32) several miRNAs (miR-16-5p, -17-5p, -20a-5p). During the end of the first and the second trimester (~week 12 - 24) fetuin-A, visfatin, omentin, ficolin-3 and leptin. In the third trimester they found the following metabolites to play a role: plasminogen activator inhibitor-1 (PAI-1), fibroblast growth factors-21 and -23,

fetuin-B, ceramide, follistatin and aspartame (11). Elevated levels of the following metabolites were found in GDM: afamin, visfatin, hs-CRP, interleukin-6, leptin miR-16-5p, miR-17-5p, miR-20a-5p, miR-21-3p and miR-19a/b-3p. The following metabolites were demonstared to have decreased levels in cases of GDM: adiponektin, sexual hormon binding globulin (SHBG), fetuin-A, Ficolin-3, Omentin-1, miR-29a, miR-132 and miR-222 (11).

Samples of maternal urine may also contain factors that may be of predictive and diagnostic value regarding early detection of GDM risk. Elevated levels of valine, 2-and 3- hydroxybutanoic acid, alanine, L-Tryptophan, and serotonin were measured in maternal urine samples (39).

The following metabolites have also been studied for their potential value regarding early risk assessment of GDM: 1,5 Anhydroglucitol and adiponectin. Regarding GDM adiponektin levels <8.9 µg/ml in maternal serum samples before gestational week 15 presents an odds ratio of 3.3. However, 1,5 anhydroglucitol was not detected to have such a discernible threshold to predict GDM with adequate sensitivity or specificity. However, levels were shown to be markedly decreased during the first trimester in pregnancies with later onset GDM (40).

All the methods above were not sensitive or specific enough to a reliable risk estimation – a more effective method is needed to improve GDM prediction.

1.3. The role of oxidative-nitrative stress

When the equilibrium between the synthesis of free radicals and production of countering antioxidants is disturbed, oxidative and nitrative stress develop. Free radicals are characterized by marked reactivity; they also include reactive oxygen (ROS), nitrogen and chlorine species. Hydroxyl- and peroxyl radicals and superoxide anions are some of the most well-known ROS (41). Damage caused by the free radicals may be counteracted by antioxidants completely or to a degree only. There is a wide variety of measurable parameters to characterize total antioxidant capacity (TAC). Such a well-established parameter is calculated to characterize the complex equilibrium between Cu(II)-bathocuproinedisulfonic acid (reagent) and Cu(I)-bathocuproinedisulfonic acid. The complex Cu(I)-bathocuproinedisulfonic acid is produced by reducing capacity of the plasma and its active substances (42). There are further parameters that characterize TAC:

total radical-trapping antioxidant parameter, trolox equivalent antioxidant capacity and the ferric reducing ability of plasma (43).

Degree of oxidative stress and consequently an elevation in circulating ROS is expected to rise during pregnancy. Trophoblast cells are exposed to a low oxygen environment (ca 18 Hgmm) during the early phases of gestation (between pregnancy weeks 8 and 10). As the trophoblast plugs dissolve by the end of the first trimester (gestational weeks 10-12), the placenta becomes perfused continuously under low-flow conditions by oxygenated blood. Blood flow in the intervillous space is elevated, and a consequential rise in oxygen pressure (around 60 Hgmm) occurs. Optimal conditions for fetal growth are ensured in part by elevated metabolic rate. This is however consequently followed by elevated levels of both antioxidants and oxidative stress in the placental tissue (44).

A well-known underlying cause of oxidative stress is hyperglycemia (41). Metabolic abnormalities and complications associated with diabetes are strongly associated to oxidative stress and the overproduction of mitochondrial superoxides (45). Several complications or adverse events seen in pregnancy have been linked to increased levels of oxidative stress markers (compared to levels in a physiological pregnancy): pregnancy loss (46), GDM (41) and preeclampsia (47). Both T2DM and GDM has been shown to be associated with an increase in oxidative stress markers, especially ROS, and the dysfunction of antioxidant pathways (41). Hypotheses have been proposed stating that oxidative - nitrative stress may be more part of an underlying cause and not an early consequence in GDM. Overproduction and increased release of inflammatory mediators and ROS has been linked to placental and adipose tissue dysfunction. This overproduction leads to dysfunction of the endothelium in GDM and in preeclampsia. In some cases, damage to the vasculature may be permanent and endothelial phenotype may even be altered – this naturally carries long-term adverse consequences. Increased ROS production also mediates a variety of vascular responses: vascular remodeling, activation of matrix metalloproteinase, cellular apoptosis, and smooth muscle hypertrophy. The IkappaB kinase complex undergoes oxidation because of this overproduction and kappa B nuclear factor levels are increased. This leads to the transcription of inflammatory mediators that cause endothelial dysfunction: a vascular cell adhesion molecule (VCAM-1) and inflammatory cells, intracellular adhesion molecules (ICAM-1) TNF-α, i.e., interleukin-6. During a physiological pregnancy these processes are well-regulated,

however GDM and preeclampsia disorganizes these pathways. A link may be hypothesized between overproduction of ROS in GDM and endothelial dysfunction (48). We may also hypothesize that different from a normal oxidative-nitrative stress measured at the end of the first trimester may also be proved to be a be an effective marker in the early detection of GDM.

Immunologically active cell membranes express urokinase plasminogen activator receptors (uPAR)-these are receptors that are bound to the cell membrane, and they play a role in various physiological and pathological pathways i.e., inflammatory, and immunological processes (49). UPAR is also expressed on the membrane of syncytiotrophoblast cells (they play a pivotal role during implantation) (50). Soluble uPAR (SuPAR) is derived from the cell membrane. Inflammatory processes, diabetes mellitus (DM) and consequential complications have all been linked to increased SuPAR levels (51), however its role in pregnancy has been investigated. It has been established that their levels are not only increased during the first trimester but also in preeclampsia and perhaps in other conditions as well (52). However, the possible link between GDM and SuPAR has not been previously investigated.

1.4. The role of steroids

1.4.1. Steroid metabolism and the placenta

The placenta may be a highly complex organ that produces a wide range of hormones. There are special circumstances and considerations regarding the placenta: (1) it is the largest endocrine organ in the body; (2) it's presence is transient and limited to the duration of the pregnancy; (3) it is an "imperfect" hormone-producing organ - it is dependent on the presence of a variety of precursors from both the fetal and the maternal sides (53). Beside acting as endocrine organ it also serves other purposes as well: immunological protection of and nutrition for the fetus. The double layer of cells lining the placenta connect through the desmosomes; cytotrophoblasts line the inner side syntitiotrophoblasts (54). The two layers differ regarding hormone production; syntitiotrophoblasts produce steroids; both the cytotrophoblasts and the syntitiotrophoblasts produce protein and peptide hormones (55).

The placental hormones are primarily released into the maternal bloodstream. The placenta expresses hormone receptors to regulate both growth and function (56). By

gestational week 7 steroid production capacity of the trophoblast cells is increased to such an extent that it becomes sufficient to provide for the pregnancy. Steroid production dominates from gestational week 12 this is the so-called “luteoplacental shift”: the placenta becomes responsible for maintaining the luteal body.

The placenta is not a classical “perfect” endocrine organ – it may be “imperfect” because it is incapable of independently producing certain hormones, i.e., regarding steroid production: cholesterol from acetate. For this reason it relies heavily on the maternal and somewhat on the fetal fetal precursors of cholesterol (57). The placenta can transform cholesterol to pregnolon to progesteron, it is however incapable of producing estrogens or androgens due to the lack of 17,10-desmolase and 17alpha-hydroxilase. Only the initial and the final steps of estrogen synthesis are performed by the placenta, and androgens are not produced here – leaving it dependent regarding precursors on maternal and fetal sources. Using dehydroepiandrosterone (DHEA) from the mater and the fetus testosterone and androstendion and then estron and estradiol are produced. In a daily cycle the adrenal gland of the fetus produces 100-200 mg of steroid precursors in its cortex – this secures around 90% of the total synthesis of estriol (58). Estetrol is an estrogenic steroid molecule synthesized exclusively by the fetal liver during human pregnancy and reaching the maternal circulation through the placenta (59). It has strong liver-protective, antioxidative, neurogenic and angiogenic effects (60). Recently also described the effect of estetrol on endothelial healing after carotid artery injuries in vivo (61), - consequently estetrol improves endothelial healing and slowers vascular aging Physiological intrauterine effects of estetrol are under further investigations.

The placenta produces steroids: the development of GDM later during the pregnancy is profoundly impacted by the physiological or pathological functioning of the placenta (11, 62). We try to predict these occurrences from the alterations found steroid metabolite levels at week 12.

1.4.2. Glucose and steroids metabolism

There is an interaction between maternal and fetal adrenal cortices during pregnancy **(Figure 1.).** The role of the placenta is to ensure optimal production of placental steroid hormones. The adrenal glands produce the main precursors of estrogen, namely DHEA and DHEA-S (the sulphated form). The fetal liver may then modify these precursors (16-

alpha hydroxylase enzyme), which leads to the synthesis of 16-hydroxy-DHEA and DHEA-S. Using the same precursors the placenta produces estradiol, estron and estriol which is associated with the production of testosterone and androstenedion as intermediate metabolites. Insulin resistance in non-pregnant women is associated with changes occurring in the androgen hormone metabolism (63). Possibilities regarding GDM prediction using these metabolites has previously been investigated to some extent. Increased testosterone level values were measured in a study in pregnancies where the women developed GDM later (64). The placenta expresses 11-beta-hydroxysteroid dehydrogenases 1 and 2 and corticotropin releasing hormone, which are responsible for the regulation of the interactions occurring between the cortisol metabolism of the fetus and the mother (65). In GDM, cortisol levels have been measured to be elevated during the second trimester of the pregnancy (65, 66), however the possible predictive role of cortisol and cortisol related metabolites has not been investigated during the first trimester.

The figure shows the steroid biosynthesis of the adrenal cortex **(Figure 1.)**

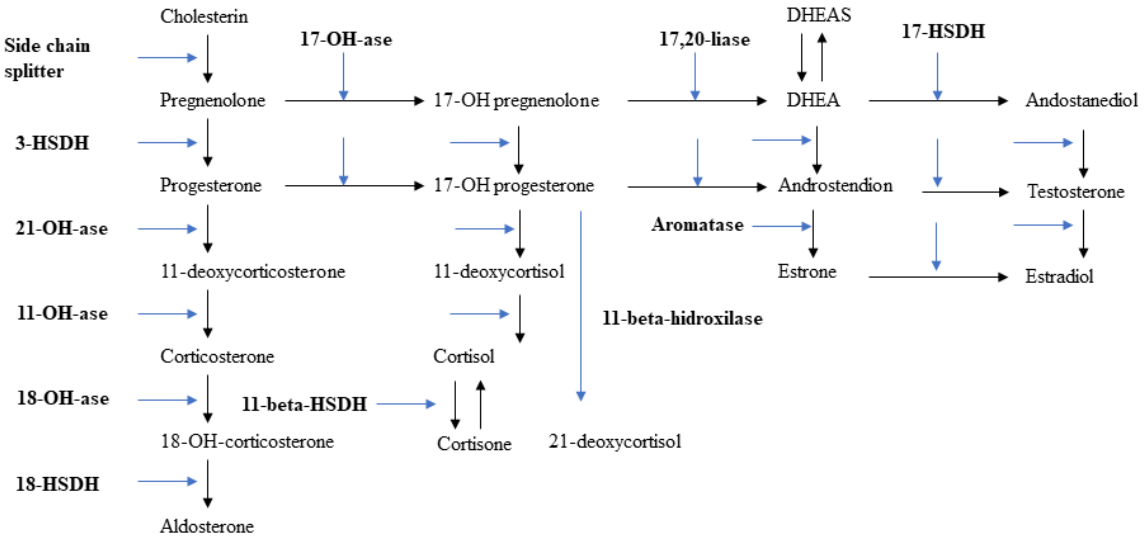

Figure 1. Biosynthesis of steroid hormones within the adrenal cortex. OH-ase-hydroxylase, OH-hydroxy, HSDH-hydroxysteroid dehydrogenase, DHEA-S-dehydroepiandrosterone sulfate, DHEA-dehydroepiandrosterone (67).

2. OBJECTIVES

Increased oxidative-nitrative stress is characteristic not only in pathologic, but also in healthy pregnancy. High uterine artery pulsatility index at the end of the first trimester is associated with altered placentation and elevated risk for adverse pregnancy outcomes.

There is no current guideline or assessment tool available for estimating early risk (11+0 and 13+6 gestational week) regarding gestational diabetes mellitus but there is a need for it, like in preeclampsia. If we would know the risk, appropriate treatment could reduce gestational and later complications.

The aims of our study were:

1. to examine the relationship of systemic oxidative-nitrative stress and uterine artery pulsatility index in the first trimester and their correlation to pregnancy outcomes.

2. in the first trimester we collected patient baseline data, we measured routine laboratory parameters, oxidative-nitrative stress markers and steroid metabolites to examine their correlation to the development of GDM later during pregnancy, to identify possible novel early markers for GDM prediction.

3. METHODS

3.1. Measuring oxidative stress and pulsatility of the uterine artery in early phase pregnancies

3.1.1. Patient inclusion criteria

Between 27. May 2016. and 12. December 2017., a prospective type of observational study was conducted at the Department of Obstetrics and Gynecology together with the Department of Physiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary. Ethical approval was confirmed by the Scientific and Research Ethics Committee of the Hungarian Medical Research Council (43102-2/2014/EKU (425/2014) 49768-1/2015/EKU (392/2015). All included patients gave informed consent.

Methods of inclusion: during the early phase of pregnancy – namely at the 12th week ultrasound (gestation week 12-13) – otherwise healthy patients (between the ages of 18-40) were randomly chosed and offered the opportunity to participate in the study. Women suffering from hypertension, obesity (BMI over 30), malignant tumors, diabetes mellitus, chronic inflammatory diseases or having twin pregnancies were excluded. Transabdominal ultrasonography was performed using GE Volusion E8 (Boston, MA, USA), color Doppler method was used for obtaining flowmetric data regarding the uterine artery at a location of 1 cm from branching-off of the iliac artery. The following equation was used to calculate pulsatility index:

$$PI = \frac{\text{peak systolic velocity (PSV) - end diastolic velocity (EDV)}}{\text{time avaraged velocity (TAV)}}$$

Using the mean of the right and left artery pulsatility index calsulated from the measured data two groups were formed; one was the low UtAPI group, (UtAPI <2.3), (n=31) and the other group of pregnant women was the high UtAPI group, (n=30). We determined the cut off point of the calculated values at 90% percentile of that measured in the European population (68).

