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MicroRNAs associados aos mecanismos fisiopatológicos da Diabetes *Mellitus* Gestacional

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UNIVERSIDADE FEDERAL DE GOIÁS
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PEDRO HENRIQUE COSTA MATOS DA SILVA

MicroRNAs associados aos mecanismos fisiopatológicos da Diabetes *Mellitus* Gestacional

Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Ciências da Saúde, da Faculdade de Medicina da Universidade Federal de Goiás para obtenção do título de Mestre em Ciências da Saúde.
Área de concentração: Patologia, Clínica e Tratamento das Doenças Humanas.
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ATA DE DEFESA DE DISSERTAÇÃO

Ata nº **15/2023** da sessão de Defesa de Dissertação de **Pedro Henrique Costa Matos da Silva**, que confere o título de Mestre em Ciências da Saúde, na área de concentração em Patologia, Clínica e Tratamento das Doenças Humanas.

Aos dezenove dias do mês de agosto de dois mil e vinte e três, a partir das 09:00h, no Auditório do Instituto de CIências Biológicas II - Campus Samambaia, realizou-se a sessão pública de Defesa de Dissertação intitulada **"MICRORNAS ASSOCIADOS AOS MECANISMOS FISIOPATOLÓGICOS DA DIABETES MELLITUS GESTACIONAL"**. Os trabalhos foram instalados pela Orientadora, Professora Doutora **Angela Adamski da Silva Reis** (FM/UFG) com a participação dos demais membros da Banca Examinadora: Professor Doutor **Leonardo Ribeiro Soares** (FM/UFG), membro titular interno; Professor Doutor **Leonardo Pereira Franchi** (ICB/UFG), membro titular externo. Durante a arguição os membros da banca não fizeram sugestão de alteração do título do trabalho. A Banca Examinadora reuniu-se em sessão secreta a fim de concluir o julgamento da Dissertação, tendo sido o candidato **aprovado** pelos seus membros. Proclamados os resultados pela Professora Doutora **Angela Adamski da Silva Reis**, Presidente da Banca Examinadora, foram encerrados os trabalhos e, para constar, lavrou-se a presente ata que é assinada pelos Membros da Banca Examinadora, aos dezenove dias do mês de agosto de dois mil e vinte e três.

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Dedico essa dissertação aos meus pais, Geneci Maria da Costa Matos e Weliton Laurêncio da Silva, e a minha irmã Ana Célia Costa Matos da Silva

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SUMÁRIO

TABELAS E FIGURAS

SÍMBOLOS, SIGLAS E ABREVIATURAS

ADA	*American Diabetes Association*
AT2	Receptor de Angiotensina II
BCL2	Regulador de apoptose BCL2
BVS	Biblioteca Virtual em Saúde
CDC42	*Cell Division Cycle 42*
DNA	Ácido desoxirribonucleico
DM1	Diabetes *Mellitus* tipo 1
DM2	Diabetes *Mellitus* tipo 2
DMG	Diabetes *Mellitus* Gestacional
E2F1	*E2F Transcription Factor 1*
EMBASE	Base de dados eletrônica da editora Elsevier
ERa	Receptor de estrogênio alfa
Exp5	Proteína exportina-5 (Exp5)
GLUT4	Transportador de glicose tipo 4
HAPO	*Hyperglycemia and Adverse Pregnancy Outcomes*
hCG	Gonadotrofina coriônica humana
HIF1A	*Hypoxia-inducible factor 1 subunit alpha*
hPL	Lactogênio placentário humano
IADPSG	*International Association of the Diabetes and Pregnancy Study Groups*
IGF-1	*Insulin-Like Growth Factor 1*
IRS1	*Insulin Receptor Substrate 1*

IRS2	*Insulin Receptor Substrate 2*
JBI	*Joanna Briggs Institute*
MEDLINE	*Medical Literature Analysis and Retrieval System Online*
MeSH	*Medical Subject Headings*
miRNAs	microRNAs
mRNA	RNA mensageiro
OMS	Organização Mundial da Saúde
PAI-1	Inibidor do ativador de plasminogênio 1
PEO	*Population, Exposure, Outcome*
pré-miRNA	Precursor do miRNA (pré-miRNA)
pri-miRNA	miRNA primário
PRISMA	*Preferred Reporting Items for Systematic Reviews and Meta-Analyses*
PROSPERO	*International Prospective Register of Ongoing Systematic Reviews*
PTEN	*Phosphatase and tensin homolog*
PubMed	Serviço da Biblioteca Nacional de Medina dos Estados Unidos para acesso gratuito ao Medline
qRT-PCR	*Polymerase chain reaction via quantitative reverse transcriptase*
RISC	Complexo de silenciamento induzido por RNA
RS	Revisão sistemática
TOTG	Teste Oral de Tolerância à Glicose
VEGFA	*Vascular endothelial growth factor A*

AGTR2	Receptor de angiotensina II tipo 2
BCL2	*B-cell lymphoma 2*
BVS	Biblioteca Virtual em Saúde
DM	Diabetes Mellitus
DM1	Diabetes Mellitus tipo 1
DM2	Diabetes Mellitus tipo 2
DMG	Diabetes Mellitus Gestacional
DNA	Ácido desoxirribonucleico
ERα	Receptor de estrogênio α
GLUT4	Transportador de glicose 4
HIF-1α	Fator induzível por Hipóxia 1 alfa
IADPSG	*International Association of the Diabetes and Pregnancy Study Groups*
IGF-1	Fator de crescimento semelhante à insulina 1
IRS1	Substrato 1 do receptor de insulina
IRS2	Substrato 2 do receptor de insulina
JBI	*The Joanna Briggs Institute*
MeSH	*Medical Subject Heading*
miRNAs	MicroRNAs
OMS	Organização Mundial da Saúde
PEO	Population, Exposure, Outcome
PRISMA	Relatório Preferenciais para Revisões Sistemáticas e Meta-análises

RESUMO

A diabetes mellitus gestacional (DMG) é uma frequente durante a gravidez, podendo ser diagnosticada desde o início do pré-natal. Estudos têm destacado o papel dos microRNAs (miRNAs) no mecanismo fisiopatológico e como possíveis biomarcadores para o diagnóstico e tratamento da DMG. miRNAs são uma classe de pequenos RNAs não codificantes, os quais desempenham um papel na expressão gênica pós-transcricional. Portanto, este estudo teve como objetivo realizar uma revisão sistemática de miRNAs associados ao DMG, para construir um painel de miRNAs associado à DMG. Foi realizado uma pesquisa bibliográfica nas bases de dados PubMed/Medline, Biblioteca Virtual da Saúde (BVS), Web of Science e EMBASE, selecionando estudos observacionais em inglês, sem restrição de tempo. O protocolo está registrado na plataforma PROSPERO (número CRD42021291791). Cinquenta e cinco estudos foram incluídos nesta revisão sistemática, nos achados foram identificados 82 miRNAs alterados na DMG. Além disso, quatro miRNAs foram mais frequentemente regulados positivamente na DMG (mir-16-5p, mir-20a-5p, mir-222-3p e mir-330-3p). A desregulação desses miRNAs está associada a mecanismos de homeostase do ciclo celular, crescimento e proliferação de células β pancreáticas, captação e metabolismo de glicose, secreção e resistência à insulina. Portanto, identificar miRNAs associados a DMG e elucidar seus principais mecanismos auxiliará na caracterização e definição de possíveis biomarcadores para o diagnóstico, tratamento e manejo da DMG.

Palavras chaves: DMG; Diabetes; miRNA; Genética

ABSTRACT

Gestational diabetes mellitus (GDM) is a common condition during pregnancy and can be diagnosed from the beginning of prenatal care. Studies have highlighted the role of microRNAs (miRNAs) in the pathophysiological mechanism and as possible biomarkers for the diagnosis and treatment of GDM. miRNAs, a class of small non-coding RNAs, play a role in post-transcriptional gene expression. Therefore, this study aimed to perform a systematic review of miRNAs associated with GDM, to build a panel of miRNAs. A bibliographic search was carried out in PubMed/Medline, Virtual Health Library (VHL), Web of Science and EMBASE databases, selecting observational studies in English, without time restriction. The protocol is registered on the PROSPERO platform (number CRD42021291791). Fifty-five studies were included in this systematic review, and 82 altered miRNAs in DMG were identified. Furthermore, four miRNAs were more frequently upregulated in GDM (mir-16-5p, mir-20a-5p, mir-222-3p and mir-330-3p). The dysregulation of these miRNAs is associated with mechanisms of cell cycle homeostasis, growth and proliferation of pancreatic β cells, glucose uptake and metabolism, insulin secretion and resistance. Therefore, identifying miRNAs associated with GDM and elucidating its main mechanisms will improve the characterization and definition of possible biomarkers for the diagnosis, treatment, and manage of GDM.

Keywords: GDM; Diabetes; miRNA; genetic

1. INTRODUÇÃO

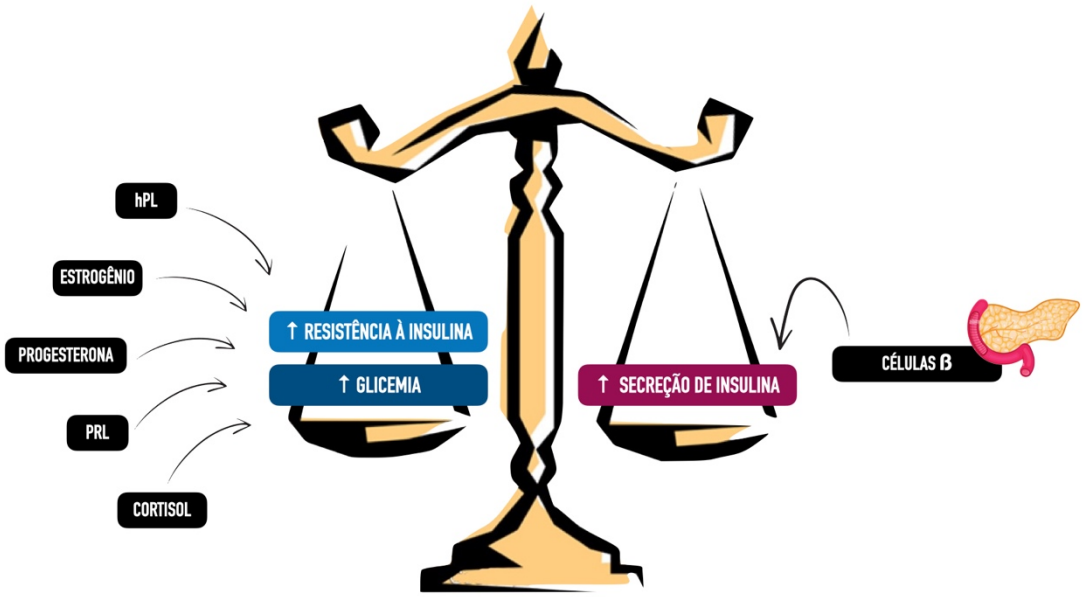
1.1 FISIOPATOLOGIA DA DIABETES *MELLITUS* GESTACIONAL E O PAPEL DOS MICRORNAS

Após a fertilização, o zigoto passa por mitoses sucessivas, conhecidas como clivagem. Ao atingir a cavidade uterina, o embrião está na fase de blastocisto e ocorre o processo de implantação do embrião no endométrio (nidificação). Uma vez que a implantação ocorre, o trofoblasto se prolifera rapidamente e se divide em duas camadas com funções distintas: o citotrofoblasto e o sinciciotrofoblasto (MOORE, 2016). O citotrofoblasto é responsável pela invasão placentária, para ancorar a placenta à decídua e miométrio, enquanto o sinciciotrofoblasto é um epitélio especializado que reveste as vilosidades placentárias, responsável pelo transporte de gases, nutrientes, metabólitos e produção de hormônios como estrogênio, progesterona, glicocorticoides, lactogênio placentário humano (hPL) e gonadotrofina coriônica humana (hCG), que participam do controle metabólico fetal, materno e placentário. (HERRICK E BORDONI, 2022; MOORE,2016).

O corpo da mulher passa por uma série de mudanças para fornecer nutrientes e oxigênio ao embrião/feto durante a gravidez. As alterações metabólicas no corpo materno são basicamente: resistência à insulina, aumento do catabolismo e anabolismo facilitado. Esse mecanismo adaptativo é mediado pelo hormônio lactogênio placentário (hPL), que reduz a sensibilidade materna à insulina; e pelo estrogênio, progesterona, cortisol e prolactina, que possuem ação hiperglicêmica (PARRETTINI, 2020).

Portanto, a gravidez é fisiologicamente caracterizada pela resistência à insulina e hiperinsulinemia. Em circunstâncias normais, observa-se proliferação das células β pancreáticas quando estimuladas pelos níveis elevados de glicose no sangue e o aumento da secreção de insulina garante a homeostase da glicose (Figura 1). Quando há disfunção das células β, o efeito compensatório é perdido e a hiperglicemia durante a gravidez se torna evidente. Assim, a adaptação inadequada das células β à resistência à insulina pode ser o principal mecanismo fisiopatológico subjacente à resistência e ao aumento da glicose relacionados à Diabetes Mellitus Gestacional (DMG). A lesão das células β pancreáticas, devido à sua destruição ou alteração na proliferação e crescimento, e/ou anormalidades que resultam em resistência à ação da insulina podem predispor ao desenvolvimento de DMG em algumas pacientes (ERNST, 2011).

Figura 1. Mecanismo de compensação entre os hormônios contra insulínicos e o aumento da secreção de insulina pelas células beta.



hPL: hormônio lactogênio placentário; PRL: prolactina.

Fonte: elaborado pelo autor.

Consequentemente, compreender a fisiopatologia da DMG envolve conhecer os processos celulares envolvidos na disfunção do ciclo das células β pancreáticas e as vias metabólicas envolvidas na resistência à insulina. Vários fatores podem estar envolvidos nesse processo, como fatores ambientais e de regulação da expressão gênica (ERNST, 2011; LORENZO-ALMORÓS, 2019).

A expressão gênica pode ser influenciada por meio de mutações ou polimorfismos no DNA ou por fatores reguladores da expressão gênica pós-transcricional. Foram reconhecidas mutações no receptor de insulina, fator de crescimento semelhante à insulina-2, glicocinase, fator nuclear hepático-4A, inibidor do ativador de plasminogênio 1 (PAI-1) e receptor de melatonina 1B, entre outros. Alguns deles são variantes genéticas encontradas na diabetes mellitus tipo 2 (DM2) (LORENZO-ALMORÓS, 2019). A regulação gênica pós-transcricional está relacionada a mecanismos capazes de interferir nos RNAs, sendo um desses mecanismos os microRNAs (miRNAs).

Estes são uma classe de RNAs endógenos não codificadores com aproximadamente 22 nucleotídeos que atuam como controladores da expressão gênica pós-transcricional, inibindo a tradução de RNAs mensageiros (mRNA) ou degradando-os. Eles regulam mais de um mRNA alvo, estimando-se que controlem a expressão de cerca de 30% dos genes codificadores de proteínas. As principais funções conhecidas dos miRNAs são a regulação da proliferação e diferenciação celular, apoptose, resposta ao estresse e regulação transcricional (YING, 2008).

A participação dos miRNAs no ciclo das células β pancreáticas ou nas vias de sinalização envolvidas na resistência à insulina pode estar relacionada à patogênese da DMG. Vários estudos investigaram a regulação dos miRNAs circulantes e placentários e sua adaptação metabólica associada à DMG. No entanto, um painel de biomarcadores para a DMG usando miRNAs ainda não foi proposto (LIU, 2021), e destaca-se a necessidade de aprimorar a compreensão da fisiopatologia desta doença associado à influência dos miRNAs com a DMG. Nesse sentido, esta dissertação, no contexto do conhecimento existente, revisou sistematicamente os mecanismos de ação dos microRNAs envolvidos na DMG e identificou aqueles que que se destacaram como biomarcadores potenciais.

1.2 EPIDEMIOLOGIA, DIAGNÓSTICO E TRATAMENTO DA DMG

Segundo a *American Diabetes Association* (ADA), a DMG é definida como qualquer grau de intolerância à glicose com início ou reconhecimento pela primeira vez durante a gravidez. Porém, isso não exclui a possibilidade de que intolerância à glicose não reconhecida possa ter antecedido ou começado concomitantemente com a gravidez. A hiperglicemia durante a gravidez pode ser transitória ou persistir após o nascimento da criança, apresentando-se como um fator de risco independente para o desenvolvimento futuro da DM2 (ADA, 2003).

De acordo com a Federação Internacional de Diabetes, a prevalência global da DMG é em média de 14% (CARRACHER, 2018), variando de 1,8% a 31% dependendo da população avaliada e dos critérios diagnósticos adotados entre os países (MCLNTYRE, 2019; SBD, 2019). A DMG é a doença metabólica mais comum durante a gravidez, ocorrendo em 3% a 25% das gestações, e sua incidência na população tem aumentado, sendo associada ao aumento da obesidade (BRANCHETEIN, 2000; SACKS, 2012; YANG E WU, 2022).

