

MESTRADO

Histopathological lesions of the placenta in pregnancies affected by diabetes: A correlation between metformin versus insulin

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DISSERTAÇÃO



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Declaration of Integrity

I, Andrea Castro Corrallo, hereby declare that this dissertation, titled "Histopathological lesions of the placenta in pregnancies affected by diabetes: A correlation between metformin versus insulin," is the result of my own original work and that it has not been submitted, in whole or in part, for any other degree or qualification at this or any other institution.

All sources of information have been duly acknowledged and referenced. All quotations have been distinguished from my own writing and properly attributed.

Furthermore, to enhance the grammatical quality and clarity of the text, I have utilized AI-powered language assistance tools, such as Grammarly. The application of these tools was strictly limited, used mainly to improve grammar, spelling, and punctuation. The intellectual content, research, analysis, and conclusions presented herein are entirely my own.

Andrea Castro Corrallo

Abstract

Background: The global rise in diabetes prevalence during pregnancy is contributing to increased maternal and neonatal morbidity. While metformin effectively controls glycemia in both type 2 diabetes and [gestational diabetes mellitus \(GDM\)](#) and offers practical benefits compared to insulin, its widespread adoption as a first-line treatment is constrained by concerns over placental transfer and potential impacts on placental function, fetal development and offspring's long-term health effects.

Objective: This study aims to assess the association between maternal diabetes pharmacological therapy (metformin versus insulin) and placental histopathology in term singleton pregnancies.

Methods: A retrospective study was conducted of term singleton diabetic pregnancies delivered from January 2019 to October 2023. Maternal and neonatal electronic medical records along with placental histopathology reports were reviewed, identifying 105 pregnancies that met the inclusion criteria (59 treated with metformin and 46 with insulin). Placental lesions were categorized based on the 2014 Amsterdam Consensus. Relevant gestational clinical data, primary neonatal outcomes, and placental histological lesions were compared between the two treatment groups.

Results: Maternal anthropometric profiles and comorbidities were comparable between the treatment groups, with the exception of a higher prevalence of pre-gestational obesity in the metformin group and a significantly increased rate of thyroid disease during pregnancy in the insulin group. Women in the insulin group exhibited significantly higher glycated hemoglobin (HbA 1c) levels during pregnancy compared to those in the metformin group. There were no significant differences in neonatal complications associated with diabetes in either type 2 or gestational diabetes cases. However, pregnancies with type 1 diabetes, which were managed exclusively with insulin, demonstrated greater maternal gestational weight gain, higher rates of large-for-gestational-age neonates, and elevated fetoplacental weight ratios. Fetal inflammatory response and chronic deciduitis were the only placental lesions significantly more frequent in the group treated with metformin among all cases of diabetes during pregnancy.

Keywords: Diabetes, Pregnancy, Metformin, Insulin, Placental Histopathology

Resumo

Contexto: O aumento global da prevalência de diabetes durante a gravidez está a contribuir para o aumento da morbilidade materna e neonatal. Embora a metformina controle eficazmente a glicemia tanto na diabetes tipo 2 como na [diabetes gestacional \(DG\)](#) e ofereça benefícios práticos em comparação com a insulina, a sua adoção generalizada como tratamento de primeira linha é limitada por preocupações em relação à sua passagem pela barreira placentária e aos potenciais impactos na função da placenta, no desenvolvimento fetal e na saúde do concepto a longo prazo.

Objetivo: Este estudo visa avaliar a associação entre a terapêutica farmacológica da diabetes materna (metformina versus insulina) e a histopatologia da placenta em gravidezes de termo com feto único.

Métodos: Foi realizado um estudo retrospectivo de gravidezes diabéticas de termo com feto único com nascimento entre janeiro de 2019 e outubro de 2023. Foram revistos os registos clínicos eletrónicos hospitalares maternos e neonatais, bem como os relatórios de histopatologia placentária, tendo sido identificadas 105 gravidezes que cumpriam os critérios de inclusão (59 tratadas com metformina e 46 com insulina). As lesões placentárias foram categorizadas com base no Consenso de Amesterdão de 2014. Os dados clínicos gestacionais, os resultados neonatais e as lesões histológicas placentárias foram comparados entre os dois grupos de tratamento.

Resultados: Os perfis antropométricos maternos e as comorbidades foram comparáveis entre os grupos de tratamento, com exceção de uma maior prevalência de obesidade pré-gestacional no grupo da metformina e uma taxa significativamente mais elevada de doença da tiroide durante a gravidez no grupo da insulina. As mulheres no grupo da insulina apresentaram níveis significativamente mais elevados de hemoglobina glicada (HbA 1c) durante a gravidez em comparação com as do grupo da metformina. Não foram encontradas diferenças significativas nas complicações neonatais associadas à diabetes, tanto nos casos de diabetes tipo 2 como nos de diabetes gestacional. No entanto, as gravidezes com diabetes tipo 1, que foram manejadas exclusivamente com insulina, demonstraram um maior aumento de peso gestacional materno, taxas mais elevadas de recém-nascidos grandes para a idade gestacional e relação de peso feto-placentários elevados. Resposta inflamatória fetal e decíduite crónica foram as únicas lesões placentárias significativamente mais frequentes no grupo tratado com metformina.

Palavras-chave: Diabetes, Gravidez, Metformina, Insulina, Histopatologia placentária

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Andrea Castro Corrallo

*“Until I began to learn to draw,
I was never much interested in looking at art.”*

Richard P. Feynman

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Abbreviations

ACOG American College of Obstetricians and Gynecologists. [2](#)

ADA American Diabetes Association. [2](#)

AKT Protein Kinase B. [7](#)

AMP Adenosine Monophosphate. [9](#)

AMPK AMP-activated protein kinase. [7](#), [9](#), [10](#)

ATP Adenosine Triphosphate. [9](#), [10](#)

BMI body mass index. [21](#), [24](#)

BW/PW birth weight-to-placental weight ratio. [14](#)

cAMP cyclic Adenosine Monophosphate. [9](#)

CI Confidence Interval. [21](#), [22](#), [23](#)

DG diabetes gestacional. [v](#)

DM diabetes mellitus. [1](#)

E1 estrone. [5](#)

E2 estradiol. [5](#)

E3 estriol. [5](#)

FBP1 Fructose-1,6-bisphosphatase 1. [9](#)

FIGO International Federation of Gynecology and Obstetrics. [2](#)

FPR feto-placental weight ratio. [25](#), [26](#)

FVM Fetal Vascular Malperfusion. [12](#)

GDM gestational diabetes mellitus. [iii](#), [xv](#), [1](#), [2](#), [3](#), [4](#), [8](#), [11](#), [24](#), [25](#), [26](#), [33](#), [34](#), [35](#), [36](#), [39](#), [40](#), [41](#)

GH growth hormone. [3](#), [4](#)

hCG human chorionic gonadotropin. [1](#)

hPGH Human placental growth hormone. [4](#)

hPL human placental lactogen. [3](#), [4](#)

IADPSG International Association of the Diabetes and Pregnancy Study Groups. [18](#)

IGF insulin-like growth factor. [3](#), [4](#)

IL-6 interleukin 6. [3](#), [7](#), [10](#)

IQR Interquartile Range. [19](#), [21](#), [24](#)

IRS insulin receptor substrate. [4](#), [5](#), [7](#)

IUGR Intrauterine Growth Restriction. [12](#)

LPS lipopolysaccharide. [10](#)

mTOR mammalian target of rapamycin. [10](#)

NAD⁺ Nicotinamide adenine dinucleotide. [10](#)

NADH Nicotinamide adenine dinucleotide + hydrogen. [10](#)

NF- κ B nuclear factor-kappa B. [10](#)

NICU Neonatal Intensive Care Unit. [8](#), [22](#)

OR odds ratios. [22](#), [23](#)

PGH placental growth hormone. [3](#), [4](#)

PRL prolactin. [3](#), [4](#)

PRLR prolactin receptor. [3](#)

SD Standard Deviation. [19](#)

SOCS3 cytokine signaling 3. [7](#)

SPSS Statistical Package for the Social Sciences. [19](#)

STAT5 Signal transducer and activator of transcription 5. [4](#), [7](#)

TNF- α Tumor Necrosis Factor alpha. [3](#), [6](#), [7](#), [10](#)

UCI umbilical cord coiling index. [19](#)

VUE Villitis of Unknown Etiology. [xi](#), [13](#)

1 Introduction

The placenta, a transient but vital organ, plays a central role in ensuring successful pregnancy outcomes. It functions as the interface for maternal-fetal exchange of nutrients, gases, and waste products, and is critical for endocrine and immunological functions to support fetal development [50, 26]. The placenta facilitates the maternal-fetal exchange, regulating the transfer of nutrients from the maternal to the fetal circulation and removing waste products from the fetal circulation [50]. Furthermore, the placenta is critical for hormone production, including [human chorionic gonadotropin \(hCG\)](#), progesterone, and estrogen, essential for pregnancy maintenance and fetal development [50]. It also participates in immune modulation, protecting the fetus from maternal immune responses while allowing for selective transfer of maternal antibodies [26]. However, in diabetic pregnancies, these intricate placental functions are disrupted [57].

[diabetes mellitus \(DM\)](#) represents a significant global health challenge, characterized by hyperglycemia resulting from defects in insulin secretion or action [12]. Classified primarily into Type 1, Type 2, and [gestational diabetes mellitus \(GDM\)](#), each type presents unique pathophysiological features and clinical management strategies [12]. The global prevalence of diabetes is alarmingly high, with a particularly concerning rise in [GDM](#) incidence. In 2017, the estimated worldwide number of adults living with diabetes mellitus was 425 million (8.8% of the total population) [12]. Worryingly, in the same year, 16.2% of worldwide live births were affected by some degree of hyperglycemia during pregnancy, and 86.4% of these cases were attributed to [GDM](#) [12]. Pregestational diabetes (Type 1 and Type 2 [DM](#) preceding pregnancy) also contributes significantly to adverse pregnancy outcomes. Diabetes in pregnancy is associated with a spectrum of maternal and fetal complications, including increased risks of preeclampsia, preterm birth, cesarean delivery, macrosomia (excessive fetal growth), shoulder dystocia, neonatal hypoglycemia, hyperbilirubinemia, and even stillbirth [37, 43].

Histopathological studies of placentas from diabetic pregnancies reveal a range of characteristic macroscopic and microscopic changes [12]. Increased placental weight is a common finding, often associated with fetal macrosomia [14]. Macroscopic alterations in placentas from [GDM](#) pregnancies include changes in surface area and volume, as well as increased central thickness and number of cotyledons [12, 49]. Microscopic alterations in diabetic placentas include villous immaturity, characterized by delayed maturation of placental villi, and an increased number of syncytial knots, indicative of syncytiotrophoblast stress [17, 23]. Other documented histological alterations include fibrinoid necrosis, representing areas of placental tissue damage, and thickening of the placental basement membrane, potentially hindering nutrient transfer [39, 1]. Moreover,

diabetes can disrupt placental vascularization, leading to both hypervascularity and hypovascularity, impacting blood flow and exchange capacity [40]. Histological markers of oxidative stress and inflammation are also prominent features in placentas from diabetic pregnancies [1, 76]. In GDM placentas, histological changes include an increased volume of intervillous space and terminal villi, syncytiotrophoblast number, fibrinoid areas, and glycogen deposits [12].

Current treatment strategies for diabetes in pregnancy aim to achieve optimal glycemic control and mitigate adverse outcomes. The initial management generally consisted of lifestyle modifications (nutritional counselling and exercise) followed by pharmacological treatment when necessary [9]. Divergent recommendations in clinical guidelines still conflict over whether Insulin and Metformin present equal treatment options for type 2 and gestational diabetes.

The American Diabetes Association (ADA) and American College of Obstetricians and Gynecologists (ACOG) [4, 3] recommend insulin as a first-line agent to treat hyperglycemia. It is also advised that, if Metformin is used pre-conception, by the end of the first trimester, should be discontinued, due to its ability to cross the placenta to the fetus and the lack of long-term safety data [4]. However, the International Federation of Gynecology and Obstetrics (FIGO) [35], the UK NICE guidelines [56] and, since 2022, the European health authorities endorsed the use of Metformin throughout pregnancy for pre-gestational and gestational diabetes, either as mono-therapy or alongside insulin, as a safe and effective option, if lifestyle modification alone fails to achieve maternal glucose control [16].

The present study compared the placental histopathological features of term singleton diabetic pregnancies managed with metformin versus insulin, and correlated these findings with key maternal and perinatal outcomes. By elucidating placental adaptations to varying glycemic control strategies, this work seeks to provide a new perspective and contribute to a more informed therapeutic decision-making.

To better understand the challenges of managing diabetes in pregnancy, it is crucial to first examine the complex physiological and hormonal changes that lead to insulin resistance during gestation. The following sections will provide a detailed review of the current literature on these mechanisms.

1.1 Literature Review

1.1.1 Physiological Mechanisms Leading to Insulin Resistance in Pregnancy

Pregnancy induces progressive insulin resistance, a normal metabolic adaptation that reduces maternal peripheral glucose uptake, thereby increasing glucose availability for the fetus [74]. This is paralleled by compensatory increases in maternal pancreatic beta-cell mass, insulin synthesis, and secretion to maintain glucose homeostasis [63, 62]. Together, these mechanisms prioritize placental glucose transfer for fetal use while regulating maternal glucose levels [63, 58]. This

physiological process is driven by a complex interplay of gestational hormones (e.g., [human placental lactogen \(hPL\)](#), [placental growth hormone \(PGH\)](#), progesterone, estrogens, cortisol) and inflammatory cytokines. While the exact mechanisms are not fully understood, these factors alter insulin signaling pathways, ensuring fetal glucose supply at the cost of progressively reduced maternal insulin sensitivity [74].

1.1.2 Hormonal Influences on Insulin Resistance

1.1.2.1 Human Placental Lactogen (hPL)

[human placental lactogen \(hPL\)](#) is a peptide hormone produced by placental syncytiotrophoblast cells. Structurally similar to pituitary [growth hormone \(GH\)](#) and [prolactin \(PRL\)](#), it belongs to the somatotropin family. It primarily binds to [PRL](#) receptors [PRLR](#), exhibiting lower affinity for [GH](#) receptors [62, 58]. Detectable in maternal plasma by six weeks of gestation, [hPL](#) levels increase linearly, peaking at 5000–7000 ng/mL around the 30th week. Near term, its secretion reaches remarkably high levels, the greatest known of any human hormone (1g / day), and is rapidly cleared from maternal circulation following placental delivery [63, 62].

Considered a "diabetogenic" hormone with both insulin-like and anti-insulin effects, [hPL](#) contributes to the altered insulin sensitivity during pregnancy, resulting in increased maternal insulin resistance and reduced glucose utilization. This metabolic shift is counterbalanced by a concomitant increase in pancreatic insulin secretory capacity, also driven by [hPL](#) [63]. Whereas in fetal tissues, [hPL](#) stimulates [insulin-like growth factor \(IGF\)](#) production and enhances the supply and uptake of key nutrients for fetal growth and development [30].

Studies using isolated rodent pancreatic islets indicate that [PRLR](#) signaling, activated by placental lactogens and prolactin, enhances glucose-induced insulin secretion. This effect is linked to the upregulation of [PRLRs](#) in pancreatic β -cells during pregnancy. Evidence indicates that this [PRLR](#) signaling is a key mediator of critical pregnancy-associated adaptations—including increased β -cell proliferation, survival, and insulin secretion, alongside a lowered glucose threshold for insulin release—which are essential for maintaining maternal glycemic control [58]. In human studies, [hPL](#) promotes lipolysis, increasing free fatty acid availability to spare glucose for fetal use.

By interacting with hypothalamic [PRL](#) receptors, [hPL](#) likely promotes increased appetite and food intake. Furthermore, it is also thought to play a role in inducing and maintaining the physiological state of hyperleptinemia and relative leptin resistance during pregnancy, which contributes to hyperphagia despite increased adipose tissue [63]. While physiological in pregnancy due to increased syncytiotrophoblast synthesis, hyperleptinemia is generally associated with insulin resistance and compensatory hyperinsulinemia, partly due to an insulin-leptin feedback loop. This loop involves insulin stimulating leptin secretion from adipose tissue, and leptin, in turn, stimulating insulin release. Moreover, leptin may increase the risk of [GDM](#) by accentuating physiological IR through the placental release of pro-inflammatory cytokines like [TNF- \$\alpha\$](#) and [IL-6](#), which impair intracellular insulin signaling and its peripheral actions [11].