3.1.2. Study protocol

Patient history - both personal (obstretics and internal medice) and familiar were taken. Blood samples were taken to check rutin lab parameters, and the following things were

isolated from them: serum, plasma, and mononuclear leukocyte fractions. Both the serum and the plasma samples were appropriately frozen and stored at a temperature of -80°C. Histopaque-1077 was used as per guideline for gradient centrifugation to isolate circulating mononuclear cells (Sigma-Aldrich, St. Louis, MO, USA). Smears fixed with methanol were prepared using the cell-suspension, these were stored at a temperature of 4°C until the following processing step was indicated. To track pregnancy complications, labor circumstances, and anthropometric data of the newborns, follow-up was continued to birth. Labor circumstances could not be obtained in six of our cases - 4 in the low UtAPI group and 2 from the high UtAPI group.

3.1.3. Measuring oxidative-nitrative stress markers

Colorimetry method (OxyStat assay - Biomedica, Wien, Austria) was used to measure the total plasma peroxide (PRX) concentration of the serum samples; this is a marker of systemic oxidative stress. OxiSelect™. Total Antioxidant Capacity (TAC) Assay Kit (Cell Biolabs Inc., San Diego, CA, USA) was used to determine plasma total antioxidant capacity levels of the samples. To measure nitrative stress horseradish peroxidase (HRP) conjugated anti-3-nitrotyrosine (3-NT) antibodies (OxiSelect™ Nitrotyrosine Elisa Kit, Cell Biolabs Inc., San Diego, CA, USA) were used by competitive ELISA method. Intracellular nitrative stress was measured on the leukocyte smears (fixed with methanol) using anti-nitrotyrosine rabbit polyclonal antibodies (Abcam, Cambridge, UK) (1:80, 4°C, overnight). To avoid aspecific marking the smears were incubated for one hour in 15% normal horse serum at room temperature. Horseradish peroxidase–conjugated horse anti-rabbit immunoglobulin (Vector Laboratories, Burlingame, CA, USA) (30 min, room temperature) was used for secondary marking. The markings were visualized using brown stain - diaminobenzidine (6 min, room temperature, brown color) (Vector Laboratories). Counter-stained using blue stain hematoxylin (Vector Laboratories). Using a magnification of 200x (Zeiss-Imager.A1 light microscope, 20x/0.45 objective, AxioCam MRc5 camera, AxioVision – Rel. 4.8 software; Carl Zeiss Microscopy GmbH, Jena, Germany) five images – considered as representative - were taken of each smear. The ratio of positive-stained area (cellular only) was compared to the total cellular area – this was performed by a blinded researcher using ImageJ software (MBF ImageJ, NIH, Bethesda, MA, USA); a minimum of 300 cells were evaluated by the experimenter on

every smear. If the sample was inadequate for sample analysis – due to hemolyzation of the serum/plasma or low cell count – it resulted a lower amount of data. To analyze the potential additive value of measuring PRX regarding the correlation between birthweight and UtAPI, a new, previously unused parameter was defined and calculated: the ratio of UtAPI to plasma PRX; PIPX= (UtAPI/PRX) * 100.

3.1.4. Statistical analysis

Data regarding normal distribution are presented as mean ± SEM. Non-Gaussian distribution was presented as median [IQR] following logarithmic transformation (TAC). Two-tailed unpaired Student's t-test was implemeted to determine the statistical significance the two patient groups. Chi-square test was used to determine nominal variables. Pearson's test was used to define correlations between the analyzed variables. Missing data was treated as such. Significancy level was determined at $p<0.05$. SPSS 22.0 and Graphpad Prism 6.0 softwares were used to perform the above statistical analysis.

3.2. Prediction of gestational diabetes mellitus in the first trimester

3.2.1. Patient history, sampling

A prospective cohort study was performed between 2010 and 2012 at the Department of Obstetrics and Gynecology at the University of Debrecen Medical and Health Science Centre, Debrecen, Hungary and at the Department of Obstetrics and Gynecology at the Andras Josa County and Teaching Hospital, Nyíregyháza, Hungary. In total 2545 pregnant women (between 11 (+0 days) and 13 (+6 days) weeks of gestation) were recruited. The following parameters were registered as part of the study: maternal characteristics, data from the screening ultrasound of the first trimester and medical history. Blood (serum and plasma) and urine samples were also taken at the same visit, and these were stored at –80°C in an accredited biobank to be available for further study. Pregnancies were followed-up until birth regarding the following complications of pregnancy: small for gestational age newborns, preeclampsia, gestational diabetes mellitus and macrosomia. Approval for the study was obtained from the appropriate local

ethics committee (identification number: DEOEC RKEB/IKEB 3092-2010). Women agreeing to participate in the study gave informed consent in writing.

3.2.2. Current study

As a result of the collaboration of the Biobank of Debrecen University and Semmelweis University, the GIPS (GDM and IUGR Prediction Study), a retrospective observational study was created. Our aim was to identify novel early risk assessment factors that may be measured and used for screening of GDM and IUGR (ethical approval: ETT TUKEB 4/4414-4/2020/EKU). Debrecen University provided samples (serum and plasma), clinical data, routine laboratory parameters (CRP, hepatic function, glucose, fructosamine, and creatine kinase), pregnancy outcomes, labor circumstances and newborn parameters of 55 healthy controls and 55-55 patients who subsequently developed GDM or IUGR. GDM patients were selected based on NICE criteria: fasting glucose level ≥ 5.6 mmol/l and/or 120' glucose level ≥ 7.8 mmol/l. Inclusion criteria included age between 18 and 40 years, exclusion citeria were as follows: hypertension, malignant tumors, diabetes mellitus, chronic inflammatory diseases, class II. obesity (BMI over 35 kg/m^2) and twin pregnancies. As part of the GIPS study, we introduce the results of both control and the GDM groups regarding early prediction of GDM.

3.2.3. Groups

Data from two patient groups was analyzed in the GIPS study: healthy controls (C), (n=55) versus those who developed GDM (D) (n=55) later during the pregancy.
N was less than 55 in certain cases due to missing data or because the value was below the technical reference level.

3.2.4. Determination of oxidative-nitrative stress related parameters

Total serum peroxide (PRX) concentration, reflecting systemic oxidative stress, was determined from serum samples using colorimetric method - OxyStat assay (Biomedica, Wien, Austria). Serum total antioxidant capacity was measured from serum samples by commercially available assay kit (OxiSelect™ Total Antioxidant Capacity (TAC) Assay Kit, Cell Biolabs Inc., San Diego, CA, USA). Oxidative index (OI) was calculated from the ratio of oxidative stress and TAC. Sandwich ELISA technique (SuPAR human ELISA

kit, BioVendor, Brno, Czech Republic) was used to determine Soluble Plasminogen Activator Urokinase Receptor (SuPAR) levels. Nitrative stress was determined by measuring plasma levels of 3-nitrotyrosine (NT) using competitive ELISA based on horseradish peroxidase conjugated anti-3-NT antibodies (OxiSelect™ Nitrotyrosine Elisa Kit, Cell Biolabs Inc., San Diego, CA, USA).

3.2.5. Measuring steroid levels

Steroid levels were measured at the Department of Laboratory Medicine, Semmelweis University. Using reversed-phase liquid chromatography-tandem mass spectrometry (LC-MS/MS) that following parameters were assayed: androstenedione, aldosterone, dehydroepiandrosterone, 11-deoxycorticosterone, dehydroepiandrosterone sulfate, 11-deoxycortisol, dihydrotestosterone, 21-deoxycortisol, 17alpha-hydroxypregnenolone, corticosterone, 17alpha-hydroxyprogesterone, cortisol, pregnenolone, cortisone, testosterone, and progesterone. Both multiple reaction monitoring and electrospray ionization were performed. Except for the measurement of cortisone, the mass spectrometer was used in the positive mode.

The method developed and validated at our laboratory (Laboratory of Mass Spectrometry and Separation Technology, Department of Laboratory Medicine, Semmelweis University, Budapest, Hungary-described previously) was used to assay the 16 steroid substances listed above (69). In brief 200 µL of serum was diluted using 600 µLs of methanol containing the internal standards. Samples were vortexed and centrifuged. 700 µL supernatant was diluted with 1300 µL of water. A solid phase extraction plate (96-well Phenomenex Strata-X 60 mg) was preconditioned by dripping the mixture of 900 µL methanol and 900 µL water (3:1, v/v) through (Gen-Lab Kft., Budapest, Hungary). Once diluted the supernatants were added onto the plate and at a slow rate were vacuum drawn through. Slots were washed with 2x900 µL water and methanol 3:1 (v/v) and after dried by propeling nitrogen (5.0 pure) through the packings. Finally, elution of the analytes was performed by the application of 2x900 µL acetonitrile and methanol 1:1 (v/v). Following drying of the eluates the residues were reconstituted by adding 50 µL methanol and water 1:1 (v/v). Two-dimensional liquid chromatographic separation was performed afterwards by making a serial connection of a Phenomenex Kinetex XB-C18 50x2x1 mm, 1.7 µm and a Kinetex Biphenyl 50x2.1 mm, 1.7 µm stationary phase (Gen-Lab Kft., Budapest,

Hungary). Mobile phases consisted of water and methanol, these both contained 0.1% formic acid. Gradient programs were also run. Quantifation was based on $1/x^2$-weighted linear calibration using Chromsystems® 6PLUS1 Multilevel Serum Calibrator Set MassChrom® Steroid Panel 1 and Panel 2 (ABL&E-Jasco Magyarország Kft., Budapest, Hungary). Internal controls (Chromsystems® MassCheck Serum Steroid Panel 1 and Panel 2, Levels I-III, ABL&E-Jasco Magyarország Kft.) were assayed at the beginning of each batch. Known and biologically relevant quantities of pregnenolone and 17-alpha-hydoxypregnenolone were spiked to the Panel 1 calibrators and internal controls.

3.2.6. Statistical analysis

Either the two-tailed unpaired Student's t-test or - in case of non-normal distribution - the Mann-Whitney test was used to analyze statistical significance between the groups. Pearson's test was used to determine correlation between the parameters. The one non-normally distributed parameter (SuPAR) was logarithmized and logarithmic values showed normal distribution. Chi-square test was used to determine nominal variables. To analyze the predictive power of both previously known and potentially novel risk factors regarding prediction of GDM, multivariate logistic regression models were used. Missing data was treated as such. $p<0.05$ was considered significant. Normal distribution data are presented as mean ± SD. Graphpad Prism 6.0 and SPSS 22.0 softwares were used for statistical analysis.

4. RESULTS

4.1. Oxidative stress and uterine artery pulsatility index (UtAPI) in early pregnancy

4.1.1. Clinical parameters and anthropometric data in low and high UtAPI groups

As a grouping variable UtAPI in the high resistance group was measured to be significantly higher **(Figure 2/A.)**.

BMI and age did not differ between the analyzed groups. Parity, number of miscarriages and gravidity were significantly lower in the high UtAPI group **(Table 1.).** Lactate dehydrogenase (LDH) was significantly lower in the higher UtAPI group **(Table 1.).**

Our study groups demonstared similarity regarding the following clinical parameters: liver enzymes, plasma creatinine, hemoglobin, glucose levels and CRP **(Table 1.)**.

Table 1. Antropomorphic parameters and clinical data of women.

Age and BMI matched study groups were created. In the high UtAPI group parity, gravity and the number of previous miscarriages were lower. In the high UtAPI group LDH level was significantly lower Other clinical laboratory parameters did not demonstrate significant differences between the two groups. Two-tailed unpaired Student's t-test. Values are the means±SEM. (70).

Variable	*low UtAPI (n = 31)*	*high UtAPI (n = 30)*	*Significance*
Anamnestic data			
Age (years)	32.00 ± 0.66	31.00 ± 0.73	ns
(min-max)	*(26-39)*	*(22-38)*	
BMI (kg/m^2)	21.60 ± 0.71	22.60 ± 0.39	ns
Gravidity	1.60 ± 0.27	0.80 ± 0.19	$p<0.05$
(min-max)	*(0-6)*	*(0-4)*	
Parity	1.00 ± 0.19	0.40 ± 0.15	$p<0.05$
(min-max)	(0-4)	(0-4)	
Miscarriages	0.50 ± 0.15	0.10 ± 0.07	$p<0.05$
Clinical parameters			
LDH (U/l)	152.66 ± 2.75	144.70 ± 2.47	$p<0.05$
Hemoglobin (g/l)	128.73 ± 1.53	126.92 ± 1.56	ns
Hematocrite (l/l)	0.38 ± 0.00	0.37 ± 0.00	ns
Glucose (mmol/l)	4.60 ± 0.12	4.63 ± 0.14	ns
Triglycerides (mmol/l)	1.40 ± 0.10	1.45 ± 0.73	ns
HDL (mmol/l)	1.87 ± 0.05	1.77 ± 0.05	ns
LDL (mmol/l)	3.31 ± 0.13	3.30 ± 0.10	ns
Bilirubin (µmol/l)	8.34 ± 0.79	7.80 ± 0.42	ns
Creatinine (µmol/l)	47.77 ± 1.55	47.43 ± 1.25	ns
AST (U/l)	18.45 ± 0.53	18.70 ± 0.83	ns
ALT (U/l)	14.58 ± 0.88	15.77 ± 1.62	ns
GGT (U/l)	13.32 ± 0.78	13.17 ± 0.96	ns
CRP (U/l)	6.27 ± 0.70	5.99 ± 0.60	ns
ALP (U/l)	56.81 ± 2.89	53.60 ± 2.25	ns

4.1.2. Oxidative-nitrative stress in low and high UtAPI groups

Systemic oxidative stress, characterized by plasma total peroxide level was significantly lower in the high UtAPI group **(Figure 2/B)**, while TAC was significantly higher in this group **(Figure 2/C)**. PIPX is calculated using UtAPI and PRX values as follows: PIPX=(UtAPI/PRX) *100. These values were found to be significantly higher in the high UtAPI group **(Figure 2/D)**. Both serum and mononuclear cell NT levels were found to be similar in the groups **(Figure 3/A, B)**.