O diagnóstico da DMG pode ser realizado ao longo da gravidez desde o início do pré-natal. No entanto, geralmente é realizado no segundo ou terceiro trimestre da gravidez (24 a 28 semanas), o que pode causar riscos à mãe e ao feto (LIU, 2021). Novos biomarcadores para o primeiro trimestre poderiam aumentar a sensibilidade diagnóstica e diminuir a incidência de complicações.

A síndrome da angústia respiratória neonatal, a hipoglicemia neonatal, a hipocalcemia, a hiper bilirrubinemia, a policitemia e a cardiomiopatia são complicações neonatais de curto prazo relacionadas à DMG (DURNWALD, 2022; METZGER, 2019; YANG E WU, 2022). Além disso, crianças nascidas de mães com DMG têm um risco aumentado de doenças metabólicas e cerebrovasculares na vida adulta. Também existem complicações maternas, gestacionais e fetais relacionadas à DMG, como pré-eclâmpsia, prematuridade, distocia de ombro, macrossomia, polidrâmnio e natimortos (YANG E WU, 2022).

A ausência de um consenso para os valores de referência no diagnóstico da DMG, além de sua relação com os resultados perinatais, motivou o estudo *Hyperglycemia and Adverse Pregnancy Outcomes* (HAPO). Em 2010, a *International Association of the Diabetes and Pregnancy Study Groups* (IADPSG) realizou uma reunião com várias sociedades médicas em todo o mundo, utilizando os resultados do HAPO como base para os critérios diagnósticos da DMG. Assim, definiu-se que o diagnóstico seria estabelecido quando pelo menos um dos valores do Teste Oral de Tolerância à Glicose (TOTG) com 75g de glicose, realizado entre 24 e 28 semanas, fosse ≥ 92 mg/dL em jejum, ≥ 180 mg/dL na primeira hora e ≥ 153 mg/dL na segunda hora (FEBRASGO, 2019; SBD, 2019).

Considerando a necessidade de padronizar os critérios diagnósticos da DMG, a Organização Mundial da Saúde (OMS) em 2013 revogou a antiga recomendação (1999) e atualizou os critérios da OMS com base na IADPSG, com a diferença de que os critérios são válidos para qualquer momento da

gravidez e que o valor do TOTG de duas horas com 75g deve estar entre 153 e 199 mg/dL para o DMG, uma vez que valores ≥ 200 mg/dL são característicos do diagnóstico de diabetes *melliuts* prévio (SBD, 2019; WHO, 2014).

Após os critérios da IADPSG, houve um aumento significativo no número de mulheres classificadas com DMG. Na própria coorte do estudo HAPO, a prevalência da DMG aumentaria para 17,8%. Assim, estudos têm sido propostos para analisar a melhor alternativa para o diagnóstico da DMG, de acordo com as características de cada população, bem como os recursos disponíveis (FEBRASGO, 2019). Recomenda-se que a triagem da DMG seja universal, e o teste diagnóstico deve ser o melhor possível para o local. O teste com a melhor sensibilidade e especificidade é o TOTG com os critérios da IADPSG. No entanto, uma reanálise do Estudo Brasileiro da DMG, considerando os mesmos critérios, observou que 86% dos casos diagnosticados pelo TOTG poderiam ser identificados apenas pela avaliação da glicemia de jejum, uma vez que apresentaram um valor ≥ 92 mg/dL (NICOLOSI, 2020).

O tratamento da DMG começa com dieta e exercícios físicos, considerando contraindicações obstétricas (CAUGHEY E TURRENTINE, 2018; ILJAS, 2017). Se os níveis glicêmicos permanecerem elevados após duas semanas de dieta, deve-se iniciar o tratamento farmacológico (antidiabéticos orais e/ou terapia com insulina) (ADA, 2019). A avaliação da glicemia deve ser realizada desde o diagnóstico até o pós-parto, independentemente do tratamento utilizado. A melhor forma de avaliação ao longo da gravidez é a autovigilância da glicemia capilar com glicosímetro de

punção digital, que está associada a uma redução nos efeitos adversos perinatais. As metas para o controle glicêmico durante a gravidez são as seguintes: em jejum: <95 mg/dl; após uma hora: <140 mg/dl; após duas horas: <120 mg/dl (ADA, 2022).

A frequência para obtenção da glicemia capilar variará de acordo com o tratamento recomendado e a disponibilidade financeira e técnica do local, preferencialmente quatro a sete vezes ao dia. As medidas pós-prandiais devem ser feitas a partir do início da refeição, e a avaliação uma hora após a refeição é aquela que melhor reflete os valores de picos pós-prandiais e está mais associada ao risco de macrossomia (VECIANA, 1995). Pacientes que usam insulina devem manter a glicemia de jejum acima de 70 mg/dl e a glicemia pós-prandial acima de 100 mg/dl para evitar a hipoglicemia (ACOG, 2018).

1.3 BIOGÊNESE DE MICRORNAS E MECANISMO DE AÇÃO

Os miRNAs são pequenos RNAs endógenos, com aproximadamente 17 a 25 nucleotídeos, não codificantes, mas que atuam como reguladores da expressão gênica pós-transcricional. Os miRNAs podem inibir a transcrição do mRNA ou degradá-lo, dependendo se o pareamento é total ou parcial, interferindo na síntese de proteínas (JONAS E IZAURRALDE, 2015; YANG E WU, 2022). Um miRNA pode atingir mais de um mRNA e, portanto, estima-se que eles controlem cerca de 30% dos genes que codificam proteínas (LEWIS, 2005; LIU, 2021).

Os miRNAs foram descritos pela primeira vez em *Caenorhabditis elegans*, em 1993, e estavam envolvidos na divisão celular do estágio larval desse nematóide (LEE, 1993; REINHART, 2000). Atualmente, mais de 2.500 miRNAs foram descritos em humanos e seu estudo tem ganhado relevância devido ao seu envolvimento em doenças como o câncer (CHATTERJEE, 2017). O papel dos miRNAs no câncer vem sendo investigado desde 2002, quando foi descrita pela primeira vez a diminuição da expressão de miR-15 e miR-16 em pacientes com leucemia linfocítica crônica. A regulação da expressão gênica pelos miRNAs envolve a regulação da proliferação e diferenciação celular, apoptose, resposta ao estresse e regulação transcricional (CALIN, 2002).

Estudos indicam que a placenta humana expressa mais de 500 miRNAs, sendo que apenas alguns deles também são expressos em outros tecidos, e variações na expressão podem alterar o mecanismo de adaptação metabólica materna (MORALES-PRIETO, 2014). A caracterização dos miRNAs durante

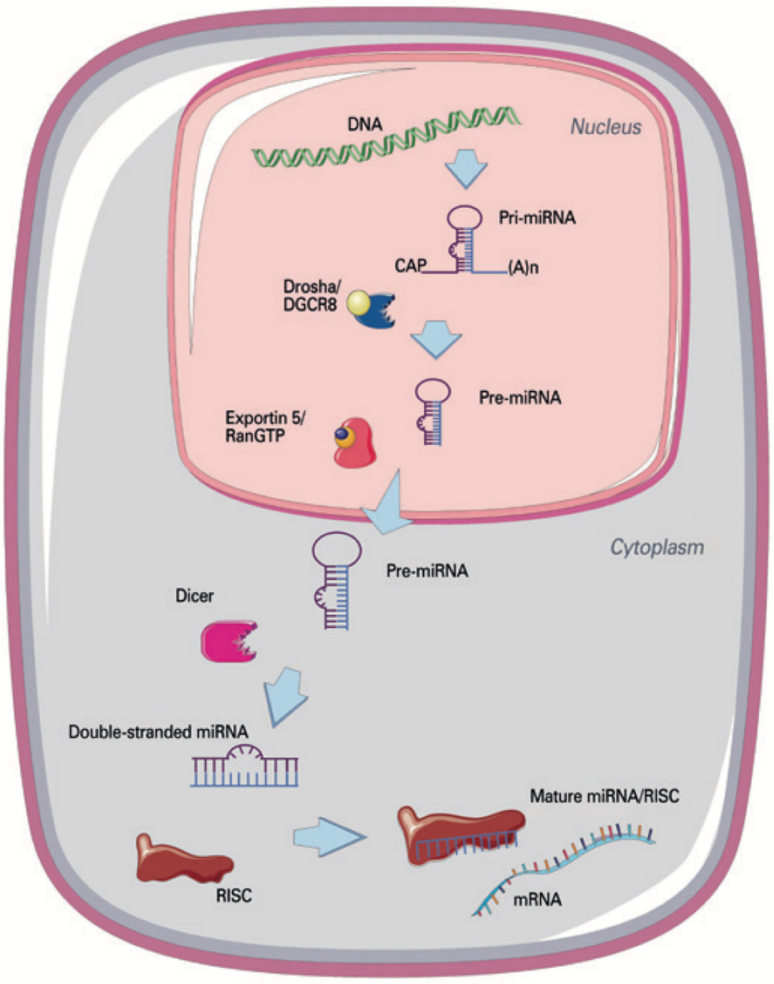
a gravidez é necessária para melhor compreender os mecanismos regulatórios da gravidez saudável e complicada. Devido ao diagnóstico da DMG geralmente ser realizado entre 24 e 28 semanas, os pesquisadores têm buscado identificar biomarcadores para o diagnóstico da DMG, sendo os miRNAs considerados como grande potencial de biomarcadores no primeiro trimestre, principalmente devido à sua alta estabilidade e acessibilidade em fluidos corporais (ILJAS, 2017; LIU, 2021).

A biogênese dos miRNAs têm início com a transcrição por RNA polimerase II ou III, a primeira transcreve os genes miRNA intragênicos e seus genes hospedeiros e a segunda transcreve os miRNAs intergênicos e seus promotores (AMARAL, 2010; GUARINO, 2018). O miRNA primário (pri-miRNA) gerado é um transcrito poliadenilado de aproximadamente 1.000 nucleotídeos com uma estrutura conformacional semelhante a um *"hairpin"*. No núcleo, os pri-miRNAs são clivados por um complexo enzimático que inclui a enzima Drosha e seu cofator DGCR8. A estrutura resultante é o precursor do miRNA (pré-miRNA), que contém aproximadamente 60 nucleotídeos, e é posteriormente exportado para o citoplasma por meio da proteína exportina-5 (Exp5) (AMARAL, 2010; HA E KIM, 2014).

No citoplasma, o pré-miRNA será processado pela enzima Dicer em um miRNA de fita dupla, uma dessas fitas será incorporada no complexo de silenciamento induzido por RNA (RISC), constituindo um miRNA maduro, enquanto a fita complementar será degradada (AMARAL, 2010; HA E KIM, 2014). A fita guia permanece no complexo RISC, formando o complexo miRNA-RISC, que irá identificar o mRNA alvo e induzir a regulação negativa (PASQUINELLI, 2012).

O reconhecimento do alvo é baseado na complementaridade entre a região 3' não traduzida do mRNA e as bases 2-8 do miRNA, conhecidas como sequência semente (WILCZYNSKA E BUSHELL, 2015). O complexo miRNA-RISC pode regular a expressão gênica por meio de dois principais mecanismos: degradação do mRNA e supressão da tradução do mRNA (AMARAL, 2010). Além disso, também foi demonstrado que os miRNAs aumentam a expressão de alguns alvos em condições específicas (PASQUINELLI, 2012). A Figura 2, sumariza a biogênese dos microRNAs.

Figura 2. Biogênese dos microRNAs.



DNA: ácido desoxirribonucleico; pri-miRNA: microRNAs primário; miRNA: microRNAs; RISC: complexo silenciador induzido por RNA; *CAP: capped*; Dicer: enzima.

Fonte: JORGE, 2021.

Além de sua função intracelular como reguladores da expressão gênica, os miRNAs podem ser encontrados extracelularmente em vários fluidos biológicos, como soro e plasma (GUARINO, 2018), ou mesmo embalados em microvesículas (por exemplo, exossomos) ou lipoproteínas (ARROYO, 2011). Os diferentes mecanismos de transporte dos miRNAs os protegem da ação de agentes de degradação, como as RNAases, tornando esses miRNAs estáveis (VALADJ, 2007).

2. OBJETIVOS

2.1. OBJETIVO GERAL

- Identificar os microRNAs associados aos mecanismos fisiopatológicos da Diabetes *Mellitus* Gestacional.

2.2. OBJETIVOS ESPECÍFICOS

- Realizar uma revisão sistemática buscando os principais microRNAs associados à Diabetes *Mellitus* Gestacional;
- Elucidar a atuação dos microRNAs na fisiopatologia da Diabetes *Mellitus* Gestacional;
- Identificar possíveis biomarcadores na patogênese da Diabetes *Mellitus* Gestacional

3. MÉTODO(S)

3.1. REGISTRO, FONTE DE DADOS E ESTRATÉGIA DE BUSCA

Com base na pergunta norteadora: "*Quais microRNAs estão associados aos mecanismos fisiopatológicos da DMG?"*; a revisão sistemática foi realizada para identificar os miRNAs associados aos mecanismos fisiopatológicos da DMG. Foi utilizado o quadro PEO (População, Exposição, Desfecho): População: mulheres grávidas com DMG; Exposição: microRNAs; Desfecho: associação de microRNAs como fator de risco para o DMG.

Esta revisão sistemática (RS) foi realizada de acordo com as diretrizes do *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) (PAGE, 2021). Para evitar duplicações, o protocolo deste estudo foi registrado *no International Prospective Register of Ongoing Systematic Reviews* (PROSPERO) (número CRD42021291791) em 17 de dezembro de 2021 (Anexo B).

A busca bibliográfica foi realizada nas bases de dados PubMed/Medline, Biblioteca Virtual em Saúde (BVS), Web of Science e EMBASE, no período de 27 de janeiro de 2022 a 15 de fevereiro de 2022, em inglês. Termos combinados catalogados em *Medical Subject Headings* (MeSH) para as palavras-chave (Gestational Diabetes OR Gestational Diabetes Mellitus OR Diabetes Induced by Pregnancy) AND (MicroRNA OR miRNA OR Circulating MicroRNA OR Cell-Free MicroRNA) foram utilizados para desenvolver a estratégia de busca, posteriormente adaptada para cada base de dados (Tabela 1).

Tabela 1. Estratégia de busca aplicada em cada base de dados.

BASE DE DADOS	ESTRATÉGIA DE BUSCA
PubMed/Medline	(("Diabetes, Gestational" OR "Diabetes Gestacional" OR "Diabetes Mellitus, Gestational" OR "Diabetes, Pregnancy Induced" OR "Diabetes, Pregnancy-Induced" OR "Gestational Diabetes" OR "Gestational Diabetes Mellitus" OR "Pregnancy-Induced Diabetes") AND ("MicroRNAs" OR "Micro RNA" OR "MicroRNA" OR "MicroRNA, Primary" OR "miRNA" OR "miRNA, Primary" OR "miRNAs" OR "pre miRNA" OR "pre-miRNA" OR "pri miRNA" OR "pri-miRNA" OR "Primary MicroRNA" OR "Primary miRNA" OR "RNA, Micro" OR "Small Temporal RNA" OR "stRNA" OR "MicroARNs" OR "miARN" OR "miARNs" OR "Micro ARN" OR "Micro ARNs" OR "MicroARN" OR "stARN" OR "Circulating MicroRNA" OR "Cell Free MicroRNA" OR "Cell-Free MicroRNA" OR "MicroRNA, Cell-Free" OR "MicroRNA, Circulating"))
Biblioteca Virtual em Saúde	("microrna") AND ("gestational diabetes") AND (mj:("Diabetes Gestacional" OR "MicroRNAs"))
Web of Science	(("Diabetes, Gestational" OR "Diabetes Mellitus, Gestational" OR "Diabetes, Pregnancy Induced" OR "Diabetes, Pregnancy-Induced" OR "Gestational Diabetes" OR "Gestational Diabetes Mellitus" OR "Pregnancy-Induced Diabetes") AND ("MicroRNAs" OR "Micro RNA" OR "MicroRNA" OR "MicroRNA, Primary" OR "miRNA" OR "miRNA, Primary" OR "miRNAs" OR "pre miRNA" OR "pre-miRNA" OR "pri miRNA" OR "pri-miRNA" OR "Primary MicroRNA" OR "Primary miRNA" OR "RNA, Micro" OR "RNA, Small Temporal" OR "Small Temporal RNA" OR "sirna" OR "stRNA" OR "Temporal RNA, Small" OR "MicroARNs"))
EMBASE	('diabetes, gestational'/exp OR 'diabetes, gestational' OR 'diabetes gestacional' OR 'diabetes, pregnancy induced' OR 'diabetes, pregnancy-induced' OR 'gestational diabetes'/exp OR 'gestational diabetes' OR 'gestational diabetes mellitus'/exp OR 'gestational diabetes mellitus' OR 'pregnancy-induced diabetes') AND ('micrornas'/exp OR 'micrornas' OR 'micro rna'/exp OR 'micro rna' OR 'microrna'/exp OR 'microrna' OR 'microrna, primary' OR 'mirna'/ exp OR 'mirna' OR 'mirna, primary' OR 'mirnas'/exp OR 'mirnas' OR 'pre mirna' OR 'pre-mirna' OR 'pri mirna' OR 'pri-mirna' OR 'primary microrna'/exp OR 'primary microrna' OR 'primary mirna' OR 'rna, micro' OR 'rna, small temporal' OR 'small temporal rna' OR 'strna' OR 'temporal rna, small' OR 'microarns' OR 'arn pequeño temporal' OR 'arn temporal pequeño' OR 'miarn' OR 'miarns' OR 'micro arn' OR 'micro arns' OR 'microarn' OR 'microarn primario' OR 'microrna primario' OR 'starn' OR 'rna pequeno temporário' OR 'rna temporário pequeno' OR 'circulating microrna'/exp OR 'circulating microrna' OR

	'cell free microrna'/exp OR 'cell free microrna' OR 'cell-free microrna'/exp OR 'cell-free microrna' OR 'microrna, cell-free' OR 'microrna, circulating' OR 'microarn circulante' OR 'microrna circulante' OR 'microrna fora da célula' OR 'microrna livre') AND ('biological marker'/dd OR 'circular ribonucleic acid'/dd OR 'glucose'/dd OR 'insulin'/dd OR 'microrna'/dd OR 'microrna 122'/dd OR 'microrna 143'/dd OR 'microrna 146a'/dd OR 'microrna 16'/dd OR 'microrna 21'/dd OR 'microrna 210'/dd OR 'microrna 222'/dd OR 'rna'/dd OR 'small interfering rna'/dd OR 'transcription factor'/ dd) AND ('congenital heart disease'/dm OR 'diabetes mellitus'/dm OR 'diabetic complication'/dm OR 'intrauterine growth retardation'/ dm OR 'macrosomia'/dm OR 'maternal diabetes mellitus'/dm OR 'pregnancy diabetes mellitus'/dm)

Fonte: elaborado pelo autor.