Placentally-derived **hPL** positively correlates with birth weight and placental mass. Accordingly, placentomegaly in maternal diabetes could elevate **hPL**, thereby stimulating maternal/fetal beta-cell expansion, boosting fetal insulin, and promoting glycogenesis, fat deposition, and further fetal growth [63]. This suggests that maternal **hPL** levels could be a biomarker for macrosomia prediction [63]. However, several studies found no direct relationship between maternal **hPL** levels and **GDM** diagnosis. Suggesting that while **hPL** contributes to metabolic adaptations in pregnancy, other factors may be more critical in **GDM** pathogenesis [63].

1.1.2.2 Human Placental Growth Hormone (**hPGH**)

Human placental growth hormone (hPGH), another key contributor to insulin resistance during pregnancy, is tonically produced and secreted by the placental syncytiotrophoblast and extravillous cytotrophoblast layers. Its maternal serum levels rise throughout gestation, gradually replacing the pulsatile secretion of pituitary growth hormone after the 15th to 20th week, rendering **GH** undetectable in maternal serum by the 24th gestational week [76, 35]. By term, **hPGH** concentrations reach 20-40 ng/ml, levels comparable to those in acromegaly and approximately 10 times higher than **GH** levels outside of pregnancy [2].

Research strongly indicates that **hPGH** exhibits potent somatogenic but weak lactogenic effects, binding **GH** receptors with high affinity and **PRL** receptors with low affinity [62, 11]. Unlike pituitary **GH**, **hPGH** secretion is not **GHRH**-regulated but rather responds to maternal hypoglycaemia, to ensure fetal glucose supply. The glucose transporter Glut1 in syncytiotrophoblast cells regulates the adjustment of **hPGH** secretion according to maternal serum glucose levels [62, 11].

Insulin resistance is induced by **hPGH** through decreased synthesis of adiponectin, an insulin-sensitizing hormone (possibly via **STAT5**-mediated promoter inhibition), and through inhibition of intracellular insulin signaling [11]. This signaling inhibition occurs as **PGH**-induced p85 subunit expression competitively blocks **insulin receptor substrate (IRS)** 1 activation of PI3K (preventing p110 heterodimerization), thus impairing GLUT4 translocation in insulin-dependent tissues [11]. Furthermore, in rodent in vitro studies, **hPGH** exhibits diabetogenic effects similar to pituitary **GH**, such as inducing hyperinsulinemia, decreasing insulin-stimulated glucose uptake and glycogen synthesis, and impairing insulin's suppression of hepatic gluconeogenesis [2].

Although these effects are not so clearly demonstrated in human pregnancy, in rodent models, **hPGH** appears to be the primary driver of maternal insulin growth factor 1 (**IGF**) levels. Suggesting an interplay within the **GH/hPGH/IGF** axis, including **IGF**-binding proteins (**IGF-BPs**), which likely couples insulin resistance with growth hormone activity during pregnancy [2].

While the physiological processes involving **hPGH** are linked to its overproduction in **GDM**, suggesting a dose-dependent relationship [11], it is also observed that reduced **hPGH** expression can decrease lipolysis and circulating **IGF-I** [62]. However, the underlying mechanisms for both these observations are not entirely elucidated.

1.1.2.3 Progesterone

Progesterone, a key hormone for pregnancy implantation and maintenance, is initially synthesized by the corpus luteum and subsequently undergoes a luteal-placental shift by the end of the second month of gestation. Thereafter, the placenta becomes the primary source, with progressive increase in maternal circulating levels until peak concentrations of approximately 130 ng/mL in the third trimester [52], and high-dose progesterone has been linked in previous studies to disturbances in glucose homeostasis [46].

Research using 3T3-L1 adipocytes has demonstrated that progesterone interferes with insulin signaling, including a reduction in *IRS*-1 expression and inhibition of insulin-stimulated tyrosine phosphorylation in many metabolic pathways. These alterations impair downstream signaling required for GLUT-4 translocation, ultimately reducing insulin-mediated glucose uptake and increasing insulin resistance [79].

Corroborating these findings, experiments using in vitro human Hep3B hepatocytes and in vivo mouse models have demonstrated that progesterone induces hepatic gluconeogenesis, leading to elevated blood glucose levels under insulin-deficient and insulin-resistant conditions. In contrast, under physiological conditions, the maintenance of normal blood glucose levels was observed. According to previous reports, this could result from the interaction of progesterone receptor membrane component 1 with GLP-1 receptor in β -cells promoting a compensatory insulin secretion [46].

1.1.2.4 Estrogen

During pregnancy, estrogen production, initially by the corpus luteum and then increasingly by the placenta, leads to a significant increase in estrogen levels (*estrone* (E1), *estradiol* (E2), and *estriol* (E3)) up to 30-fold at term and E2 rise from approximately 7 ng ml⁻¹ near the end of the first trimester, to 28-30 ng ml⁻¹ at term [53].

Studies also in 3T3-L1 adipocytes have demonstrated a *estrone* (E1) concentration-specific effect on glucose metabolism: physiological concentrations (e.g., 10⁻⁸ M) enhance insulin-induced glucose uptake, whereas the high concentrations found in pregnancy (e.g., 10⁻⁵ M) inhibit it, contributing to insulin resistance (90-Nagira). The result observed indicated that the mechanism for this inhibition by high E2 involves impairing, at least in part, the modulation of *IRS*-1 phosphorylation at [Ser307], resulting in inhibition of tyrosine phosphorylation of *IRS*-1 that leads to the attenuation of insulin signaling [54].

Conversely, estrogen receptors are also expressed in the islets of Langerhans and respond to rising estrogen levels during pregnancy. Rodent studies have identified that E2 potentiates insulin biosynthesis, enhancing the insulin secretory response to glucose and promoting resistance in β -cells from injury-stimulated apoptosis. Therefore, taking part in the maintenance of maternal glycaemia within the physiological range [53].

1.1.2.5 Cortisol

During pregnancy, maternal circulating cortisol levels increase substantially, particularly in the second and third trimesters. Driven by the placental production of large amounts of corticotropin releasing hormone, the maternal hypothalamic–pituitary–adrenal axis responds with a rise of adrenocorticotrophin hormone output by the pituitary gland, which leads to increased synthesis and release of cortisol by the adrenal cortex into maternal circulation. By the third trimester, circulating cortisol concentrations reach approximately three times non-pregnant levels. Concurrently, a progressive increase in circulating cortisol-binding globulin levels, mediated by the oestrogen, results in an increase in total cortisol levels with a higher concentration of active (biochemically 'free') cortisol [21].

Glucocorticoids, such as cortisol, severely disrupt glucose homeostasis through tissue-specific mechanisms. The activation of glucocorticoid receptors triggers distinct cellular responses that promote hyperglycemia and insulin resistance [27, 47].

Under normal conditions, hepatic gluconeogenesis and glycogenolysis are potently suppressed by insulin. However, glucocorticoid exposure promotes hepatic insulin resistance by disrupting phosphorylation of downstream effectors in the insulin-mediated gluconeogenic signalling pathway. There is also an intensification of shuttling of gluconeogenic precursors from skeletal muscle and adipose tissue, associated with the upregulation of gluconeogenic enzymes in hepatocytes. These combined effects increase hepatic glucose production and output, which remains unopposed by insulin [47].

Studies with primary cultures of human skeletal myocytes suggest that cortisol impairs insulin sensitivity by interfering with the insulin signaling cascade, specifically reducing insulin-stimulated phosphorylation of the insulin receptor substrate-1. This reduction in phosphorylation subsequently attenuates GLUT-4 translocation to the plasma membrane, thereby limiting insulin-stimulated glucose uptake by the muscle. Whereas primary cultures of human adipocytes, induced by glucocorticoid treatment, increased insulin-stimulated glucose uptake, with increasing expression and phosphorylation of insulin receptor substrate-2 while maintaining insulin receptor substrate-1 expression and phosphorylation, thereby maintaining insulin sensitization [27].

1.1.3 Adipokines

Adipokines are a broad group of cytokines secreted by white adipose tissue and the placenta during gestation that include *TNF- α* , adiponectin, leptin, resistin, and others. With increasing interest, many studies related to pregnancy, obesity, and diabetes have investigated the effects of adipokines on the development and modulation of insulin resistance. However, the mechanisms involved in most adipokines require further studies and are still not fully understood [33].

Analysis of human maternal plasma and placental cotyledon identified that *TNF- α* levels were the most significant independent predictor of insulin sensitivity, when compared to leptin, cortisol, and reproductive hormones in a multivariate stepwise regression analysis. Circulating levels of *TNF- α* declined in early pregnancy and increased during the third trimester, paralleling changes in

insulin resistance. Although $\text{TNF-}\alpha$ is synthesized and secreted by both the placenta and adipose tissue, data obtained from the cotyledon in vitro analysis suggest that $\text{TNF-}\alpha$ the late-gestational increase appears to be predominantly due to placental production [42].

Findings from cultured hepatocytes [24], skeletal muscle cell [18] and adipocytes [36] indicated that tumor necrosis factor $\text{TNF-}\alpha$ impaired insulin signalling by promoting serine phosphorylation of insulin receptor substrate (IRS)-1 and IRS-2, and also decreased insulin receptor tyrosine kinase activity, thus reducing insulin-stimulated glucose uptake [24, 18, 36].

Adiponectin is the most abundant and almost exclusive adipose tissue adipokine released [33, 68]. In contrast to $\text{TNF-}\alpha$, adiponectin can be considered as an insulin-sensitizing and anti-inflammatory hormone that enhances skeletal muscle glucose uptake and suppresses hepatic glucose production by activating AMP-activated protein kinase (AMPK). However, evidence indicates that $\text{TNF-}\alpha$, other cytokines, and placental growth hormone inhibit adiponectin expression and release in adipocytes, possibly via STAT5 protein activation and binding to adiponectin promoter sites, contributing to the progressive decline in adiponectin circulating levels with advancing pregnancy [6, 33, 10].

Leptin is an adipokine synthesized by adipose tissue; however, during pregnancy, it is primarily produced by the syncytiotrophoblast with peak levels mid-gestation that remain elevated until postpartum. Although hyperleptinaemia is physiological during pregnancy, maternal obesity or excessive weight gain can further elevate leptin levels and contribute to further maternal fat mass gain, aggravating the insulin resistance and a compensatory hyperinsulinaemia [68]. As insulin promotes leptin secretion by white adipose tissue, this creates a vicious cycle that exacerbates insulin resistance [48]. Proposed mechanism associated with higher leptin levels includes increased placental release of tumor necrosis factor- α and interleukin 6 (IL-6), which impairs insulin signalling and reduces its peripheral effects [6].

Resistin is a hormone synthesized primarily by the adipose cells during adipogenesis and is upregulated in obesity. During pregnancy, the placenta becomes the primary source of resistin production, with secretion progressively increasing and peaking in the third trimester [48, 68]. Described as an insulin resistance-inducing hormone, it impairs pancreatic β -cell function by inducing suppressor of cytokine signaling 3 (SOCS3) expression and inhibiting AKT phosphorylation, thereby reducing insulin release. Moreover, it decreases insulin sensitivity in skeletal muscle, adipose tissue, and liver, by interfering with GLUT4 translocation and glucose uptake [6].

1.2 Metformin

Metformin (dimethylbiguanide hydrochloride) is a biguanide, orally administered, that improves insulin sensitivity with a glucose-lowering effect [43]. As a constituent of many herbal remedies, the prescription of biguanides in Europe for diabetes mellitus symptoms can be traced back to the Middle Ages [69]. Metformin was synthesized in the 1920s and introduced in the late 1950s for the treatment of non-insulin-dependent diabetes mellitus [69]. Although metformin itself demonstrated a favorable safety profile, other biguanides presented an increased risk of lactic acidosis and

cardiovascular mortality, prompting, in the late 1970s, the United States and many other countries to withdraw the entire class from clinical use [69, 6].

After 20 years of safe and effective use in Europe, metformin was approved for prescription use in the United States in 1995. In 2009, became first-line pharmacological therapy for type 2 diabetes in a joint consensus of the American Diabetes Association and the European Association for the Study of Diabetes, which was subsequently endorsed by numerous national and international guidelines [69, 55, 6].

Regarding the use of metformin during pregnancy, in 1979, a study involving 60 women with gestational diabetes reported the use of metformin during the second and third trimester after dietary failure, particularly in overweight patients. The findings indicated satisfactory outcomes related to maternal glycemic control and perinatal morbidity and mortality [15]. Subsequently, metformin for the management of gestational diabetes gained progressive clinical traction and research interest. In 2009, the International Diabetes Federation recognized metformin as a treatment option for gestational diabetes, prompting updates in other international guidelines in the following years [69, 45].

Studies comparing the use of metformin and insulin for GDM have shown maternal and neonatal benefits, such as reduced gestational weight gain and lower risk of pregnancy-induced hypertension, decreased risks of macrosomia, neonatal hypoglycemia, and NICU admission. These advantages were achieved with no increased risk of preeclampsia, preterm birth, or cesarean delivery [7, 13].

Systematic reviews and large-scale case-control studies have also consistently found no evidence that first-trimester exposure to metformin increases the risk of significant congenital anomalies [13, 28]. Metformin is usually well tolerated, but gastrointestinal adverse effects are common, including nausea, vomiting, diarrhea, abdominal bloating and pain, especially during initiation of the therapy. Typically, these are transient, mild symptoms [7].

However, concern persists as metformin is not metabolized, is distributed into most tissues, excreted unchanged in maternal urine, and can cross the placental barrier, reaching clinically relevant concentrations in both placental and fetal tissues (50%~100% of maternal levels). This exposure could potentially influence placental and fetal physiology through direct or indirect pathways. Therefore, its use in diabetic insulin-independent pregnancies continues to vary among country-specific protocols [43, 17, 17].

1.2.1 Pharmacokinetics of metformin

Although decades of clinical application and extensive research, the precise mechanism underlying metformin's actions remains complex and not fully elucidated [78]. After oral administration, metformin accumulates preferably in the gastrointestinal tract, liver, and kidney, while circulating levels remain comparatively lower [31]. Metformin primarily antihyperglycaemic effect improves glycemic control by suppressing hepatic gluconeogenesis and glucose output, thereby reducing fasting glycemia without increasing basal insulin levels or inducing clinically significant hypoglycemia or weight gain. Additional mechanisms in the intestine have been proposed, such as

the suppression of intestinal gluconeogenesis, increased glucose uptake, promotion of anaerobic metabolism, and increased glucagon-like peptide-1 secretion [78, 6, 25].

The impact of metformin on systemic glucose uptake is still a subject of debate. Some studies indicate an increase in insulin-stimulated glucose uptake and glycogen synthesis. In contrast, other studies have found no improvement in peripheral insulin sensitivity and suggest that relevant results may depend on supra-therapeutic doses of metformin than those used in clinical settings [25, 31]. Moreover, studies have demonstrated improvements in blood lipid profiles, reduced coagulability, and attenuation of inflammation [6, 31].

1.2.1.1 Hepatic glucose production

Current evidence, although still highly debated, suggests that hepatic gluconeogenesis suppression involves four distinct pathways that collectively reduce glucose production in hepatocytes. A proposed mechanism of metformin action involves the inhibition of mitochondrial complex I activity, which reduces mitochondrial respiration and the [ATP]:[AMP] ratio, thereby promoting a cytosolic increase in AMP levels. Elevated AMP activates AMPK, which inhibits gluconeogenic gene expression and promotes lipid oxidation. Elevated AMP also antagonizes hepatic glucagon signaling by preventing glucagon-stimulated production of cAMP and also allosterically inhibits FBP1, thereby directly inhibiting the gluconeogenic pathway from pyruvate. Metformin would also hinder cytosolic enzyme activity and alter cytosolic redox by raising [NADH]:[NAD⁺] ratio, thereby impairing gluconeogenesis from glycerol and lactate [44, 31].

1.2.1.2 Glucose metabolism in the intestine

High levels of metformin are retained in the intestine, where it may inhibit mitochondrial complex I activity and suppress gluconeogenesis in enterocytes, analogous to its effects in hepatocytes. Gluconeogenesis in the intestine corresponds to 5-7% of systemic glucose production. By increasing AMP levels, metformin activates AMPK in enterocytes, leading to a subsequent reallocation of the GLUT2 transporter to the apical and basolateral membranes, thereby enhancing glucose uptake from the circulation and intestinal lumen. The inhibition of mitochondrial respiration increases glucose utilization through anaerobic glycolysis, and the enterocyte produces lactate. The lactate is subsequently reconverted to glucose in the liver through a futile intestinal-liver cycle, increasing energy expenditure [31, 81]. In addition, AMPK activation can also increase GLP1 secretion by intestinal L-cells and, through this hormone, reduce food intake, increase insulin release, and inhibit glucagon secretion, contributing to glucose homeostasis [31].