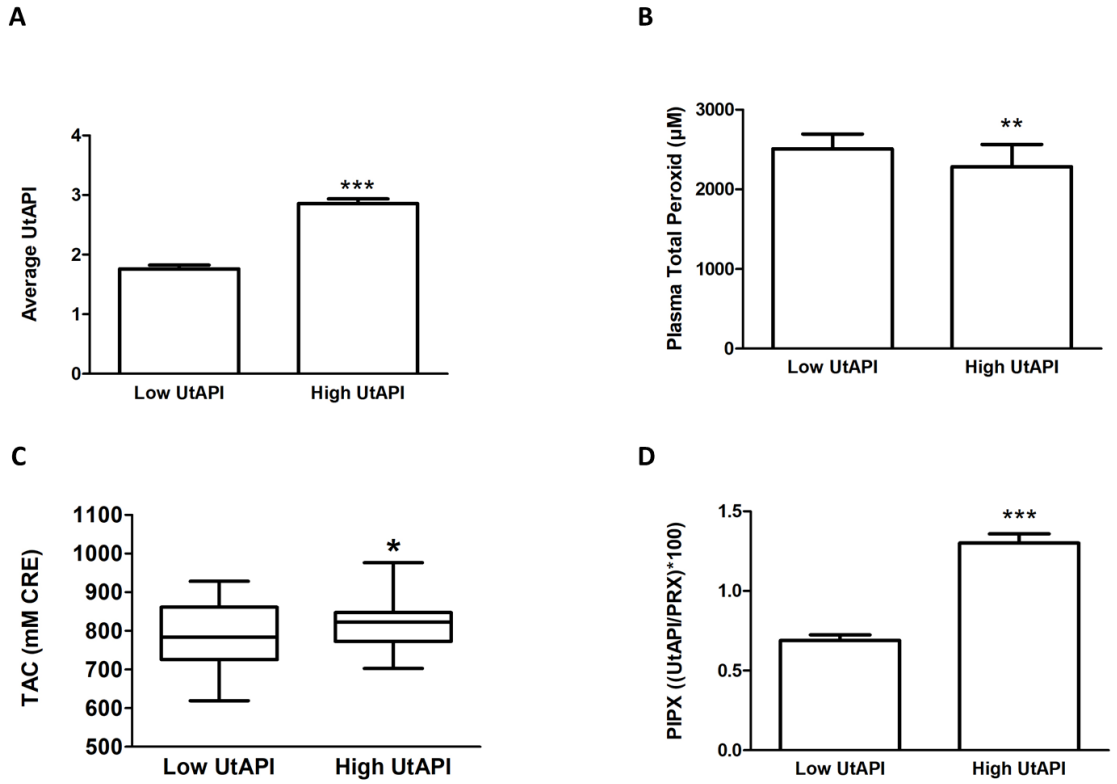

Figure 2. Pulsatility index and oxidative stress markers.

Panel **A**: Mean pulsatility index of the uterine artery. (n=31;30) Panel **B**: The high UtAPI group demonstrated significantly lower plasma total peroxide levels. (n=22;22); Panel **C**: Significantly higher levels of plasma TAC were in the high UtAPI group. (n=29;27) Panel **D**: PIPX was calculated using UtAPI and PRX values – this proved to be significantly higher in the high UtAPI group. (n=22;22). Two-tailed unpaired Student's t-test. Data are presented as mean±SEM, or Median [IQR] in case of TAC. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$ (70).

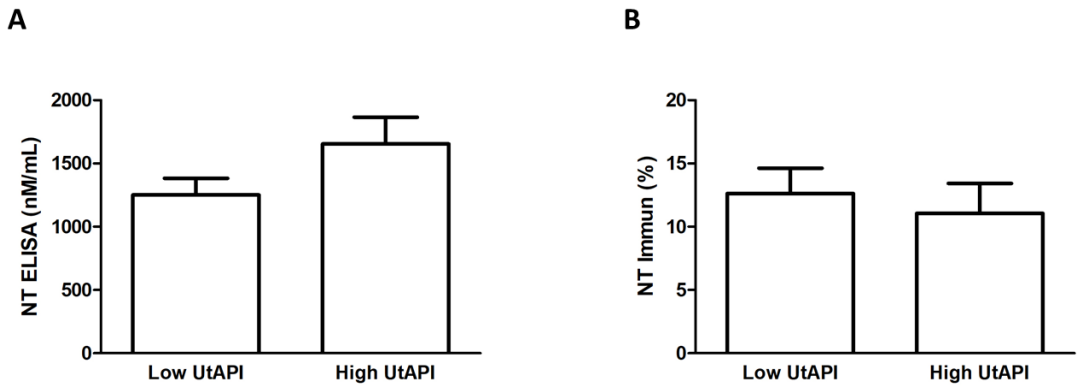

Figure 3. Pulsatility index and nitrative stress markers.

Panel **A** and **B**: Similar levels of serum and mononuclear cell 3-nitrotyrosine were found in the two study groups. (n = 21; 28 and n = 16; 14). Two-tailed unpaired Student's t-test. Data are presented as means ± SEM (70).

4.1.3. Pregnancy outcomes and anthropometric data of newborns in low and high UtAPI groups

The rate of Cesarean section was similar in the two groups. All newborns were in the normal body parameter range; however, newborns of the high UtAPI group had significantly lower birthweight and chest circumference than ones of the low UtAPI group. There was no difference in gestational weeks at labour, therefore these differences were not due to earlier delivery **(Table 2.)**.

Table 2. Pregnancy outcomes and anthropometric data of newborns in low and high UtAPI groups.

The rate of Cesarean sections and the development of GDM did not differ between the two groups. The high UtAPI newborns demonstrated lower birthweight and chest circumference values despite there being no difference regarding gestational age between the mothers at labor. Two-tailed unpaired Student's t-test. Values are the means±SEM. (70).

Variable	*low UtAPI (n = 31)*	*high UtAPI (n = 30)*	*Significance*
Pregnancies' outcome			
Cesarean section (n)	13	11	ns
GDM (n)	3	3	
Bodyweight (g)	3517.41 ± 77.02	3316.79 ± 63.76	$p<0.05$
Gestational week	39.80 ± 0.19	39.30 ± 0.20	ns
Chest circumference (cm)	34.41 ± 0.29	33.57 ± 0.26	$p<0.05$
Head circumference (cm)	35.00 ± 0.26	34.39 ± 0.30	ns
Apgar 1'	9.44 ± 0.11	9.61 ± 0.09	ns
Apgar 5'	10.00 ± 0.00	10.00 ± 0.00	ns

4.1.4. Correlations between oxidative-nitrative stress, UtAPI and clinical data in the total study cohort

PRX showed negative correlation to UtAPI and a positive correlation to the birthweight of the newborns **(Table 3.)**. However, TAC demonstrated a negative correlation to the number of previous pregnancies and a positive correlation to serum bilirubin and creatinine levels **(Table 3.)**. Intracellular NT immunohistochemical staining intensity demonstrated a positive correlation to measured γGT levels, and to the occurrence of Cesarean sections.

The negative correlation of UtAPI to birthweight could be improved in our cohort by combining UtAPI measurements with plasma PRX levels and calculating their ratio (PIPX) **(Table 4.)**.

Table 3. Clinical parameters correlating to systemic oxidative-nitrative stress markers measured on the 12-13th weeks of gestation.

Plasma total peroxide level positively correlated to birthweight. Plasma total antioxidant capacity negatively correlated to the number of previous healthy pregnancy and positively to serum bilirubin and creatinine levels. Intracellular tyrosine nitration of circulating mononuclear cells positively correlated to γGT levels and the occurrence of Cesarean section (70).

Independent variable: Plasma Total Peroxide			
Dependent variable	*r*	*p*	*n*
Birthweight	*0,342*	*0,031*	*40*
UtAPI	*-0.428*	*0.004*	*44*
Independent variable: Log (Plasma TAC)			
Dependent variables	*r*	*p*	*n*
Gravidity	-0.323	0.015	56
Bilirubin	0.268	0.045	56
Creatinine	0.357	0.007	56
Independent variable: Leukocyte NT			
Dependent variables	*r*	*p*	*n*
γGT	0.407	0.026	30
Cesarean section	0.391	0.048	26

Table 4. Correlation of birthweight of the newborns to UtAPI, plasma total peroxide level and their combined variable PIPX (UtAPI/PRX*100).

All analyzed parameters showed correlation to birthweight; however, the strongest correlation could be found in case of PIPX (70).

Independent variable: UtAPI			
Dependent variable	*r*	*p*	*n*
Birthweight	-0.347	0.009	55
Independent variable: PIPX			
Dependent variable	*r*	*p*	*n*
Birthweight	-0.450	0.004	40

4.2. Gestational diabetes mellitus – prediction in the first trimester

4.2.1. Patient characteristics in early pregnancy - at the end of the first trimester

Patients who developed GDM in our cohort were 3.2 years older on average, they also demonstrated both higher body weight measurements (by 9.04 kg on average) and BMI values (by 3.2 kg/m^2 on average) **(Table 5.)**. Control and GDM groups did not differ significantly regarding height, gravidity, parity, and weight gain during pregnancy **(Table 5.)**.

Table 5. Physiological parameters measured in early pregnancy (on the 12th week of gestation)

Age, body weight and BMI measurements were significantly higher in the GDM group compared to controls. Height, parity, gravidity, and weight gain during pregnancy were similar in the two groups. Two-tailed unpaired Student's t-test. Values are the means±SD.(67).

Variable	*Control (n=55)*	*GDM (n=55)*	*Significance*
Age (years)	27.78 ± 3.47	30.98 ± 4.66	p<0.001
Body weight (kg)	66.63 ± 8.86	75.67 ± 15.69	p<0.001
Height (cm)	165.07 ± 6.46	164.85 ± 6.72	ns
BMI (kg/m^2)	24.55 ± 2.83	27.75 ± 5.10	p<0.001
Weight gain (kg)	10.69 ± 4.07	10.76 ± 5.19	ns
Gravidity	1.90 ± 0.95	2.38 ± 1.63	ns
Parity	0.85 ± 0.85	1.24 ± 1.35	ns

BMI, body mass index; GDM, gestational diabetes mellitus.

4.2.2. Glucose, CRP, fructosamine, liver function and creatin kinase

The two study groups did not differ significantly regarding CRP, liver function and creatine kinase levels and neither did fasting glucose measurements. Levels of fructosamine were found in a normal range, however, these values were markedly higher in the GDM group compared to controls **(Table 6.)**.

Table 6. CRP, liver function and markers of glucose metabolism at the end of the first trimester

While fructosamine levels were measured to be higher amongst the later GDM women, the rest of the analyzed laboratory parameters were found to be similar between the two study groups. Two-tailed unpaired Student's t-test. Values are shown as means±SD. (67).

Variable	*Control (n=55)*	*GDM (n=55)*	*Significance*
Glucose (mmol/l)	4.47 ± 0.81	4.68 ± 1.13	ns
Fructosamine (µmol/l)	200.93 ± 13.53	214.02 ± 22.98	p<0.001
Creatine kinase (U/l)	53.25 ± 21.94	52.48 ± 18.67	ns
CRP (mg/l)	6.38 ± 6.26	8.62 ± 8.40	ns
ALP (U/l)	59.20 ± 14.20	58.91 ± 14.20	ns
LDH (U/l)	146.22 ± 27.20	139.94 ± 33.80	ns
AST (U/l)	15.66 ± 3.65	15.20 ± 3.51	ns
ALT (U/l)	12.58 ± 5.00	13.49 ± 5.49	ns
GGT (U/l)	12.89 ± 6.85	14.50 ± 9.05	ns

ALP, alkaline phosphatase; CRP, c-reactive protein; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase.

4.2.3. Oxidative – nitrative stress parameters and SuPAR

Serum total peroxide levels did not demonstrate differences between the groups, TAC was measured to be significantly higher in the GDM group, however oxidative stress index (calculated from serum total peroxide levels and TAC) did not demonstrate differences between the groups **(Figure 4. A, B, C)**. Plasma 3-nitrotyrosin levels did not differ between the two study groups in our series **(Figure 4. D)**. The later GDM women had significantly lower serum SuPAR levels compared to the controls **(Figure 4. E)**.

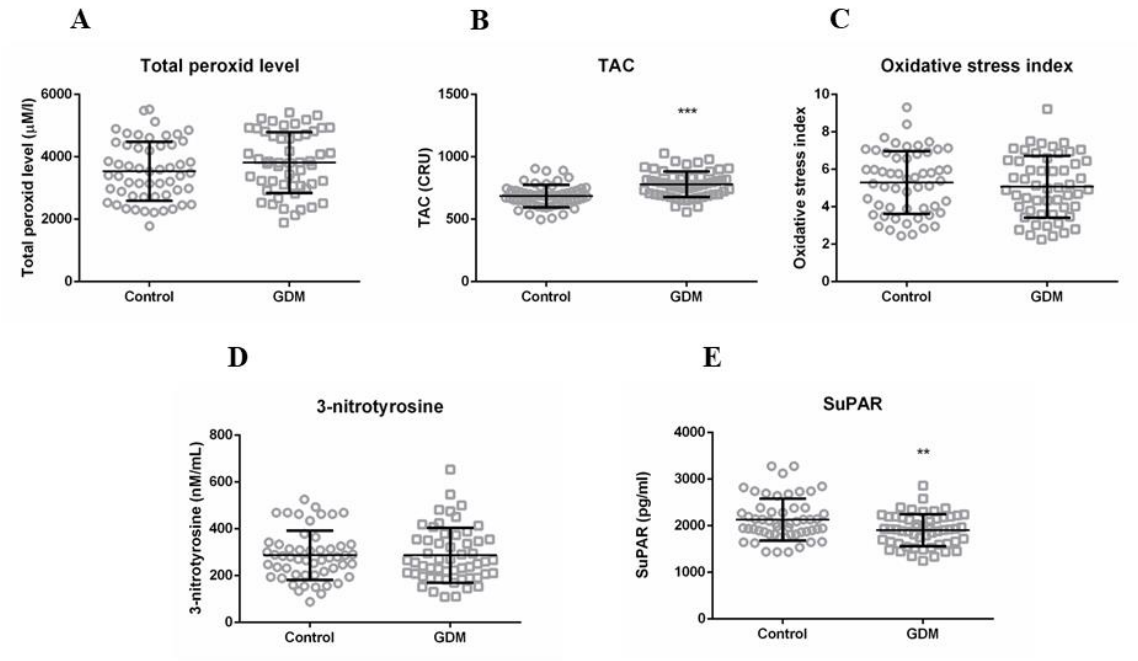

Figure 4. Oxidative – nitrative stress parameters and SuPAR.

Panel **A:** Serum total peroxide level. The oxidative stress in the serum was similar in the two groups (n=54 and n=53). Panel **B:** Total antioxidant capacity (TAC). GDM was associated with more pronounced TAC (n=55 and n=55). Panel **C:** Oxidative stress index. The oxidative stress index did not differ between the control and the GDM groups (n=54 and n=53). Panel **D:** Plasma levels of 3-nitrotyrosine (NT). The nitrative stress did not differ between the control and GDM groups (n=53 and n=55). Panel **E:** Soluble urokinase plasminogen activator receptor (SuPAR). The GDM group showed significantly reduced serum SuPAR level (n=55 and n=54). Data are shown as mean±SD; two-tailed unpaired Student's t-test and Mann-Whitney test for Oxystat and SuPAR. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ Control vs. GDM (67).

4.2.4. Steroid metabolites

Androgens

Serum levels of DHEA-S (which is the most key adrenal androgen metabolite) were significantly lower in the GDM group. The key ovarian metabolite – testosterone. was found to be significantly higher in the GDM group. The physiologically active metabolite of ovarian testosterone, namely dihydrotestosterone, was decreased in the GDM group leading to a demonstrably decreased DHT/T ratio **(Figure 5. A, B, C, D)**.