Critérios de inclusão dos estudos nesta revisão sistemática: Estudos observacionais de pesquisa original que identificaram miRNAs em mulheres grávidas com DMG e em controles (mulheres grávidas sem DMG); sem restrições de idade; publicados em inglês; sem restrição do ano de publicação do estudo. Como critérios de exclusão, foram estabelecidos: estudos que não abordaram o tema da pesquisa; e com outro desenho de estudo.

3.2. SELEÇÃO DOS ESTUDOS

Dois revisores (PHCMS e LS) selecionaram e identificaram independentemente os artigos em todas as etapas da RS. Os artigos identificados nas bases de dados foram importados para a plataforma *Rayyan* (OUZZANI, 2016) para otimizar a análise. Inicialmente, o título e o resumo foram lidos (Fase I), em seguida, os artigos selecionados na primeira triagem foram lidos na íntegra (Fase II). Em ambas as fases, os artigos foram avaliados de acordo com os critérios de inclusão e exclusão pré-estabelecidos.

3.3. AVALIAÇÃO DO RISCO DE VIÉS

O risco de viés metodológico foi avaliado utilizando a *The Joanna Briggs Institute (JBI) Critical Assessment Tool* (INSTITUTE TJB, 2022) para cada desenho de estudo (Anexo D). Todos os artigos selecionados para inclusão na revisão sistemática foram submetidos a uma avaliação rigorosa. Essa ferramenta é composta por perguntas com as possíveis respostas: "Sim", "Não", "Não aplicável" e "Incerto".

Uma ferramenta de avaliação crítica foi aplicada a cada tipo de estudo: estudos caso-controle, estudos de coorte, estudos transversais. Os artigos que tiveram 100% das respostas "sim" foram considerados de baixo risco de viés; aqueles que pontuaram de 70 a 99% "sim" foram considerados de risco moderado; e aqueles com menos de 70% de respostas "sim" foram excluídos do estudo, sendo considerados de alto risco de viés metodológico. As divergências de opinião entre os revisores foram discutidas e resolvidas por consenso.

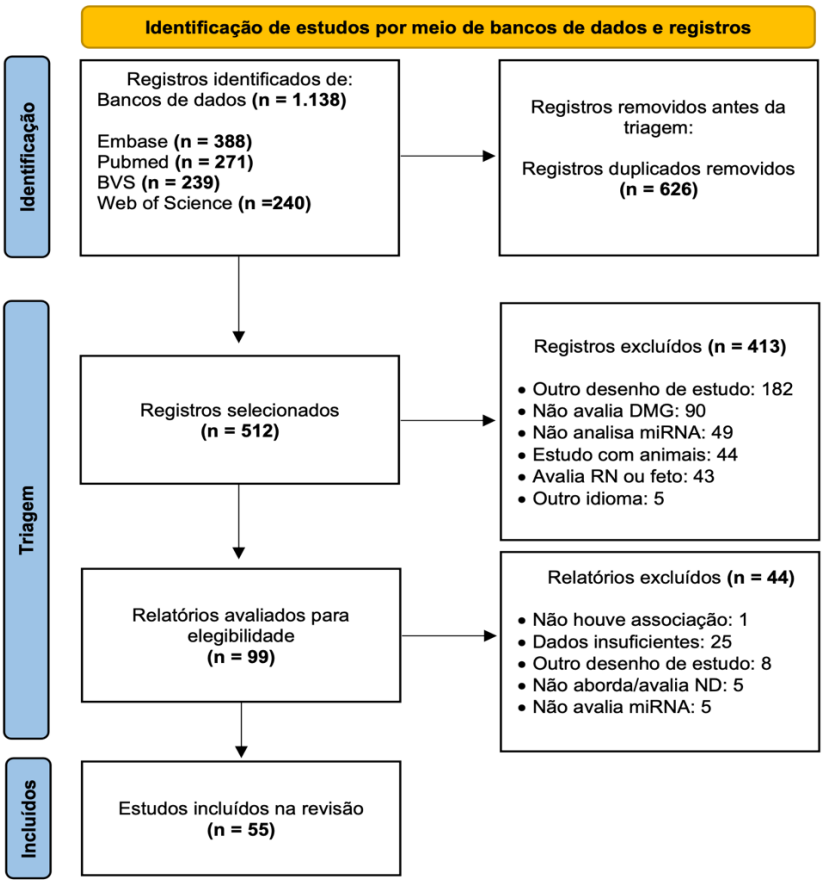
3.4. EXTRAÇÃO E SÍNTESE DE DADOS

Estudos que avaliam miRNAs carecem de análises estatísticas mais robustas, a maioria descreve apenas o tipo de regulação identificada e se houve diferença significativa nessa regulação entre os grupos avaliados (geralmente considerando $p<0,05$). Assim, esta revisão sistemática utilizou uma abordagem qualitativa e descritiva para análise dos dados, extraindo os seguintes dados: (1) autores e ano de publicação; (2) desenho do estudo; (3) país ou continente onde o estudo foi realizado; (4) tamanho da amostra; (5) idade média; (6) tempo gestacional; (7) tecido analisado; (8) técnica de identificação de miRNA; (9) miRNA; (10) regulação; (11) valor de p. Os dados extraídos foram importados para uma planilha pré-definida do Excel, e os autores dos estudos com dados faltantes foram contatados. A extração de dados foi realizada de forma independente por dois revisores, e as divergências entre os revisores foram resolvidas por consenso.

4. RESULTADOS

Após a busca inicial, foram identificados 1.138 artigos nas bases de dados, sendo que 626 duplicatas foram excluídas, resultando em 512 artigos para a fase I. Com base na leitura dos títulos e resumos (fase I), foram excluídos 413 artigos por não atenderem aos critérios de inclusão pré-estabelecidos. Portanto, a fase II compreendeu a leitura completa dos 99 artigos selecionados. Quarenta e quatro foram excluídos e 55 foram incluídos nesta revisão sistemática (Figura 3).

Figura 3. Fluxograma PRISMA demonstrando o processo de exclusão ou inclusão de estudos nesta revisão sistemática.



Embase: base de dados eletrônica da editora Elsevier; PubMed: serviço da Biblioteca Nacional de Medicina dos Estados Unidos para acesso gratuito ao Medline; BVS: Biblioteca Virtual em Saúde.

Fonte: adaptado de PAGE, 2021.

Tabela 2. Dados extraídos dos estudos incluídos na revisão sistemática.

AUTOR, ANO	TIPO DE ESTUDO	LOCAL	TAMANHO DA AMOSTRA	IDADE MÉDIA	IDADE GESTACIONAL (SEMANAS)	TIPO DE AMOSTRA	MÉTODO	miRNA	REGULAÇÃO	valor-P
KE, 2022	Caso-controle	China	Caso: 70 Controle: 70	Caso: 30.33 ± 2.25 Controle: 30.04 ± 2.25	Caso: 39.12 ± 1.15 Controle: 38.76 ± 1.06	Sangue	qRT-PCR	miR-134-5p	*UP*	<0.001
WANG, 2021	Caso-controle	China	Caso: 26 Controle: 23	Caso: 30.6 ± 4.4 Controle: 29.2 ± 3.5	Caso: 38.1 ± 1.2 Controle: 39.4 ± 1.2	Placenta	RT-PCR	miR-6869-5p	*DOWN*	<0.001
LIU, 2021	Caso-controle	China	Caso: 110 Controle: 78	Caso: 33,118±3,402 Controle: 32,385±3,579	Caso: 25.591±1.757 Controle: 25.218±2.196	Sangue	qRT-PCR	miR-1323	*UP*	<0.05
ZHANG, 2021	Estudo transversal	China	Caso: 36 Controle: 37	20-40 anos	37- 41	Placenta	qRT-PCR	miR-125b; miR-144; miR-543	*UP*	<0.001
LI, 2021	Caso-controle	China	Caso: 30 Controle: 30	Caso: 24-39 Controle: 24-39	24-28 Semanas	Plasma e Placenta	qRT-PCR	miR-345-3p	*DOWN*	<0.01
GUAN, 2020	Caso-controle	Gana	Caso: 137 Controle: 158	Caso: 30.94 ± 3.45 Controle: 29.67 ± 3.64	Caso: 272.22 ± 6.40 Controle: 274.41 ± 8.02 (dias)	Placenta	qRT-PCR	miR-21	*DOWN*	<0.01
DENG, 2020	Caso-controle	China	Caso: 68 Controle: 55	Caso:32,65 ± 4,63 Controle: 31,27 ± 4,01	Caso: 39.09 ± 1.11 Controle: 38.98 ± 1.05	Plasma	qRT-PCR	miR-29a; miR-29b	*DOWN*	<0.001
ZHAO, 2020	Caso-controle	China	Caso: 33 Controle: 20	ND	Caso: 24-26 Controle: 37-41	Placenta	qRT-PCR	miR-140-3p	*UP*	<0.05
XIAO, 2020	Caso-controle	China	Caso: 30 Controle: 10	ND	ND	Sangue	qRT-PCR	miR-330-3p	*UP*	<0.001
TANG, 2020	Caso-controle	China	Caso: 20 Controle:20	Caso: 29.4 ± 1.2 Controle: 29.6 ± 0.4	Caso: 38.7 ± 0.6 Controle: 39.0 ± 1.0	Placenta	qRT-PCR	miR-17; miR-195; miR-257	*DOWN; UP; UP*	<0.01
HOCAOGLU, 2019	Caso-controle	Turquia	Caso: 19 Controle: 28	Caso: 30.4 ± 4.6 Controle: 28.1 ± 5.8	Caso: 33.5 ± 3.6 Controle: 33 ± 4.1	Sangue	qRT-PCR	miR-21-3p	*DOWN*	0.008
STIRM, 2018	Caso-controle	Alemanha	Caso: 30 Controle: 30	Caso: 31±4 Controle: 32±4	Caso: 27.0±2.3 Controle:27.6±2.37	Sangue	qRT-PCR	miRNA-340	*UP*	0.02
WANDER, 2017	Coorte	EUA	Caso: 36 Controle: 80	Caso: 34.3 ± 3.6 Controle: 32.9 ± 4.4	Caso: 15.1 ± 2.9 Controle: 16.5 ± 2.3	Sangue	qRT-PCR	miR-21-3p; miR-155-5p	ND	0.005 0.028
HOCAOGLU, 2020	Caso-controle	Turquia	Caso: 14 Controle: 27	Caso: 30.4 ± 4.4 Controle: 27.9 ± 5.5	Caso: 33.5 ± 3.5 Controle: 33.1 ± 4.1	Sangue	qPCR	miR-155-5p	*DOWN*	0.04
HUA, 2021	Caso-controle	China	Caso: 30 Controle: 38	Caso: 30.27 ± 6.38 Controle: 33.21 ± 8.17	Caso: 38.12 ± 1.65 Controle: 37.54 ± 1.31	Sangue	qRT-PCR	miR-377-3p	*UP*	<0.01
SHEN, 2021	Caso-controle	China	Caso: 25 Controle: 30	ND	ND	Sangue	qRT-PCR	miR-181d	*UP*	<0.01

BALCI, 2020	Caso-controle	Turquia	Caso: 30 Controle: 30	Caso: 32.3 Controle: 29.9	Caso: 26.9 Controle: 27.4	Plasma	qRT-PCR	miR-7-5p	*UP*	0.02
JI, 2020	Caso-controle	China	Caso: 20 Controle: 20	Caso: 35.30±4.37 Controle: 32.30±3.40	Caso: 74.38±8.58 Controle: 71.50±11.72	Sangue	qRT-PCR	miR-193b	*DOWN*	<0.001
TU, 2020	Caso-controle	China	Caso: 76 Controle: 73	27,02±3,17	ND	Sangue	qRT-PCR	miR-409-5p	*UP*	<0.05
PFEIFFER, 2020	Cohort	Espanha	Caso: 31 Controle: 29	Caso: 31.9 ± 1.8 Controle: 31.0 ± 3.6	Caso: 39.1 ± 1.3 Controle: 39.2 ± 1.2	Sangue	qRT-PCR	miR-330-3p	*UP*	0.003
ZHU, 2015	Caso-controle	China	Caso: 10 Controle: 10	Caso: 30.03 ± 3.56 Controle: 26.67 ± 4.59	Caso: 17.66 ± 0.85 Controle: 18.17 ± 0.93	Sangue	qRT-PCR	miR-16-5p; miR-17-5p; miR-19a-3p; miR-19b-3p; miR-20a-5p	*UP*	5.36E-11; 1.10E-10; 6.57E-43; 1.73E-74; 5.27E-37
ZHAO, 2011	Caso-controle	China	Caso: 24 Controle: 24	Caso: 28.79±2.21 Controle: 29.46±1.89	Caso: 17.40±0.70 Controle: 17.16±0.79	Plasma	qRT-PCR / chip TLDA	miR-132; miR-29a; miR-222	*DOWN*	0.042; 0.032; 0.041
WNAG, 2019	Caso-controle	China	Caso: 100 Controle: 100	ND	ND	Sangue	qRT-PCR	miRNA-19a; miRNA-19b	*UP*	ND
GILLET, 2019	Coorte	Canada	Caso: 23 Controle: 46	Caso: 29.8 ±5.3 Controle: 27.9 ±4.4	Caso: 10.5 ±2.5 Controle: 10.6 ±2.4	Sangue	qRT-PCR	miR–122-5p; miR–132-3p; miR–1323; miR–136-5p; miR–182-3p; miR–210-3p; miR–29a-3p; miR–29b-3p; miR-342-3p; miR-520h	*UP*	0.01; 0.03; 0.03; 0.03; 0.01; 0.02; 0.03; 0.04; 0.008; 0.03
WANG, 2019	Caso-controle	China	Caso: 48 Controle: 46	Caso: 29.86 ± 0.94 Controle: 30.02 ± 0.89	ND	Placenta	qRT-PCR	miR-657	*UP*	<0.001
WANG, 2019	Caso-controle	China	Caso: 30 Controle: 29	Caso: 28-40 Controle: 29-38	Caso: 37.9±1.1 Controle: 39.2±1.1	Placenta	qRT-PCR	miR-657	*UP*	<0.01
LI, 2015	Caso-controle	China	Caso: 10 Controle: 10	21-37 Anos	ND	Placenta	qRT-PCR	miR-508-3p; miR-27a; miR-9; miR-137; miR-92a; miR-33a; miR-30d; miR-362-5p; miR-502-5p	*UP;* *DOWN;* *DOWN;* *DOWN;* *DOWN;* *DOWN;* *DOWN;* *DOWN;* *DOWN*	<0.01; <0.05; <0.05; <0.05; <0.05; <0.05; <0.05; <0.05; <0.05