1.2.1.3 Peripheral glucose uptake

The mechanism underlying metformin's increase in whole-body sensitivity to insulin might be associated with multiple biological pathways. Extensive research has demonstrated that metformin increases GLUT4 expression and enhances its translocation to the plasma membrane in skeletal muscle, adipose tissue, and endometrial tissue, thereby increasing peripheral glucose uptake.

Current data suggest these effects are mediated via **AMPK** activation, modulation of the insulin signaling pathway, and epigenetic modifications, including regulation of microRNAs and activation of transcription factors [32].

1.2.1.4 Anti-inflammatory effects

A significant focus of research on the nonglycemic actions of metformin has been its role in immune modulation and control of inflammation. These effects are mainly mediated through activation of **AMPK**, although **AMPK**-independent mechanisms have also been described [38].

Metformin, by inhibiting mitochondrial complex I, activates **AMPK**, which in turn suppresses **nuclear factor-kappa B (NF- κ B)** and **mammalian target of rapamycin (mTOR)** signaling [38]. It is described that **AMPK** promotes SIRT1 activity through regulation of the **NAD⁺⁺/NADH** ratio and SIRT1 inhibits **NF- κ B** and the expression of **TNF- α** , **IL-6** [71]. In a study using adipose-derived mesenchymal stem cells, metformin enhanced the immunomodulatory capacity of these cells via the **AMPK/mTOR/STAT1 (sirtuin)** pathway and improved their ability to inhibit pro-inflammatory T-cell proliferation and cytokine secretion [38].

In experiments with adipocytes in cell culture, metformin was shown to significantly reduce pro-inflammatory response, in an **AMPK**-independent manner, by increasing the expression of **PFKFB3/ iPFK2**. This gene expression resulted in a significant decrease in the mRNA levels of **IL-1 β** and **TNF- α** , suppressing adipocyte inflammatory responses even in the presence of **lipopolysaccharide (LPS)**, a potent pro-inflammatory stimulus [61].

In a rat model of olanzapine-induced insulin resistance, metformin markedly decreased plasma levels of **TNF- α** , **IL-1 β** , and **IL-6**, and its protective effect was associated with reduced macrophage infiltration and inhibition of M1 polarization in white adipose tissue; however, the metabolic pathway involved is not described [29].

Overall, metformin exhibits apparent anti-inflammatory activity in experimental models; however, clinical results are less consistent and appear to depend on patient and treatment factors [38].

1.2.1.5 Placental response to metformin

Only a limited number of studies have investigated the direct effects of metformin on placental metabolism and structure. It is proposed that metformin modulates placental metabolism by reducing glucose uptake and shifting energy production from oxidative phosphorylation to glycolysis. A potential protective effect of metformin in the placenta is also described, involving improvements in angiogenesis, reduction of inflammation, and mitigation of oxidative stress [73, 22].

An experiment in human trophoblast from pregnancies with gestational diabetes treated with metformin has demonstrated, in vivo, fetal plasma levels approximately 1.5 times higher than maternal levels, and placental tissue levels with a linear relationship with maternal plasma concentrations. In vitro exposure of trophoblasts to clinically relevant metformin concentrations reduced basal mitochondrial respiration and **ATP** production, while simultaneously decreasing oxidative

stress at the level of gene expression. The authors cautioned, however, that impaired basal respiration and ATP synthesis may compromise placental bioenergetics needs and biosynthetic functions, given the organ's increasing energy demands throughout pregnancy [72].

Complementary evidence from a rat model of [gestational diabetes mellitus \(GDM\)](#) demonstrated that metformin treatment attenuated placental overgrowth, normalizing placental weight and restoring the typical mature architecture of the placenta to levels comparable with those of non-diabetic controls. Through histological, immunohistochemical, and molecular analyses, the study revealed that metformin modulated proangiogenic pathways, including upregulation of VEGF, KLF2, and PlGF, to levels observed in healthy placentas. Furthermore, the metformin group improved redox balance, with significantly increased total antioxidant capacity and suppressed oxidative stress markers, including endothelial nitric oxide synthase, nitric oxide, and malondialdehyde, compared to the [GDM](#) group [22].

1.3 Study Limitations in Placental Research

Animal model studies, while valuable, are limited by significant discrepancies in placental structure, function, and metabolic responses across species. Similarly, in vitro studies fail to replicate the complex maternal–fetal metabolic environment oscillations and dynamic adaptations to support progressive fetal growth demands that occur throughout gestation. These models are further complicated by methodological heterogeneity, which limits reproducibility and the interpretation of results [19, 5].

The current understanding of placental physiology and diabetes-related alterations primarily derives from studies using human placentas obtained in the late second and third trimesters, as ethical constraints limit access to early gestation tissue. However, older histopathologic studies examining the effects of diabetes on the human placenta may no longer reflect recent clinical observations due to advancements in pregnancy surveillance, improvement in glycemic control, and increasing incidence of obesity and type 2 diabetes [5].

The adoption of standardized gross and histological examinations, supported by description protocols with consistent terminology established by the Amsterdam Placental Workshop Group Consensus, has enhanced the reproducibility of placental analysis and strengthened clinical-pathological correlations with maternal health and fetal outcomes [65, 5].

1.4 Histopathological Markers of Placental Dysfunction

In 2014, a group of 26 perinatal and placental pathologists attended and actively participated in the workshop held in Amsterdam, Netherlands, to establish standardized guidelines for placental examination, including sampling strategies, lesion definitions, and diagnostic criteria for major histopathological lesions (Based on the Amsterdam Placental Workshop Group Consensus, published in 2016) [41].

1.4.1 Maternal Vascular Malperfusion

The features in this classification indicate impaired maternal blood flow to the placenta. Gross features include placental hypoplasia, with low weight and/or thin cord (<10th centile for gestational age), any infarction in pre-term placenta or central infarction compromising more than 5% at term, and retroplacental hemorrhage, with blood accumulation on the maternal surface. Microscopically, the decidua is dissected, and the overlying intervillous space is compressed. Histologic findings consist of villous development disturbance, featured by:

- Accelerated villous maturation, with short hypermature villi for gestational age, usually accompanied by increased syncytial knots and intervillous fibrin.
- Distal villous hypoplasia, defined by the involvement of more than 30% of a parenchymal slide with thin and elongated appearing villi, with increased syncytial knots.
- Decidual vasculopathy, including atherosclerosis, fibrinoid necrosis, absence of spiral artery remodeling, and arterial thrombosis.
- Collectively, these lesions reflect chronic uteroplacental insufficiency and are strongly associated with preeclampsia, chronic hypertension, [Intrauterine Growth Restriction \(IUGR\)](#), and pre-term birth.

1.4.2 Fetal Vascular Malperfusion

Describe the compromise of fetal blood flow within the placental vasculature. Segmental lesion, characterized by chorionic or stem villous vessel thrombotic occlusion or stem vessel obliteration, affects small territories. Alternatively, an [Fetal Vascular Malperfusion \(FVM\)](#) with global involvement suggests that umbilical blood circulation is partially obstructed, and a diffuse placental impairment is observed. Both patterns result in downstream disruption of villous development, including avascular villi, villous stromal-vascular karyorrhexis, and intramural fibrin deposition.

Avascular villi are terminal villi that present no capillaries and are characterized by bland hyaline fibrosis. The classification depends on the number of compromised villi foci. The presence of nonocclusive intramural fibrin deposition must be specified by whether it involves large fetal vessels with fibrin or fibrinoid deposition, indicating recent injury, or presents signs of calcification, indicating a more remote lesion. The villous stromal-vascular karyorrhexis criteria are based on the extension of focal breakdown of fetal vascular cells in the presence of intact trophoblast.

Noticeably, [FVM](#) was observed in both live-born and stillborn infants, and therefore, its presence alone does not establish causality for intrauterine demise. Nonetheless, careful characterization of the type and location of vascular thrombosis provides valuable insights into the severity of fetal circulatory compromise and potential contribution to adverse outcomes.

1.4.3 Delayed Villous Maturation

Described after 34-36 gestational weeks, and characterized by a predominance of a monotonous villous population with centrally positioned capillaries and reduced vasculosyncytial membranes. It reflects impaired terminal villous maturation and resembles the architecture of pre-term gestation, presenting clinical relevance when these features involve more than one-third of a full-thickness parenchymal section.

1.4.4 Inflammatory Lesions

There was an agreement during the Consensus that ascending intrauterine infection is a significant indication for placental pathology evaluations, with emphasis on the correlation between histological chorioamnionitis and the clinical presentation, which would not always be present.

Accurate documentation of placental inflammation requires a careful description of its topography and features, as well as determination of its chronicity, which enables the distinction between maternal and fetal inflammatory responses. This differentiation is clinically meaningful, as fetal inflammatory lesions are more strongly associated with adverse neonatal outcomes, whereas chronic and acute inflammatory patterns carry distinct clinical implications.

Subchorionitis, characterized by neutrophilic infiltration limited to the subchorionic space or beneath the chorion laeve, is regarded as an early manifestation of infection. Involvement of the choriodecidual interface reflects infection of the amniotic fluid and is reported as chorioamnionitis. The guidelines for grading and staging inflammation specify the occurrence and level of maternal response and/or fetal response, reflecting clinical relevance. Notably, describing which fetal vessels are affected is also relevant, as umbilical arteritis is associated with higher cytokine levels and worse neonatal outcomes compared to venous involvement.

1.4.5 Villitis of Unknown Etiology VUE

Villitis of Unknown Etiology (VUE) is a chronic lymphohistiocytic inflammation of chorionic villi in the absence of a viral or acute infection cause. Distinguishes in low-grade form, when fewer than 10 contiguous villi are inflamed in at least two foci, from high-grade **VUE**, when inflammation affects more than 10 contiguous villi across multiple foci. A diffuse pattern is diagnosed when more than 30% of all distal villi are involved.

Closely related to other chronic inflammatory reactions, **VUE** is often associated with chronic deciduitis and chronic intervillitis. The inflammatory cells may also compromise fetoplacental circulation through vessel wall injury, stem cell obliteration, and the development of contiguous avascular villi. Precise documentation of lesion extension and distribution is clinically relevant, as high-grade villitis is associated with adverse outcomes, such as fetal growth restriction, neurodevelopmental impairment, and risk of recurrence.

1.5 Placental Alterations and Lesions in Maternal Diabetes

The most consistent placental abnormalities described in diabetic pregnancies include increased placental weight, delayed villous maturation, and enhanced angiogenesis [37, 66, 5].

Placental efficiency is commonly estimated by the [birth weight-to-placental weight ratio \(BW/PW\)](#), which reflects the placental tissue's capacity to sustain the fetal requirements for nutrients. Placentas from diabetic mothers are often heavier than those from normoglycemic pregnancies; however, the [BW/PW](#) ratio is often preserved, suggesting an adaptive response to maintain fetal growth [59].

At the same time, it is reported that diabetes interferes with the normal progress of distal villous maturation. By 32 gestational weeks, over 80% of distal villi are expected to be immature, whereas, at term, delayed villous maturity is determined by more than 30% prevalence of villous immaturity. The placental lesion is characterized by enlarged villi with centrally placed capillaries, thickened trophoblast, and reduced vasculosyncytial membranes. Therefore, it increases the diffusion distance between maternal and fetal circulations, diminishing gas and nutrient transfer. Additionally, stromal fibrosis, basement membrane thickening, villous edema, and syncytial knot formation may also be found in diabetic placentas, further compromising exchange efficiency and contributing to adverse outcomes, including late stillbirth [41, 5, 66, 64].

The fetal hyperinsulinemia, a consequence of maternal hyperglycemia, promotes accelerated fetal growth by enhancing glucose aerobic metabolism, thereby increasing fetal oxygen demand. The primary placental adaptive response to compensate for this is angiogenic remodeling, which expands the exchange surface and facilitates oxygen transfer. A key regulator of placental vascular remodeling is fetal chronic hypoxia, with insulin as an additional contributing factor. However, excessive metabolic stress may lead to abnormal villous vascular expansion, characterized by excessive branching capillaries or chorangiosis [5, 20].

Other placental abnormalities, such as chronic villitis, fibrinoid deposition, syncytial knot, fetal vascular malperfusion, and calcification, are also reported more frequently in diabetic pregnancies, even under reasonable maternal glycemic control. Although these lesions are not specific to diabetes, their increased prevalence reflects the heightened susceptibility of the placenta to diabetic conditions [5].

2 Aims and Objectives

In light of divergent recommendations from major international guidelines on the use of metformin as an alternative to insulin for managing diabetes in pregnancy, this study aimed to provide a comprehensive understanding of its comparative effects on the placenta—a critical determinant of fetal health. The study was therefore designed to rigorously compare the placental and clinical outcomes of metformin versus insulin treatment across the spectrum of diabetic pregnancies (type 1, type 2, and gestational).

The pre-specified objectives were as follows:

Primary Objective: To compare the frequency of histopathological placental lesions between women treated with metformin versus those treated with insulin.

Secondary Objectives: To assess and compare a range of key maternal and neonatal clinical outcomes between the two treatment groups.

Furthermore, a subgroup analysis restricted to women with gestational diabetes mellitus was aimed at isolating treatment effects from the confounding influence of pre-existing disease. Conversely, the smaller sample size limits the statistical power, only for this analysis to provide an exploratory insight into the comparative effects of metformin versus insulin in a more pathologically homogeneous population.

3 Materials and Methods

The following sections detail the study design, population selection criteria, data collection procedures for maternal and neonatal information, placental analysis protocols, and the statistical methods used for data analysis.

3.1 Study Design

Following the institutional human research ethical committee approval (approval number: 236/2023), a comparative cross-sectional retrospective study was conducted over the period of January 2019 and October 2023 at the Departments of Gynecology-Obstetrics, Pediatrics, and Pathology of the University Hospital Center of São João, Porto, Portugal.

3.2 Study Population

The study included women with term singleton pregnancies complicated by type 1 diabetes, type 2 diabetes, or gestational diabetes who delivered at our institution during the study period. Eligibility was restricted to cases receiving pharmacological treatment with either metformin or insulin and with placentas for which histopathological reports were available.

Pregnancies managed exclusively with lifestyle modification, treated with combined metformin and insulin, complicated by fetal malformations, or lacking pathology reports were excluded.

During the study period, 1,037 singleton term births from mothers with diabetes, without chromosomal abnormalities or fetal malformations, were recorded. Of these, 317 placentas were submitted for pathological examination, and 261 pathology reports were retrieved. Comparative analyses were performed on the 105 eligible cases: 59 treated with metformin and 46 with insulin.

To minimize potential bias due to the higher prevalence of pregestational diabetes in the insulin group (25 cases in the Insulin group and only 2 in the Metformin group), a secondary comparative analysis was performed, restricting the population to the 78 cases of gestational diabetes, comprising 57 treated with metformin and 21 treated with insulin.

3.3 Maternal Data Survey

Maternal clinical and anthropometric data were obtained from electronic medical records across the course of pregnancy. All patient information was anonymized and coded prior to analysis.

Diabetes diagnosis criteria: Diabetes during pregnancy was diagnosed according to IADPSG established criteria: fasting plasma glucose $\geq 92 \text{ mg/dL}$ in the first trimester, random plasma glucose $\geq 200 \text{ mg/dL}$, HbA1c $\geq 6.5\%$, or a 75 g oral glucose tolerance test performed between 24-28 weeks of gestation with at least one abnormal value (fasting glucose $\geq 92 \text{ mg/dL}$, 1-h glucose $\geq 180 \text{ mg/dL}$, or 2-h glucose $\geq 153 \text{ mg/dL}$) [51].

Glycemic control criteria: Glycemic control criteria control was defined according to the ADA Standards of Care, 2024, which recommend a target glycosylated haemoglobin (HbA1c) $< 6\%$ ($< 42 \text{ mmol/mol}$). Pregnancies that exceeded this threshold at any time were classified as uncontrolled diabetes [4].

Pre-gestational body mass index criteria: Pre-gestational body mass index was classified according to World Health Organization criteria: underweight ($< 18.5 \text{ kg/m}^2$), normal weight ($18.5\text{-}24.9 \text{ kg/m}^2$), overweight ($25\text{-}29.9 \text{ kg/m}^2$), and obesity ($\geq 30.0 \text{ kg/m}^2$) [80].

3.4 Neonatal Data Survey

Neonatal clinical and anthropometric data were collected from electronic medical records at birth and during the early postnatal period. Outcomes included hypoglycemia, jaundice, transient tachypnea, respiratory distress syndrome with hypoxemia, thrombocytopenia, polycythemia, meconium-stained amniotic fluid, and neonatal intensive care unit admission. All data were anonymized and linked to maternal codes.