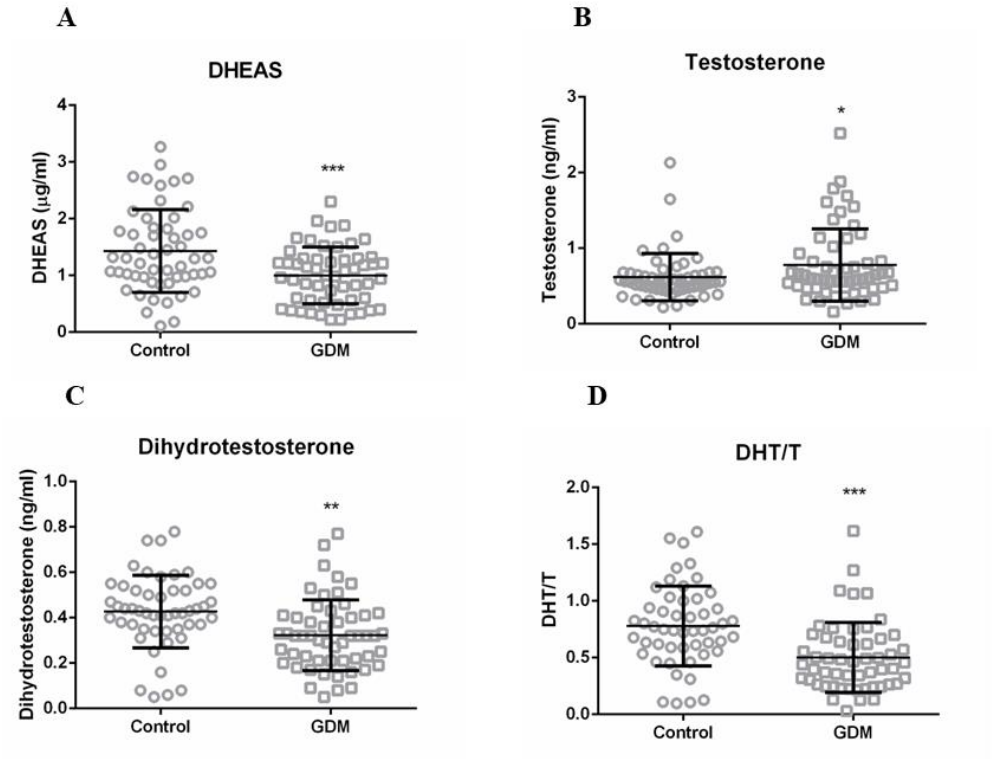

Figure 5. Androgens

Panel **A:** Dehydroepiandrosterone sulfate (DHEA-S). The level of DHEA-S was significantly lower in the gestational diabetes mellitus (GDM) group (n=54 and n=55). Panel **B:** Testosterone (T). GDM was associated with more pronounced level of testosterone (n=54 and n=55). Panel **C:** Dihydrotestosterone (DHT). The level of DHT was significantly decreased in GDM group (n=51 and n=54). Panel **D:** DHT/T ratio. The DHT/T ratio was significantly lower in GDM group (n=51 and n=54). Data are shown as mean±SD; two-tailed unpaired Student's t-test. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ Control vs. GDM (67).

Further adrenal steroids: mineralo-and glucocorticoid metabolites

Women in the GDM group were found to have lower cortisol and elevated cortisone levels compared to the control group. The GDM group also demonstrated elevated 21-deoxycortisol levels (a metabolite derived from 17α-hydroxyprogesterone). 11-deoxycorticosterone (a mineralocorticoid and an aldosterone precursor) was significantly lower in the GDM group **(Figure 6. A, B, C, D)**.

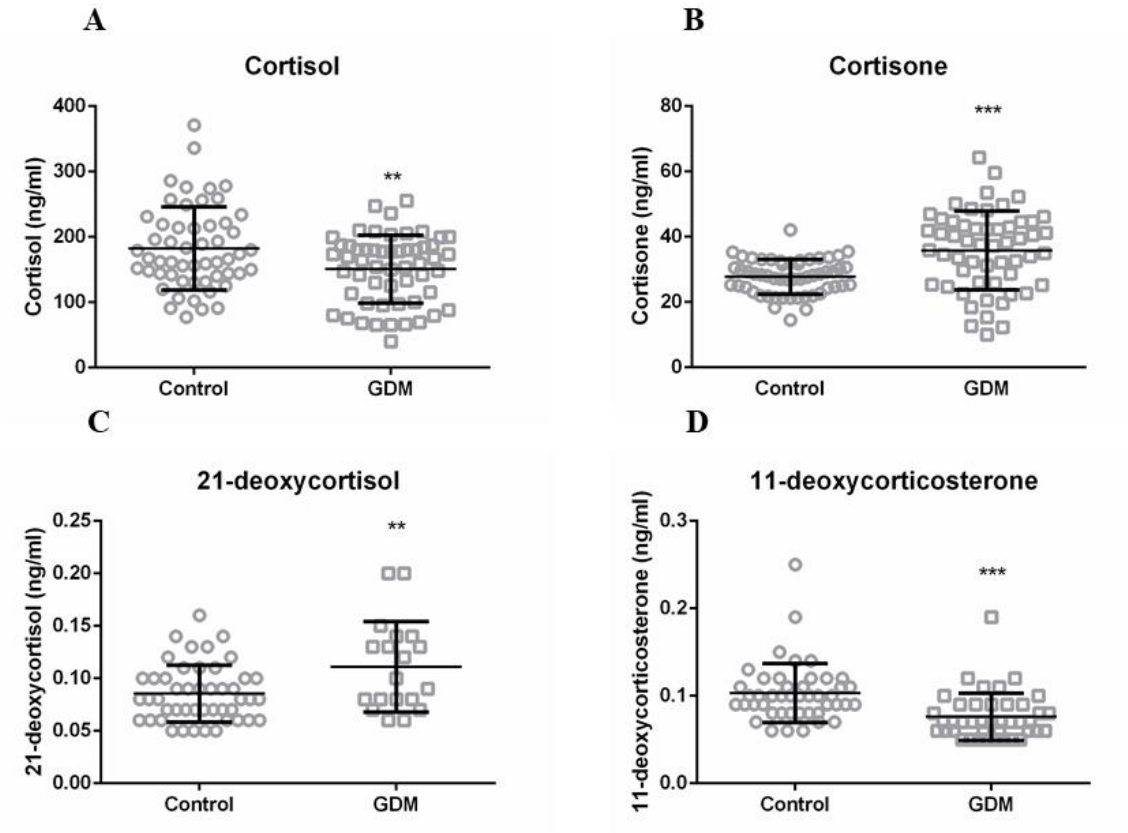

Figure 6. Further steroids

Panel **A:** The GDM group showed significantly lower levels of cortisol (n=54 and n=55). Panel **B:** Cortisone levels were increased in the GDM group (n=54 and n=55). Panel **C:** 21-deoxycortisol levels were found to be significantly higher in the GDM group compared to the control group (n=47 and n=19). Panel **D:** 11-deoxycorticosterone was measured be significantly lower in the GDM group compared to the control group (n=45 and n=41). Data are shown as mean±SD; two-tailed unpaired Student's t-test. **: $p < 0.01$, ***: $p < 0.001$ Control vs. GDM (67).

4.2.5. Correlation analysis of „classical" GDM risk factors and potential new candidates for marking risk

GDM women had elevated plasma total antioxidant capacity, and this showed a positive correlation with BMI. SuPAR levels were measured to be lower in the GDM group and they did not correlate with any of the classical or the analyzed risk factors. Women who developed GDM later-on during the pregnancy had decreased levels of DHEA-S and DHT; and they also showed a negative correlation with maternal age. DHT and serum fructosamine levels correlated negatively. When analyzing androgen hormones, testosterone was found not to have a significant correlation to the measured parameters. The GDM women had decreased cortisol level, and they showed a negative correlation with gravidity and parity. In our series cortison levels did not demonstrate any significant correlations. GDM women also had lower 11-deoxycorticosterone levels; they also correlated negatively with BMI **(Table 7.)**.

Table 7. Correlation of known risk factors of GDM to TAC, SuPAR and steroid hormone metabolites that were found significantly different in the GDM group compared to controls

TAC showed a positive correlation with BMI. DHEA-S correlated negatively with maternal age. DHT correlated negatively with maternal age and with serum fructosamine levels. Serum cortisol correlated negatively with gravidity and with parity also. 11-deoxycorticosterone showed a negative correlation with BMI; Statistical analysis: Pearson correlations; Level of significance $p<0.05$. TAC-total antioxidant capacity, SuPAR-soluble urokinase activator receptor, DHEA-S-dehydroepiandrosterone sulfate, T-total testosterone, DHT-dihydrotestosterone (67).

	Age (years)	**BMI (kg/m^2)**	**Gravidity**	**Parity**	**Fructosamine (µmol/l)**
TAC (CRU)	p=0.24 r=0.11	**p=0.01 r=0.32**	p=0.23 r=0.12	p=0.46 r=0.076	p=0.94 r=-0.007
SuPAR (pg/ml)	p=0.13 r=-0.15	p=0.81 r=-0.02	p=0.17 r=-0.13	p=0.18 r=-0.13	p=0.07 r=-0.17
DHEAS (ng/ml)	**p=0.01 r=-0.25**	p=0.75 r=-0.03	p=0.12 r=-0.15	p=0.07 r=-0.17	p=0.88 r=-0.02
T (ng/ml)	p=0.44 r=-0.08**.**	p=0.06 r=0.18	p=0.35 r=-0.09	p=0.41 r=-0.08	p=0.09 r=-0.16
DHT (ng/ml)	**p=0.035 r=-0.24**	p=0.58 r=-0.05**.**	p=0.23 r=-0.12**.**	p=0.25 r=-0.11	**p=0.015 r=-0.24**
21-deoxycortisol (ng/ml)	p=0.76 r=0.04	p=0.08 r=0.22	p=0.38 r=-0.11	p=0.36 r=-0.11	p=0.12 r=0.19
Cortisol (ng/ml)	p=0.11 r=-0.15	p=0.11 r=-0.16	**p=0.024 r=-0.22**	**p=0.01 r=-0.24**	p=0.72 r=-0.04
Cortisone (ng/ml)	p=0.74 r=0.03	p=0.37 r=0.09	p=0.08 r=-0.17	p=0.08 r=-0.17	p=0.51 r=0.07
11-deoxycorticosterone (ng/ml)	p=0.07 r=-0.2	**p=0.001 r=-0.35**	p=0.49 r=-0.08	p=0.26 r=-0.12	p=0.82 r=0.03

4.2.6. Classic risk factors vs proposed new candidates for assesment of risk – findings regarding potential additional predictive value

Each factor that was considered as a potential candidate regarding risk assessment underwent a multivariate logistic regression model analysis. Classically known GDM risk factors (i.e.: age, BMI and fructosamine levels) were also analyzed and they demonstrated a significant correlation to GDM in our cohort. The baseline model included these three elements only, and it demonstrated a significant correlation to the development of GDM later during the pregnancy (R^2=0.464, p<0.001). Each parameter was also proven to be an independent risk factor (p<0.01 for each variable). TAC was integrated into the model analysis (age, BMI, fructosamine, TAC) this increased predictive power: R^2=0.597 (p<0.001). In this scenario all four analyzed variables were found to be significant independent determinants (p<0.05) regarding GDM. Similarly, to TAC, all examined novel markers except for 21-deoxycortisol were demonstrated to have a significant additive value to the baseline logistic regression model. They also all proved to be strong independent determinants. When including all these parameters the predictive power of the integrated model altered as follows: suPAR: R^2=0.514; testosterone: R^2=0.526; DHT: R^2=0.512; DHEA-S: R^2=0.530; cortison: R^2=0.619; 11-deoxycorticosterone: R^2=0.495 (p<0.001 for all models, and p<0.05 for all variables in each model). When performing a stepwise forward multivariate logistic regression model analysis that included not only the traditional but also all the novel markers, the highest predictive power that could be achieved was R^2=0.943 (p<0.001). This model included the following risk factor candidates: fructosamine, cortison, cortisol, 11-deoxycorticosterone and SuPAR, this model was shown to have a specificity of 96.6% and a sensitivity of 97.5%, respectively.

4.2.7. Maternal and neonatal pregnancy outcomes

Higher body weight, height, head, and-chest circumferences, were found in the GDM newborn group. **(Table 8)**.

A higher number of cesarean sections were performed in the GDM group (p<0.001) – the calculated relative risk from the date was 2.067 (Confidence Interval was: 1.266 to 3.375). GDM newborns were also found to demonstrate hypoglycemia (p<0.01) more frequently

– the calculated relative risk from the data was 1.146 (Confidence Interval was 1.036 to 1.268).

Table 8. Neonatal pregnancy outcomes

Higher body weight, height, head, and-chest circumferences, were found in the GDM newborn group. Two-tailed unpaired Student's t-test. Values are the means±SD (67).

Variable	*Control (n=55)*	*GDM (n=55)*	*Significance*
Birth weight (g)	3266.90 ± 236.1	4587.10 ± 630.80	p<0.001
Birth height (cm)	51.24 ± 1.72	52.84 ± 2.29	p<0.001
Head circumference (cm)	34.42 ± 1.46	35.61 ± 1.46	p<0.001
Chest circumference (cm)	33.42 ± 1.38	35.58 ± 1.85	p<0.001

5. DISCUSSION

In case of many diseases, risk assessment in the early stages of pregnancy (week 12 of gestation) is an important and nowadays highlighted part of the clinicians' work. For example, assessing risk regarding preeclampsia involves gathering data regarding the following: (1) maternal characteristics (i.e. weight, age, the occurrence of preeclampsia in previous pregnancies; (2) Results from the Doppler ultrasound; the uterine artery pulsatility index; (3) certain biomarkers - placental growth factor (PIGF), pregnancy-associated plasma protein A (PAPP-A), and soluble fms-like tyrosine kinase-1 (sFlt-1) (33). Should the risk of preeclampsia be calculated to be high the pregnancy itself is monitored more closely; also, certain additional data is collected more frequently regarding both urine protein levels and blood-pressure values. Preventive therapeutic measures may also be taken by initiating the administration of low dose acetyl-salicylate (150 mg) before the 16th gestational week (34). The rate of early detection by screening is 90% regarding risk assessment for early and 75% for preterm preeclampsia with a false-positive rate of 10% (35).