SØRENSEN, 2021	Caso-controle	Europa	Caso: 41 Controle: 41	Caso: 32.7 ± 4 Controle: 33.2 ± 3.8	Caso: 40 ± 39.3 Controle: 40.1 ± 1.1	Sangue	qRT-PCR	miR-16-5p; miR-29a-3p; miR-134-5p	*UP*	0.008; 0.004; 0.046
XU, 2017	Caso-controle	China	Caso: 25 Controle: 25	ND	ND	Sangue	qRT-PCR	miR-503	*UP*	<0.01
HE, 2017	Caso-controle	China	Caso: 20 Controle: 20	ND	ND	Sangue	qRT-PCR	miR-494	*DOWN*	<0.01
SEBASTIANI 2017	Coorte	Itália	Caso: 21 Controle: 10	Caso: 35.57 ± 5.63 Controle: 32.80 ± 5.16	ND	Sangue	qRT-PCR	miR-330-3p	*UP*	0.01
FILARDI, 2022	Coorte	Itália	Caso: 12 Controle: 12	Caso: 36.4 ± 4.6 Controle: 34.9 ± 5.1	ND	Plasma	qRT-PCR	miR-222-3p; miR-409-3p	*UP*	0.05; 0.01
YU, 2021	Caso-controle	China	Caso: 123 Controle: 123	Caso: 31.23 ± 2.31 Controle: 30.84 ± 2.95	Caso: 39.35 ± 3.81 Controle: 39.52 ± 2.47	Plasma	qRT-PCR	miR-96-5p	*DOWN*	0.01
ZHOU, 2019	Caso-controle	China	Caso: 108 Controle: 50	Caso: 30.89 ± 3.45 Controle: 30.10 ± 2.79	Caso: 25.20 ± 1.23 Controle: 25.32 ± 1.45	Sangue e placenta	qRT-PCR	miR-132	*DOWN*	<0.001
ABDELTAWAB 2020	Caso-controle	Egito	Caso: 109 Controle: 103	Caso: 29.9 ±6.28 Controle: 29.7 ±5.85	ND	Sangue	qRT-PCR	miR-223	*UP*	<0.001
SHI, 2014	Caso-controle	China	Caso: 13 Controle: 13	Caso: 27.62 ± 3.10 Controle: 27.85 ± 3.36	ND	Tecido adiposo	qRT-PCR	miR-222	*UP*	<0.01
MA, 2019	Caso-controle	China	Caso: 30 Controle: 26	Caso: 27.6 Controle: 26.9	ND	Sangue	qRT-PCR	miR-214	*DOWN*	<0.05
SUN, 2020	Caso-controle	China	Caso: 204 Controle: 202	Caso: 30.91 ± 0.38 Controle: 29.68 ± 0.53	Caso: 271.68 ± 11.47 Controle: 274.37 ± 8.10 (dias)	Placenta	qRT-PCR	miR-29b	*DOWN*	<0.05
YOFFE, 2019	Caso-controle	Espanha e Itália	Caso: 23 Controle: 20	Caso: 34.0 (32.5–37.5) Controle: 34.5 (32.0–37.2)	Caso: 40.3 (38.4–40.7) Controle: 39.9 (39.4–40.7)	Plasma	qRT-PCR	miR-223; miR-23a	*UP*	1.4×10^{-7}; 0.019
ZHANG, 2020	Caso-controle	China	Caso: 30 Controle: 30	ND	ND	Sangue	qRT-PCR	miR-770-5p	*UP*	<0.01
WEN, 2021	Caso-controle	China	Caso: 32 Controle: 48	Caso: 32.71±5.26 Controle: 29.13±4.22	Caso: 28.33±2.81 Controle: 29.10±2.32	Sangue	qRT-PCR	miR-520h	*UP*	<0.001
ZHANG, 2018	Caso-controle	China	Caso: 5 Controle: 5	ND	ND	Placenta	qRT-PCR	miR-9-5p	*DOWN*	<0.01
PHEIFFER, 2018	Caso-controle	África do Sul	Caso: 28 Controle: 53	Caso: 29.5 ±6.2 Controle: 28.6 ±6.4	ND	Sangue	qRT-PCR	miR-20a-5p; miR-222-3p	*DOWN*	0.038; 0.027
FENG, 2020	Caso-controle	China	Caso: 12 Controle: 12	ND	ND	Sangue	qRT-PCR	miR-33a-5p	*UP*	<0.01
ZHANG, 2020	Caso-controle	China	Caso: 112 Controle: 58	Caso: 31.59 ± 3.93 Controle: 31.14 ± 3.94	Caso: 24.85 ± 1.69 Controle: 24.47 ± 2.15	Sangue e placenta	qRT-PCR	miR-136	*UP*	<0.001
ZHANG, 2021	Caso-controle	China	Caso: 166 Controle: 196	Caso: 31.03 ± 3.63 Controle: 29.70 ± 3.83	ND	Placenta	qRT-PCR	miR-30d-5p	*DOWN*	<0.01

WANG, 2020	Caso-controle	China	Caso: 102 Controle:102	Caso: 29.8 ± 3.2 Controle: 29.5 ± 2.8	Caso: 27.0 ± 1.6 Controle: 26.8 ± 1.1	Sangue	qRT-PCR	miR-195-5p	*UP*	<0.01
CAO, 2016	Caso-controle	China	Caso: 193 Controle: 202	Caso: 30.93 ± 3.45 Controle: 29.68 ± 3.66	Caso: 272.22 ± 6.39 Controle: 274.41 ± 8.03 (dias)	Placenta	qRT-PCR	miR-98	*UP*	<0.05
CAO, 2017	Caso-controle	China	Caso: 85 Controle: 72	Caso: 26.8 ± 3.5 Controle: 26.4 ± 3.6	Caso: 25.8 ± 2.5 Controle: 26.1 ± 1.2	Sangue	qRT-PCR	miR-16-5p; miR-17-5p; miR-20a-5p	*UP*	<0.01
WANG, 2021	Caso-controle	China	Caso: 53 Controle: 46	Caso: 29.8 ± 0.4 Controle: 29.2 ± 0.6	Caso: 39.9 ± 0.1 Controle: 39.0 ± 0.2	Sangue	qRT-PCR	miR-574-5p; miR-3135b	*DOWN*	<0.0001; 0.002
OOSTDAM, 2020	Caso-controle	México	Caso: 27 Controle: 34	Caso: 29.93±6.03 Controle: 26.06±5.28	ND	Urina	qRT-PCR	miR-516-5p; miR-517-3p; miR-518-5p; miR-222-3p; miR-16-5p	*DOWN;* *DOWN;* *DOWN;* *DOWN;* *UP*	<0.05; <0.05; <0.01; <0.01; <0.01
PENG, 2018	Caso-controle	China	Caso: 11 Controle: 12	Caso: 29.91 ± 1,385 Controle: 29.08 ± 1,305	ND	Sangue	qRT-PCR	miR-137	UP	0.0073
TAGOMA, 2018	Caso-controle	Estônia	Caso: 13 Controle: 9	Caso: 31.1 ± 4.2 Controle: 28.1 ± 4.5	23-31 semanas	Sangue	qRT-PCR	let-7e-5p; let-7g-5p; miR-100-5p; miR-101-3p; miR-146a-5p; miR-195-5p; miR-222-3p; miR-23b-3p; miR-30b-5p; miR-30c-5p; miR-30d-5p; miR-342-3p; miR-423-5p	UP	0.03; 0.01; 0.04; 0.03; 0.03; 0.03; 0.03; 0.02; 0.04; 0.02; 0.03; 0.04; 0.02
MONFARED, 2018	Caso-controle	Iran	Caso: 30 Controle: 30	ND	ND	Plasma	qRT-PCR	miR-135a	DOWN	0.001
WANG, 2021	Caso-controle	China	Caso: 5 Controle: 5	ND	ND	Placenta	qRT-PCR	miR-190b	UP	<0.001

UP: *up-regulated*; DOWN: *down-regulated*; ND: não descrito; qRT-PCR*: Polymerase chain reaction via quantitative reverse transcriptase*.

Fonte: elaborado pelo autor

Dos estudos incluídos, 48 foram estudos de caso-controle, seis foram estudos de coorte e um foi um estudo transversal. A idade média dos grupos de caso e controle variou entre 20 e 40 anos. Um total de 2.749 casos (gestantes com DMG) e 2.710 controles (gestantes sem DMG) foram analisados, totalizando 5.459 indivíduos. As amostras coletadas e analisadas foram sangue, plasma, placenta, tecido adiposo e urina (exossomo urinário) (Tabela 2).

Além disso, os estudos foram homogêneos em termos de avaliação da qualidade metodológica, alcançando individualmente um mínimo de 70% de respostas positivas, sendo considerados de baixo ou moderado risco de viés (Tabela 3). Para estudos de caso-controle, foram avaliados 10 parâmetros, para estudos de coorte 11 e para estudos transversais 8 parâmetros. A maioria dos estudos de caso-controle relatou que não houve identificação de possíveis fatores de confusão (questão 06) e, consequentemente, a não aplicabilidade da questão 07, que diz respeito às estratégias para lidar com fatores de confusão.

Tabela 3. Resumo das respostas para cada estudo incluído na avaliação de viés de risco.

REFERÊNCIA	QUESTÕES										
	01	02	03	04	05	06	07	08	09	10	11
KE, 2022[1]	S	S	S	S	S	N	NA	S	S	S	NA
WANG, 2021[1]	S	S	S	S	S	N	NA	S	S	S	NA
LIU, 2021[1]	S	S	S	S	S	N	NA	S	S	S	NA
ZHANG, 2021 [3]	S	S	S	S	N	NA	S	S	NA	NA	NA
LI, 2021[1]	S	S	S	S	S	N	NA	S	S	S	NA
GUAN, 2020[1]	S	S	S	S	S	N	NA	S	S	S	NA
DENG, 2020[1]	S	S	S	S	S	N	NA	S	S	S	NA
ZHAO, 2020[1]	S	S	S	S	S	S	S	S	S	S	NA
XIAO, 2020[1]	S	S	S	S	S	N	NA	S	S	S	NA
TANG, 2020[1]	S	S	S	S	S	N	NA	S	S	S	NA
HOCAOGLU, 2019[1]	S	S	S	S	S	N	NA	S	S	S	NA
STIRM, 2018[1]	S	S	S	S	S	S	S	S	S	S	NA
WANDER, 2017[2]	S	S	S	N	NA	S	S	S	S	S	S
HOCAOGLU, 2020[1]	S	S	S	S	S	S	S	S	S	S	NA
HUA, 2021[1]	S	S	S	S	S	N	NA	S	S	S	NA
SHEN, 2021[1]	S	S	S	S	S	N	NA	S	S	S	NA
BALCI, 2020[1]	S	S	S	S	S	N	NA	S	S	S	NA
JI, 2020[1]	S	S	S	S	S	N	NA	S	S	S	NA
TU, 2020[1]	S	S	S	S	S	N	NA	S	S	S	NA
PFEIFFER, 2020[2]	S	S	S	N	NA	S	S	S	S	S	S
ZHU, 2015[1]	S	S	S	S	S	S	S	S	S	S	NA
ZHAO, 2011[1]	S	S	S	S	S	S	S	S	S	S	NA
WNAG, 2019[1]	S	S	S	S	S	N	NA	S	S	S	NA
GILLET, 2019[2]	S	S	S	S	S	N	S	S	S	S	S
WANG, 2019[1]	S	S	S	S	S	S	S	S	S	S	NA
WANG, 2019[1]	S	S	S	S	S	N	NA	S	S	S	NA
LI, 2015[1]	S	S	S	S	S	N	NA	S	S	S	NA
SØRENSEN, 2021[2]	S	S	S	S	S	N	S	S	S	S	S
XU, 2017[1]	S	S	S	S	S	N	NA	S	S	S	NA
HE, 2017[1]	S	S	S	S	S	N	NA	S	S	S	NA

REFERÊNCIA	QUESTÕES										
	01	02	03	04	05	06	07	08	09	10	11
SEBASTIANI, 2017[2]	S	S	S	S	S	N	S	S	S	S	S
FILARDI, 2022[2]	S	S	S	N	NA	S	S	S	S	S	S
YU, 2021[1]	S	S	S	S	S	N	NA	S	S	S	NA
ZHOU, 2019[1]	S	S	S	S	S	N	NA	S	S	S	NA
ABDELTAWAB, 2020[1]	S	S	S	S	S	N	NA	S	S	S	NA
SHI, 2014[1]	S	S	S	S	S	N	NA	S	S	S	NA
MA, 2019[1]	S	S	S	S	S	N	NA	S	S	S	NA
SUN, 2020[1]	S	S	S	S	S	N	NA	S	S	S	NA
YOFFE, 2019[1]	S	S	S	S	S	N	NA	S	S	S	NA
ZHANG, 2020[1]	S	S	S	S	S	N	NA	S	S	S	NA
WEN, 2021[1]	S	S	S	S	S	N	NA	S	S	S	NA
ZHANG, 2018[1]	S	N	S	S	S	N	NA	S	S	S	NA
PHEIFFER, 2018[1]	S	S	S	S	S	N	NA	S	S	S	NA
FENG, 2020[1]	S	S	S	S	S	N	NA	S	S	S	NA
ZHANG, 2020[1]	S	S	S	S	S	N	NA	S	S	S	NA
ZHANG, 2021[1]	S	S	S	S	S	N	NA	S	S	S	NA
WANG, 2020[1]	S	S	S	S	S	S	S	S	S	S	NA
CAO, 2016[1]	S	S	S	S	S	N	NA	S	S	S	NA
CAO, 2017[1]	S	S	S	S	S	N	NA	S	S	S	NA
WANG, 2021[1]	S	S	S	S	S	N	NA	S	S	S	NA
OOSTDAM, 2020[1]	S	S	S	S	S	N	NA	S	S	S	NA
PENG, 2018[1]	S	S	S	S	S	N	NA	S	S	S	NA
TAGOMA, 2018[1]	S	S	S	S	S	N	NA	S	S	S	NA
MONFARED, 2018[1]	S	N	S	S	S	N	NA	S	S	S	NA
WANG, 2021[1]	S	S	S	S	S	N	NA	S	S	S	NA

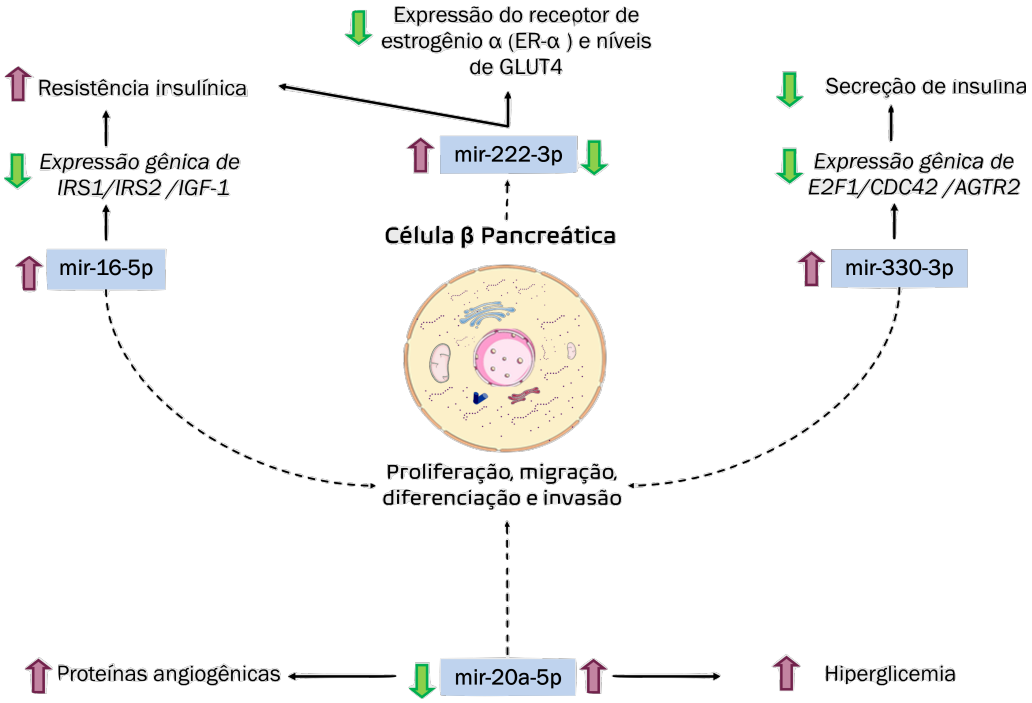
[1] Caso-controle; [2] Coorte; [3] Estudo Transversal.
S sim; N não; NA não aplicável.

Fonte: Elaborado pelo autor

Nesta revisão sistemática, foram identificados 82 miRNAs, destacando os mais citados na literatura por estarem desregulados na DMG (mir-16-5p, mir-20a-5p, mir-222-3p e mir-330-3p). De acordo com os resultados, os miRNAs identificados foram encontrados com regulação positiva e/ou negativa na DMG, considerando $p<0,05$ como significativo. Como mencionado anteriormente, os estudos que avaliam os miRNAs geralmente carecem de análises estatísticas mais robustas, muitas vezes descrevendo apenas se houve diferença significativa e o tipo de regulação do miRNA entre os grupos avaliados.

Dentre os miRNAs mais frequentemente encontrados na literatura, mir-16-5p (CAO, 2017; OOSTDAM, 2020; SØRENSEN, 2021; ZHU, 2015;) e mir-330-3p (PFEIFFER, 2020; SEBASTIANI, 2017; XIAO, 2020) apresentaram regulação positiva, enquanto mir-20a-5p e mir-222-3p apresentaram regulação positiva e negativa em diferentes estudos (CAO, 2017; FILARDI, 2022; OOSTDAM, 2020; PHEIFFER, 2018; TAGOMA, 2018; ZHU, 2015) (Tabela 4). Em geral, a desregulação desses miRNAs está associada a mecanismos de inflamação, crescimento e proliferação das células β pancreáticas, captação e metabolismo da glicose, secreção e resistência à insulina (Figura 4). A função biológica e regulação dos miRNAs encontrados na revisão sistemática estão demonstrados na Tabela 5.

Figura 4. O papel dos principais miRNAs na fisiopatologia da DMG.