Birthweight classification. Neonatal birth weight was assessed according to the INTERGROWTH-21st standards, with infants classified as small ($< 10\text{th}$ percentile), appropriate ($10\text{th}\text{-}89\text{th}$ percentile), or large ($\geq 90\text{th}$ percentile) according to gestational age and sex [77].

3.5 Placental Analysis Criteria

After delivery, placentas were collected, processed, and sampled for macroscopic and histopathological evaluation. Analyses were performed in accordance with the Amsterdam Placental Workshop Group Consensus Statement [41]. Experienced histopathologists performed the placenta examination using light microscopy and were blinded to pharmacological treatment in cases of

type 2 and gestational diabetes. Clinical data available to the pathologist included maternal parity, pregnancy-related or specific pathological conditions, neonatal birth weight, Apgar score, and gestational age at delivery.

Gross morphological findings compared between metformin and insulin groups included placental weight, weight percentile for gestational age [60], greatest chorionic disk diameter, feto-placental weight ratio, umbilical cord insertion site, number of umbilical blood vessels, and umbilical cord coiling **umbilical cord coiling index (UCI)**. Placentas with sampling defects were excluded from the statistical analysis of weight and feto-placental weight ratio. For disk diameter measurement, cases with Incomplete placenta or that presented succenturiate or accessory lobes were also excluded from statistical evaluation [67].

The **UCI** was classified according to the criteria: hypocoiled cord (< 0.07 coils/cm), normocoiled cord ($0.07 - 0.3$ coils/cm), and hypercoiled cord (> 0.3 coils/cm) [75]. Histological lesions of placental tissue were compared between the metformin and insulin groups, including maternal vascular malperfusion, fetal vascular malperfusion, inflammatory lesions (chorioamnionitis, maternal inflammatory response, fetal inflammatory response, villitis, deciduitis), villous stromal-vascular lesions (corangiosis), villous maturation disorders, and evidence of meconium exposure.

3.6 Statistical Analysis

All statistical analyses were performed using **Statistical Package for the Social Sciences (SPSS)** version 28.0 [34]. Continuous variables were expressed as mean $\pm$ **Standard Deviation (SD)** or median with **Interquartile Range (IQR)**, depending on the data distribution. Categorical variables were presented as frequencies (n) and percentages (%). Comparisons between groups (metformin vs. Insulin) for continuous variables were conducted using the independent *t-test* or the *Mann-Whitney U test*, following assessment of normality using the *Kolmogorov-Smirnov test*. Categorical variables were compared between the two groups using the *Chi-square test* (χ^2) and *Fisher's exact test*, with *Phi* or *Cramer's V* employed to assess the strength and direction of associations. Statistical significance was defined at *p-value* < 0.05 (two-tailed).

4 Results

4.1 Diabetes diagnosis (Table 4.1)

In the study population, the distribution of diabetes types differed markedly between treatment groups. As expected, all cases of type 1 diabetes (47.8% of the insulin group) were treated with insulin. Gestational diabetes predominated in the metformin group (96.6%) and accounted for less than half of the insulin group (45.7%). Type 2 diabetes was less frequently observed in both cases (3.4%) in the metformin group and 3 cases (6.5%) in the insulin group. No cases with missing data were identified.

4.1.1 Maternal Demographic, Anthropometric, and Clinical Characteristics (Table 4.1)

Maternal clinical profiles were broadly comparable between the metformin and insulin-treated groups, with no significant differences in age, gravidity, previous parity ($p = 0.684$, $p = 0.948$ and $p = 0.989$ respectively), smoking or comorbidities including hypertensive disorders, cardiac, pulmonary, renal disease or anemia, during pregnancy ($p = 0.869$, $p = 0.100$, $p = 0.438$, $p = 0.227$, $p = 0.438$ and $p = 0.693$ respectively). Thyroid disease, however, was significantly more prevalent among women treated with insulin (71.4%) compared with those receiving metformin (28.6%) ($\chi^2(1, N = 105) = 10.84$, $p < 0.001$). Additionally, intrapartum maternal fever was reported exclusively in the metformin group (11.9%), with a significant difference, but low association (Fisher's test, $p = 0.017$, $\Phi = -0.236$).

Pre-pregnancy BMI differed significantly between hypoglycemic treatment groups, with a moderate overall association ($\chi^2(2, N = 105) = 9.004$, $p = 0.011$, Cramer's $V = 0.293$). Women in the insulin group were more frequently classified within the normal BMI range ($\chi^2(1, N = 105) = 8.919$, $p = 0.003$, $\Phi = 0.291$). In contrast, those in the metformin group showed a modest association with obesity ($\chi^2(1, N = 105) = 4.645$, $p = 0.031$, $\Phi = 0.210$). In contrast, gestational weight gain was significantly greater in the insulin group (median 14.4 kg, IQR 7.4) compared with the metformin group (median 9.7 kg, IQR 7.8), with differences confirmed in both central tendency and distribution (Mann-Whitney $U = 1782.5$, $p < 0.001$, 95% CI $[-7.400, -1.900]$; Kolmogorov-Smirnov $Z = 1.588$, $p = 0.013$).

Glycemic control was strongly associated with treatment group ($\chi^2(1, N = 100) = 43.778$, $p < 0.001$, $\Phi = 0.662$). The majority of women receiving metformin maintained HbA1c levels

< 6.0% throughout pregnancy (86.4%), whereas those treated with insulin had markedly higher odds of uncontrolled HbA1c ($\geq 6.0\%$) (69.5% vs. 6.8%), corresponding to an odds ratio of 31.38 (95% CI: 9.40 – 104.68).

4.1.2 Perinatal and neonatal outcomes (Tables 4.2 and 4.3)

Gestational age at delivery, sex of neonate, neonatal length, and head circumference results indicated no significant difference ($p = 0.100$, $p = 0.232$, $p = 0.160$, and $p = 0.139$, respectively). However, mean birth weight was significantly lower in the metformin group (3197.0 ± 495.9 g) compared with the insulin group (3562 ± 534.6 g) (t -Test (103) = 0.485, $p = < 0.001$, 95% CI: -565.12, -164, 93). Birth weight percentiles also differed significantly, with a moderate association ($\chi^2(2, N = 105) = 10.896$, $p = 0.004$, Cramer's $V = 0.322$).

Perinatal outcomes did not differ significantly between treatment groups. Median APGAR scores at first, fifth, and tenth minutes were uniformly high (9.0, 10, and 10, respectively), with no significant intergroup variation ($p = 0.320$, $p = 0.334$, and $p = 0.073$). Similarly, the frequency of NICU admission ($p = 0.748$), neonatal hypoglycemia ($p = 0.100$), jaundice ($p = 0.521$), and jaundice requiring phototherapy ($p = 0.486$) was comparable between the groups. Other neonatal complications, such as transient tachypnea, respiratory distress syndrome with hypoxemia, meconium-stained amniotic fluid, thrombocytopenia, and polycythemia, were infrequent in both groups and showed no statistically significant differences.

4.1.3 Placental Morphometry, Fetoplacental Ratio and Umbilical Cord Features (Table 4.4)

Mean placental weight was higher in the insulin group (504.7 ± 114.0 g) than in the metformin group (464.1 ± 95.3 g), showing a trend toward significance (t -Test (99) = 0.417, $p = 0.059$). A complementary percentile-based analysis for gestational age confirmed no significant differences between groups in the lowest decile ($\chi^2(1, N = 101) = 0.528$, $p = 0.467$) or within the 10th-89th percentile range ($\chi^2(1, N = 101) = 0.318$, $p = 0.573$). Placental weight in the highest decile was more frequent in the insulin group (17.4%) compared with the metformin group (5.1%), likewise indicating a trend without statistical significance (Fisher's exact test, $p = 0.058$).

Notably, the feto-placental weight ratio differed significantly between groups with a strong association ($\chi^2(2, N = 105) = 13.004$, $p = 0.002$, Cramer's $V = 0.359$). Despite no statistical difference in feto-placental weight Ratios below the 10th percentile (Fisher's exact test, $p = 0.237$), ratios within the 10th-89th percentile were more frequent in the metformin group, with over seven-fold higher odds compared to the insulin group ($\chi^2(1, N = 105) = 9.229$, $p < 0.001$, $\Phi = 0.296$; OR = 7.12, 95% CI: 2.19, 23.48). In contrast, ratios above the 90th percentile were significantly less common in the metformin group, with nearly a 90% reduction in odds ($\chi^2(1, N = 105) = 10.036$, $p = 0.002$, $\Phi = -0.309$; OR = 0.11, 95% CI: 0.02 – 0.54).

No statistically significant differences were found between the groups regarding the greatest placental diameter ($p = 0.516$), umbilical cord insertion ($p = 0.621$), number of umbilical vessels ($p = 1.000$), and umbilical cord coiling index ($p = 0.940$).

4.1.4 Placental Inflammatory Lesions (Table 4.5)

The metformin group exhibited a higher frequency of fetal inflammatory response (37.3%) compared with the insulin group (19.6%), with a small but statistically significant association ($\chi^2(1, N = 105) = 3.647, p = 0.048, \Phi = 0.193$). Metformin exposure increased the odds of a fetal inflammatory response by more than two-fold, although the confidence interval suggests a borderline effect (OR = 2.44, 95% CI : 0.99 – 6.00).

A similar association was found with chronic deciduitis, which occurred at a significantly higher frequency in the metformin group compared to the insulin group (42.4% vs. 21.7%; $\chi^2(1, N = 105) = 5.146, p = 0.026, \Phi = 0.217$). Women treated with metformin exhibited 2.64 times higher odds of developing chronic deciduitis (OR = 2.64, 95% CI : 1.10, 6.63).

The metformin and insulin groups did not differ significantly in other placental inflammatory lesions that were also analysed, including acute and/or chronic inflammation (chorioamnionitis, chorionitis, subchorionitis, villitis, or deciduitis; $p = 0.192$), chronic inflammation alone ($p = 0.192$), acute inflammation ($p = 0.939$), maternal inflammatory response ($p = 0.154$), or chronic villitis ($p = 0.703$). Chronic chorioamnionitis occurred infrequently, with two cases in the metformin group and five in the insulin group ($p = 0.236$). Chronic chorionitis was observed only in the metformin group (five cases), suggesting a nonsignificant trend toward a higher frequency compared with insulin ($p = 0.066, \Phi = 0.197$). In contrast, chronic subchorionitis was absent in both groups.

4.1.5 Placental Villous and Intervillous Pathology (Table 4.6)

No statistically significant association was found between the treatment groups and the patterns of placental villous maturation. The incidence of delayed villous maturation was comparable between the metformin (61.0%) and insulin (67.4%) groups ($p = 0.320$), as were the frequencies of adequate (35.6% vs. 21.7%; $p = 0.159$) and accelerated villous maturation (3.4% vs. 6.5%; $p = 0.649$).

Likewise, the analysis of the incidences of chorangiosis (33.9% vs. 23.9%) and intervillous thrombosis (18.6% vs. 17.4%) revealed no significant differences between the metformin and insulin groups ($p = 0.266$ and $p = 0.869$, respectively). Other pathologies, including massive perivillous fibrin deposition, distal villous hypoplasia, and chronic histiocytic intervillitis, were rare in both groups, precluding meaningful statistical comparison.

4.1.6 Other Placental Finding (Table 4.6)

Histological evidence of placental meconium exposure showed no significant association with treatment allocation ($p = 0.970$), occurring in nearly identical proportions in the metformin (47.5%)

and insulin (47.8%) groups.

4.2 Secondary Analysis: Gestational Diabetes Mellitus Subgroup

The primary analysis of the total cohort revealed a discrepancy in the distribution of diabetes subtypes between the treatment arms (Table 4.1). The metformin group consisted almost entirely of women with gestational diabetes mellitus (96.6%), whereas the insulin group included a total of women with type 1 diabetes (representing 47.8% of the insulin group population).

Given this heterogeneity, a pre-specified secondary analysis restricted to the GDM cohort was conducted to enable a more direct and homogeneous comparison. Separate analyses for other diabetes subtypes were not performed, as patients with type 1 diabetes ($n = 22$) were managed exclusively with insulin and those with type 2 diabetes ($n = 5$) were too few for meaningful comparison, enabling a comparison of placental and clinical outcomes for the GDM-only cohort (Metformin, $n = 57$; Insulin, $n = 21$).

The following results compare the placental and clinical outcomes for the GDM-only cohort (Metformin, $n = 57$; insulin, $n = 21$).

4.2.1 Other Placental Finding (Table 4.7)

In the gestational diabetes-only subgroup, maternal demographic and clinical characteristics were well balanced between the metformin and insulin groups, with no significant differences in age, gravidity, parity, smoking status, or hypertensive disorders (all $p > 0.05$, Table 4.7). Only a few cases of cardiac and pulmonary disease were reported, and no renal disease occurred in either group, precluding meaningful statistical comparisons for these conditions.

Conversely, the prevalence of thyroid disease was significantly higher in the insulin group. This was a robust finding, with the proportion of women with thyroid disease in the insulin group being significantly greater than in the metformin group in both the primary analysis ($p < 0.001$) and the GDM-only subgroup (38.1% vs. 10.3%; $p = 0.009$).

The previously significant associations between pharmacological treatment group and both pre-pregnancy BMI and gestational weight gain were no longer observed in the GDM-only analysis. Median pre-pregnancy BMI did not differ significantly between women treated with metformin (32.0 kg/m^2 , IQR 9.0) and those treated with insulin (29.7 kg/m^2 , IQR 8.7). Similarly, gestational weight gain was comparable between the metformin group (9.7 kg, IQR 8.8) and the insulin group (11.2 kg, IQR 7.4). The associations weakening likely reflect the influence of type 1 diabetes cases in the overall cohort, who were treated exclusively with insulin and characterized by both lower pre-pregnancy BMI (mean 23.4 kg/m^2 , IQR 4.9) and higher gestational weight gain (mean 15.7 kg, IQR 7.3).

In the primary analysis, metformin treatment was significantly associated with intrapartum maternal fever (11.9% vs. 0%; $p = 0.012$). Although the direction and magnitude of this effect

were unchanged in the **GDM**-only subgroup (12.3% vs. 0%), the association lost statistical significance ($p = 0.059$). This likely reflects the limited statistical power resulting from the smaller sample size.

In contrast, the association between metformin treatment and superior glycemic control was robust, remaining highly significant in both the primary analysis ($p < 0.001$) and the **GDM**-only analysis ($p = 0.003$). Within the **GDM** subgroup, a significantly higher proportion of women in the metformin group achieved glycemic control compared to those treated with insulin (87.7% vs. 61.9%).

4.2.2 Perinatal and neonatal outcomes (Table 4.8)

Perinatal and neonatal outcomes were also comparable between the treatment groups in the **GDM** subgroup. No significant differences were observed in gestational age at birth, neonatal anthropometric measures (length and head circumference), Apgar scores at 1, 5, and 10 minutes, or admission to the Neonatal Intensive Care Unit (all $p > 0.05$, Table 4.8). Furthermore, the incidence of other neonatal morbidities, including hypoglycemia, respiratory distress, and jaundice, was low and did not differ between the groups.

The significant difference in birth weight observed in the total cohort was no longer present in the **GDM**-only analysis, where mean birth weight was comparable between the metformin (3180.2 ± 493.0 g) and insulin (3283.8 ± 408.0 g) groups ($p = 0.393$). This suggests the initial finding was an artifact of the study population, driven by the significantly higher birth weights of infants born to mothers with type 1 diabetes (median 3865.0 g), who were managed exclusively with insulin.

The only new significant association to emerge in the **GDM**-only subgroup was in neonatal sex. In contrast to the primary analysis, where no difference was observed ($p = 0.232$), a significant association with neonatal sex emerged in the **GDM**-only subgroup. The proportion of male neonates was significantly higher in the insulin group than in the metformin group (76.2% vs. 49.1%; $p = 0.032$). Given the multiple secondary outcomes examined, this isolated finding should be interpreted with caution, as it may represent a chance finding.

4.2.3 Placental Morphometry, Fetoplacental Ratio, Umbilical Cord Features, and Vascular Pathology (Table 4.9)

Differences in placental characteristics observed in the total cohort were attenuated mainly in the **GDM**-only subgroup, suggesting that they were primarily driven by underlying population heterogeneity. In the primary analysis, the insulin group exhibited a trend toward higher mean placental weight ($p = 0.059$) and a significantly different **feto-placental weight ratio (FPR)** distribution, including a higher proportion of high **FPR** (≥ 90 th percentile) ($p = 0.002$). However, neither of these differences was evident in the **GDM**-only analysis, where the mean placental weight ($p = 0.778$) and the **FPR** distribution ($p > 0.10$ for all categories) were comparable between groups. The attenuation of these findings is explained by the confounding influence of the insulin-treated type 1

diabetes cohort, which was characterized by both markedly higher mean placental weights and a distinct [FPR](#) profile.