There is a definite clinical need for an early risk assessment tool in case of the following adverse events associated with pregnancy: intrauterine growth restriction (IUGR), low birth weight, and gestational diabetes mellitus (GDM). It would we highly desirable to effectively screen for these occurrences in the early stages of pregnancy (up to the 12th week of gestation) as this would allow the clinician to intervene – either by more closely monitoring the pregnancy or by implementing preventative or therapeutic measures (71).

Right on the verge of the first and second trimester physiological placentation may be measured via a well-established clinical marker, namely the marked decrease in the pulsatility index of the uterine artery. As the uteroplacental vasculature develops to fulfill its specialized role (to support and satisfy the growing nutritional and oxygen requirements of the fetus) a decline may be observed regarding this parameter, namely the appearance of expanded, high flow, low resistance vessels (72). Anomalies of placentation may occur, when this process is delayed, and this may be considered a red flag or even a warning sign regarding the onset of placental dysfunction later during the pregnancy. Measuring the blood flow of the uterine artery at the end of the first trimester by Doppler ultrasound and calculating both pulsatility index and resistance values is

widely accepted and practiced routine in obstetrics. Regarding preeclampsia and IUGR it has even been proposed to have a predictive value. Values exceeding the 95 percentile may be considered to be pathognostic for the development of preeclampsia later (73-76). The correlation between elevated resistance index or UtAPI and low birth weight and especially IUGR has been researched by many (30, 77-79).

Many physiological as well as pathological occurrences lead to the production of both nitrogen and oxygen derived reactive species, these act as mediators in various biological pathways. Overproduction of these species, however, causes oxidative-nitrative stress; this is the basis for the pathogenesis of a wide variety of diseases (80, 81). Physiological pregnancy is associated with an increase in oxidative stress levels; however, this increase is markedly more pronounced both in the placenta and the blood plasma in pathological occurrences such as preeclampsia, GDM or IUGR (82, 83). By the onset of the third trimester, physiological pregnancy is characterized by elevated nitrative stress levels; however, in association with the above listed pathologies this may occur earlier on and be more marked (84, 85).

A meta-analysis conducted regarding randomized controlled studies revealed that the risk of GDM may be lowered effectively with the timely implementation of preventative and therapeutic measures; this is especially true if the initial risk is calculated to be high (i.e. 20%). Lifestyle changes implemented before and during the pregnancy – such as modifying diet and performing regular exercise (50-60 minutes twice a week during the pregnancy) - may decrease this calculated risk of GDM by as much as 20% (86). Decreasing BMI from excess weight or obesity to normal also decreases risk of GDM (87, 88). However, the beneficial effects regarding prevention of later onset of GDM by adapting a light exercise routine in the second trimester has not been proven to be effective (89-91).

There is an effective clinical tool for the early risk assessment of preeclampsia, however as to date there is no first trimester evidence-based risk assessment tool for the prediction of later onset GDM. The moderate predictive values of the following parameters have been described: BMI, maternal age, parity, large for gestational age fetus or having a

history of GDM during previous pregnancies (92-94). A series of retrospective studies – with designs like ours – have proposed certain biomarkers as having further predictive value regarding GDM. The most proposed markers - fasting and post-load glucose levels and HbgA1c - have failed to effectively improve rates of prediction (95, 96). Biomarkers associated with inflammatory processes (TNF-alpha, CRP, etc.), liver function (GGT, ALT, etc.) (97), blood trace elements and vitamins (vitamin A, vitamin D, selenium) (98-100), markers characterizing platelets (101), adipokines (leptin, adiponectin) (97), specific miRNAs (102) and numerous combinations of routine laboratory parameters have all previously been studied; they yielded maximum specificity and sensitivity levels of around 60-77% (103, 104).

The goal of our observational study was to analyze the relationship between UtAPI and systemic oxidative-nitrative stress at the 12-13th gestational week of a healthy pregnancy, and to examine their correlation to neonatal characteristics (i.e. birth weight). We also aimed to study the relationship between the development of GDM and oxidative-nitrative stress marker levels, SuPAR, androgen and other corticosteroid hormones and their derivatives at the end of the first trimester to attempt to pinpoint possible novel early markers of GDM.

5.1. Uterine artery pulsatility index and oxidative - nitrative stress in early pregnancy

High-UtAPI lead to significantly lesser chest circumference and birth weight in newborns even though gestational week values did not differ at birth from the low-UtAPI group. UtAPI was even found to correlate negatively regarding newborn birth weight values even in normal weight neonates. This would suggest even during physiological pregnancy UtAPI measured right between the first and the second trimester may have predictive value regarding adverse placental function and fetal development. As placentation is only one of several factors that influence fetal growth, measuring UtAPI at the end of the first trimester only carries a low predictive values regarding low birth weight, SGA and IUGR in the otherwise low-risk population (30) – as has been demonstrated by numerous systematic reviews and meta-analyses (105-107).

Therefore, during early pregnancies, it is advisable to measure other parameters beside UtAPI and to study their correlations and relationships to be able to predict SGA and IUGR more effectively. This was the rationale for our measuring not only UtAPI but oxidative-nitrative stress also in our cohort.

The plasma PRX (indicative of oxidative stress) levels were demonstrated to be significantly lower in the high UtAPI group compared to the low UtAPI group. TAC was significantly greater in the high UtAPI group compared to low UtAPI group. Initial placentation is heavily influenced by oxidative stress in the first trimester, oxygen tension triples by the end of the first trimester, leading to a consequent elevation of ROS levels. This in turn, effects development of the placenta further. The placenta attempts to compensate for the elevation in oxidative stress by increasing the amount of cellular antioxidants (108). The high UtAPI group - where oxidative stress was low - may demonstrate that this oxidative burst was either impaired or delayed. The negative correlation found between UtAPI and oxidative stress, and the positive relationship between UtAPI and birth weight in healthy pregnancies may also support this hypothesis. Further analysis of the effects and correlations of ROS and pathological occurrences or outcomes during and after pregnancies is necessary regarding pathological pregnancies. This is the rationale behind the development of a more sensitive and effective preventive tool like PIPX, which involves both classical and more novel oxidative stress parameters. PIPX is a variable that was developed to reflect the simultaneous effects of UtAPI and plasma PRX (as they both demonstrated a correlation with low birth weight in newborns). PIPX is calculated from these parameters using the following formula: (UtAPI/PRX)*100. The correlation between PIPX and low birthweight was found to be stronger than when measuring UtAPI alone. This suggests that including oxidative stress level values when estimating fetal development improves the predictive value of UtAPI.

The trophoblast cells effect a secondary invasion into the placenta at the time of this oxidative burst (109); this is associated with consequential cellular degradation, which leads to elevated levels of LDH in the serum. In our cohort LDH concentration was found to be lower when measured at the end of the first trimester in the high UtAPI group. It is our hypothesis that lower LDH levels represent a decrease regarding trophoblast invasion. Our research group also demonstrated previously that even following a physiological pregnancy, oxidative stress remains elevated even after three years (110). Having

undergone a higher number of pregnancies may also lead to elevated oxidative stress levels in low-resistance pregnant women. This hypothesis may also be supported by the finding of our study that TAC correlates negatively to the previous number of pregnancies.

The low UtAPI group was shown to have higher scores regarding both gravidity and parity. Previous studies have also revealed that muliparity has a protective effect regarding the development of high UtAPI. In our cohort gravidity and parity were both higher in the low UtAPI group supporting the thesis that multiparous women (those whose physiology has had previous experience in placentation) may be more effective and successful regarding trophoblast invasion and circulatory adaptation during a next pregnancy (111).

Even though oxidative stress was measured to be decreased in high UtAPI group, nitrative stress values did not differ between the two groups in our study. The interaction between protein tyrosine residues and peroxynitrite leads to the production of nitrotyrosine; while the reactions between nitric oxide and superoxide anions produce peroxynitrite (112). When taking these pathways into consideration we may expect nitrotyrosine levels to adapt to and follow the changes occurring in PRX concentration. Previous data in the literature has, however failed to confirm, or demonstrate such a correlation either in under pathological or in healthy conditions (110, 113). A positive correlation was demonstrated between nitrative stress (intracellular) and serum GGT values. There are numerous studies that concluded that GGT may be a possible marker of both oxidative stress and inflammation. A study published by Simona Bo et al. found that NT and serum GGT correlate positively (114). A novel fact was revealed by our study in this field: intracellular NT levels correlated positively with the necessity to perform a Cesarean section.

5.2. First trimester prediction of GDM

Randomly selected women – who completed a physiological or a GDM pregnancy were included in our cohort. At the end of the first trimester (11 (+0 days) and 13 (+6 days) weeks of gestation) possible novel GDM prediction markers were recorded. Our result, showing that the women who later developed GDM during the pregnancy were characterized by higher age, body weight, BMI, and serum fructosamine values; this

proved to be in accordance with previous data from the literature regarding GDM pregnancies (115, 116)

5.2.1. First trimester SuPAR and oxidative-nitrative stress markers in GDM

Our results showed that serum total antioxidant capacity (TAC) was significantly increased at the end of the first trimester in women who developed GDM later during the pregnancy, without a significant change in plasma total peroxide levels (PRX) and oxidative stress index calculated by PRX/TAC.

Elevated serum glucose levels are associated with increased oxidative-nitrative stress levels in both type 1 and type 2 DM. Type 2 DM and consequentially occurring complications have been linked to decreased antioxidant levels (117-119). Increased oxidative and nitrative stress has also been described to be associated with the development of insulin resistance and with the dysfunction of the beta cells themselves. Several studies have focused on the correlation between oxidative and nitrative stress marker levels in both physiological pregnancies and in GDM. These studies demonstrated that oxidative stress increases near term even in physiological conditions, however, GDM pregnancies demonstrate markedly greater oxidative-nitrative stress levels (120). The limitation of these studies is that they were conducted in the third trimester of pregnancy following the established diagnosis of GDM; maximum around the 16-18th gestational week in the high-risk population. In a study involving GDM pregnancies conducted by Hongwei Lia et al demonstrated that between 16th and 20th and the 32nd to 36th week of gestation 8-iso-prostaglandin F2α, advanced oxidative protein products and protein carbonyl were markedly elevated in GDM (121). Based on the findings of K. A. Sudharshana Murthy et al by their study conducted in high GDM risk patients, those who developed GDM had elevated levels of both pro-inflammatory and oxidative stress markers between gestational weeks 24 to 28 and 12 to 16. The GDM group also demonstrated significantly elevated levels of the following pro-inflammatory agents: cytokines interleukin-8 and interleukin-6 (122). Clinically established GDM has been proven to be accompanied by a significant decrease regarding TAC levels (123, 124) by a number of publications, however diet and antioxidant intake has also been described to reduce the abnormal glucose levels during pregnancy (43). However, Ulduz Zamani-

Ahari et al. measured TAC levels to be elevated in the saliva of women during GDM pregnancies (125).

Results from our cohort demonstrate that at the end of the first trimester TAC increases significantly in pregnancies where GDM manifests later, while PRX levels do not alter significantly. The changes in the ratio of PRX/TAC – an index indicative of oxidative stress-, suggests that there is an increase in the production of reactive free radical agents, but also in the endogenous production of compensatory antioxidants. PRX and TAC – known to carry risk regarding GDM – both demonstrated a positive correlation with BMI values. This suggests a correlation between excess weight and higher oxidative stress levels. The multiple logistic linear regression model revealed that an increase in TAC levels is independently associated with the development of GDM later during the pregnancy.

Serum SuPAR levels are well-known and documented to be elevated both in cases of T2DM and in preeclampsia (51, 126). In our study at the end of the first trimester the level of serum SuPAR was decreased in women who later developed manifest GDM during their pregnancies. The logistic regression analysis revealed that the decrease in serum suPAR levels is an independent risk factor regarding the later onset of GDM. We suppose a biphasic change of SuPAR in GDM pregnancy; lower levels at the first trimester and higher in the third, but this is a novel result, no one examined it before us. Therefore, further analysis and research is necessary to reveal the relationships and correlations between serum suPAR levels and the development of clinically manifest GDM.

5.2.2. The role of glucocorticoids

Alterations regarding glucocorticoid levels in the maternal serum at the end of the first trimester were analyzed in our cohort. 21-deoxycortisol and cortisone were increased in the GDM group, while cortisol level was decreased. Impaired glucose tolerance during pregnancy has long been linked to an increase in cortisol and cortisol derivatives; as most of the steroid hormones are contra-insular, diabetogenic hormones, they affect the function of the beta cells themselves regardless of pregnancy. These hormones

demonstrate a marked elevation during pregnancy, leading to adverse effects regarding glucose tolerance. The focus of previous studies has been the measurement and analysis of steroid hormone biosynthesis and tryptophan metabolites in urine samples from women with manifest GDM (127).

These are data available regarding steroid metabolites collected from women with manifest GDM during the third trimester. These data however were not collected for screening or risk assessment purposes. The following results were found in this group: an increased clearance of tetrahydroaldosterone-3-glucuronide, 21-deoxycortisol 5-androstene-3b,16b,17a-triol, 11-oxo-androsterone-glucuronide, cortolone-3-glucuronide, (127). Active cortisol is transformed into inactive cortisone by the 11beta-hydroxysteroid dehydrogenase enzyme (11β-HSDs) is responsible for transforming active cortisol into. At term both cortisone and cortisol levels were measured via high performance liquid chromatography. Significantly higher values were found in the maternal serum of GDM patients, while cortisone levels did not differ between the groups (65). In manifest GDM 11β-HSD1 levels were decreased while 11β-HSD2 were increased when measured in samples from the placenta (65). Gestational weeks 11-14 were chosen for sampling plasma in our study; cortisol levels decreased while cortisone levels increased in the later GDM women and these are independent risk factors regarding GDM. Changing cortisol and cortisone levels from early to late pregnancy in GDM might sign altered placental function from the time of placentation.

5.2.3. Androgens

In our study, later GDM patients' DHEA-S level was significantly lower at the end of the first trimester. This indicates that patients who develop GDM later during their pregnancy may have altered sulfatase or sulfotransferase enzyme functions at this stage of the pregnancy. Testosterone level was significantly increased in the GDM group compared to the controls. Elevated testosterone levels were associated with decreased DHT levels and reduced DHT/T ratio, suggesting saturation or reduced capacity of the 5-alpha reductase enzyme. Possible correlations between maternal insulin resistance and androgen levels measured in the fetus, mRNA and proteins linked to placental androgen production were previously investigated by Morisset et al. Dihydrotestosterone, total testosterone and dehydroepiandrosterone were measured in maternal plasma samples

following a glucose tolerance test (75g per of glucose intake) performed at gestational week 26.1 ± 3.7. A significant and inverse correlation was found between plasma testosterone and DHEA levels and insulin sensitivity index (Matsuda); this correlation was significant even following correction for differences in BMI. A positive correlation was found between testosterone and DHEA levels and HOMA-IR. Serum glucose levels – measured at 120 minutes during the glucose tolerance test –, and both the HOMA-IR and the Matsuda (insulin sensitivity) indexes correlated significantly with maternal testosterone levels. However, mRNA and gene expression of the proteins associated with steroid production in the placenta did not show a significant difference compared to maternal insulin resistance (128). Testosterone production in the placenta was shown to be increased in women who developed GDM - Uzalec et al. found this to be caused by a decrease in aromatase (a rate-limiting enzyme in the conversion process from androgens to estrogen) activity in the placenta; most probably due to a noticeable decrease in protein expression in the placenta. However, mRNA expression remained unaltered regarding the aromatase gene (129). As mentioned above, we suggest a reduced functional capacity of the 5-alpha reductase enzyme. The finding that in GDM women the ratio of 5-alpha-tehtrahydro cortisol to tetrahydrocortisol was reduced in urine samples would appear to support this hypothesis (130).