IRS1/IRS2: *insulin receptor substrate 1 and 2*; IGF-1: *insulin-like growth factor 1*; ERα: *estrogen receptor α*; GLUT 4: *glucose transporter type 4*; E2F1: E2F *transcription factor 1*; CDC42: *protein Cell Division Cycle 42*; AGTR2: *angiotensin II receptor type 2.*

Fonte: elaborado pelo autor

Tabela 4. Principais miRNAs e suas regulações encontrados na revisão sistemática.

REFERÊNCIA	mir-16-5p	mir-330-3p	mi-20a-5p	mir-222-3p
XIAO, 2020		↑		
PFEIFFER, 2020		↑		
ZHU, 2015	↑		↑	
SØRENSEN, 2021	↑			
SEBASTIANI, 2017		↑		
FILARDI, 2022				↑
PHEIFFER, 2018			↓	↓
CAO, 2017	↑		↑	
OOSTDAM, 2020	↑			↓
TAGOMA, 2018				↑

↑ *up-regulated;* ↓ *down-regulated*

Fonte: elaborado pelo autor

Tabela 5. Função biológica e regulação dos miRNAs encontrados na revisão sistemática.

miRNA	FUNÇÃO BIOLÓGICA	REGULAÇÃO	REFERÊNCIA
let-7e-5p	diferenciação osteoblástica e formação óssea	↑	TAGOMA, 2018
let-7g-5p	inibição da proliferação de células mamárias	↑	TAGOMA, 2018
miR-7-5p	regulação da sensibilidade à insulina	↑	BALCI, 2020
miR-9	desenvolvimento e função neuronal de mamíferos	↓	LI, 2015
miR-9-5p	regulação da glicólise aeróbia e expressão do complexo mitocondrial	↓	ZHANG, 2018
miR-16-5p	tem como alvo os IRS1 e 2 e media IGF-1, que está intimamente relacionado à resistência à insulina	↑	CAO, 2017 OOSTDAM, 2020 SØRENSEN, 2021 ZHU, 2015
miR-17	atividade antidiabética por efeito anti-inflamatório em macrófagos	↓	TANG, 2020
miR-17-5p	melhora os desequilíbrios metabólicos da insulina e da glicose sanguínea	↑	CAO, 2017 ZHU, 2015
miR-19a	promove a proliferação celular e angiogênese regulando a via *PI3K/AKT,* conhecida como via de sinalização da insulina	↑	WANG, 2019
miR-19a-3p	regula a secreção de insulina, enquanto suprime a apoptose das células β pancreáticas	↑	ZHU, 2015
miR-19b	regula a apoptose celular	↑	WANG, 2019
miR-19b-3p	regula o sistema imunológico e nervoso	↑	ZHU, 2015
miR-20a-5p	melhora a captação de glicose e alivia a hipertrofia cardíaca diabética, fibrose, inflamação e apoptose	↑	CAO, 2017 ZHU, 2015
		↓	PHEIFFER, 2018
miR-21	mantém os níveis de glicose baixos em pacientes com complicações diabéticas	↓	GUAN, 2020
miR-21-3p	regula o processo de cicatrização de lesões diabéticas	↓	HOCAOGLU, 2019
		ND	WANDER, 2017

miR-23a	regula a resposta inflamatória		YOFFE, 2019
miR-23b-3p	promove alta memória metabólica celular induzida por glicose na retinopatia diabética		TAGOMA, 2018
miR-27a	promove a resistência à insulina		LI, 2015
miR-29a	atua como um importante regulador do metabolismo da glicose estimulada pela insulina		DENG, 2020 ZHAO, 2011
miR–29a-3p	regulador do receptor do IGF-1		GILLET, 2019 SØRENSEN, 2021
miR-29b	regula o remodelamento e enrijecimento da aorta no diabetes		DENG, 2020 SUN, 2020
miR–29b-3p	promove proliferação celular, apoptose, fibrose e inflamação		GILLET, 2019
miR-30b-5p	induz a angiogênese anormal		TAGOMA, 2018
miR-30c-5p	induz inflamação mediada por macrófagos e vias de sinalização pró-aterosclerose		TAGOMA, 2018
miR-30d	induz a produção de insulina		LI, 2015
miR-30d-5p	regula a proliferação, migração e capacidade de captação de glicose das células trofoblastos coriônicas humanas		TAGOMA, 2018
			ZHANG, 2021
miR-33a	regula a via de sinalização da insulina e o metabolismo da glicose		LI, 2015
miR-33a-5p	relacionado à diminuição do crescimento celular (célula β) e produção de insulina		FENG, 2020
miR-92a	modulação da homeostase vascular		LI, 2015
miR-96-5p	alivia os efeitos antiproliferativos induzidos por condições de glicose elevada sobre trofoblastos		YU, 2021
miR-98	protege as células do núcleo pulposo contra a apoptose		CAO, 2016

miR-100-5p	promove angiogênese durante o processo de implantação	↑	TAGOMA, 2018
miR-101-3p	associado à produção de insulina, sobrevivência e morte de células β pancreáticas	↑	TAGOMA, 2018
miR–122-5p	suprime a resistência à insulina	↑	GILLET, 2019
miR-125b	modula a secreção de insulina	↑	ZHANG, 2021
miR-132	promover a proliferação de células trofoblastos	↓	ZHAO, 2011 ZHOU, 2019
miR–132-3p	relacionados à resistência à insulina e β função celular	↑	GILLET, 2019
miR-134-5p	regular o comportamento das células trofoblastas na pré-eclâmpsia, como apoptose, migração e invasão	↑	KE, 2022 SØRENSEN, 2021
miR-135a	relacionados à proliferação e migração de células	↓	MONFARED, 2018
miR-136	alivia a viabilidade celular inibida induzida pelo tratamento com glicose elevada	↑	ZHANG, 2020
miR–136-5p	relacionados à proliferação, migração e invasão celular	↑	GILLET, 2019
miR-137	participa da interação entre células endoteliais e monócitos	↑	PENG, 2018
		↓	LI, 2015
miR-140-3p	importante regulador da osteoblastogênese	↑	ZHAO, 2020
miR-144	regula a proliferação e a apoptose	↑	ZHANG, 2021
miR-146a-5p	inibir a resposta inflamatória e a apoptose	↑	TAGOMA, 2018
miR-155-5p	promove fibrose das células do túbulo proximal	↓	HOCOAGLU, 2020
		ND	WANDER, 2017
miR-181d	modula o processo de sinalização da insulina, viabilidade celular e apoptose em células β pancreáticas	↑	SHEN, 2021
miR–182-3p	relacionados à proliferação e migração de células musculares lisas vasculares	↑	GILLET, 2019

miR-190b	inibe a proliferação de células β pancreáticas e a secreção de insulina	↑	WANG, 2021
miR-193b	suprime a apoptose e a autofagia	↓	JI, 2020
miR-195	aumenta a transição epitélio-mesenquimal e a permeabilidade celular	↑	TANG, 2020
miR-195-5p	relaciona-se com a viabilidade e proliferação celular, e apoptose	↑	TAGOMA, 2018 WANG, 2020
miR–210-3p	reduz a viabilidade celular e promove a apoptose das células β pancreáticas	↑	GILLET, 2019
miR-214	envolvido na resposta imune celular, regulando a ativação, proliferação e diferenciação celular	↓	MA, 2019
miR-222	regula o receptor de estrogênio (ER) e o transportador de glicose 4 (GLUT4)	↑	SHI, 2014
		↓	ZHAO, 2011
miR-222-3p	relaciona-se com o metabolismo da glicose na gravidez	↑	FILARDI, 2022 TAGOMA, 2018
		↓	OOSTDAM, 2020 PHEIFFER, 2018
miR-223	modulador da diferenciação mieloide humana	↑	ABDELTAWAB, 2020 YOFFE, 2019
miR-257	ND	↑	TANG, 2020
miR-330-3p	envolvido na secreção de insulina e β crescimento e proliferação celular, ativando proteínas envolvidas no ciclo celular e crescimento celular	↑	PFEIFFER, 2020 SEBASTIANI, 2017 XIAO, 2020
miR-340	associado à hiperinsulinemia induzida	↑	STIRM, 2018
miR-342-3p	indução de disfunção vascular	↑	GILLET, 2019 TAGOMA, 2018
miR-345-3p	regula o crescimento celular, apoptose, migração e invasão	↓	LI, 2021
miR-362-5p	promove proliferação celular e inibição da apoptose	↓	LI, 2015
miR-377-3p	regula a expressão VEGF	↑	HUA, 2021
miR-409-3p	relaciona-se com a desregulação imunológica na diabetes autoimune	↑	FILARDI, 2022

miR-409-5p	correlacionado com a resistência à insulina	↑	TU, 2020
miR-423-5p	alivia lesões podocitárias mediadas por glicose elevada	↑	TAGOMA, 2018
miR-494	diminuição da secreção de insulina e proliferação celular, e aumento da apoptose	↓	HE, 2017
miR-502-5p	medeia a resistência à insulina	↓	LI, 2015
miR-503	prejudica a angiogênese restauradora	↑	XU, 2017
miR-508-3p	promove a proliferação celular e inibe a apoptose	↑	LI, 2015
miR-516-5p	inibe a proliferação celular	↓	OOSTDAM, 2020
miR-517-3p	promove disfunção do trofoblasto	↓	OOSTDAM, 2020
miR-518-5p	aumenta o dano das células endoteliais vasculares induzido por hipóxia	↓	OOSTDAM, 2020
miR-520h	inibir a viabilidade celular e promover a apoptose celular	↑	GILLET, 2019 WEN, 2021
miR-543	regula a alta fibrose induzida por glicose e autofagia	↑	ZHANG, 2021
miR-574-5p	regulador metabólico para lipídios séricos e glicose sanguínea	↓	WANG, 2021
miR-657	promove polarização de macrófagos	↑	WANG, 2019
miR-770-5p	inibe a proliferação celular e promove apoptose	↑	ZHANG, 2020
miR–1323	promove a viabilidade das células trofoblastos sob condições de alta glicose	↑	GILLET, 2019 LIU, 2021
miR-3135b	regulador metabólico para lipídios séricos e glicose sanguínea	↓	WANG, 2021
miR-6869-5p	envolvidos na manutenção do equilíbrio microambiental da placenta	↓	WANG, 2021

ND: não descrito; ↑: up-regulated; ↓: down-regulated.

5. DISCUSSÃO

Os miRNAs têm sido estudados como potenciais biomarcadores da DMG, o conhecimento sobre sua regulação e função, bem como sua adaptação metabólica a esta doença, têm sido investigados e podem ajudar a aumentar a compreensão da patogênese da doença (LIU, 2021) Nesta revisão sistemática, identificamos 82 miRNAs alterados na DMG. Destes, quatro foram os mais citados: mir-16-5p e mir-330-3p estavam *up-regulated*, e mir-20a-5p e mir-222-3p foram encontrados *up-regulated* e *down-regulated,* respectivamente.

De acordo com Gao e Zhao (2020), mir-16-5p controla a proliferação, migração e invasão celular, afetando o ciclo celular e promovendo a apoptose. Além disso, esse miRNA modula a via de sinalização PI3K/Akt, um importante regulador do ciclo celular que inclui genes como *Pi3Kr1, Pi3kr3, mTOR e Mapk3*, entre outros. A superexpressão dessa via tem sido associada à DM e à DMG (KWON, 2014).

Outros alvos deste miRNA são os genes *Insulin Receptor Substrate 1 e 2 (IRS1/IRS2) e Insulin-Like Growth Factor 1 (IGF-1),* que estão intimamente relacionados à resistência à insulina, uma condição característica da diabetes (CAO, 2017; KWON 2014). Além disso, a superexpressão deste miRNA em pacientes com DMG no segundo trimestre de gestação demonstrou uma correlação com a subexpressão de *IRS1* e *IRS2*, levando a uma sinalização

anormal da via *Wnt/β-catenina*, importante para o desenvolvimento embrionário e a homeostase dos tecidos adultos (GENG, 2014).

Por outro lado, mir-330-3p está associado à proliferação, diferenciação e secreção de insulina, sendo altamente expresso no DMG relacionado à alta concentração de glicose. Também atua como um regulador central da homeostase do ciclo celular. Assim, a superexpressão deste miRNA em pacientes com DMG pode contribuir para a disfunção das células β pancreáticas, alterando a proliferação e o crescimento dessas células (PFEIFFER, 2020; SEBASTIANI, 2017).

Estudos revelaram que os genes *E2F Transcription Factor 1* (E2F1) e *Cell Division Cycle 42* (CDC42) são alvos do mir-330-3p. Ambos estão envolvidos no crescimento e na proliferação das células β e no controle da secreção de insulina. Portanto, a baixa expressão desses genes causada pelo aumento dos níveis de miRNA pode comprometer a proliferação das células β e a secreção de insulina (PFEIFFER, 2020; SEBASTIANI, 2017).

Além disso, o receptor de Angiotensina II (AT2) também foi identificado como alvo deste miRNA; esse gene atua durante o desenvolvimento do pâncreas na fase embrionária e é um possível mediador da regeneração das células β no pâncreas adulto. Assim, hipotetiza-se que altos níveis do mir-330-3p possam inibir a neogênese pancreática por meio da subexpressão de *AT2*, causando um defeito na regeneração do pâncreas endócrino sob alta demanda metabólica (SEBASTIANI, 2017).

Ademais, nesta revisão sistemática, Zhu et al. (2015) e Cao *et al*. (2017) identificaram a superexpressão de mir-20a-5p, enquanto Pheiffer *et al.* (2018)

encontraram a subexpressão desse miRNA no DMG. mir-20a-5p pertence ao cluster mir-17-92 e está associado à angiogênese (PHEIFFER, 2018). Na DMG, a expressão de proteínas angiogênicas é aumentada, como o *Vascular endothelial growth factor A* (VEGFA), o *Hypoxia-inducible factor 1 subunit alpha* (HIF1A) (LI, 2015) *Phosphatase and tensin homolog* (PTEN) (LI, 2015) e o regulador de apoptose BCL2 (BCL2) (MAGEE, 2014). Esse fato corrobora o efeito regulatório da expressão diminuída de mir-20a-5p encontrado por Pheiffer *et al.* (2018).

Zhu *et al.* (2015) também associaram mir-20a-5p às vias de sinalização da insulina, MAPK, TGF-β e mTOR. A via de sinalização PI3K/Akt, que regula o ciclo celular, a homeostase da glicose e a sinalização da insulina, bem como a proteína FoxO, que também regula a via insulina/PI3K/Akt, foram consideradas alvos do mir-20a-5p. Assim, este miRNA pode ser considerado como um possível biomarcador da DMG, e a alteração de sua expressão pode interferir nessas vias, gerando a hiperglicemia observada em pacientes com DMG (PHEIFFER, 2018).

A via de sinalização MAPK desempenha um papel no desenvolvimento de lesões vasculares, como a diabetes (NISHIYAMA, 2004), e sua sinalização anormal foi identificada em complicações da gravidez (ZHU, 2015). Além disso, a via de sinalização TGF-β tem sido associada à pré-eclâmpsia (PERUCCI, 2014). Por outro lado, a via de sinalização mTOR controla o equilíbrio energético (KUMAR, 2010), e o bloqueio dessas vias pode contribuir para o desenvolvimento da DMG (ZHU, 2015).

Por fim, mir-222-3p foi identificado por Tagoma *et al.* (2018) e Filardi *et al*. como superexpresso, enquanto Oostdam *et al.* (2020) e Pheiffer *et al.* o encontraram subexpresso na DMG. O mir-222-3p pertence ao cluster mir-222, abundante no plasma durante as semanas 24-28 de gestação, e é um miRNA placentário que atua na proliferação das células estromais endometriais (FILARDI, 2022; QIAN, 2009), regulando a expressão do receptor de estrogênio alfa (ERα) na resistência à insulina induzida por estrogênio no DMG (PHEIFFER, 2018; SHI, 2014), e está fortemente relacionado ao metabolismo da glicose na gravidez, impactando profundamente o peso ao nascer (FILARDI, 2022).

Além disso, altos níveis deste miRNA foram encontrados no tecido adiposo de pacientes com DMG, correlacionando-se negativamente com os níveis de ER-α e transportador de glicose tipo 4 (GLUT4) (SHI, 2014) Mulheres com DMG têm níveis mais altos de estradiol em comparação com mulheres saudáveis, e o estradiol e o ERα atuam na GLUT4, tornando-se reguladores críticos da obesidade e resistência à insulina (FILARDI, 2022).

Portanto, identificar possíveis biomarcadores da DMG tem um valor clínico significativo para desenvolver estratégias de diagnóstico e possivelmente prevenir complicações obstétricas e materno-fetais (LIU, 2021) No entanto, estudos sobre reconhecimento precoce, critérios diagnósticos, possíveis biomarcadores e alvos terapêuticos para a DMG mostram resultados controversos, principalmente devido a diferenças étnicas, geográficas, genéticas e fatores ambientais, e critérios diagnósticos (LIU, 2021; LONG, 2013; YANG, 2015).

Um miRNA pode regular até 200 mRNAs, indicando que os miRNAs podem regular individualmente diferentes processos biológicos (YU, 2021) Assim, sugere-se que os miRNAs sejam utilizados como biomarcadores em painéis de miRNAs ou algoritmos de avaliação de risco e não como biomarcadores individuais para o diagnóstico da DMG (PHEIFFER, 2018).