4.2.4 Placental Inflammatory Lesions (Table 4.10)

In the primary analysis of the total cohort, metformin treatment was associated with a significantly higher frequency of both fetal inflammatory response (37.3% vs. 19.6%; $p = 0.048$) and chronic deciduitis (42.4% vs. 21.7%; $p = 0.026$). While the direction of these associations remained consistent in the [GDM](#)-only subgroup, neither retained statistical significance ($p = 0.056$ and $p = 0.100$, respectively), a finding likely attributable to the reduced statistical power of the smaller sample. The frequencies of all other placental inflammatory lesions examined were comparable between the treatment groups in both primary and subgroup analyses (all $p > 0.05$).

4.2.5 Placental Villous and Intervillous Pathology and Other Placental Findings (Table 4.11)

Finally, no significant differences were observed between the treatment groups for a range of other placental pathologies in either the primary or [GDM](#)-only analyses. This included patterns of villous maturation, the prevalence of chorangiosis, intervillous thrombosis, and histological evidence of meconium exposure (all $p > 0.05$). Other rare lesions, such as massive perivillous fibrin deposition, distal villous hypoplasia, and chronic histiocytic intervillitis, were reported too infrequently in either group for meaningful statistical comparison.

Maternal Demographic, Anthropometric, and Clinical Characteristics	Metformina (N=59)	Insulina (n=46)	p-value
Diabetes diagnosis, n (%)			
Gestational diabetes	57 (96.6)	21 (45.7)	
Type 1 diabetes mellitus	0 (0.0)	22 (47.8)	
Type 2 diabetes mellitu	2 (3.4)	3 (6.5)	
Missing	0 (0.0)	0 (0.0)	
Maternal age at delivery (years), median (IQR) / mean (±SD)	34.9 (±5.6)	34.4 (±5.3)	0.684
Missing, n(%)	0 (0.0)	0 (0.0)	
Gravidity, n(%)			
Primigravida	24 (40.7)	19 (41.3)	0.948
Multigravida	35 (59.3)	27 (58.7)	
Missing	0 (0.0)	0 (0.0)	
Previous parity, n(%)			
Nulliparous	29 (49.2)	22 (47.8)	0.989
Primiparous	21 (35.6)	17 (37.0)	
Multiparous	9 (15.3)	7 (15.2)	
Missing	0 (0.0)	0 (0.0)	
Pre-pregnancy BMI (kg/m²), median (IQR) / mean (±SD)	32.0 (10.1)	25.6 (8.7)	<0.001 /
Missing, n (%)	0 (0.0)	0 (0.0)	0.008
Pre-pregnancy BMI, n (%)			
Normal	12 (20.3)	22 (47.8)	0.011
Overweight	14 (23.7)	8 (17.4)	
Obesity	33 (55.9)	16 (34.8)	
Missing	0 (0.0)	0 (0.0)	
Normal pre-pregnancy BMI, n (%)			
Present	12 (20.3)	22 (47.8)	0.003
Absent	47 (79.7)	24 (52.2)	
Missing	0 (0.0)	0 (0.0)	
Overweight pre-pregnancy BMI, n (%)			
Present	14 (23.7)	8 (17.4)	0.429
Absent	45 (76.4)	38 (82.6)	
Missing	0 (0.0)	0 (0.0)	
Obesity pre-pregnancy BMI, n (%)			
Present	33 (55.9)	16 (34.8)	0.031
Absent	26 (44.1)	30 (65.2)	
Missing	0 (0.0)	0 (0.0)	
Gestational weight gain (kg), median (IQR) / mean (±SD)	9.7 (7.8)	14.4 (7.4)	<0.001 /
Missing, n (%)	3 (5.1%)	0 (0.0)	0.013
Glycated hemoglobin tests during pregnancy, n (%)			
Controlled (all values <6.0%)	51 (86.4)	13 (28.3)	<0.001
Uncontrolled (any value ≥ 6.0%)	4 (6.8)	32 (69.5)	
Missing	4 (6.8)	1 (2.2)	
Intrapartum maternal fever, n (%)			
Present	7 (11.9)	0 (0.0)	0.017
Absent	52 (88.1)	46 (100.0)	
Missing	0 (0.0)	0 (0.0)	
Smoking, n (%)			
Present	11 (18.6)	8 (17.4)	0.869
Absent	48 (81.4)	38 (82.6)	
Missing	0 (0.0)	0 (0.0)	
Hypertensive disorders, n (%)			
Present	17 (28.8)	7 (15.2)	0.100
Absent	42 (71.2)	39 (84.8)	
Missing	0 (0.0)	0 (0.0)	
Cardiac disease, n (%)			
Present	0 (0.0)	1 (2.2)	0.438
Absent	59 (100.0)	45 (97.8)	
Missing	0 (0.0)	0 (0.0)	
Pulmonary disease, n (%)			
Present	5 (8.5)	1 (2.2)	0.227
Absent	54 (91.5)	45 (97.8)	
Missing	0 (0.0)	0 (0.0)	
Renal disease, n (%)			
Present	0 (0.0)	1 (2.2)	0.438
Absent	59 (100.0)	45 (97.8)	
Missing	0 (0.0)	0 (0.0)	
Thyroid disease, n (%)			
Present	6 (10.2)	17 (37.0)	<0.001
Absent	53 (89.8)	29 (63.0)	
Missing	0 (0.0)	0 (0.0)	
Anemia, n (%)			
Present	4 (6.8)	2 (4.3)	0.693
Absent	55 (93.2)	44 (95.7)	
Missing	0 (0.0)	0 (0.0)	

Table 4.1: Maternal Demographic, Anthropometric, and Clinical Characteristics

Perinatal and neonatal outcomes	Metformina (N=59)	Insulina (n=46)	p-value
Gestational age at delivery (days), median (IQR) / mean ($\pm$SD)*	272.7 ($\pm$ 5.5)	272.0 (5.0)	0.100 /
Missing, n(%)	0 (0.0)	0 (0.0)	0.152
*Gestational age at delivery (equivalent in weeks)	38.9 ($\pm$ 0.8)	38.6 (0.7)	
Sex of neonate, n(%)			
Female	30 (50.8)	18 (39.1)	0.232
Male	29 (49.2)	28 (60.9)	
Missing	0 (0.0)	0 (0.0)	
First minute APGAR score, median (IQR) / mean ($\pm$SD)	9.0 (0.0)	9.0 (0.0)	0.320 /
Missing, n(%)	0 (0.0)	0 (0.0)	0.968
Fifth minute APGAR score, median (IQR) / mean ($\pm$SD)	10.0 (0.0)	10.0 (0.0)	0.334 /
Missing, n(%)	0 (0.0)	0 (0.0)	0.992
Tenth minute APGAR score, median (IQR) / mean ($\pm$SD)	10.0 (0.0)	10.0 (0.0)	0.073 /
Missing, n(%)	0 (0.0)	0 (0.0)	1.000
Birth weight (g), median (IQR) / mean ($\pm$SD)	3197.0 ($\pm$ 495.4)	3562.0 ($\pm$ 534.6)	<0.001
Missing, n(%)	0 (0.0)	0 (0.0)	
Birth weight percentile for gestational age, n (%)			
<10	7 (11.9)	2 (4.3)	0.004
≥ 10 e ≤ 90	43 (72.9)	24 (52.2)	
>90	9 (15.2)	20 (43.5)	
Missing	0 (0.0)	0 (0.0)	
Birth weight <P10 gestational age, n (%)			
Present	7 (11.9)	2 (4.3)	0.293
Absent	52 (88.1)	44 (95.7)	
Missing	0 (0.0)	0 (0.0)	
Birth weight ≥ 10 e ≤ 90 gestational age, n (%)			
Present	43 (72.9)	24 (52.2)	0.028
Absent	16 (27.1)	22 (47.8)	
Missing	0 (0.0)	0 (0.0)	
Birth weight >P90 gestational age, n (%)			
Present	9 (15.2)	20 (43.5)	0.001
Absent	50 (84.7)	26 (56.5)	
Missing	0 (0.0)	0 (0.0)	
Neonatal length (cm), median (IQR) / mean ($\pm$SD)	49.1 ($\pm$ 2.3)	50.0 (2.6)	0.160
Missing, n(%)	0 (0.0)	0 (0.0)	
Head circumference (cm), median (IQR) / mean ($\pm$SD)	34.5 (2.0)	34.9 ($\pm$ 1.4)	0.139 /
Missing, n(%)	0 (0.0)	0 (0.0)	0.654
NICU admission, n (%)			
Present	9 (15.3)	6 (13.0)	0.748
Absent	50 (84.7)	40 (87.0)	
Missing	0 (0.0)	0 (0.0)	
Hypoglycemia, n (%)			
Present	3 (5.1%)	7 (15.2)	0.100
Absent	56 (94.9)	39 (84.8)	
Missing	0 (0.0)	0 (0.0)	
Jaundice, n (%)			
Present	22 (37.3)	20 (43.5)	0.521
Absent	37 (62.7)	26 (56.5)	
Missing	0 (0.0)	0 (0.0)	
Jaundice requiring phototherapy, n (%)			
Present	12 (20.3)	12 (26.1)	0.486
Absent	47 (79.7)	34 (73.9)	
Missing	0 (0.0)	0 (0.0)	
Transient tachypnea, n (%)			
Present	2 (3.4)	3 (6.5)	0.652
Absent	57 (96.6)	43 (93.5)	
Missing	0 (0.0)	0 (0.0)	
Respiratory distress syndrome with hypoxemia, n (%)			
Present	2 (3.4)	2 (4.3)	1.000
Absent	57 (96.6)	44 (95.7)	
Missing	0 (0.0)	0 (0.0)	
Meconium-stained amniotic fluid, n (%)			
Present	4 (6.8)	2 (4.3)	0.693
Absent	55 (93.2)	44 (95.7)	
Missing	0 (0.0)	0 (0.0)	
Thrombocytopenia, n (%)			
Present	2 (3.4)	1 (2.2)	1.000
Absent	57 (96.6)	45 (97.8)	
Missing	0 (0.0)	0 (0.0)	
Polycythemia, n (%)			
Present	1 (1.7)	1 (2.2)	1.000
Absent	58 (98.3)	45 (97.8)	
Missing	0 (0.0)	0 (0.0)	

Table 4.2: Perinatal and neonatal outcomes (Part 1)

Placental Morphometry & Fetoplacental Ratio	Metformina (N=59)	Insulina (n=46)	p-value
Feto-placental weight ratio, n (%)			
<10th percentile (low)	2 (3.4)	5 (10.9)	0.002
10th–89th percentile (appropriate)	52 (88.1)	29 (63.0)	
≥90th percentile (high)	2 (3.4)	11 (23.9)	
Missing (including sampling defect)	3 (5.1)	1 (2.2)	
Feto-placental weight <10th percentile (low), n (%)			
Present	2 (3.4)	5 (10.9)	0.237
Absent	54 (91.5)	40 (87.0)	
Missing (including sampling defect)	3 (5.1)	1 (2.2)	
Feto-placental weight 10th–89th percentile (appropriate), n (%)			
Present	52 (88.1)	29 (63.0)	<0.001
Absent	4 (6.8)	16 (34.8)	
Missing (including sampling defect)	3 (5.1)	1 (2.2)	
Feto-placental weight ≥90th percentile (high), n (%)			
Present	2 (3.4)	11 (23.9)	0.002
Absent	54 (91.5)	34 (73.9)	
Missing (including sampling defect)	3 (5.1)	1 (2.2)	
Placental weight (g), median (IQR) / mean (±SD)	462.5 (±98.1)	503.0 (±114.8)	0.059
Missing, n(%)	3 (5.1)	1 (2.2)	
Placental weight percentile, n (%)			
<10th percentile (low)	20 (33.9)	13 (28.2)	0.113
10th–89th percentile (appropriate)	33 (55.9)	24 (52.2)	
≥90th percentile (high)	3 (5.1)	8 (17.4)	
Missing, n(%)	3 (5.1)	1 (2.2)	
Placental weight <10th percentile (low), n (%)			
Present	20 (33.9)	13 (28.3)	0.467
Absent	36 (61.0)	32 (69.6)	
Missing (including sampling defect)	3 (5.1)	1 (2.2)	
Placental weight within 10th–89th percentile (appropriate), n (%)			
Present	33 (55.9)	24 (52.2)	0.573
Absent	23 (39.0)	21 (45.7)	
Missing (including sampling defect)	3 (5.1)	1 (2.2)	
Placental weight ≥90th percentile (high), n (%)			
Present	3 (5.1)	8 (17.4)	0.058
Absent	53 (89.8)	37 (80.4)	
Missing (including sampling defect)	3 (5.1)	1 (2.2)	
Greatest placental diameter (cm), median (IQR) / mean (±SD)	19.0 (3.0)	19.0 (2.5)	0.516 /
Missing, n(%)	5 (8.5)	3(6.5)	

Table 4.3: Perinatal and neonatal outcomes (Part 2)

Placental Morphometry & Fetoplacental Ratio	Metformina (N=59)	Insulina (n=46)	p-value
Feto-placental weight ratio, n (%)			
<10th percentile (low)	2 (3.4)	5 (10.9)	0.002
10th–89th percentile (appropriate)	52 (88.1)	29 (63.0)	
≥90th percentile (high)	2 (3.4)	11 (23.9)	
Missing (including sampling defect)	3 (5.1)	1 (2.2)	
Feto-placental weight <10th percentile (low), n (%)			
Present	2 (3.4)	5 (10.9)	0.237
Absent	54 (91.5)	40 (87.0)	
Missing (including sampling defect)	3 (5.1)	1 (2.2)	
Feto-placental weight 10th–89th percentile (appropriate), n (%)			
Present	52 (88.1)	29 (63.0)	<0.001
Absent	4 (6.8)	16 (34.8)	
Missing (including sampling defect)	3 (5.1)	1 (2.2)	
Feto-placental weight ≥90th percentile (high), n (%)			
Present	2 (3.4)	11 (23.9)	0.002
Absent	54 (91.5)	34 (73.9)	
Missing (including sampling defect)	3 (5.1)	1 (2.2)	
Placental weight (g), median (IQR) / mean (±SD)	462.5 (±98.1)	503.0 (±114.8)	0.059
Missing, n(%)	3 (5.1)	1 (2.2)	
Placental weight percentile, n (%)			
<10th percentile (low)	20 (33.9)	13 (28.2)	0.113
10th–89th percentile (appropriate)	33 (55.9)	24 (52.2)	
≥90th percentile (high)	3 (5.1)	8 (17.4)	
Missing, n(%)	3 (5.1)	1 (2.2)	
Placental weight <10th percentile (low), n (%)			
Present	20 (33.9)	13 (28.3)	0.467
Absent	36 (61.0)	32 (69.6)	
Missing (including sampling defect)	3 (5.1)	1 (2.2)	
Placental weight within 10th–89th percentile (appropriate), n (%)			
Present	33 (55.9)	24 (52.2)	0.573
Absent	23 (39.0)	21 (45.7)	
Missing (including sampling defect)	3 (5.1)	1 (2.2)	
Placental weight ≥90th percentile (high), n (%)			
Present	3 (5.1)	8 (17.4)	0.058
Absent	53 (89.8)	37 (80.4)	
Missing (including sampling defect)	3 (5.1)	1 (2.2)	
Greatest placental diameter (cm), median (IQR) / mean (±SD)	19.0 (3.0)	19.0 (2.5)	0.516 / 0.910
Missing, n(%)	5 (8.5)	3 (6.5)	
Umbilical Cord Features	Metformina (N=59)	Insulina (n=46)	p-value
Umbilical cord insertion, n (%)			
Central	9 (15.3)	11 (23.9)	0.621
Paracentral	30 (50.8)	20 (43.5)	
Paramarginal	14 (23.7)	12 (26.1)	
Marginal	6 (10.2)	3 (6.5)	
Missing	0 (0.0)	0 (0.0)	
Number of umbilical vessels, n (%)			
Two	1 (1.7)	1 (2.2)	1.000
Three	58 (98.3)	45 (97.8)	
Missing	0 (0.0)	0 (0.0)	
Umbilical cord coiling index, n (%)			
Hypocoiled (UCI <0.07)	0 (0.0)	0 (0.0)	0.940
Normocoiled (UCI 0.07–0.30)	32 (54.2)	28 (60.9)	
Hypercoiled (UCI >0.30)	11 (18.7)	10 (21.7)	
Missing	16 (27.1)	8 (17.4)	
Placental Maternal & Fetal Vascular Pathology	Metformina (N=59)	Insulina (n=46)	p-value
Maternal vascular malperfusion, n(%)			
Present	26 (44.1)	25 (54.3)	0.296
Absent	33 (55.9)	21 (45.7)	
Missing	0 (0.0)	0 (0.0)	
Fetal vascular malperfusion, n (%)			
Present	2 (3.4)	2 (4.3)	1.000
Absent	57 (96.6)	44 (95.7)	
Missing	0 (0.0)	0 (0.0)	
Hypoxic-ischemic changes, n(%)			
Present	11 (18.7)	8 (17.4)	0.869
Absent	48 (81.4)	38 (82.6)	
Missing	0 (0.0)	0 (0.0)	