There is a definite need for further research regarding the effects of changes in androgen levels during the first trimester of pregnancy and the pathogenesis of GDM, results from our study suggest that these alterations may play a significant role regarding the prediction of later onset GDM.

5.2.4. The role of mineralocorticoids

Our study revealed that in later GDM women, the level of 11-deoxycorticosterone, measured at the end of the first trimester, decreased significantly. Possible correlations between 11-deoxycorticosterone level and the development of GDM have not been examined previously. However, studies have described an association between increased levels of 11-deoxycorticosterone and aldosterone in pre-diabetes and the prevalence of T2DM (however, the correlation of T2DM and 11-deoxycorticosterone did not remain significant level statistically following correction) (131).

5.2.5. Prediction of later onset GDM

In our study cohort SuPAR, TAC, 21-deoxycortisol, cortisol, cortisone, DHT, testosterone, DHEA-S, and 11-deoxycorticosterone measured at the end of the first trimester, all be significant contributing factors regarding predictive power of GDM compared to using classical risk factors only as independent determinants. In our current study we utilized all available parameters to build a logistic regression model. This enhanced GDM prediction model has a predictive power of R^2=0.943. To date, the predictive power of this model is superior to any previously published one, as regarding prediction of later onset GDM it provides a specificity index of 96.6% and a sensitivity of 97.5%. This model included the following risk factor candidates: fructosamine, cortison, cortisol, 11-deoxycorticosterone and SuPAR. Application of this model allows prediction GDM between gestational week 24-28 at the end of the first trimester. This provides an opportunity for either slowing or limiting the progression of GDM or in certain cases even preventing development of GDM altogether. The prevention or better management of GDM by timely intervention has a significant beneficial impact regarding the cardiometabolic risk of both mother and offspring.

5.3. Limitations and strength of our study

Regarding UtAPI and oxidative-nitrative stress markers: the high variability of these markers during pregnancy must be considered a limitation of our cohort. It also led to difficulties when determining distinct cutoff values regarding these markers in pathological outcomes and adverse events during pregnancies. Combined parameters, such as PIPX (calculated from the measured values of PRX and UtAPI) and PRX, may rightly be expected to improve predictive values.

Possible novel markers were integrated with previously defined ones to improve GDM prediction rates in early pregnancy, namely at the end of the first trimester. Two promising fields of markers were chosen as focal points: steroid hormone and oxidative stress metabolites. Analysis of our data resulted in the proposal of nine possible novel markers and the building of a promisingly efficient early prediction model. However, the women being all from the Caucasian race is a limitation that must be acknowledged. Our

early prediction model itself is unique and precise GDM risk assessment in the first trimester.

6. CONCLUSIONS

After analyzing the results from our study, we propose the following conclusions:

1. Measuring oxidative stress and pulsatility of the uterine artery in early phase pregnancies

-In our cohort high UtAPI alone (with no additional risk factors) resulted in normal range but lower weight newborns.

-LDH and plasma oxidative stress (associated with abnormalities of placentation) were however decreased in our high UtAPI group.

-In further support of this hypothesis, we also found that a combined parameter (PIPX: including both UtAPI and PRX- a marker of oxidative stress) strengthens correlation to birthweight. Higher uterine artery pulsatility index and lower oxidative stress in the first trimester – during the placentation process, are predict lower birthweight.

2. Regarding later onset GDM the following novel risk prediction markers – measured at the end of the first triemster- were identified: SuPAR, TAC, DHEA S, testosterone DHT, cortisone, cortisol, 21-deoxycortisol and 11-deoxycorticosterone.

- When measured at the end of the first trimester women who had GDM pregnancies had significantly higher serum TAC levels and decreased serum SuPAR levels.
- In the later onset GDM group cortisol levels were decreased, while both cortisone and 21-deoxycortisol levels were increased.
- The GDM group demonstrated decreased DHEA-S and dihydrotestosterone levels, and DHT/T ratios, while serum testosterone levels were increased.
- In the GDM group 11-deoxycorticosterone levels were observed to be lower.

-By including both previously known “classical” risk factors and the novel ones, we were successful in building an effective logistic regression model for early prediction of GDM. This enhanced GDM prediction model is superior to any previously published one with a specificity index of 96.6% and a sensitivity of 97.5%. This model included the following risk factor candidates: fructosamine, cortison, cortisol, 11-deoxycorticosterone and SuPAR.

7. SUMMARY

Aims/hypothesis: the goal of our study was to analyze the correlation between UtAPI and systemic oxidative-nitrative stress at the end of the first trimester. We also aimed to identify novel early, first trimester prediction markers for GDM.

Methods: Healthy pregnant women at the end of the first trimester were divided into two groups: the high (UtAPI≥2.3) (n=30) and the low (n=31) UtAPI groups (Doppler ultrasound was used to measure UtAPI). Anthropometric data, rutin lab parameters, pregnancy outcomes and labor circumstances were monitored. Nitrotyrosine levels, total peroxide levels and TAC were measured to indicate systemic oxidative-nitrative stress.

We made a case-control study what based on a study cohort of a Hungarian biobank containing the biological samples, anthropometric data and follow ups from 2545 pregnant women. Oxidative-nitrative stress related parameters, steroid hormone, and metabolits were measured in the serum/plasma samples collected at the end of the first trimester from 55-55 randomly selected control and later GDM women.

Results: The high UtAPI group demonstrated significantly lower plasma total peroxide and higher TAC levels; this was also associated with lower values regarding birthweight. Pregnant women, who later developed GDM, were older and had higher body mass indexes. The GDM group was demonstrated to have higher levels of the following markers in their serum/plasma samples: TAC, fructosamine, testosterone, 21-deoxycortisol; cortisone. Meanwhile DHT, DHEA-S, 11-deoxycorticosterone and cortisol levels decreased. A forward stepwise multivariate logistic regression model was used to predict later GDM with a specificity of 96.6% and sensitivity of 97.5% (included variables: fructosamine, cortisol, cortisone, 11-deoxycorticosterone, SuPAR).

Conclusions: Based on our results we may conclude that between gestational weeks of 12 to 13 high UtAPI in correlation with lower systemic oxidative stress may be considered to have predictive value regarding the birthweight of healthy newborns. Later onset GDM – between gestational weeks 24 and 28 - may accurately be predicted based on the measurement of the following parameters at the end of the first trimester: oxidative stress markers, steroid hormones, and metabolites. Early risk prediction provides the opportunity for targeted prevention and an early treatment plan for later onset GDM.

8. REFERENCES

1. Caballero Sanz S, Nozaleda Pastor G, Garcia-Tizon Larroca S. First-Trimester Biochemical Screening For Low Birth Weight: Clinical Effectiveness of Low Pregnancy-Associated Plasma Protein-A and High Thyroid-Stimulating Hormone. Clinical laboratory. 2018;64(9):1501-8.
2. Martins JG, Biggio JR, Abuhamad A. Society for Maternal-Fetal Medicine Consult Series #52: Diagnosis and management of fetal growth restriction: (Replaces Clinical Guideline Number 3, April 2012). Am J Obstet Gynecol. 2020;223(4):B2-b17.
3. Mlynarczyk M, Chauhan SP, Baydoun HA, Wilkes CM, Earhart KR, Zhao Y, et al. The clinical significance of an estimated fetal weight below the 10th percentile: a comparison of outcomes of <5th vs 5th-9th percentile. Am J Obstet Gynecol. 2017;217(2):198.e1-.e11.
4. Mari G. Fetal growth restriction: Evaluation and management. Lockwood CJ, Levine D, editors: UpToDate. ; 2022.
5. Chew LC, Verma RP. Fetal Growth Restriction. StatPearls. Treasure Island (FL): StatPearls Publishing
Copyright © 2022, StatPearls Publishing LLC.; 2022.
6. Eggenhuizen GM, Go A, Koster MPH, Baart EB, Galjaard RJ. Confined placental mosaicism and the association with pregnancy outcome and fetal growth: a review of the literature. Hum Reprod Update. 2021;27(5):885-903.
7. Kanter D, Lindheimer MD, Wang E, Borromeo RG, Bousfield E, Karumanchi SA, et al. Angiogenic dysfunction in molar pregnancy. Am J Obstet Gynecol. 2010;202(2):184 e1-5.
8. Jeyabalan A. Epidemiology of preeclampsia: impact of obesity. Nutr Rev. 2013;71 Suppl 1:S18-25.
9. Lisonkova S, Joseph KS. Incidence of preeclampsia: risk factors and outcomes associated with early- versus late-onset disease. Am J Obstet Gynecol. 2013;209(6):544 e1- e12.
10. Uzan J, Carbonnel M, Piconne O, Asmar R, Ayoubi JM. Pre-eclampsia: pathophysiology, diagnosis, and management. Vasc Health Risk Manag. 2011;7:467-74.

11. Lorenzo-Almorós A, Hang T, Peiró C, Soriano-Guillén L, Egido J, Tuñón J, et al. Predictive and diagnostic biomarkers for gestational diabetes and its associated metabolic and cardiovascular diseases. Cardiovasc Diabetol. 2019;18(1):140.

12. ACOG Practice Bulletin No. 190: Gestational Diabetes Mellitus. Obstet Gynecol. 2018;131(2):e49-e64.

13. Solomon CG, Willett WC, Carey VJ, Rich-Edwards J, Hunter DJ, Colditz GA, et al. A prospective study of pregravid determinants of gestational diabetes mellitus. JAMA. 1997;278(13):1078-83.

14. Kim C, Liu T, Valdez R, Beckles GL. Does frank diabetes in first-degree relatives of a pregnant woman affect the likelihood of her developing gestational diabetes mellitus or nongestational diabetes? Am J Obstet Gynecol. 2009;201(6):576.e1-6.

15. Hedderson MM, Williams MA, Holt VL, Weiss NS, Ferrara A. Body mass index and weight gain prior to pregnancy and risk of gestational diabetes mellitus. Am J Obstet Gynecol. 2008;198(4):409.e1-7.

16. Hedderson MM, Gunderson EP, Ferrara A. Gestational weight gain and risk of gestational diabetes mellitus. Obstet Gynecol. 2010;115(3):597-604.

17. Gibson KS, Waters TP, Catalano PM. Maternal weight gain in women who develop gestational diabetes mellitus. Obstet Gynecol. 2012;119(3):560-5.

18. Carreno CA, Clifton RG, Hauth JC, Myatt L, Roberts JM, Spong CY, et al. Excessive early gestational weight gain and risk of gestational diabetes mellitus in nulliparous women. Obstet Gynecol. 2012;119(6):1227-33.

19. Getahun D, Fassett MJ, Jacobsen SJ. Gestational diabetes: risk of recurrence in subsequent pregnancies. Am J Obstet Gynecol. 2010;203(5):467.e1-6.

20. Feng Y, Zhao Z, Fu D, Gao W, Zhang F. Maternal and neonatal outcomes after energy-restricted diet for women with gestational diabetes mellitus: A systematic review and meta-analysis of randomized controlled trials. Medicine (Baltimore). 2021;100(14):e25279.

21. Chatzakis C, Sotiriadis A, Tsakmaki E, Papagianni M, Paltoglou G, Dinas K, et al. The Effect of Dietary Supplements on Oxidative Stress in Pregnant Women with Gestational Diabetes Mellitus: A Network Meta-Analysis. Nutrients. 2021;13(7).

22. Ding Q, Hu Y, Fu Y, Qian L. Systematic review and meta-analysis of the correlation between intestinal flora and gestational diabetes mellitus. Ann Palliat Med. 2021;10(9):9752-64.

23. Guariguata L, Linnenkamp U, Beagley J, Whiting DR, Cho NH. Global estimates of the prevalence of hyperglycaemia in pregnancy. Diabetes Res Clin Pract. 2014;103(2):176-85.

24. International Association of D, Pregnancy Study Groups Consensus P, Metzger BE, Gabbe SG, Persson B, Buchanan TA, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care. 2010;33(3):676-82.

25. Shah NS, Wang MC, Freaney PM, Perak AM, Carnethon MR, Kandula NR, et al. Trends in Gestational Diabetes at First Live Birth by Race and Ethnicity in the US, 2011-2019. Jama. 2021;326(7):660-9.

26. Hugh O, Williams M, Turner S, Gardosi J. Reduction of stillbirths in England from 2008 to 2017 according to uptake of the Growth Assessment Protocol: 10-year population-based cohort study. Ultrasound Obstet Gynecol. 2021;57(3):401-8.

27. Gosling RG, Dunbar G, King DH, Newman DL, Side CD, Woodcock JP, et al. The quantitative analysis of occlusive peripheral arterial disease by a non-intrusive ultrasonic technique. Angiology. 1971;22(1):52-5.

28. Stuart B, Drumm J, FitzGerald DE, Duignan NM. Fetal blood velocity waveforms in normal pregnancy. British journal of obstetrics and gynaecology. 1980;87(9):780-5.

29. Bhide A, Acharya G, Baschat A, Bilardo CM, Brezinka C, Cafici D, et al. ISUOG Practice Guidelines (updated): use of Doppler velocimetry in obstetrics. Ultrasound Obstet Gynecol. 2021;58(2):331-9.

30. Sarmiento A, Casasbuenas A, Rodriguez N, Angarita AM, Sarmiento P, Sepulveda W. First-trimester uterine artery Doppler velocimetry in the prediction of birth weight in a low-risk population. Prenatal diagnosis. 2013;33(1):21-4.

31. Drouin O, Boutin A, Paquette K, Gasse C, Guerby P, Demers S, et al. First-Trimester Uterine Artery Doppler for the Prediction of SGA at Birth: The Great Obstetrical Syndromes Study. Journal of obstetrics and gynaecology Canada : JOGC = Journal d'obstetrique et gynecologie du Canada : JOGC. 2018;40(12):1592-9.