Nesta revisão sistemática, encontramos 55 artigos que analisaram a alteração de miRNAs na DMG, identificando 82 miRNAs alterados. A expressão desses miRNAs variou dependendo do tipo de amostra utilizada e das diferentes idades gestacionais, o que pode explicar as diferenças nos resultados. Adicionalmente, esses miRNAs foram associados a vários mecanismos, como a homeostase do ciclo celular, crescimento e proliferação de células β pancreáticas, captação e metabolismo da glicose, secreção e resistência à insulina. Certamente, os miRNAs são biomarcadores potenciais para o diagnóstico precoce da DMG. No entanto, mais pesquisas são necessárias para elucidar seu valor diagnóstico e preditivo, bem como seus mecanismos patogênicos na gravidez.

6. CONCLUSÃO

A DMG é uma das doenças mais frequentes durante a gravidez, podendo ser diagnosticada desde o início do pré-natal. Novos biomarcadores para o primeiro trimestre, como miRNAs, poderiam aumentar a sensibilidade diagnóstica e diminuir a incidência de complicações.

Esta dissertação trata-se revisão sistemática (RS) realizada com pesquisas bibliográficas nas bases de dados PubMed/Medline, Biblioteca Virtual da Saúde (BVS), Web of Science e EMBASE. O protocolo está registrado na plataforma PROSPERO (número CRD42021291791).

Além disso, nesta RS, encontramos 55 artigos que analisaram a alteração de miRNAs na DMG, identificando 82 miRNAs alterados. Destes, vários miRNAs foram associados com mecanismos específicos, como: homeostase do ciclo celular, crescimento e proliferação de células β pancreáticas, captação e metabolismo de glicose, secreção de insulina e resistência. Os miRNAs mais frequentemente desregulados em DMG foram: mir-16-5p, mir-20a-5p, mir-222-3p, and mir-330-3p.

Algumas das considerações pertinentes sobre os estudos avaliados são: i) O local dos estudos são em sua grande maioria na China, o que pode limitar a generalização dos resultados para outras populações; ii) A idade gestacional avançada das pacientes avaliadas em alguns estudos pode dificultar a avaliação dos miRNAs como biomarcador precoces; iii) A ausência de variáveis de avaliação de risco nos estudos podem limitar a utilização dos

miRNAs como biomarcadores; iv) Alguns miRNAs alterados encontrados na DMG ainda persistem sem correlação fisiopatológica.

Sendo assim, sugere-se que os miRNAs sejam usados como biomarcadores em painéis de miRNA ou algoritmos de avaliação de risco, e não como biomarcadores individuais para o diagnóstico de DMG. Certamente, os miRNAs são potenciais biomarcadores para o diagnóstico precoce da DMG. Porém, mais pesquisas são necessárias para elucidar seu valor diagnóstico, preditivo e seus mecanismos patogênicos na gravidez.

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ANEXO A: PUBLICAÇÃO

Journal of *Personalized Medicine*

MDPI

Systematic Review

MicroRNAs Associated with the Pathophysiological Mechanisms of Gestational Diabetes Mellitus: A Systematic Review for Building a Panel of miRNAs

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Abstract: miRNAs, a class of small non-coding RNAs, play a role in post-transcriptional gene expression. Therefore, this study aimed to conduct a systematic review of miRNAs associated with GDM to build a panel of miRNAs. A bibliographic search was carried out in the PubMed/Medline, Virtual Health Library (VHL), Web of Science, and EMBASE databases, selecting observational studies in English without time restriction. The protocol was registered on the PROSPERO platform (number CRD42021291791). Fifty-five studies were included in this systematic review, and 82 altered miRNAs in GDM were identified. In addition, four miRNAs were most frequently dysregulated in GDM (mir-16-5p, mir-20a-5p, mir-222-3p, and mir-330-3p). The dysregulation of these miRNAs is associated with the mechanisms of cell cycle homeostasis, growth, and proliferation of pancreatic β cells, glucose uptake and metabolism, insulin secretion, and resistance. On the other hand, identifying miRNAs associated with GDM and elucidating its main mechanisms can assist in the characterization and definition of potential biomarkers for the diagnosis and treatment of GDM.

Keywords: GDM; diabetes; miRNA; personalized medicine

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1. Introduction

Gestational Diabetes Mellitus (GDM) is defined as any degree of glucose intolerance first diagnosed during pregnancy [1]. Hyperglycemia during pregnancy can be transient or persist after birth, presenting itself as an independent risk factor for the future development of Type 2 Diabetes Mellitus (T2DM) [2]. According to the International Diabetes Federation, the global prevalence of GDM averages 14% [3], ranging from 1.8–31% depending on the population evaluated and the diagnostic criteria adopted between countries [4]. GDM is the most common metabolic disease during pregnancy, occurring in 3–25% of pregnancies, and its incidence in the population has increased along with T2DM and obesity [5].

There is no universally accepted standard for screening or diagnosing GDM. Guidelines from local medical organizations are followed. However, the test with better sensitivity and specificity is the oral glucose tolerance test (OGTT) with 75 g of glucose, considering values between 153 and 199 mg/dL for GDM [6]. GDM diagnosis can be performed throughout pregnancy from the beginning of prenatal care. However, it is usually performed in the second or third trimester of pregnancy (24–28 weeks), which can cause risks to the mother and the fetus [7]. Short-term adverse outcomes are observed, such as hypoglycemia, hypoxia, respiratory distress syndrome, higher rates of preeclampsia, and large for gestational age or macrosomic newborns, among others [2,5,8]. In addition, children born from mothers with GDM have an increased risk for metabolic and cerebrovascular diseases in adult age [5].

Pregnancy stresses the body and promotes physiological changes to ensure the proper growth of the embryo/fetus. Adaptations and/or dysregulations during pregnancy are performed by placental hormones and increased levels of cortisol and progesterone [9]. Additionally, it is reported that molecules such as placenta-derived microRNAs (miRNAs) may be involved in these adaptations. Variations in the expression of these miRNAs may indicate changes in the maternal metabolic adaptation mechanism [7,10].

miRNAs are a class of endogenous non-coding RNAs with approximately 22 nucleotides, which act as regulators of post-transcriptional gene expression, inhibiting the translation of messenger RNAs (mRNA) or degrading them [5,11]. miRNAs regulate more than one target mRNA, and studies previously described that occurs a control of the expression to an average of 30% of protein-coding genes [7,12]. The main well-known functions of miRNAs are the regulation of cell proliferation and differentiation, apoptosis, stress response, and transcriptional regulation [7].

Studies indicate that the human placenta expresses more than 500 miRNAs, and only some of these are also expressed in other tissues [13]. Therefore, the characterization of miRNAs during pregnancy is necessary to understand better the regulatory mechanisms of healthy and complicated pregnancy [14]. Currently, studies have sought to identify biomarkers for the diagnosis of GDM before 24–28 weeks of gestation, and these miRNAs have revealed great potential as biomarkers for GDM in the early trimester, mainly due to their high stability and accessibility in body fluids [15,16].

Additionally, investigating the regulation of placental and circulating miRNAs and their metabolic adaptation associated with GDM can improve diagnostic, therapeutic, and personalized prognosis [7]. Thus, understanding the functions of miRNAs can improve broadening the insight into the etiology and pathophysiology of GDM and identify possible biomarkers with clinical value to elaborate diagnostic strategies and prevent obstetric and maternal–fetal complications.

2. Materials and Methods

2.1. Registration, Data Source, and Search Strategy

Based on the guiding question, "Which microRNAs are associated with the pathophysiological mechanisms of Gestational Diabetes Mellitus (GDM)?", the systematic review was carried out to identify the miRNAs associated with the pathophysiological mechanisms of GDM. We used the PEO (Population, Exposure, Outcome) framework: Population: pregnant woman with GDM; Exposure: microRNAs; Outcome: association of microRNAs as a risk factor for GDM.

This systematic review was carried out according to the Preferred Report Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [17]. To avoid duplication, this study had the protocol registered in the International Prospective Register of Systematic Reviews (PROSPERO) (number CRD42021291791) on 17 December 2021 (File S1).

The literature search was carried out in PubMed/Medline, Virtual Health Library (VHL), Web of Science, and EMBASE databases from 27 January 2022 to 15 February 2022 in the English language. Combined terms cataloged in Medical Subject Headings (MeSH) for the keywords (Gestational Diabetes OR Gestational Diabetes Mellitus OR Diabetes Induced by Pregnancy) AND (MicroRNA OR miRNA OR Circulating MicroRNA OR Cell-Free MicroRNA) were used to develop the search strategy, later adapted for each database (Table 1).

Table 1. Search strategy applied in each database.

Database	Search Strategy
PubMed/Medline	(("Diabetes, Gestational" OR "Diabetes Gestacional" OR "Diabetes Mellitus, Gestational" OR "Diabetes, Pregnancy Induced" OR "Diabetes, Pregnancy-Induced" OR "Gestational Diabetes" OR "Gestational Diabetes Mellitus" OR "Pregnancy-Induced Diabetes") AND ("MicroRNAs" OR "Micro RNA" OR "MicroRNA" OR "MicroRNA, Primary" OR "miRNA" OR "miRNA, Primary" OR "miRNAs" OR "pre miRNA" OR "pre-miRNA" OR "pri miRNA" OR "pri-miRNA" OR "Primary MicroRNA" OR "Primary miRNA" OR "RNA, Micro" OR "Small Temporal RNA" OR "stRNA" OR "MicroARNs" OR "miARN" OR "miARNs" OR "Micro ARN" OR "Micro ARNs" OR "MicroARN" OR "stARN" OR "Circulating MicroRNA" OR "Cell Free MicroRNA" OR "Cell-Free MicroRNA" OR "MicroRNA, Cell-Free" OR "MicroRNA, Circulating"))
Virtual Health Library	("microrna") AND ("gestational diabetes") AND (mj:("Diabetes Gestacional" OR "MicroRNAs"))
Web of Science	(("Diabetes, Gestational" OR "Diabetes Mellitus, Gestational" OR "Diabetes, Pregnancy Induced" OR "Diabetes, Pregnancy-Induced" OR "Gestational Diabetes" OR "Gestational Diabetes Mellitus" OR "Pregnancy-Induced Diabetes") AND ("MicroRNAs" OR "Micro RNA" OR "MicroRNA" OR "MicroRNA, Primary" OR "miRNA" OR "miRNA, Primary" OR "miRNAs" OR "pre miRNA" OR "pre-miRNA" OR "pri miRNA" OR "pri-miRNA" OR "Primary MicroRNA" OR "Primary miRNA" OR "RNA, Micro" OR "RNA, Small Temporal" OR "Small Temporal RNA" OR "sirna" OR "stRNA" OR "Temporal RNA, Small" OR "MicroARNs"))
EMBASE	('diabetes, gestational'/exp OR 'diabetes, gestational' OR 'diabetes gestacional' OR 'diabetes, pregnancy induced' OR 'diabetes, pregnancy-induced' OR 'gestational diabetes'/exp OR 'gestational diabetes' OR 'gestational diabetes mellitus'/exp OR 'gestational diabetes mellitus' OR 'pregnancy-induced diabetes') AND ('micrornas'/exp OR 'micrornas' OR 'micro rna'/exp OR 'micro rna' OR 'microrna'/exp OR 'microrna' OR 'microrna, primary' OR 'mirna'/exp OR 'mirna' OR 'mirna, primary' OR 'mirnas'/exp OR 'mirnas' OR 'pre mirna' OR 'pre-mirna' OR 'pri mirna' OR 'pri-mirna' OR 'primary microrna'/exp OR 'primary microrna' OR 'primary mirna' OR 'rna, micro' OR 'rna, small temporal' OR 'small temporal rna' OR 'strna' OR 'temporal rna, small' OR 'microarns' OR 'arn pequeño temporal' OR 'arn temporal pequeño' OR 'miarn' OR 'miarns' OR 'micro arn' OR 'micro arns' OR 'microarn' OR 'microarn primario' OR 'microrna primario' OR 'starn' OR 'rna pequeno temporário' OR 'rna temporário pequeno' OR 'circulating microrna'/exp OR 'circulating microrna' OR 'cell free microrna'/exp OR 'cell free microrna' OR 'cell-free microrna'/exp OR 'cell-free microrna' OR 'microrna, cell-free' OR 'microrna, circulating' OR 'microarn circulante' OR 'microrna circulante' OR 'microrna fora da célula' OR 'microrna livre') AND ('biological marker'/dd OR 'circular ribonucleic acid'/dd OR 'glucose'/dd OR 'insulin'/dd OR 'microrna'/dd OR 'microrna 122'/dd OR 'microrna 143'/dd OR 'microrna 146a'/dd OR 'microrna 16'/dd OR 'microrna 21'/dd OR 'microrna 210'/dd OR 'microrna 222'/dd OR 'rna'/dd OR 'small interfering rna'/dd OR 'transcription factor'/dd) AND ('congenital heart disease'/dm OR 'diabetes mellitus'/dm OR 'diabetic complication'/dm OR 'intrauterine growth retardation'/dm OR 'macrosomia'/dm OR 'maternal diabetes mellitus'/dm OR 'pregnancy diabetes mellitus'/dm)

As criteria for inclusion of studies in this systematic review, we used: observational studies with original research in the area of human and medical genetics that identified miRNAs in pregnant women with GDM and controls (pregnant women without GDM); no age restrictions; published in English; without the restriction of the year of publication of the study. For exclusion, the following criteria were established: Studies that did not address the research topic; and with another study design.

2.2. Selection of Studies

Two reviewers (PHCMS and LS) independently selected and identified articles at all stages of the systematic review. The articles identified in the databases were imported into the Rayyan platform [18] to optimize the analysis. Initially, the title and abstract were read (phase I), then the articles selected in the first screening were read in full (phase II).

In both phases, the articles were evaluated according to the pre-established inclusion and exclusion criteria.

2.3. Risk of Bias Assessment

The risk of methodological bias was assessed using The Joanna Briggs Institute (JBI) Critical Assessment Tool [19] for each study design. All articles selected for inclusion in the systematic review underwent rigorous evaluation. This tool is composed of questions with the possible answers: "Yes", "No", "Not applicable", and "Unclear".

A critical assessment tool was applied to each type of study: case-control studies, cohort studies, and cross-sectional studies. Those articles that had 100% of the answers "yes" were considered at low risk of bias; those who scored 70–99% "yes" were considered moderate risk; and those with less than 70% of "yes" answers were excluded from the study, being considered at high risk of methodological bias. Differences of opinion between reviewers were discussed and resolved by consensus.

2.4. Data Extraction and Synthesis

Studies evaluating miRNAs lack more robust statistical analyses; most describe only the type of regulation identified and whether there was a significant difference in this regulation between the evaluated groups (usually considering $p < 0.05$). Thus, this systematic review used a qualitative and descriptive approach for data analysis, extracting the following data: (1) authors and year of publication; (2) study design; (3) country or continent where the study was performed; (4) sample size; (5) mean age; (6) gestational time; (7) tissue analyzed; (8) microRNA identification technique; (9) microRNA; (10) regulation; (11) *p*-value. The extracted data were imported into a predefined Excel worksheet, and authors of studies with missing data were contacted. Data extraction was performed independently by two reviewers, and disagreements between reviewers were resolved by consensus.

3. Results

After the initial search, 1138 articles were identified in the databases, and 626 duplicates were excluded, resulting in 512 articles for phase I. Based on the reading of titles and abstracts (phase I), 413 articles were excluded because they did not meet the pre-established inclusion criteria. Therefore, phase II comprised the complete reading of the 99 selected articles. Forty-four were excluded, and 55 were included in this systematic review (Figure 1).

Of the included studies, 48 were case-control studies, six were cohort studies, and one was a cross-sectional study. The selected studies were published between 2011 and 2022. The mean age of the case and control groups ranged between 20 and 40 years. A total of 2749 cases (pregnant women with GDM) and 2710 controls (pregnant women without GDM) were analyzed, totaling 5459 individuals. The samples collected and analyzed were blood, plasma, placenta, adipose tissue, and urine (urinary exosome) (Table 2).

Additionally, the studies were homogeneous in terms of methodological quality assessment, individually reaching a minimum of 70% of positive responses, being considered at low or moderate risk of bias (Table 3). For case-control studies, 10 parameters were evaluated, cohorts 11, and cross-sectional studies 8 parameters, respectively. Most case-control studies reported that there was no identification of possible confounding factors (question 6—Q6), and, consequently, the non-applicability of question 7 (Q7) declared strategies to deal with confounding factors.