Table 4.4: Placental Morphometry, Fetoplacental Ratio, and Umbilical Cord Features

Placental Inflammatory Lesions	Metformina (N=59)	Insulina (n=46)	p-value
Acute and/or chronic inflammation, n (%) (chorioamnionitis, chorionitis, subchorionitis, villitis, deciduitis)			
Present	43 (72.9)	28 (60.9)	0.192
Absent	16 (27.1)	18 (39.1)	
Missing	0 (0.0)	0 (0.0)	
Chronic inflammation, n (%) (chorioamnionitis, chorionitis, subchorionitis, villitis, deciduitis)			
Present	28 (47.5)	16 (34.8)	0.192
Absent	31 (52.5)	30 (65.2)	
Missing	0 (0.0)	0 (0.0)	
Acute inflammation, n (%) (chorioamnionitis, chorionitis, subchorionitis, villitis, deciduitis)			
Present	15 (25.4)	12 (26.1)	0.939
Absent	44 (74.6)	34 (73.9)	
Missing	0 (0.0)	0 (0.0)	
Chronic chorioamnionitis, n (%)			
Present	2 (3.4)	5 (10.9)	0.236
Absent	57 (96.6)	41 (89.1)	
Missing	0 (0.0)	0 (0.0)	
Chronic chorionitis, n (%)			
Present	5 (8.5)	0 (0.0)	0.066
Absent	54 (91.5)	46 (100.0)	
Missing	0 (0.0)	0 (0.0)	
Chronic subchorionitis, n (%)			
Present	0 (0.0)	0 (0.0)	N/A
Absent	59 (100.0)	46 (100.0)	
Missing	0 (0.0)	0 (0.0)	
Maternal inflammatory response, n (%)			
Present	26 (44.1)	14 (30.4)	0.154
Absent	33 (55.9)	32 (69.6)	
Missing	0 (0.0)	0 (0.0)	
Fetal inflammatory response, n (%)			
Present	22 (37.3)	9 (19.6)	0.048
Absent	37 (62.7)	37 (80.4)	
Missing	0 (0.0)	0 (0.0)	
Chronic villitis (VUE), n (%)			
Present	12 (20.3)	8 (17.4)	0.703
Absent	47 (79.7)	38 (82.6)	
Missing	0 (0.0)	0 (0.0)	
Chronic deciduitis, n (%)			
Present	25 (42.4)	10 (21.7)	0.026
Absent	34 (57.6)	36 (78.3)	
Missing	0 (0.0)	0 (0.0)	

Table 4.5: Placental Inflammatory Lesions

Placental Villous & Intervillous Pathology	Metformina (N=59)	Insulina (n=46)	p-value
Placental maturity			
Immature	36 (61.0)	31 (67.4)	N/A
Adequate	21 (35.6)	10 (21.7)	
Accelerated	2 (3.4)	3 (6.5)	
Missing	0 (0.0)	2 (4.3)	
Delayed Villous Maturation			
Present	36 (61.0)	31 (67.4)	0.320
Absent	23 (39.0)	13 (28.3)	
Missing	0 (0.0)	2 (4.3)	
Adequate Villous Maturation			
Present	21 (35.6)	10 (21.7)	0.159
Absent	38 (64.4)	34 (73.9)	
Missing	0 (0.0)	2 (4.3)	
Accelerated Villous Maturation			
Present	2 (3.4)	3 (6.5)	0.649
Absent	57 (96.6)	41 (89.1)	
Missing	0 (0.0)	2 (4.3)	
Massive perivillous fibrin deposition, n (%)			
Present	0 (0.0)	1 (2.2)	0.438
Absent	59 (100.0)	45 (97.8)	
Missing	0 (0.0)	0 (0.0)	
Chorangiomas, n (%)			
Present	20 (33.9)	11 (23.9)	0.266
Absent	39 (66.1)	35 (76.1)	
Missing	0 (0.0)	0 (0.0)	
Distal villous hypoplasia, n (%)			
Present	1 (1.7)	0 (0.0)	1.000
Absent	58 (98.3)	46 (100.0)	
Missing	0 (0.0)	0 (0.0)	
Intervillous thrombosis, n (%)			
Present	11 (18.6)	8 (17.4)	0.869
Absent	48 (81.4)	38 (82.6)	
Missing	0 (0.0)	0 (0.0)	
Chronic histiocytic intervillitis, n (%)			
Present	1 (1.7)	0 (0.0)	1.000
Absent	58 (98.3)	46 (100.0)	
Missing	0 (0.0)	0 (0.0)	
Other Finding	Metformina (N=59)	Insulina (n=46)	p-value
Meconium, n (%)			
Present	28 (47.5)	22 (47.8)	0.970
Absent	31 (52.5)	24 (52.2)	
Missing	0 (0.0)	0 (0.0)	

Table 4.6: Placental Villous, Intervillous, and Other Pathological Findings

Maternal Demographic, Anthropometric, and Clinical Characteristics	Metformina (N=57)	Insulina (n=21)	p-value
Diabetes diagnosis, n (%)			
Gestational diabetes	57 (100)	21 (0.0)	
Type 1 diabetes mellitus	0 (0.0)	0 (0.0)	
Type 2 diabetes mellitu	0 (0.0)	0 (0.0)	
Missing	0 (0.0)	0 (0.0)	
Maternal age at delivery (years), median (IQR) / mean ($\pm$SD)	34.8 ($\pm$ 5.6)	35.0 (6.5)	0.778 /
Missing, n(%)	0 (0.0)	0 (0.0)	0.716
Gravidity, n(%)			
Primigravida	24 (42.1)	5 (23.8)	0.138
Multigravida	33 (57.9)	16 (76.2)	
Missing	0 (0.0)	0 (0.0)	
Previous parity, n(%)			
Nulliparous	29 (50.9)	7 (33.3)	0.373
Primiparous	21 (36.8)	11 (52.4)	
Multiparous	7 (12.3)	3 (14.3)	
Missing	0 (0.0)	0 (0.0)	
Pre-pregnancy BMI (kg/m²), median (IQR) / mean ($\pm$SD)	32.0 (9.9)	29.7 (8.7)	0.113 /
Missing, n (%)	0 (0.0)	0 (0.0)	0.211
Pre-pregnancy BMI, n (%)			
Normal	12 (21.1)	7 (33.3)	0.533
Overweight	13 (22.8)	4 (19.0)	
Obesity	32 (56.1)	10 (47.6)	
Missing	0 (0.0)	0 (0.0)	
Normal pre-pregnancy BMI, n (%)			
Present	12 (21.1)	7 (33.3)	0.262
Absent	45 (78.9)	14 (66.7)	
Missing	0 (0.0)	0 (0.0)	
Overweight pre-pregnancy BMI, n (%)			
Present	13 (22.8)	4 (19.0)	0.721
Absent	44 (77.2)	17 (81.0)	
Missing	0 (0.0)	0 (0.0)	
Obesity pre-pregnancy BMI, n (%)			
Present	32 (56.1)	10 (47.6)	0.503
Absent	25 (43.9)	11 (52.4)	
Missing	0 (0.0)	0 (0.0)	
Gestational weight gain (kg), median (IQR) / mean ($\pm$SD)	9.7 (8.8)	11.2 (7.4)	0.539
Missing, n (%)	3 (5.3)	0 (0.0)	
Glycated hemoglobin tests during pregnancy, n (%)			
Controlled (all values <6.0%)	50 (87.7)	13 (61.9)	0.003
Uncontrolled (any value $\geq$ 6.0%)	3 (5.3)	7 (33.3)	
Missing	4 (7.0)	1 (4.8)	
Intrapartum maternal fever, n (%)			
Present	7 (12.3)	0 (0.0)	0.180
Absent	50 (87.7)	21 (100.0)	
Missing	0 (0.0)	0 (0.0)	
Smoking, n (%)			
Present	11 (19.3)	4 (19.0)	1.000
Absent	46 (80.7)	17 (81.0)	
Missing	0 (0.0)	0 (0.0)	
Hypertensive disorders, n (%)			
Present	17 (29.8)	2 (9.5)	0.064
Absent	40 (70.2)	19.0 (90.5)	
Missing	0 (0.0)	0 (0.0)	
Cardiac disease, n (%)			
Present	0 (0.0)	1 (4.8)	0.269
Absent	57 (100.0)	20 (95.2)	
Missing	0 (0.0)	0 (0.0)	
Pulmonary disease, n (%)			
Present	5 (8.8)	1 (4.8)	1.000
Absent	52 (91.2)	20 (95.2)	
Missing	0 (0.0)	0 (0.0)	
Renal disease, n (%)			
Present	0 (0.0)	0 (0.0)	N/A
Absent	57 (100.0)	21 (100.0)	
Missing	0 (0.0)	0 (0.0)	
Thyroid disease, n (%)			
Present	6 (10.5)	8 (38.1)	0.009
Absent	51 (89.5)	13 (61.9)	
Missing	0 (0.0)	0 (0.0)	
Anemia , n (%)			
Present	3 (5.3)	1 (4.8)	1.000
Absent	54 (94.7)	20 (95.2)	
Missing	0 (0.0)	0 (0.0)	

Table 4.7: Maternal Characteristics in the [gestational diabetes mellitus \(GDM\)](#) Subgroup

Perinatal and neonatal outcomes	Metformina (N=57)	Insulina (n=21)	p-value
Gestational age at delivery (days), median (IQR) / mean ($\pm$ SD)*	274.0 (8.0)	273.0 (5.0)	0.901 /
Missing, n(%)	0 (0.0)	0 (0.0)	0.552
*Gestational age at delivery (equivalent in weeks)	39.1 (1.1)	39.0 (0.7)	
Sex of neonate, n(%)			
Female	29 (50.9)	5 (23.8)	0.032
Male	28 (49.1)	16 (76.2)	
Missing	0 (0.0)	0 (0.0)	
First minute APGAR score, median (IQR) / mean ($\pm$ SD)	9.0 (0.0)	9.0 (0.0)	0.224 /
Missing, n(%)	0 (0.0)	0 (0.0)	0.949
Fifth minute APGAR score, median (IQR) / mean ($\pm$ SD)	10.0 (0.0)	10.0 (0.0)	0.063 /
Missing, n(%)	0 (0.0)	0 (0.0)	0.700
Tenth minute APGAR score, median (IQR) / mean ($\pm$ SD)	10.0 (0.0)	10.0 (0.0)	0.287 /
Missing, n(%)	0 (0.0)	0 (0.0)	1.000
Birth weight (g), median (IQR) / mean ($\pm$ SD)	3180.2 ($\pm$ 493.2)	3283.8 ($\pm$ 408.6)	0.393
Missing, n(%)	0 (0.0)	0 (0.0)	
Birth weight percentile for gestational age, n (%)			
<10	7 (12.3)	1 (4.8)	N/A
≥ 10 e ≤ 90	42 (73.7)	17 (81.0)	
>90	8 (14.0)	3 (14.3)	
Missing	0 (0.0)	0 (0.0)	
Birth weight <P10 gestational age, n (%)			
Present	7 (12.3)	1 (4.8)	0.437
Absent	50 (87.7)	20 (95.2)	
Missing	0 (0.0)	0 (0.0)	
Birth weight ≥ 10 e ≤ 90 gestational age, n (%)			
Present	42 (73.7)	17 (81.0)	0.507
Absent	15 (26.3)	4 (19.0)	
Missing	0 (0.0)	0 (0.0)	
Birth weight >P90 gestational age, n (%)			
Present	8 (14.0)	3 (14.3)	1.000
Absent	49 (86.0)	18 (85.7)	
Missing	0 (0.0)	0 (0.0)	
Neonatal length (cm), median (IQR) / mean ($\pm$ SD)	49.0 ($\pm$ 2.3)	49.3 ($\pm$ 1.5)	0.566
Missing, n(%)	0 (0.0)	0 (0.0)	
Head circumference (cm), median (IQR) / mean ($\pm$ SD)	34.5 (2.0)	35.0 (3.0)	0.551 /
Missing, n(%)	0 (0.0)	0 (0.0)	0.839
NICU admission, n (%)			
Present	8 (14.0)	0 (0.0)	0.100
Absent	49 (86.0)	21 (100.0)	
Missing	0 (0.0)	0 (0.0)	
Hypoglycemia, n (%)			
Present	3 (5.3)	1 (4.8)	1.000
Absent	54 (94.7)	20 (95.2)	
Missing	0 (0.0)	0 (0.0)	
Jaundice, n (%)			
Present	22 (38.6)	7 (33.3)	0.670
Absent	35 (61.4)	14 (66.7)	
Missing	0 (0.0)	0 (0.0)	
Jaundice requiring phototherapy, n (%)			
Present	12 (21.1)	3 (14.3)	0.747
Absent	45 (78.9)	18 (85.7)	
Missing	0 (0.0)	0 (0.0)	
Transient tachypnea, n (%)			
Present	2 (3.5)	0 (0.0)	1.000
Absent	55 (96.5)	21 (100.0)	
Missing	0 (0.0)	0 (0.0)	
Respiratory distress syndrome with hypoxemia, n (%)			
Present	2 (3.5)	0 (0.0)	1.000
Absent	55 (96.5)	21 (100.0)	
Missing	0 (0.0)	0 (0.0)	
Meconium-stained amniotic fluid, n (%)			
Present	3 (5.3)	1 (4.8)	1.000
Absent	54 (94.7)	20 (95.2)	
Missing	0 (0.0)	0 (0.0)	
Thrombocytopenia, n (%)			
Present	2 (3.5)	0 (0.0)	1.000
Absent	55 (96.5)	21 (100.0)	
Missing	0 (0.0)	0 (0.0)	
Polycythemia, n (%)			
Present	1 (1.8)	0 (0.0)	1.000
Absent	56 (98.2)	21 (100.0)	
Missing	0 (0.0)	0 (0.0)	