32. Feng Y, Zheng H, Fang D, Mei S, Zhong W, Zhang G. Prediction of late-onset fetal growth restriction using a combined first- and second-trimester screening model. Journal of gynecology obstetrics and human reproduction. 2022;51(2):102273.
33. Poprawski G, Wender-Ozegowska E, Zawiejska A, Brazert J. [Modern methods of early screening for preeclampsia and pregnancy-induced hypertension--a review]. Ginekol Pol. 2012;83(9):688-93.
34. Choi YJ, Shin S. Aspirin Prophylaxis During Pregnancy: A Systematic Review and Meta-Analysis. Am J Prev Med. 2021;61(1):e31-e45.
35. Chaemsaithong P, Sahota DS, Poon LC. First trimester preeclampsia screening and prediction. Am J Obstet Gynecol. 2022;226(2S):S1071-S97 e2.
36. Benaiges D, Flores-Le Roux JA, Marcelo I, Mañé L, Rodríguez M, Navarro X, et al. Is first-trimester HbA1c useful in the diagnosis of gestational diabetes? Diabetes Res Clin Pract. 2017;133:85-91.
37. Farrar D, Simmonds M, Bryant M, Lawlor DA, Dunne F, Tuffnell D, et al. Risk factor screening to identify women requiring oral glucose tolerance testing to diagnose gestational diabetes: A systematic review and meta-analysis and analysis of two pregnancy cohorts. PloS one. 2017;12(4):e0175288.
38. Zhao Y, Zhao Y, Fan K, Jin L. Serum uric acid in early pregnancy and risk of gestational diabetes mellitus: a cohort study of 85,609 pregnant women. Diabetes & metabolism. 2021:101293.
39. Leitner M, Fragner L, Danner S, Holeschofsky N, Leitner K, Tischler S, et al. Combined Metabolomic Analysis of Plasma and Urine Reveals AHBA, Tryptophan and Serotonin Metabolism as Potential Risk Factors in Gestational Diabetes Mellitus (GDM). Front Mol Biosci. 2017;4:84.
40. Corcoran SM, Achamallah N, Loughlin JO, Stafford P, Dicker P, Malone FD, et al. First trimester serum biomarkers to predict gestational diabetes in a high-risk cohort: Striving for clinically useful thresholds. Eur J Obstet Gynecol Reprod Biol. 2018;222:7-12.
41. Chen X, Scholl TO. Oxidative stress: changes in pregnancy and with gestational diabetes mellitus. Curr Diab Rep. 2005;5(4):282-8.

42. Gosmaro F, Bagnati M, Berto S, Bellomo G, Prenesti E. Measurement of total antioxidant capacity of human plasma: setting and validation of the CUPRAC-BCS method on routine apparatus ADVIA 2400. Talanta. 2013;115:526-32.
43. Daneshzad E, Tehrani H, Bellissimo N, Azadbakht L. Dietary Total Antioxidant Capacity and Gestational Diabetes Mellitus: A Case-Control Study. Oxid Med Cell Longev. 2020;2020:5471316.
44. Chiarello DI, Abad C, Rojas D, Toledo F, Vazquez CM, Mate A, et al. Oxidative stress: Normal pregnancy versus preeclampsia. Biochim Biophys Acta Mol Basis Dis. 2020;1866(2):165354.
45. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010;107(9):1058-70.
46. Zejnullahu VA, Zejnullahu VA, Kosumi E. The role of oxidative stress in patients with recurrent pregnancy loss: a review. Reprod Health. 2021;18(1):207.
47. D'Souza V, Rani A, Patil V, Pisal H, Randhir K, Mehendale S, et al. Increased oxidative stress from early pregnancy in women who develop preeclampsia. Clin Exp Hypertens. 2016;38(2):225-32.
48. McElwain CJ, Tuboly E, McCarthy FP, McCarthy CM. Mechanisms of Endothelial Dysfunction in Pre-eclampsia and Gestational Diabetes Mellitus: Windows Into Future Cardiometabolic Health? Front Endocrinol (Lausanne). 2020;11:655.
49. Zhang Q, Li L, Chen H, Zhang G, Zhu S, Kong R, et al. Soluble urokinase plasminogen activator receptor associates with higher risk, advanced disease severity as well as inflammation, and might serve as a prognostic biomarker of severe acute pancreatitis. J Clin Lab Anal. 2020;34(3):e23097.
50. Liu S, Zheng Q, Cui XY, Dai KX, Yang XS, Li FS, et al. Expression of uPAR in human trophoblast and its role in trophoblast invasion. Int J Clin Exp Pathol. 2015;8(11):14325-34.
51. Rasmussen LJH, Petersen JEV, Eugen-Olsen J. Soluble Urokinase Plasminogen Activator Receptor (suPAR) as a Biomarker of Systemic Chronic Inflammation. Front Immunol. 2021;12:780641.
52. Odden N, Roland MC, Lorentzen B, Morkrid L, Henriksen T. PP071. suPAR levels in normal- and preeclamptic pregnancies. Pregnancy Hypertens. 2013;3(2):93.

53. Noyola-Martínez N, Halhali A, Barrera D. Steroid hormones and pregnancy. Gynecol Endocrinol. 2019;35(5):376-84.
54. Aplin JD, Jones CJ, Harris LK. Adhesion molecules in human trophoblast - a review. I. Villous trophoblast. Placenta. 2009;30(4):293-8.
55. Pidoux G, Gerbaud P, Cocquebert M, Segond N, Badet J, Fournier T, et al. Review: Human trophoblast fusion and differentiation: lessons from trisomy 21 placenta. Placenta. 2012;33 Suppl:S81-6.
56. Pandey Y, Pooja AR, Devi HL, Jalmeria NS, Punetha M, Kumar S, et al. Expression and functional role of IGFs during early pregnancy in placenta of water buffalo. Theriogenology. 2021;161:313-31.
57. Pasqualini JR, Chetrite GS. The formation and transformation of hormones in maternal, placental and fetal compartments: biological implications. Horm Mol Biol Clin Investig. 2016;27(1):11-28.
58. Kaludjerovic J, Ward WE. The Interplay between Estrogen and Fetal Adrenal Cortex. J Nutr Metab. 2012;2012:837901.
59. Holinka CF, Diczfalusy E, Coelingh Bennink HJ. Estetrol: a unique steroid in human pregnancy. J Steroid Biochem Mol Biol. 2008;110(1-2):138-43.
60. Tskitishvili E, Pequeux C, Munaut C, Viellevoye R, Nisolle M, Noel A, et al. Estrogen receptors and estetrol-dependent neuroprotective actions: a pilot study. J Endocrinol. 2017;232(1):85-95.
61. Davezac M, Zahreddine R, Buscato M, Smirnova NF, Febrissy C, Laurell H, et al. The different natural estrogens promote endothelial healing through distinct cell targets. JCI Insight. 2023;8(5).
62. Carter AM. Evolution of placental function in mammals: the molecular basis of gas and nutrient transfer, hormone secretion, and immune responses. Physiological reviews. 2012;92(4):1543-76.
63. Azziz R, Carmina E, Chen Z, Dunaif A, Laven JS, Legro RS, et al. Polycystic ovary syndrome. Nat Rev Dis Primers. 2016;2:16057.
64. Gozukara YM, Aytan H, Ertunc D, Tok EC, Demirturk F, Sahin S, et al. Role of first trimester total testosterone in prediction of subsequent gestational diabetes mellitus. J Obstet Gynaecol Res. 2015;41(2):193-8.

65. Ma R, Liu J, Wu L, Sun J, Yang Z, Yu C, et al. Differential expression of placental 11beta-hydroxysteroid dehydrogenases in pregnant women with diet-treated gestational diabetes mellitus. Steroids. 2012;77(7):798-805.

66. Feng Y, Feng Q, Qu H, Song X, Hu J, Xu X, et al. Stress adaptation is associated with insulin resistance in women with gestational diabetes mellitus. Nutr Diabetes. 2020;10(1):4.

67. Gerszi D, Orosz G, Torok M, Szalay B, Karvaly G, Orosz L, et al. Risk estimation of gestational diabetes mellitus in the first trimester. J Clin Endocrinol Metab. 2023. dgad301. doi: 10.1210/clinem/dgad301.

68. Valent S, Nemeth J, Sara L, Gidai J, Toth P, Schaff Z, et al. High early uterine vascular resistance values increase the risk of adverse pregnancy outcome independently from placental VEGF and VEGFR1 reactivities. Eur J Obstet Gynecol Reprod Biol. 2011;156(2):165-70.

69. Karvaly G, Kovacs K, Meszaros K, Kocsis I, Patocs A, Vasarhelyi B. The comprehensive characterization of adrenocortical steroidogenesis using two-dimensional ultra-performance liquid chromatography - electrospray ionization tandem mass spectrometry. J Pharm Biomed Anal. 2018;153:274-83.

70. Gerszi D, Penyige A, Mezei Z, Sarai-Szabo B, Benko R, Banyai B, et al. Evaluation of oxidative/nitrative stress and uterine artery pulsatility index in early pregnancy. Physiol Int. 2021;107(4):479-90.

71. Salomon LJ, Alfirevic Z, Da Silva Costa F, Deter RL, Figueras F, Ghi T, et al. ISUOG Practice Guidelines: ultrasound assessment of fetal biometry and growth. Ultrasound Obstet Gynecol. 2019;53(6):715-23.

72. Gomez O, Figueras F, Fernandez S, Bennasar M, Martinez JM, Puerto B, et al. Reference ranges for uterine artery mean pulsatility index at 11-41 weeks of gestation. Ultrasound Obstet Gynecol. 2008;32(2):128-32.

73. Pijnenborg R, Bland JM, Robertson WB, Brosens I. Uteroplacental arterial changes related to interstitial trophoblast migration in early human pregnancy. Placenta. 1983;4(4):397-413.

74. Jauniaux E, Greenwold N, Hempstock J, Burton GJ. Comparison of ultrasonographic and Doppler mapping of the intervillous circulation in normal and abnormal early pregnancies. Fertil Steril. 2003;79(1):100-6.

75. McLeod L. How useful is uterine artery Doppler ultrasonography in predicting pre-eclampsia and intrauterine growth restriction? CMAJ. 2008;178(6):727-9.

76. Gonzalez-Gonzalez NL, Gonzalez-Davila E, Gonzalez Marrero L, Padron E, Conde JR, Plasencia W. Value of placental volume and vascular flow indices as predictors of intrauterine growth retardation. Eur J Obstet Gynecol Reprod Biol. 2017;212:13-9.

77. Martin AM, Bindra R, Curcio P, Cicero S, Nicolaides KH. Screening for pre-eclampsia and fetal growth restriction by uterine artery Doppler at 11-14 weeks of gestation. Ultrasound Obstet Gynecol. 2001;18(6):583-6.

78. Nidhi Sharma1* SS, Krishnamurthy Jayashree1, Kulasekaran Nadhamuni3, Meenakshi Subbiah3 and Vijayaraghavan Rajagopalan4. Prediction of Intrauterine Growth Restriction in High
Pulsatility Index of Uterine Artery Br J Med Med Res 2017;22(2):1-6.

79. K. Melchiorre, K. Leslie, F. Prefumo, A. Bhide, Thilaga B. First-trimester uterine artery Doppler indices in the prediction of small-for-gestational age pregnancy and intrauterine growth restriction. Ultrasound Obstet Gynecol. 2009;33(5):524–9.

80. Auten RL, Davis JM. Oxygen toxicity and reactive oxygen species: the devil is in the details. Pediatr Res. 2009;66(2):121-7.

81. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci. 2008;4(2):89-96.

82. Sanchez-Aranguren LC, Prada CE, Riano-Medina CE, Lopez M. Endothelial dysfunction and preeclampsia: role of oxidative stress. Frontiers in physiology. 2014;5:372.

83. Cuffe JS, Xu ZC, Perkins AV. Biomarkers of oxidative stress in pregnancy complications. Biomark Med. 2017;11(3):295-306.

84. Lacza EMHRMKVSLKS. Nitrative stress and poly(ADP-ribose) polymerase activation in healthy and gestational diabetic pregnancies. Diabetologia. 2009;52:1935-43.

85. Ramirez-Emiliano J, Fajardo-Araujo ME, Zuniga-Trujillo I, Perez-Vazquez V, Sandoval-Salazar C, Ornelas-Vazquez JK. Mitochondrial content, oxidative, and nitrosative stress in human full-term placentas with gestational diabetes mellitus. Reprod Biol Endocrinol. 2017;15(1):26.

86. Guo XY, Shu J, Fu XH, Chen XP, Zhang L, Ji MX, et al. Improving the effectiveness of lifestyle interventions for gestational diabetes prevention: a meta-analysis and meta-regression. Bjog. 2019;126(3):311-20.

87. Burke AE, Bennett WL, Jamshidi RM, Gilson MM, Clark JM, Segal JB, et al. Reduced incidence of gestational diabetes with bariatric surgery. J Am Coll Surg. 2010;211(2):169-75.

88. Magro-Malosso ER, Saccone G, Di Mascio D, Di Tommaso M, Berghella V. Exercise during pregnancy and risk of preterm birth in overweight and obese women: a systematic review and meta-analysis of randomized controlled trials. Acta Obstet Gynecol Scand. 2017;96(3):263-73.

89. Russo LM, Nobles C, Ertel KA, Chasan-Taber L, Whitcomb BW. Physical activity interventions in pregnancy and risk of gestational diabetes mellitus: a systematic review and meta-analysis. Obstet Gynecol. 2015;125(3):576-82.

90. Sanabria-Martínez G, García-Hermoso A, Poyatos-León R, Álvarez-Bueno C, Sánchez-López M, Martínez-Vizcaíno V. Effectiveness of physical activity interventions on preventing gestational diabetes mellitus and excessive maternal weight gain: a meta-analysis. Bjog. 2015;122(9):1167-74.

91. Kennelly MA, Ainscough K, Lindsay KL, O'Sullivan E, Gibney ER, McCarthy M, et al. Pregnancy Exercise and Nutrition With Smartphone Application Support: A Randomized Controlled Trial. Obstet Gynecol. 2018;131(5):818-26.

92. Sun Y, Shen Z, Zhan Y, Wang Y, Ma S, Zhang S, et al. Effects of pre-pregnancy body mass index and gestational weight gain on maternal and infant complications. BMC Pregnancy Childbirth. 2020;20(1):390.

93. Lee KW, Ching SM, Ramachandran V, Yee A, Hoo FK, Chia YC, et al. Prevalence and risk factors of gestational diabetes mellitus in Asia: a systematic review and meta-analysis. BMC Pregnancy Childbirth. 2018;18(1):494.

94. McIntyre HD, Catalano P, Zhang C, Desoye G, Mathiesen ER, Damm P. Gestational diabetes mellitus. Nat Rev Dis Primers. 2019;5(1):47.

95. Saeedi M, Hanson U, Simmons D, Fadl H. Characteristics of different risk factors and fasting plasma glucose for identifying GDM when using IADPSG criteria: a cross-sectional study. BMC Pregnancy Childbirth. 2018;18(1):225.