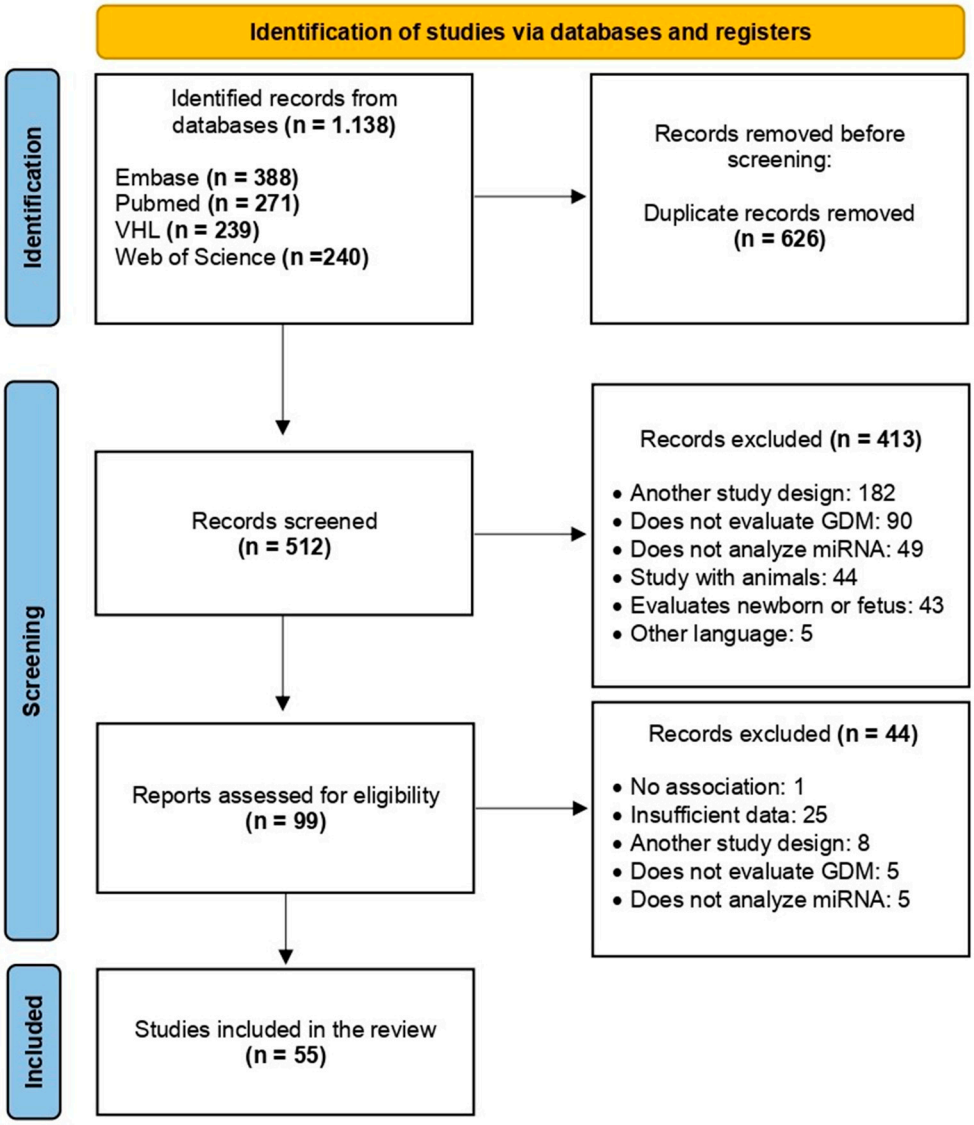

Figure 1. PRISMA flow chart demonstrating the process of exclusion or inclusion of studies in this systematic review. Adapted from: [17].

In this systematic review, 82 miRNAs were identified, highlighting the most cited in the literature due it was deregulated in GDM (mir-16-5p, mir-20a-5p, mir-222-3p, and mir-330-3p). According to the results, the identified miRNAs were down- and/or up-regulated in the GDM, considering $p < 0.05$ as significant. Studies evaluating miRNAs lack more robust statistical analyses, often describing whether there was a significant difference and the type of miRNA regulation between the evaluated groups.

Among those most frequently found in the literature, mir-16-5p [40,47,68,70] and mir-330-3p [28,39,50] were up-regulated, and mir-20a-5p and mir-222-3p were up- and down-regulated in different studies [40,51,62,68,70,72] (Figure 2). In general, the dysregulation of these miRNAs is associated with mechanisms of inflammation, growth, and proliferation of pancreatic β cells, glucose uptake and metabolism, insulin secretion, and resistance (Table 4).

Table 2. Data extracted from studies included in the systematic review.

Authors And Year of Publication	Study Design	Country or Continent	Sample Size	Mean Age	Gestational Time	Sample Type	miRNA Identification Technique	miRNA	Regulation	*p*-Value
[20]	Case-control	China	Case: 70 Control: 70	Case: 30.33 ± 2.25 Control: 30.04 ± 2.25	Case: 39.12 ± 1.15 Control: 38.76 ± 1.06 (weeks)	Blood	qRT-PCR	miR-134-5p	Up-regulation	<0.001
[21]	Case-control	China	Case: 26 Control: 23	Case: 30.6 ± 4.4 Control: 29.2 ± 3.5	Case: 38.1 ± 1.2 Control: 39.4 ± 1.2 (weeks)	Placenta	RT-PCR	miR-6869-5p	Down-regulation	<0.001
[22]	Case-control	China	Case: 110 Control: 78	Case: 33,118 ± 3402 Control: 32,385 ± 3579	Case: 25.591 ± 1.757 Control: 25.218 ± 2.196	Blood	qRT-PCR	miR-1323	Up-regulation	<0.05
[23]	Cross-sectional	China	Case: 36 Control: 37	20–40 Years	37–41 Weeks	Placenta	qRT-PCR	miR-125b; miR-144; miR-543	Up-regulation	<0.001
[24]	Case-control	China	Case: 30 Control: 30	Case: 24–39 Control: 24–39	24–28 Weeks	Plasma and Placenta	qRT-PCR	miR-345-3p	Down-regulation	<0.01
[25]	Case-control	Gana	Case: 137 Control: 158	Case: 30.94 ± 3.45 Control: 29.67 ± 3.64	Case: 272.22 ± 6.40 Control: 274.41 ± 8.02 (Days)	Placenta	qRT-PCR	miR-21	Down-regulation	<0.01
[26]	Case-control	China	Case: 68 Control: 55	Case:32.65 ± 4.63 Control: 31.27 ± 4.01	Case: 39.09 ± 1.11 Control: 38.98 ± 1.05 (weeks)	Plasma	qRT-PCR	miR-29a; miR-29b	Down-regulation	<0.001
[27]	Case-control	China	Case: 33 Control: 20	ND	Case: 24–26 weeks Control: 37–41 weeks	Placenta	qRT-PCR	miR-140-3p	Up-regulation	<0.05
[28]	Case-control	China	Case: 30 Control: 10	ND	ND	Blood	qRT-PCR	miR-330-3p	Up-regulation	<0.001
[29]	Case-control	China	Case: 20 Control: 20	Case: 29.4 ± 1.2 Control: 29.6 ± 0.4	Case: 38.7 ± 0.6 Control: 39.0 ± 1.0	Placenta	qRT-PCR	miR-17; miR-195; miR-257	Down-regulation; Up-regulation; Up-regulation	<0.01
[30]	Case-control	Turkey	Case: 19 Control: 28	Case: 30.4 ± 4.6 Control: 28.1 ± 5.8	Case: 33.5 ± 3.6 Control: 33 ± 4.1	Blood	qRT-PCR	miR-21-3p	Down-regulation	0.008
[31]	Case-control	Germany	Case: 30 Control: 30	Case: 31 ± 4 Control: 32 ± 4	Case: 27.0 ± 2.3 Control:27.6 ± 2.37	Blood	qRT-PCR	miRNA-340	Up-regulation	0.02
[32]	Cohort	United States	Case: 36 Control: 80	Case: 34.3 ± 3.6 Control: 32.9 ± 4.4	Case: 15.1 ± 2.9 Control: 16.5 ± 2.3	Blood	qRT-PCR	miR-21-3p; miR-155-5p	ND	0.005; 0.028
[33]	Case-control	Turkey	Case: 14 Control: 27	Case: 30.4 ± 4.4 Control: 27.9 ± 5.5	Case: 33.5 ± 3.5 Control: 33.1 ± 4.1	Blood	qPCR	miR-155-5p	Down-regulation	0.04
[34]	Case-control	China	Case: 30 Control: 38	Case: 30.27 ± 6.38 Control: 33.21 ± 8.17	Case: 38.12 ± 1.65 Control: 37.54 ± 1.31	Blood	qRT-PCR	miR-377-3p	Up-regulation	<0.01
[35]	Case-control	China	Case: 25 Control: 30	ND	ND	Blood	qRT-PCR	miR-181d	Up-regulation	<0.01
[36]	Case-control	Turkey	Case: 30 Control: 30	Case: 32.3 Control: 29.9	Case: 26.9 Control: 27.4	Plasma	qRT-PCR	miR-7-5p	Up-regulation	0.02
[37]	Case-control	China	Case: 20 Control: 20	Case: 35.30 ± 4.37 Control: 32.30 ± 3.40	Case: 74.38 ± 8.58 Control: 71.50 ± 11.72	Blood	qRT-PCR	miR-193b	Down-regulation	<0.001
[38]	Case-control	China	Case: 76 Control: 73	27.02 ± 3.17	ND	Blood	qRT-PCR	miR-409-5p	Up-regulation	<0.05
[39]	Cohort	Spain	Case: 31 Control: 29	Case: 31.9 ± 1.8 Control: 31.0 ± 3.6	Case: 39.1 ± 1.3 Control: 39.2 ± 1.2	Blood	qRT-PCR	miR-330-3p	Up-regulation	0.003

Table 2. *Cont.*

Authors And Year of Publication	Study Design	Country or Continent	Sample Size	Mean Age	Gestational Time	Sample Type	miRNA Identification Technique	miRNA	Regulation	*p*-Value
[40]	Case-control	China	Case: 10 Control: 10	Case: 30.03 ± 3.56 Control: 26.67 ± 4.59	Case: 17.66 ± 0.85 Control: 18.17 ± 0.93	Blood	qRT-PCR	miR-16-5p; miR-17-5p; miR-19a-3p; miR-19b-3p; miR-20a-5p	Up-regulation	5.36×10^{-11}; 1.10×10^{-10}; 6.57×10^{-43}; 1.73×10^{-74}; 5.27×10^{-37}
[41]	Case-control	China	Case: 24 Control: 24	Case: 28.79 ± 2.21 Control: 29.46 ± 1.89	Case: 17.40 ± 0.70 Control: 17.16 ± 0.79	Plasma	qRT-PCR/ chip TLDA	miR-132; miR-29a; miR-222	Down-regulation	0.042; 0.032; 0.041
[42]	Case-control	China	Case: 100 Control: 100	ND	ND	Blood	qRT-PCR	miRNA-19a; miRNA-19b	Up-regulation	ND
[43]	Cohort	Canada	Case: 23 Control: 46	Case: 29.8 ± 5.3 Control: 27.9 ± 4.4	Case: 10.5 ± 2.5 Control: 10.6 ± 2.4	Blood	qRT-PCR	miR-122-5p; miR-132-3p; miR-1323; miR-136-5p; miR-182-3p; miR-210-3p; miR-29a-3p; miR-29b-3p; miR-342-3p; miR-520h	Up-regulation	0.01; 0.03; 0.03; 0.03; 0.01; 0.02; 0.03; 0.04; 0.008; 0.03
[44]	Case-control	China	Case: 48 Control: 46	Case: 29.86 ± 0.94 Control: 30.02 ± 0.89	ND	Placenta	qRT-PCR	miR-657	Up-regulation	<0.001
[45]	Case-control	China	Case: 30 Control: 29	Case: 28–40 Control: 29–38	Case: 37.9 ± 1.1 Control: 39.2 ± 1.1	Placenta	qRT-PCR	miR-657	Up-regulation	<0.01
[46]	Case-control	China	Case: 10 Control: 10	21–37 Years	ND	Placenta	qRT-PCR	miR-508-3p; miR-27a; miR-9; miR-137; miR-92a; miR-33a; miR-30d; miR-362-5p; miR-502-5p	Up-regulation; down-regulation; down-regulation; down-regulation; down-regulation; down-regulation; down-regulation; down-regulation; down-regulation	<0.01; <0.05; <0.05; <0.05; <0.05; <0.05; <0.05; <0.05; <0.05
[47]	Case-control	Europe	Case: 41 Control: 41	Case: 32.7 ± 4 Control: 33.2 ± 3.8	Case: 40 ± 39.3 Control: 40.1 ± 1.1	Blood	qRT-PCR	miR-16-5p; miR-29a-3p; miR-134-5p	Up-regulation	0.008; 0.004; 0.046
[48]	Case-control	China	Case: 25 Control: 25	ND	ND	Blood	qRT-PCR	miR-503	Up-regulation	<0.01

Table 2. *Cont.*

Authors And Year of Publication	Study Design	Country or Continent	Sample Size	Mean Age	Gestational Time	Sample Type	miRNA Identification Technique	miRNA	Regulation	*p*-Value
[49]	Case-control	China	Case: 20 Control: 20	ND	ND	Blood	qRT-PCR	miR-494	Down-regulation	<0.01
[50]	Cohort	Italy	Case: 21 Control: 10	Case: 35.57 ± 5.63 Control: 32.80 ± 5.16	ND	Blood	qRT-PCR	miR-330-3p	Up-regulation	0.01
[51]	Cohort	Italy	Case: 12 Control: 12	Case: 36.4 ± 4.6 Control: 34.9 ± 5.1	ND	Plasma	qRT-PCR	miR-222-3p; miR-409-3p	Up-regulation	0.05; 0.01
[52]	Case-control	China	Case: 123 Control: 123	Case: 31.23 ± 2.31 Control: 30.84 ± 2.95	Case: 39.35 ± 3.81 Control: 39.52 ± 2.47	Plasma	qRT-PCR	miR-96-5p	Down-regulation	0.01
[53]	Case-control	China	Case: 108 Control: 50	Case: 30.89 ± 3.45 Control: 30.10 ± 2.79	Case: 25.20 ± 1.23 Control: 25.32 ± 1.45	Blood and placenta	qRT-PCR	miR-132	Down-regulation	<0.001
[54]	Case-control	Egypt	Case: 109 Control: 103	Case: 29.9 ± 6.28 Control: 29.7 ± 5.85	ND	Blood	qRT-PCR	miR-223	Up-regulation	<0.001
[55]	Case-control	China	Case: 13 Control: 13	Case: 27.62 ± 3.10 Control: 27.85 ± 3.36	ND	Adipose tissue	qRT-PCR	miR-222	Up-regulation	<0.01
[56]	Case-control	China	Case: 30 Control: 26	Case: 27.6 Control: 26.9	ND	Blood	qRT-PCR	miR-214	Down-regulation	<0.05
[57]	Case-control	China	Case: 204 Control: 202	Case: 30.91 ± 0.38 Control: 29.68 ± 0.53	Case: 271.68 ± 11.47 Control: 274.37 ± 8.10 (days)	Placenta	qRT-PCR	miR-29b	Down-regulation	<0.05
[58]	Case-control	Spain and Italy	Case: 23 Control: 20	Case: 34.0 (32.5–37.5) Control: 34.5 (32.0–37.2)	Case: 40.3 (38.4–40.7) Control: 39.9 (39.4–40.7)	Plasma	qRT-PCR	miR-223; miR-23a	Up-regulation	1.4×10^{-7}; 0.019
[59]	Case-control	China	Case: 30 Control: 30	ND	ND	Blood	qRT-PCR	miR-770-5p	Up-regulation	<0.01
[60]	Case-control	China	Case: 32 Control: 48	Case: 32.71 ± 5.26 Control: 29.13 ± 4.22	Case: 28.33 ± 2.81 Control: 29.10 ± 2.32	Blood	qRT-PCR	miR-520h	Up-regulation	<0.001
[61]	Case-control	China	Case: 5 Control: 5	ND	ND	Placenta	qRT-PCR	miR-9-5p	Down-regulation	<0.01
[62]	Case-control	South Africa	Case: 28 Control: 53	Case: 29.5 ± 6.2 Control: 28.6 ± 6.4	ND	Blood	qRT-PCR	miR-20a-5p; miR-222-3p	Down-regulation	0.038; 0.027
[63]	Case-control	China	Case: 12 Control: 12	ND	ND	Blood	qRT-PCR	miR-33a-5p	Up-regulation	<0.01
[64]	Case-control	China	Case: 112 Control: 58	Case: 31.59 ± 3.93 Control: 31.14 ± 3.94	Case: 24.85 ± 1.69 Control: 24.47 ± 2.15	Blood and placenta	qRT-PCR	miR-136	Up-regulation	<0.001
[65]	Case-control	China	Case: 166 Control: 196	Case: 31.03 ± 3.63 Control: 29.70 ± 3.83	ND	Placenta	qRT-PCR	miR-30d-5p	Down-regulation	<0.01
[66]	Case-control	China	Case: 102 Control:102	Case: 29.8 ± 3.2 Control: 29.5 ± 2.8	Case: 27.0 ± 1.6 Control: 26.8 ± 1.1	Blood	qRT-PCR	miR-195-5p	Up-regulation	<0.01
[67]	Case-control	China	Case: 193 Control: 202	Case: 30.93 ± 3.45 Control: 29.68 ± 3.66	Case: 272.22 ± 6.39 Control: 274.41 ± 8.03 (days)	Placenta	qRT-PCR	miR-98	Up-regulation	<0.05
[68]	Case-control	China	Case: 85 Control: 72	Case: 26.8 ± 3.5 Control: 26.4 ± 3.6	Case: 25.8 ± 2.5 Control: 26.1 ± 1.2	Blood	qRT-PCR	miR-16-5p; miR-17-5p; miR-20a-5p	Up-regulation	<0.01
[69]	Case-control	China	Case: 53 Control: 46	Case: 29.8 ± 0.4 Control: 29.2 ± 0.6	Case: 39.9 ± 0.1 Control: 39.0 ± 0.2	Blood	qRT-PCR	miR-574-5p; miR-3135b	Down-regulation	<0.0001; 0.002

Table 2. *Cont.*

Authors And Year of Publication	Study Design	Country or Continent	Sample Size	Mean Age	Gestational Time	Sample Type	miRNA Identification Technique	miRNA	Regulation	*p*-Value
[70]	Case-control	Mexico	Case: 27 Control: 34	Case: 29.93 ± 6.03 Control: 26.06 ± 5.28	ND	Urine (urinary exosome)	qRT-PCR	miR-516-5p; miR-517-3p; miR-518-5p; miR-222-3p; miR-16-5p	Down-regulation; down-regulation; down-regulation; down-regulation; up-regulation	<0.05; <0.05; <0.01; <0.01; <0.01
[71]	Case-control	China	Case: 11 Control: 12	Case: 29.91 ± 1.385 Control: 29.08 ± 1.305	ND	Blood	qRT-PCR	miR-137	Up-regulation	0.0073
[72]	Case-control	Estonia	Case: 13 Control: 9	Case: 31.1 ± 4.2 Control: 28.1 ± 4.5	23–31 weeks	Blood	qRT-PCR	let-7e-5p; let-7g-5p; miR-100-5p; miR-101-3p; miR-146a-5p; miR-195-5p; miR-222-3p; miR-23b-3p; miR-30b-5p; miR-30c-5p; miR-30d-5p; miR-342-3p; miR-423-5p	Up-regulation	0.03; 0.01; 0.04; 0.03; 0.03; 0.03; 0.03; 0.02; 0.04; 0.02; 0.03; 0.04; 0.02
[73]	Case-control	Iran	Case: 30 Control: 30	ND	ND	Plasma	qRT-PCR	miR-135a	Down-regulation	0.001
[74]	Case-control	China	Case: 5 Control: 5	ND	ND	Placenta	qRT-PCR	miR-190b	Up-regulation	<0.001

ND: Not described; qRT-PCR: Polymerase chain reaction via quantitative reverse transcriptase.