Table 4.8: Perinatal and Neonatal Outcomes in the **GDM** Subgroup

Placental Morphometry & Fetoplacental Ratio	Metformina (N=57)	Insulina (n=21)	p-value
Feto-placental weight ratio, n (%)			
<10th percentile (low)	2 (3.5)	2 (9.5)	N/A
10th–89th percentile (appropriate)	51 (89.5)	16 (76.2)	
≥90th percentile (high)	2 (3.5)	3 (14.3)	
Missing (including sampling defect)	2 (3.5)	0 (0.0)	
Feto-placental weight <10th percentile (low), n (%)			
Present	2 (3.5)	2 (9.5)	0.305
Absent	53 (93.0)	19 (90.5)	
Missing (including sampling defect)	2 (3.5)	0 (0.0)	
Feto-placental weight 10th–89th percentile (appropriate), n (%)			
Present	51 (89.5)	16 (76.2)	0.105
Absent	4 (7.0)	5 (23.8)	
Missing (including sampling defect)	2 (3.5)	0 (0.0)	
Feto-placental weight ≥90th percentile (high), n (%)			
Present	2 (3.5)	3 (14.3)	0.126
Absent	53 (93.0)	18 (85.7)	
Missing (including sampling defect)	2 (3.5)	0 (0.0)	
Placental weight (g), median (IQR) / mean (±SD)	460.0 (141.5)	475.0 (137.5)	0.778 / 0.987
Missing, n(%)	2 (3.5)	0 (0.0)	
Placental weight percentile, n (%)			
<10th percentile (low)	20 (35.1)	9 (42.9)	N/A
10th–89th percentile (appropriate)	33 (57.9)	12 (57.1)	
≥90th percentile (high)	2 (3.5)	0 (0.0)	
Missing, n(%)	2 (3.5)	0 (0.0)	
Placental weight <10th percentile (low), n (%)			
Present	20 (35.1)	9 (42.9)	0.602
Absent	35 (61.4)	12 (57.1)	
Missing (including sampling defect)	2 (3.5)	0 (0.0)	
Placental weight within 10th–89th percentile (appropriate), n (%)			
Present	33 (57.9)	12 (57.1)	0.821
Absent	22 (38.6)	9 (42.9)	
Missing (including sampling defect)	2 (3.5)	0 (0.0)	
Placental weight ≥90th percentile (high), n (%)			
Present	2 (3.5)	0 (0.0)	1.000
Absent	53 (93.0)	21 (100.0)	
Missing (including sampling defect)	2 (3.5)	0 (0.0)	
Greatest placental diameter (cm), median (IQR) / mean (±SD)	19.0 (3.0)	18.0 (2.5)	0.322
Missing, n(%)	4 (7.0)	0 (0.0)	
Umbilical Cord Features	Metformina (N=57)	Insulina (n=21)	p-value
Umbilical cord insertion, n (%)			
Central	9 (15.8)	6 (28.6)	N/A
Paracentral	28 (49.1)	7 (33.3)	
Paramarginal	14 (24.6)	7 (33.3)	
Marginal	6 (10.5)	1 (4.8)	
Missing	0 (0.0)	0 (0.0)	
Number of umbilical vessels, n (%)			
Two	1 (1.8)	1 (4.8)	0.469
Three	56 (98.2)	20 (95.2)	
Missing	0 (0.0)	0 (0.0)	
Umbilical cord coiling index, n (%)			
Hypocoiled (UCI <0.07)	0 (0.0)	0 (0.0)	1.000
Normocoiled (UCI 0.07–0.30)	31 (54.4)	12 (57.1)	
Hypercoiled (UCI >0.30)	11 (19.3)	4 (19.0)	
Missing	15 (26.3)	5 (23.8)	
Placental Maternal & Fetal Vascular Pathology	Metformina (N=57)	Insulina (n=21)	p-value
Maternal vascular malperfusion, n(%)			
Present	25 (43.9)	13 (61.9)	0.157
Absent	32 (56.1)	8 (38.1)	
Missing	0 (0.0)	0 (0.0)	
Fetal vascular malperfusion, n (%)			
Present	2 (3.5)	0 (0.0)	1.000
Absent	55 (96.5)	21 (100.0)	
Missing	0 (0.0)	0 (0.0)	
Hypoxic-ischemic changes, n(%)			
Present	10 (17.5)	3 (14.3)	1.000
Absent	47 (82.5)	18 (85.7)	
Missing	0 (0.0)	0 (0.0)	

Table 4.9: Placental Morphometry and Vascular Pathology in the [GDM](#) Subgroup

Placental Inflammatory Lesions	Metformina (N=57)	Insulina (n=21)	p-value
Acute and/or chronic inflammation, n (%) (chorioamnionitis, chorionitis, subchorionitis, villitis, deciduitis)			
Present	41 (71.9)	13 (61.9)	0.395
Absent	16 (28.1)	8 (38.1)	
Missing	0 (0.0)	0 (0.0)	
Chronic inflammation, n (%) (chorioamnionitis, chorionitis, subchorionitis, villitis, deciduitis)			
Present	28 (49.1)	8 (38.1)	0.386
Absent	29 (50.9)	13 (61.9)	
Missing	0 (0.0)	0 (0.0)	
Acute inflammation, n (%) (chorioamnionitis, chorionitis, subchorionitis, villitis, deciduitis)			
Present	13 (22.8)	5 (23.8)	1.000
Absent	44 (77.2)	16 (76.2)	
Missing	0 (0.0)	0 (0.0)	
Chronic chorioamnionitis, n (%)			
Present	2 (3.5)	1 (4.8)	1.000
Absent	55 (96.5)	20 (95.2)	
Missing	0 (0.0)	0 (0.0)	
Chronic chorionitis, n (%)			
Present	5 (8.8)	0 (0.0)	0.316
Absent	52 (91.2)	21 (100.0)	
Missing	0 (0.0)	0 (0.0)	
Chronic subchorionitis, n (%)			
Present	0 (0.0)	0 (0.0)	N/A
Absent	57 (100.0)	21 (100.0)	
Missing	0 (0.0)	0 (0.0)	
Maternal inflammatory response, n (%)			
Present	25 (43.9)	5 (23.8)	0.106
Absent	32 (56.1)	16 (76.2)	
Missing	0 (0.0)	0 (0.0)	
Fetal inflammatory response, n (%)			
Present	21 (36.8)	3 (14.3)	0.056
Absent	36 (63.2)	18 (85.7)	
Missing	0 (0.0)	0 (0.0)	
Chronic villitis (VUE), n (%)			
Present	12 (21.1)	4 (19.0)	1.000
Absent	45 (78.9)	17 (81.0)	
Missing	0 (0.0)	0 (0.0)	
Chronic deciduitis, n (%)			
Present	25 (43.9)	5 (23.8)	0.106
Absent	32 (56.1)	16 (76.2)	
Missing	0 (0.0)	0 (0.0)	

Table 4.10: Placental Inflammatory Lesions in the **GDM** Subgroup

Placental Villous & Intervillous Pathology	Metformina (N=57)	Insulina (n=21)	p-value
Placental maturity			
Immature	34 (59.6)	14 (66.7)	N/A
Adequate	21 (36.8)	5 (23.8)	
Accelerated	2 (3.5)	1 (4.8)	
Missing	0 (0.0)	1 (4.8)	
Delayed Villous Maturation			
Present	34 (59.6)	14 (66.7)	0.411
Absent	23 (40.4)	6 (28.5)	
Missing	0 (0.0)	1 (4.8)	
Adequate Villous Maturation			
Present	21 (36.8)	5 (23.8)	0.335
Absent	36 (63.2)	15 (66.4)	
Missing	0 (0.0)	1 (4.8)	
Accelerated Villous Maturation			
Present	2 (3.5)	1 (4.8)	1.000
Absent	55 (96.5)	19 (90.5)	
Missing	0 (0.0)	1 (4.8)	
Massive perivillous fibrin deposition, n (%)			
Present	0 (0.0)	1 (4.8)	0.269
Absent	57 (100.0)	20 (95.2)	
Missing	0 (0.0)	0 (0.0)	
Chorangiomas, n (%)			
Present	19 (33.3)	5 (23.8)	0.419
Absent	38 (66.7)	16 (76.2)	
Missing	0 (0.0)	0 (0.0)	
Distal villous hypoplasia, n (%)			
Present	1 (1.8)	0 (0.0)	1.000
Absent	56 (98.2)	21 (100.0)	
Missing	0 (0.0)	0 (0.0)	
Intervillous thrombosis, n (%)			
Present	10 (17.5)	4 (19.0)	1.000
Absent	47 (82.5)	17 (81.0)	
Missing	0 (0.0)	0 (0.0)	
Chronic histiocytic intervillitis, n (%)			
Present	1 (1.8)	0 (0.0)	1.000
Absent	56 (98.2)	21 (100.0)	
Missing	0 (0.0)	0 (0.0)	
Other Finding	Metformina (N=57)	Insulina (n=21)	p-value
Meconium, n (%)			
Present	26 (45.6)	10 (47.6)	0.875
Absent	31 (54.4)	11 (52.4)	
Missing	0 (0.0)	0 (0.0)	

Table 4.11: Placental Villous and Intervillous Pathology and Other Placental Findings

5 Discussion

This study presents a comprehensive comparative analysis of metformin versus insulin in diabetic pregnancies, encompassing placental morphology and histopathology, as well as maternal and neonatal outcomes. By linking placental findings with clinical outcomes, we aimed to provide a more comprehensive understanding of how pharmacological treatment affects both maternal–fetal health and placental biology.

In the primary analysis, pregnancies affected by pre-gestational and gestational diabetes were identified. A dual placental signature: metformin was associated with a more efficient feto-placental ratio but also with a significantly increased frequency of inflammatory lesions.

The most novel and complex findings of this study relate to the placental inflammatory lesions, specifically to the more than two-fold increase in the odds of both fetal inflammatory response and chronic deciduitis after metformin exposure. This observation is intriguing, as metformin is generally considered to have systemic anti-inflammatory properties. A potential explanation is that this finding may not represent a direct pharmacological effect, but rather reflects a confounding factor. The women treated with metformin had a higher prevalence of obesity, a known pro-inflammatory state, which is reportedly associated with a significantly higher risk of placental inflammatory lesions [8, 82]. A critical challenge for further investigation is to determine whether metformin is primarily involved in placental inflammatory lesions or if it promotes improvements in placental efficiency despite this inflammatory environment.

These complex placental findings occurred in the context of clear maternal metabolic benefits. Consistent with established evidence from landmark randomized trials such as the MiG study [70], similar results were observed in this study regarding maternal and fetal outcomes. Metformin was associated with superior glycemic control compared to insulin in both analyses. Although lower gestational weight gain was significant only in the initial analysis, the direction and magnitude of this effect in the **GDM**-only subgroup analysis remained unchanged. Metformin use was also associated with intrapartum fever. Although significant, this finding should be interpreted cautiously, as intrapartum fever is multifactorial and may arise from noninfectious inflammation, epidural analgesia, or infection. Additional peripartum data would be needed to clarify the nature of this association.

Despite the concerning signal for placental inflammation, the downstream neonatal outcomes were reassuring. After accounting for the confounding effect of the heavier infants born to mothers with type 1 diabetes, neonatal outcomes in the **GDM** cohort were comparable between treatment groups, with no significant differences in common morbidities. The only new association

to emerge in the **GDM** subgroup was a higher proportion of male neonates in the insulin group, which probably represents a chance finding.

The primary strength of this study lies in its comprehensive and standardized histopathological examination of the placenta within a clinical cohort. The pre-specified subgroup analysis was crucial for dissecting the complex results. However, the retrospective, observational design is the main limitation, precluding any claims of causality. The most significant limitation was the sample size, particularly for the secondary analysis sample, which constrained the ability to draw definitive conclusions regarding the non-significant but persistent trends for placental inflammation.

6 Conclusions

This study provides critical evidence relevant to the ongoing debate among international guidelines regarding the safe use of metformin in pregnancy. Metformin is a low-cost, oral administration with minimal risk of maternal hypoglycemia and benefits from decades of research on maternal and neonatal outcomes. However, its involvement in diverse and tissue-specific metabolic pathways raises persistent uncertainties about its effects on placental and fetal tissues.

While our findings confirm metformin's efficacy for maternal glycemic control in women with [GDM](#), they also reveal a significant and previously underexplored association with placental inflammatory lesions and intrapartum fever. Although extensive long-term studies of neonatal outcomes have mainly been reassuring, the potential downstream consequences of the placental inflammatory signals identified here remain unknown.

To address these concerns, future research should prioritize large, adequately powered randomized controlled trials in which detailed placental pathology is a pre-specified primary outcome, to clarify the safety of metformin with respect to maternal, placental, and fetal inflammatory environments.

Bibliography

- [1] Rehab M Abdelhalim e Dalia M El-sammak. Morphological and histological changes in the placenta of diabetic mother. *Int J Clin Exp Med*, 5(1):1–10, 2018.
- [2] Sally K Abell, Barbora De Courten, Jacqueline A Boyle e Helena J Teede. Inflammatory and other biomarkers: role in pathophysiology and prediction of gestational diabetes mellitus. *International journal of molecular sciences*, 16(6):13442–13473, 2015.
- [3] American College of Obstetricians and Gynecologists Committee on Practice Bulletins—Obstetrics. Practice bulletin no. 180: Gestational diabetes mellitus, jul 2017.
- [4] American Diabetes Association. Management of diabetes in pregnancy: standards of care in diabetes—2024. *Diabetes Care*, 47(Supplement_1):S282–S294, 2024.
- [5] Rebecca N Baergen, Graham J Burton e Cynthia G Kaplan. *Benirschke's pathology of the human placenta*. Springer Nature, 2021.
- [6] Clifford J Bailey. Metformin: Therapeutic profile in the treatment of type 2 diabetes. *Diabetes, Obesity and Metabolism*, 26:3–19, 2024.
- [7] Le-xin Bao, Wan-ting Shi e Yu-xin Han. Metformin versus insulin for gestational diabetes: a systematic review and meta-analysis. *The Journal of Maternal-Fetal & Neonatal Medicine*, 34(16):2741–2753, 2021.
- [8] Fausta Beneventi, Camilla Bellingeri, Irene De Maggio, Chiara Cavagnoli, Silvia Fumanelli, Elisa Ligari, Giacomo Fiandrino, Stefania Cesari e Arsenio Spinillo. Placental pathologic features in obesity. *Placenta*, 144:1–7, 2023.
- [9] Katherine C Bishop, Benjamin S Harris, Brita K Boyd, Emily S Reiff, Laura Brown e Jeffrey A Kuller. Pharmacologic treatment of diabetes in pregnancy. *Obstetrical & Gynecological Survey*, 74(5):289–297, 2019.
- [10] Jens M Bruun, Aina S Lihn, Camilla Verdich, Steen B Pedersen, Søren Toubro, Arne Astrup e Bjørn Richelsen. Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. *American journal of physiology-Endocrinology and metabolism*, 285(3):E527–E533, 2003.

- [11] María José Calvo, Heliana Parra, Raquel Santeliz, Jordan Bautista, Eliana Luzardo, Nelson Villasmil, María Sofía Martínez, Maricamen Chacín, Clímaco Cano, Ana Checa-Ros et al. The placental role in gestational diabetes mellitus: A molecular perspective. *touchREVIEWS in Endocrinology*, 20(1):10, 2024.
- [12] Ivo Carrasco-Wong, Alejandra Moller, Fernanda R Giachini, Victor V Lima, Fernando Toledo, Jana Stojanova, Luis Sobrevia e Sebastián San Martín. Placental structure in gestational diabetes mellitus. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, 1866(2):165535, 2020.
- [13] Matteo Cassina, Marta Dona, Elena Di Gianantonio, Pietro Litta e Maurizio Clementi. First-trimester exposure to metformin and risk of birth defects: a systematic review and meta-analysis. *Human reproduction update*, 20(5):656–669, 2014.
- [14] Cheril Clarson, Gerald JM Tevaarwerk, Paul GR Harding, Graham W Chance e M Daria Haust. Placental weight in diabetic pregnancies. *Placenta*, 10(3):275–281, 1989.
- [15] EJ Coetzee e WPU Jackson. Metformin in management of pregnant insulin-independent diabetics. *Diabetologia*, 16(4):241–245, 1979.
- [16] Germany-BUSINESS WIRE DARMSTADT. Glucophage® is the first oral diabetes treatment approved in europe for use during pregnancy. Press Release, 2022.
- [17] G Daskalakis, S Marinopoulos, A Papapanagiotou, S Mesogitis, N Papantoniou e A Antsaklis. Placental pathology in women with gestational diabetes. *Acta obstetrica et gynecologica Scandinavica*, 87(4):403–407, 2008.
- [18] Luis F del Aguila, Kevin P Claffey e John P Kirwan. Tnf- α impairs insulin signaling and insulin stimulation of glucose uptake in c2c12muscle cells. *American Journal of Physiology-Endocrinology and Metabolism*, 276(5):E849–E855, 1999.
- [19] Gernot Desoye. The human placenta in diabetes and obesity: friend or foe? the 2017 norbert freinkel award lecture. *Diabetes care*, 41(7):1362–1369, 2018.
- [20] Gernot Desoye e Jonathan CK Wells. Pregnancies in diabetes and obesity: the capacity-load model of placental adaptation. *Diabetes*, 70(4):823–830, 2021.
- [21] Leanne Duthie e Rebecca M Reynolds. Changes in the maternal hypothalamic-pituitary-adrenal axis in pregnancy and postpartum: influences on maternal and fetal outcomes. *Neuroendocrinology*, 98(2):106–115, 2013.
- [22] Basmah M Eldakhkhny, Fatma M Ghoneim, Mona FM Soliman, Salwa M Abo El-Khair, Ayman Z Elsamanoudy, Yousef M Almoghrabi, Passant M Mohie, Fatma E Hassan e Amany A Abd Elfattah. Modulation of placental angiogenesis by metformin in a rat model of gestational diabetes. *Histochemistry and Cell Biology*, 163(1):28, 2025.