96. Fong A, Serra AE, Gabby L, Wing DA, Berkowitz KM. Use of hemoglobin A1c as an early predictor of gestational diabetes mellitus. Am J Obstet Gynecol. 2014;211(6):641 e1-7.

97. Powe CE. Early Pregnancy Biochemical Predictors of Gestational Diabetes Mellitus. Curr Diab Rep. 2017;17(2):12.

98. Mahendra A, Fall CHD. Maternal vitamin D deficiency and GDM risk: evidence for the case of investing more attention in antenatal clinics. Proc Nutr Soc. 2021:1-7.

99. Liang JW, Chen MX, Hu XA, Zhou M, Zhang Y, Wang LL. Potential Biomarkers in Early Pregnancy for Predicting Gestational Diabetes Mellitus and Adverse Pregnancy Outcomes. Clin Lab. 2021;67(8).

100. Xu W, Tang Y, Ji Y, Yu H, Li Y, Piao C, et al. The association between serum selenium level and gestational diabetes mellitus: A systematic review and meta-analysis. Diabetes Metab Res Rev. 2022;38(4):e3522.

101. Huang Y, Chen X, You ZS, Gu F, Li L, Wang D, et al. The value of first-trimester platelet parameters in predicting gestational diabetes mellitus. J Matern Fetal Neonatal Med. 2022;35(11):2031-5.

102. Dias S, Pheiffer C, Abrahams Y, Rheeder P, Adam S. Molecular Biomarkers for Gestational Diabetes Mellitus. Int J Mol Sci. 2018;19(10).

103. Francis EC, Li M, Hinkle SN, Cao Y, Chen J, Wu J, et al. Adipokines in early and mid-pregnancy and subsequent risk of gestational diabetes: a longitudinal study in a multiracial cohort. BMJ Open Diabetes Res Care. 2020;8(1).

104. Kotzaeridi G, Blatter J, Eppel D, Rosicky I, Mittlbock M, Yerlikaya-Schatten G, et al. Performance of early risk assessment tools to predict the later development of gestational diabetes. Eur J Clin Invest. 2021;51(12):e13630.

105. Parry S, Sciscione A, Haas DM, Grobman WA, Iams JD, Mercer BM, et al. Role of early second-trimester uterine artery Doppler screening to predict small-for-gestational-age babies in nulliparous women. Am J Obstet Gynecol. 2017;217(5):594.e1-.e10.

106. Tian Y, Yang X. A Review of Roles of Uterine Artery Doppler in Pregnancy Complications. Frontiers in medicine. 2022;9:813343.

107. Conde-Agudelo A, Bird S, Kennedy SH, Villar J, Papageorghiou AT. First- and second-trimester tests to predict stillbirth in unselected pregnant women: a systematic review and meta-analysis. Bjog. 2015;122(1):41-55.

108. Pereira AC, Martel F. Oxidative stress in pregnancy and fertility pathologies. Cell Biol Toxicol. 2014;30(5):301-12.

109. Zhou Y, McMaster M, Woo K, Janatpour M, Perry J, Karpanen T, et al. Vascular endothelial growth factor ligands and receptors that regulate human cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome. Am J Pathol. 2002;160(4):1405-23.

110. Horvath EM, Magenheim R, Beres NJ, Benko R, Pek T, Tabak AG, et al. Oxidative-Nitrative Stress and Poly (ADP-Ribose) Polymerase Activation 3 Years after Pregnancy. Oxid Med Cell Longev. 2018;2018:1743253.

111. Lin JH, Liang AJ, Lin QD, Liu XH, Sun LZ, Zhang WY, et al. [A multi-center study to evaluate the dynamic changes of uterine artery and umbilical artery flow in a normal pregnancy and hypertensive disorders in pregnancy]. Zhonghua Fu Chan Ke Za Zhi. 2010;45(8):583-7.

112. Dijkstra G, Moshage H, van Dullemen HM, de Jager-Krikken A, Tiebosch AT, Kleibeuker JH, et al. Expression of nitric oxide synthases and formation of nitrotyrosine and reactive oxygen species in inflammatory bowel disease. J Pathol. 1998;186(4):416-21.

113. Molnar A, Toth A, Bagi Z, Papp Z, Edes I, Vaszily M, et al. Activation of the poly(ADP-ribose) polymerase pathway in human heart failure. Mol Med. 2006;12(7-8):143-52.

114. Bo S, Gambino R, Durazzo M, Guidi S, Tiozzo E, Ghione F, et al. Associations between gamma-glutamyl transferase, metabolic abnormalities and inflammation in healthy subjects from a population-based cohort: a possible implication for oxidative stress. World J Gastroenterol. 2005;11(45):7109-17.

115. Li Y, Ren X, He L, Li J, Zhang S, Chen W. Maternal age and the risk of gestational diabetes mellitus: A systematic review and meta-analysis of over 120 million participants. Diabetes Res Clin Pract. 2020;162:108044.

116. Krystynik O, Macakova D, Cibickova L, Karasek D. Fasting Plasma Glucose and Its Relationship to Anthropometric Phenotype in Women Diagnosed with Gestational Diabetes According to IADPSG Criteria. Life (Basel). 2023;13(1).

117. Eftekharpour E, Fernyhough P. Oxidative Stress and Mitochondrial Dysfunction Associated with Peripheral Neuropathy in Type 1 Diabetes. Antioxid Redox Signal. 2022;37(7-9):578-96.

118. Luc K, Schramm-Luc A, Guzik TJ, Mikolajczyk TP. Oxidative stress and inflammatory markers in prediabetes and diabetes. J Physiol Pharmacol. 2019;70(6).

119. Najafi A, Pourfarzam M, Zadhoush F. Oxidant/antioxidant status in Type-2 diabetes mellitus patients with metabolic syndrome. J Res Med Sci. 2021;26:6.

120. Joo EH, Kim YR, Kim N, Jung JE, Han SH, Cho HY. Effect of Endogenic and Exogenic Oxidative Stress Triggers on Adverse Pregnancy Outcomes: Preeclampsia, Fetal Growth Restriction, Gestational Diabetes Mellitus and Preterm Birth. Int J Mol Sci. 2021;22(18).

121. Li H, Yin Q, Li N, Ouyang Z, Zhong M. Plasma Markers of Oxidative Stress in Patients with Gestational Diabetes Mellitus in the Second and Third Trimester. Obstet Gynecol Int. 2016;2016:3865454.

122. Sudharshana Murthy KA, Bhandiwada A, Chandan SL, Gowda SL, Sindhusree G. Evaluation of Oxidative Stress and Proinflammatory Cytokines in Gestational Diabetes Mellitus and Their Correlation with Pregnancy Outcome. Indian J Endocrinol Metab. 2018;22(1):79-84.

123. Parast VM, Paknahad Z. Antioxidant Status and Risk of Gestational Diabetes Mellitus: a Case-Control Study. Clin Nutr Res. 2017;6(2):81-8.

124. Ma H, Qiao Z, Li N, Zhao Y, Zhang S. The relationship between changes in vitamin A, vitamin E, and oxidative stress levels, and pregnancy outcomes in patients with gestational diabetes mellitus. Ann Palliat Med. 2021;10(6):6630-6.

125. Zamani-Ahari U, Zamani-Ahari S, Fardi-Azar Z, Falsafi P, Ghanizadeh M. Comparison of Total Antioxidant Capacity of Saliva in Women with Gestational diabetes mellitus and Non-diabetic Pregnant Women. J Clin Exp Dent. 2017;9(11):e1282-e6.

126. Toldi G, Biro E, Szalay B, Stenczer B, Molvarec A, Rigo J, et al. Soluble urokinase plasminogen activator receptor (suPAR) levels in healthy pregnancy and preeclampsia. Clin Chem Lab Med. 2011;49(11):1873-6.

127. Lopez-Hernandez Y, Herrera-Van Oostdam AS, Toro-Ortiz JC, Lopez JA, Salgado-Bustamante M, Murgu M, et al. Urinary Metabolites Altered during the Third Trimester in Pregnancies Complicated by Gestational Diabetes Mellitus: Relationship with Potential Upcoming Metabolic Disorders. Int J Mol Sci. 2019;20(5).

128. Morisset AS, Dube MC, Drolet R, Pelletier M, Labrie F, Luu-The V, et al. Androgens in the maternal and fetal circulation: association with insulin resistance. J Matern Fetal Neonatal Med. 2013;26(5):513-9.

129. Uzelac PS, Li X, Lin J, Neese LD, Lin L, Nakajima ST, et al. Dysregulation of leptin and testosterone production and their receptor expression in the human placenta with gestational diabetes mellitus. Placenta. 2010;31(7):581-8.

130. Manjunath-Gowda S, Charles C, Muneyyirci-Delale O, Nacharaju V. Cortisol metabolism in normal pregnancy and pregnancy associated with gestational diabetes. Fertility and Sterility Home. 2013;100(3).

131. Wei D, Liu X, Jiang J, Tu R, Qiao D, Li R, et al. Mineralocorticoids, glucose homeostasis and type 2 diabetes mellitus: The Henan Rural Cohort study. J Diabetes Complications. 2020;34(5):107558.

9. BIBLIOGRAPHY OF PUBLICATIONS

Publications related to the thesis:

Gerszi D, Orosz G, Török M, Szalay B, Karvaly G, Orosz L, Hetthéssy J, Vásárhelyi B, Török O, Horváth EM, Várbíró S.; Risk estimation of gestational diabetes mellitus in the first trimester; JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM Paper: DOI: 10.1210/clinem/dgad301, 29 p. (2023)
IF: 6.134

Gerszi D., Penyige Á., Mezei Z., Sárai-Szabó B., Benkő R., Bányai B., Demendi C., Ujvári E., Várbíró S., Horváth E.M.; Evaluation of oxidative/nitrative stress and uterine artery pulsatility index in early pregnancy; PHYSIOLOGY INTERNATIONAL 107: 4 pp. 479-490. , 12 p. (2020)
IF: 2.09

Publications not related to the thesis:

Tarszabó Róbert, Bányai Bálint, Ruisanchez Éva, Péterffy Borbála, Korsós-Novák Ágnes, Lajtai Krisztina, Sziva Réka Eszter, **Gerszi Dóra**, Hosszú Ádám, Benkő Rita, Benyó Zoltán, Horváth Eszter Mária, Masszi Gabriella, Várbíró Szabolcs; Influence of Vitamin D on the Vasoactive Effect of Estradiol in a Rat Model of Polycystic Ovary Syndrome; INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 22: 17 Paper: 9404 , 12 p. (2021)
IF: 6.208

Sipos Miklós, Péterffy Borbála, Sziva Réka Eszter, Magyar Péter, Hadjadj Leila, Bányai Bálint, Süli Anita, Soltész-Katona Eszter, **Gerszi Dóra**, Kiss Judit, Szekeres Mária, Nádasy György L., Horváth Eszter Mária, Várbíró Szabolcs; Vitamin D Deficiency Cause Gender Specific Alterations of Renal Arterial Function in a Rodent Model; NUTRIENTS 13: 2 Paper: 704 , 13 p. (2021)
IF: 6.706

Sipos Miklós, **Gerszi Dóra**, Dalloul Hicham, Bányai Bálint, Sziva Réka Eszter, Kollarics Réka, Magyar Péter, Török Marianna, Ács Nándor, Szekeres Mária, Nádasy György L., Hadjadj Leila, Horváth Eszter Mária, Várbíró Szabolcs; Vitamin D Deficiency and Gender Alter Vasoconstrictor and Vasodilator Reactivity in Rat Carotid Artery; INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 22 Paper: 8029 , 12 p. (2021)
IF: 6.208

Lajtai K., Tarszabó R., Bányai B., Péterffy B., **Gerszi D.**, Ruisanchez É., Sziva R. E., Korsós-Novák Á., Benkő R., Hadjadj L., Benyó Z., Horváth E. M., Masszi G., Várbíró S.; Effect of Vitamin D Status on Vascular Function of the Aorta in a Rat Model of PCOS; OXIDATIVE MEDICINE AND CELLULAR LONGEVITY 2021 Paper: 8865979 , 6 p. (2021)
IF: 7.310

Török Marianna, Horváth Eszter M, Monori-Kiss Anna, Pál Éva, **Gerszi Dóra**, Merkely Petra, Sayour Alex A, Mátyás Csaba, Oláh Attila, Radovits Tamás, Merkely Béla, Ács Nándor, Nádasy György L, Várbíró Szabolcs; Chronic swimming training resulted in more relaxed coronary arterioles in male and enhanced vasoconstrictor ability in female rats; JOURNAL OF SPORTS MEDICINE AND PHYSICAL FITNESS 61: 3 pp. 489-496. , 8 p. (2021)
IF: 1.669

Lajtai Krisztina, Nagy Csilla Terézia, Tarszabó Róbert, Benkő Rita, Hadjadj Leila, Sziva Réka Eszter, **Gerszi Dóra**, Bányai Bálint, Ferdinandy Péter, Nádasy György László, Giricz Zoltán, Horváth Eszter Mária, Várbíró Szabolcs; Effects of Vitamin D Deficiency on Proliferation and Autophagy of Ovarian and Liver Tissues in a Rat Model of Polycystic Ovary Syndrome; BIOMOLECULES 9: 9 Paper: 471 , 14 p. (2019)
IF: 4.082

∑IF: 40.407

10. ACKNOWLEDGEMENTS

- Thank you to my supervisors: Dr. Eszter Mária Horváth, Prof. Dr. Szabolcs Várbíró
- Thank you to the staff of Department of Laborarory Medicine, Semmelweis University: Dr. Balázs Szalay, Dr. Gellért Karvaly, Prof. Dr. Barna Vásárhelyi
- Thank you to the staff of Department of Obstetrics and Gynecology, University of Debrecen: Dr. Olga Török, Dr. Gergő Orosz, Dr. László Orosz
- Thank you to the staff of Department of Obstetrics and Gynecology, Semmelweis University: Dr. Csaba Demendi, Dr. Elemér Ujvári, Prof. Dr. Nándor Ács
- Thank you to the staff of Department of Physiology: Dr. Rita Benkő, Dr. Bálint Bányai, Prof. Dr. László Hunyady, Prof. Dr. Attila Mócsai
- Thank you to my consultants from Workgroup for Science Management, Doctoral School: Dr. Marianna Török, Dr. Judit Hetthéssy
- Thank you to other co-authors of our first manuscript: Dr. Áron Penyige, Dr. Boglárka Sárai-Szabó, Zsuzsanna Mezei
- Thank you to our funders: Hungarian National Research, Development and Innovation Office (STIA-OTKA-2021, S.V. and OTKA-PD 113022 NKFIH-FK 129206), Hungarian Hypertension Society (M.T., S.V.)
- Thank you to my family, my husband and to my little son.