- [23] Ingrid M Evers, Peter GJ Nikkels, J Wolter Sikkema e Gerard HA Visser. Placental pathology in women with type 1 diabetes and in a control group with normal and large-for gestational-age infants. *Placenta*, 24(8-9):819–825, 2003.
- [24] Revital Feinstein, Hannah Kanety, MZ Papa, B Lunenfeld e A Karasik. Tumor necrosis factor-alpha suppresses insulin-induced tyrosine phosphorylation of insulin receptor and its substrates. *Journal of Biological Chemistry*, 268(35):26055–26058, 1993.
- [25] Marc Foretz, Bruno Guigas, Luc Bertrand, Michael Pollak e Benoit Viollet. Metformin: from mechanisms of action to therapies. *Cell metabolism*, 20(6):953–966, 2014.
- [26] Abigail L Fowden, AN Sferruzzi-Perri, PM Coan, M Constancia e GJ Burton. Placental efficiency and adaptation: endocrine regulation. *The Journal of physiology*, 587(14):3459–3472, 2009.
- [27] Laura L Gathercole, Iwona J Bujalska, Paul M Stewart e Jeremy W Tomlinson. Glucocorticoid modulation of insulin signaling in human subcutaneous adipose tissue. *The Journal of Clinical Endocrinology & Metabolism*, 92(11):4332–4339, 2007.
- [28] Joanne E Given, Maria Loane, Ester Garne, Marie-Claude Addor, Marian Bakker, Bénédicte Bertaut-Nativel, Miriam Gatt, Kari Klungsoyr, Nathalie Lelong, Margery Morgan et al. Metformin exposure in first trimester of pregnancy and risk of all or specific congenital anomalies: exploratory case-control study. *bmj*, 361, 2018.
- [29] Cuilian Guo, Jinxin Liu e Huqun Li. Metformin ameliorates olanzapine-induced insulin resistance via suppressing macrophage infiltration and inflammatory responses in rats. *Biomedicine & Pharmacotherapy*, 133:110912, 2021.
- [30] Stuart Handwerger e Michael Freemark. The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development. *Journal of Pediatric Endocrinology and Metabolism*, 13(4):343–356, 2000.
- [31] Ling He. Metformin and systemic metabolism. *Trends in pharmacological sciences*, 41(11): 868–881, 2020.
- [32] Rok Herman, Nika Aleksandra Kravos, Mojca Jensterle, Andrej Janež e Vita Dolžan. Metformin and insulin resistance: a review of the underlying mechanisms behind changes in glut4-mediated glucose transport. *International journal of molecular sciences*, 23(3):1264, 2022.
- [33] Teri L Hernandez, Jacob E Friedman e Linda A Barbour. Insulin resistance in pregnancy: implications for mother and offspring. In *Insulin resistance: childhood precursors of adult disease*, páginas 67–94. Springer, 2019.
- [34] Perry R Hinton, Isabella McMurray e Charlotte Brownlow. *SPSS explained*. Routledge, 2014.

- [35] Moshe Hod, Anil Kapur, David A Sacks, Eran Hadar, Mukesh Agarwal, Gian Carlo Di Renzo, Luis Cabero Roura, Harold David McIntyre, Jessica L Morris e Hema Divakar. The international federation of gynecology and obstetrics (figo) initiative on gestational diabetes mellitus: A pragmatic guide for diagnosis, management, and care. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics*, 131:S173–S211, 2015.
- [36] Gókhán S Hotamisligil, David L Murray, Lisa N Choy e Bruce M Spiegelman. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. *Proceedings of the National Academy of Sciences*, 91(11):4854–4858, 1994.
- [37] J Huynh, D Dawson, D Roberts e R Bentley-Lewis. A systematic review of placental pathology in maternal diabetes mellitus. *Placenta*, 36(2):101–114, 2015.
- [38] Se Gwang Jang, Jaeseon Lee, Seung-Min Hong, Seung-Ki Kwok, Mi-La Cho e Sung-Hwan Park. Metformin enhances the immunomodulatory potential of adipose-derived mesenchymal stem cells through stat1 in an animal model of lupus. *Rheumatology*, 59(6):1426–1438, 2020.
- [39] E Jauniaux e GJ Burton. Villous histomorphometry and placental bed biopsy investigation in type i diabetic pregnancies. *Placenta*, 27(4-5):468–474, 2006.
- [40] M Jirkovská, T Kučera, J Kaláb, M Jadrníček, V Niedobová, J Janáček, L Kubínová, M Moravcová, Z Žižka e V Krejčí. The branching pattern of villous capillaries and structural changes of placental terminal villi in type 1 diabetes mellitus. *Placenta*, 33(5):343–351, 2012.
- [41] T Yee Khong, Eoghan E Mooney, Ilana Ariel, Nathalie CM Balmus, Theonia K Boyd, Marie-Anne Brundler, Hayley Derricott, Margaret J Evans, Ona M Faye-Petersen, John E Gillan et al. Sampling and definitions of placental lesions: Amsterdam placental workshop group consensus statement. *Archives of pathology & laboratory medicine*, 140(7):698–713, 2016.
- [42] John P Kirwan, Sylvie Hauguel-De Mouzon, Jacques Lepercq, Jean-Claude Challier, Lorraine Huston-Presley, Jacob E Friedman, Satish C Kalhan e Patrick M Catalano. Tnf- α is a predictor of insulin resistance in human pregnancy. *Diabetes*, 51(7):2207–2213, 2002.
- [43] Linnea Ladfors, Nael Shaat, Nana Wiberg, Anastasia Katararou, Kerstin Berntorp e Karl Kristensen. Fetal overgrowth in women with type 1 and type 2 diabetes mellitus. *PloS one*, 12(11):e0187917, 2017.
- [44] Traci E LaMoia e Gerald I Shulman. Cellular and molecular mechanisms of metformin action. *Endocrine reviews*, 42(1):77–96, 2021.
- [45] Joyce Leary, David J Pettitt e Lois Jovanović. Gestational diabetes guidelines in a hapo world. *Best practice & research Clinical endocrinology & metabolism*, 24(4):673–685, 2010.

- [46] Sang R Lee, Woo-Young Choi, Jun H Heo, Jiyoung Huh, Globinna Kim, Kyu-Pil Lee, Hyo-Jung Kwun, Hyun-Jin Shin, In-Jeoung Baek e Eui-Ju Hong. Progesterone increases blood glucose via hepatic progesterone receptor membrane component 1 under limited or impaired action of insulin. *Scientific reports*, 10(1):16316, 2020.
- [47] Jia-Xu Li e Carolyn L Cummins. Fresh insights into glucocorticoid-induced diabetes mellitus and new therapeutic directions. *Nature Reviews Endocrinology*, 18(9):540–557, 2022.
- [48] Gloria López Stewart. Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy: A world health organization guideline. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 2014.
- [49] Thomas R Magee, Michael G Ross, Lauren Wedekind, Mina Desai, Siri Kjos e Louiza Belkacemi. Gestational diabetes mellitus alters apoptotic and inflammatory gene expression of trophoblasts from human term placenta. *Journal of Diabetes and its Complications*, 28(4): 448–459, 2014.
- [50] Emin Maltepe e Susan J Fisher. Placenta: the forgotten organ. *Annual review of cell and developmental biology*, 31(1):523–552, 2015.
- [51] Boyd E Metzger, Steven G Gabbe, Bengt Persson, Lynn P Lowe, Alan R Dyer, Jeremy JN Oats e Thomas A Buchanan. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy: response to weinert. *Diabetes care*, 33(7):e98–e98, 2010.
- [52] Yves Morel, Florence Roucher, Ingrid Plotton, Claire Goursaud, Véronique Tardy e Delphine Mallet. Evolution of steroids during pregnancy: Maternal, placental and fetal synthesis. *Annales d'endocrinologie*, 77(2):82–89, 2016.
- [53] Angel Nadal, Paloma Alonso-Magdalena, Sergi Soriano, Ana B Ropero e Ivan Quesada. The role of oestrogens in the adaptation of islets to insulin resistance. *The Journal of physiology*, 587(21):5031–5037, 2009.
- [54] Kiyofumi Nagira, Toshiyasu Sasaoka, Tsutomu Wada, Kazuhito Fukui, Mariko Ikubo, Satoko Hori, Hiroshi Tsuneki, Shigeru Saito e Masashi Kobayashi. Altered subcellular distribution of estrogen receptor α is implicated in estradiol-induced dual regulation of insulin signaling in 3T3-L1 adipocytes. *Endocrinology*, 147(2):1020–1028, 2006.
- [55] David M Nathan, John B Buse, Mayer B Davidson, Ele Ferrannini, Rury R Holman, Robert Sherwin e Bernard Zinman. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the american diabetes association and the european association for the study of diabetes. *Diabetes care*, 32(1):193–203, 2009.

- [56] National Institute for Health and Care Excellence (NICE). Diabetes in pregnancy: management from preconception to the postnatal period, feb 2015. NICE Guideline [NG3]. Published 25 February 2015. Accessed 24 August 2025.
- [57] Scott M Nelson, Philip M Coan, Graham J Burton e Robert S Lindsay. Placental structure in type 1 diabetes: relation to fetal insulin, leptin, and igf-i. *Diabetes*, 58(11):2634–2641, 2009.
- [58] Jackson Nteeba, Kaiyu Kubota, Wenfang Wang, Hao Zhu, Jay L Vivian, Guoli Dai e Michael J Soares. Pancreatic prolactin receptor signaling regulates maternal glucose homeostasis. *Journal of Endocrinology*, 241(1):71–83, 2019.
- [59] Katherine Ogurtsova, JD da Rocha Fernandes, Y Huang, Ute Linnenkamp, L Guariguata, Nam H Cho, David Cavan, JE Shaw e LE Makaroff. Idf diabetes atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes research and clinical practice*, 128: 40–50, 2017.
- [60] H Pinar, CJ Sung, CE Oyer e DB Singer. Reference values for singleton and twin placental weights. *Pediatric Pathology & Laboratory Medicine*, 16(6):901–907, 1996.
- [61] Ting Qi, Yanming Chen, Honggui Li, Ya Pei, Shih-Lung Woo, Xin Guo, Jiajia Zhao, Xiaoxian Qian, Joseph Awika, Yuqing Huo et al. A role for pfkfb3/ipfk2 in metformin suppression of adipocyte inflammatory responses. *Journal of molecular endocrinology*, 59(1):49, 2017.
- [62] Kate Rassie, Rinky Giri, Anju E Joham, Helena Teede e Aya Mousa. Human placental lactogen in relation to maternal metabolic health and fetal outcomes: A systematic review and meta-analysis. *International Journal of Molecular Sciences*, 23(24):15621, 2022.
- [63] Kate Louise Rassie, Rinky Giri, Angela Melder, Anju Joham, Aya Mousa e Helena J Teede. Lactogenic hormones in relation to maternal metabolic health in pregnancy and postpartum: protocol for a systematic review. *BMJ open*, 12(2):e055257, 2022.
- [64] Raymond W Redline. Distal villous immaturity. *Diagnostic Histopathology*, 18(5):189–194, 2012.
- [65] Raymond W Redline. Classification of placental lesions. *American journal of obstetrics and gynecology*, 213(4):S21–S28, 2015.
- [66] Raymond W Redline, Drucilla J Roberts, Mana M Parast, Linda M Ernst, Terry K Morgan, Michael F Greene, Cynthia Gyamfi-Bannerman, Judette M Louis, Emin Maltepe, Karen K Mestan et al. Placental pathology is necessary to understand common pregnancy complications and achieve an improved taxonomy of obstetrical disease. *American journal of obstetrics and gynecology*, 228(2):187–202, 2023.

- [67] Drucilla J Roberts, Rebecca N Baergen, Theonia K Boyd, Chrystalle Katte Carreon, Virginia E Duncan, Linda M Ernst, Ona M Faye-Petersen, Ann K Folkins, Jonathon L Hecht, Amy Heerema-McKenney et al. Criteria for placental examination for obstetrical and neonatal providers. *American journal of obstetrics and gynecology*, 228(5):497–508, 2023.
- [68] Maria del Mar Roca-Rodriguez, Pablo Ramos-Garcia, Cristina Lopez-Tinoco e Manuel Aguilar-Diosdado. Significance of serum-plasma leptin profile during pregnancy in gestational diabetes mellitus: A systematic review and meta-analysis. *Journal of clinical medicine*, 11(9):2433, 2022.
- [69] Roberto Romero, Offer Erez, Maik Hüttemann, Eli Maymon, Bogdan Panaïtescu, Agustin Conde-Agudelo, Percy Pacora, Bo Hyun Yoon e Lawrence I Grossman. Metformin, the aspirin of the 21st century: its role in gestational diabetes mellitus, prevention of preeclampsia and cancer, and the promotion of longevity. *American journal of obstetrics and gynecology*, 217(3):282–302, 2017.
- [70] Janet A Rowan, William M Hague, Wanzhen Gao, Malcolm R Battin e M Peter Moore. Metformin versus insulin for the treatment of gestational diabetes. *New England Journal of Medicine*, 358(19):2003–2015, 2008.
- [71] Yan Sun, Jia Li, Na Xiao, Meng Wang, Junping Kou, Lianwen Qi, Fang Huang, Baolin Liu e Kang Liu. Pharmacological activation of ampk ameliorates perivascular adipose/endothelial dysfunction in a manner interdependent on ampk and sirt1. *Pharmacological Research*, 89: 19–28, 2014.
- [72] Jane L Tarry-Adkins, India G Robinson, Rebecca M Reynolds, Irving LMH Aye, D Stephen Charnock-Jones, Benjamin Jenkins, Albert Koulman, Susan E Ozanne e Catherine E Aiken. Impact of metformin treatment on human placental energy production and oxidative stress. *Frontiers in Cell and Developmental Biology*, 10:935403, 2022.
- [73] Jane L Tarry-Adkins, India G Robinson, Lucas C Pantaleão, Jenna L Armstrong, Benjamin D Thackray, Lorenz MW Holzner, Alice E Knapton, Sam Virtue, Benjamin Jenkins, Albert Koulman et al. The metabolic response of human trophoblasts derived from term placentas to metformin. *Diabetologia*, 66(12):2320–2331, 2023.
- [74] Giulia Tosti, Annarita Barberio, Linda Tartaglione, Alessandro Rizzi, Mauro Di Leo, Luca Viti, Angelo Sirico, Sara De Carolis, Alfredo Pontecorvi, Antonio Lanzzone et al. Lights and shadows on the use of metformin in pregnancy: from the preconception phase to breastfeeding and beyond. *Frontiers in Endocrinology*, 14:1176623, 2023.
- [75] CC Van Dijk, A Franx, MWM de Laat, HW Bruinse, GHA Visser e PGJ Nikkels. The umbilical coiling index in normal pregnancy. *The Journal of Maternal-Fetal & Neonatal Medicine*, 11(4):280–283, 2002.

- [76] Ranjana Verma, Sabita Mishra, Jagat Mohini Kaul et al. Cellular changes in the placenta in pregnancies complicated with diabetes. *Int J Morphol*, 28(1):259–64, 2010.
- [77] José Villar, Leila Cheikh Ismail, Cesar G Victora, Eric O Ohuma, Enrico Bertino, Doug G Altman, Ann Lambert, Aris T Papageorgiou, Maria Carvalho, Yasmin A Jaffer et al. International standards for newborn weight, length, and head circumference by gestational age and sex: the newborn cross-sectional study of the intergrowth-21st project. *The Lancet*, 384(9946):857–868, 2014.
- [78] Benoit Viollet, Bruno Guigas, Nieves Sanz Garcia, Jocelyne Leclerc, Marc Foretz e Fabrizio Andreelli. Cellular and molecular mechanisms of metformin: an overview. *Clinical science*, 122(6):253–270, 2012.
- [79] Tsutomu Wada, Satoko Hori, Maine Sugiyama, Eriko Fujisawa, Tetsuro Nakano, Hiroshi Tsuneki, Kiyofumi Nagira, Shigeru Saito e Toshiyasu Sasaoka. Progesterone inhibits glucose uptake by affecting diverse steps of insulin signaling in 3t3-l1 adipocytes. *American Journal of Physiology-Endocrinology and Metabolism*, 298(4):E881–E888, 2010.
- [80] World Health Organization. Body mass index – bmi, 2023. URL <https://www.who.int/data/nutrition/nlis/info/malnutrition-in-women>. Accessed 26 August 2025.
- [81] Ming Yang, Tamana Darwish, Pierre Larraufie, Debra Rimmington, Irene Cimino, Deborah A Goldspink, Benjamin Jenkins, Albert Koulman, Cheryl A Brighton, Marcella Ma et al. Inhibition of mitochondrial function by metformin increases glucose uptake, glycolysis and gdf-15 release from intestinal cells. *Scientific Reports*, 11(1):2529, 2021.
- [82] Peilin Zhang, Tsu Haymar, Farah Al-Sayyed, Sylvia Dygulski, Beata Dygulska, Arooj Devi, Sanford Lederman, Carolyn Salafia e Rebecca Baergen. Placental pathology associated with maternal age and maternal obesity in singleton pregnancy. *The Journal of Maternal-Fetal & Neonatal Medicine*, 35(25):9517–9526, 2022.

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