Table 3. Summary of responses for each study included in the risk of bias assessment.

Reference	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11
[20] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[21] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[22] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[23] [3]	Y	Y	Y	Y	N	NA	Y	Y	NA	NA	NA
[24] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[25] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[26] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[27] [1]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NA
[28] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[29] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[30] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[31] [1]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NA
[32] [2]	Y	Y	Y	N	NA	Y	Y	Y	Y	Y	Y
[33] [1]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NA
[34] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[35] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[36] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[37] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[38] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[39] [2]	Y	Y	Y	N	NA	Y	Y	Y	Y	Y	Y
[40] [1]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NA
[41] [1]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NA
[42] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[43] [2]	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y
[44] [1]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NA
[45] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[46] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[47] [2]	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y
[48] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[49] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[50] [2]	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y
[51] [2]	Y	Y	Y	N	NA	Y	Y	Y	Y	Y	Y
[52] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[53] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[54] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[55] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[56] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[57] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[58] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA

Table 3. *Cont.*

Reference	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11
[59] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[60] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[61] [1]	Y	N	Y	Y	Y	N	NA	Y	Y	Y	NA
[62] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[63] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[64] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[65] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[66] [1]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NA
[67] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[68] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[69] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[70] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[71] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[72] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA
[73] [1]	Y	N	Y	Y	Y	N	NA	Y	Y	Y	NA
[74] [1]	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	NA

[1] Case-control; [2] Cohort; [3] Cross-sectional. Y—Yes; N—No; NA—Not applicable; U—Unclear.

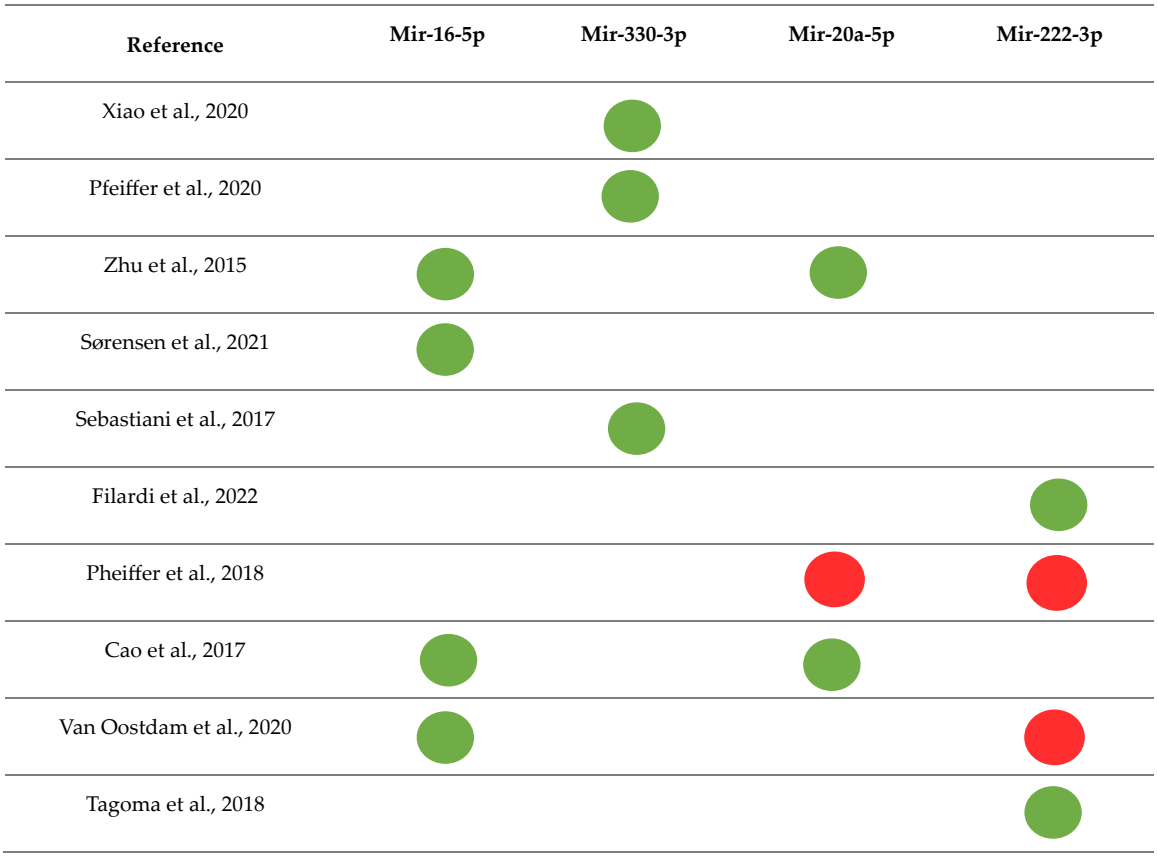

Figure 2. Main miRNAs and their regulations were found in the systematic review [28,39,40,47,50,51,62,68,70,72].

Table 4. Biological function and regulation of miRNAs found in the systematic review.

miRNA	Role/Biological Function	Regulation	Reference
let-7e-5p	Osteoblast differentiation and bone formation	↑	[72]
let-7g-5p	Inhibit mammary cell proliferation	↑	[72]
miR-7-5p	Regulates insulin sensitivity	↑	[36]
miR-9	Mammalian neuronal development and function	↓	[46]
miR-9-5p	Regulation of aerobic glycolysis and mitochondrial complex expression	↓	[61]
miR-16-5p	It targets insulin receptor substrate (IRS) proteins 1 and 2 and mediates insulin-like growth factor-I (IGF-I), which is closely related to insulin resistance	↑	[40,47,68,70]
miR-17	Anti-diabetic activity by the anti-inflammation effect on macrophage	↓	[29]
miR-17-5p	Improves insulin and blood glucose metabolic imbalances	↑	[40,68]
miR-19a	Promotes cell proliferation and angiogenesis by regulating the PI3K/AKT pathway, known as insulin signaling pathway	↑	[42]
miR-19a-3p	Regulates insulin secretion while it suppresses the apoptosis of pancreatic β cells	↑	[40]
miR-19b	Regulates cells apoptosis	↑	[42]
miR-19b-3p	Regulates the immune and nervous system	↑	[40]
miR-20a-5p	Improves glucose uptake and relieves diabetic cardiac hypertrophy, fibrosis, inflammation, and apoptosis	↑ ↓	[40,68] [62]
miR-21	Keeps glucose levels down in patients with diabetic complications	↓	[25]
miR-21-3p	Regulates the healing process of diabetic lesions	↓ ND	[30] [32]
miR-23a	Regulates the inflammatory response	↑	[58]
miR-23b-3p	Promotes high glucose-induced cellular metabolic memory in diabetic retinopathy	↑	[72]
miR-27a	Promotes insulin resistance	↓	[46]
miR-29a	Acts as an important regulator of insulin-stimulated glucose metabolism	↓	[26,41]
miR-29a-3p	Regulator of insulin-like growth factor 1 receptor	↑	[43,47]
miR-29b	Regulates aortic remodeling and stiffening in diabetes	↓	[26,57]
miR-29b-3p	Promotes cell proliferation, apoptosis, fibrosis, and inflammation	↑	[43]
miR-30b-5p	Induces abnormal angiogenesis	↑	[72]
miR-30c-5p	Induces macrophage-mediated inflammation and pro-atherosclerosis signal pathways	↑	[72]
miR-30d	Induces insulin production	↓	[46]
miR-30d-5p	Regulates human chorionic trophoblast cell proliferation, migration, and glucose uptake capacity	↑ ↓	[72] [65]
miR-33a	Regulates the insulin signaling pathway and glucose metabolism	↓	[46]
miR-33a-5p	Related to decreased cell growth (β cell) and insulin production	↑	[63]
miR-92a	Modulation of vascular homeostasis	↓	[46]
miR-96-5p	Relieves the antiproliferative effects induced by high glucose conditions on trophoblasts	↓	[52]
miR-98	Protects nucleus pulposus cells against apoptosis	↑	[67]
miR-100-5p	Promotes angiogenesis during the implantation process	↑	[72]
miR-101-3p	Associated with insulin production, survival, and death of pancreatic β cells	↑	[72]
miR-122-5p	Suppresses insulin resistance	↑	[43]
miR-125b	Modulates insulin secretion	↑	[23]
miR-132	Promote the trophoblast cell proliferation	↓	[41,53]
miR-132-3p	Related to insulin resistance and β cell function	↑	[43]
miR-134-5p	Regulate trophoblast cell behaviors in preeclampsia, such as apoptosis, migration, and invasion	↑	[20,47]
miR-135a	Related to the proliferation and migration of cells	↓	[73]
miR-136	Relieves inhibited cell viability induced by high glucose treatment	↑	[64]
miR-136-5p	Related to cellular proliferation, migration, and invasion	↑	[43]
miR-137	Participates in the interaction between endothelial cells and monocytes	↑ ↓	[71] [46]
miR-140-3p	Important regulator of osteoblastogenesis	↑	[27]

Table 4. *Cont.*

miRNA	Role/Biological Function	Regulation	Reference
miR-144	Regulates proliferation and apoptosis	↑	[23]
miR-146a-5p	Inhibit the inflammatory response and apoptosis	↑	[72]
miR-155-5p	Promotes fibrosis of proximal tubule cells	↓ ND	[33] [32]
miR-181d	Modulates the process of insulin signaling, cell viability, and apoptosis in pancreatic β cells	↑	[35]
miR-182-3p	Related to vascular smooth muscle cells proliferation and migration	↑	[43]
miR-190b	Inhibits pancreatic β cell proliferation and insulin secretion	↑	[74]
miR-193b	Suppresses apoptosis and autophagy	↓	[37]
miR-195	Increases epithelial-mesenchymal transition and cell permeability	↑	[29]
miR-195-5p	Relates to cell viability and proliferation, and apoptosis	↑	[66,72]
miR-210-3p	Reduces cell viability and promotes apoptosis of pancreatic β cells	↑	[43]
miR-214	Involved in cellular immune response, regulating cell activation, proliferation, and differentiation	↓	[56]
miR-222	Regulates estrogen receptor (ER) and glucose transporter 4 (GLUT4)	↑ ↓	[55] [41]
miR-222-3p	Relates to glucose metabolism in pregnancy	↑ ↓	[51,72] [62,70]
miR-223	Modulator of human myeloid differentiation	↑	[54,58]
miR-257	ND	↑	[29]
miR-330-3p	Involved in insulin secretion and β cell growth and proliferation, activating proteins involved in cell cycle and cell growth	↑	[28,39,50]
miR-340	Associated with induced hyperinsulinemia	↑	[31]
miR-342-3p	Induction of vascular dysfunction	↑	[43,72]
miR-345-3p	Regulates cell growth, apoptosis, migration, and invasion	↓	[24]
miR-362-5p	Promotes cell proliferation and inhibition of apoptosis	↓	[46]
miR-377-3p	Regulates VEGF expression	↑	[34]
miR-409-3p	Relates to immune dysregulation in autoimmune diabetes	↑	[51]
miR-409-5p	Correlated with insulin resistance	↑	[38]
miR-423-5p	Relieves high glucose-mediated podocyte injuries	↑	[72]
miR-494	Decreased insulin secretion and cell proliferation, and increased apoptosis	↓	[49]
miR-502-5p	Mediates insulin resistance	↓	[46]
miR-503	Impairs restorative angiogenesis	↑	[48]
miR-508-3p	Promotes cell proliferation and inhibits apoptosis	↑	[46]
miR-516-5p	Inhibits cell proliferation	↓	[70]
miR-517-3p	Promotes trophoblast dysfunction	↓	[70]
miR-518-5p	Increases hypoxia-induced vascular endothelial cell damage	↓	[70]
miR-520h	Inhibit cell viability and promote cell apoptosis	↑	[43,60]
miR-543	Regulates high glucose-induced fibrosis and autophagy	↑	[23]
miR-574-5p	Metabolic regulator for serum lipids and blood glucose	↓	[69]
miR-657	Promotes macrophage polarization	↑	[44,45]
miR-770-5p	Inhibits cell proliferation and promotes apoptosis	↑	[59]
miR-1323	Promotes the trophoblast cell viability under high glucose conditions	↑	[22,43]
miR-3135b	Metabolic regulator for serum lipids and blood glucose	↓	[69]
miR-6869-5p	Involved in maintaining the microenvironmental balance of the placenta	↓	[21]

ND: not described; ↑: up-regulated; ↓: down-regulated.

4. Discussion

miRNAs have been studied as potential biomarkers of GDM, and knowledge about their regulation and function, as well as metabolic adaptation associated with GDM, have been investigated and may help in the understanding of the pathogenesis of the disease [7]. In this systematic review, we identified 82 altered miRNAs in GDM. Of these, four were most cited, mir-16-5p and mir-330-3p were up-regulated, and mir-20a-5p and mir-222-3p were found to be up-regulated and down-regulated, respectively.

According to Gao and Zhao [75], mir-16-5p controls cell proliferation, migration, and invasion, affecting the cell cycle and promoting apoptosis. In addition, this miRNA modulates the PI3K/Akt signaling pathway, an important cell cycle regulator that includes genes such as Pi3Kr1, Pi3kr3, mTOR, and Mapk3, among others. Kwon et al. [76] observed that the up-regulation of mir-16-5p in the liver of Cmah-null mice, used as models for T2DM, can negatively regulate the insulin/PI3K-AKT signaling pathway in association with other genes. These results suggest that the impairment of insulin mechanisms may favor the development of metabolic disorders, such as chronic hyperglycemia.

Other targets of this miRNA are the genes Insulin Receptor Substrate 1 and 2 (IRS1/IRS2) and Insulin-Like Growth Factor 1 (IGF-1), which are closely related to insulin resistance, a characteristic condition of diabetes [68,76]. In addition, the up-regulation of this miRNA in GDM patients in the second trimester of gestation has demonstrated a correlation with the down-regulation of IRS1 and IRS2, leading to abnormal signaling of the Wnt/β-catenin pathway, important for embryonic development and adult tissue homeostasis [77].

Whereas mir-330-3p is associated with proliferation, differentiation, and insulin secretion and is highly expressed in GDM related to high glucose concentration. It also acts as a central regulator of cell cycle homeostasis. Thus, the up-regulation of this miRNA in patients with GDM may contribute to pancreatic β cell dysfunction, altering the proliferation and growth of these cells [39,50]. On the other hand, studies have revealed that the E2F Transcription Factor 1 (E2F1) and Cell Division Cycle 42 (CDC42) genes are targets of mir-330-3p. Both are involved in the growth and proliferation of β cells and the control of insulin secretion. Therefore, the low expression of these genes is caused by increased miRNA levels that can compromise β cell proliferation and insulin secretion [39,50].

Moreover, the Angiotensin II receptor Type 2 (AGTR2) gene was also identified as a target of this miRNA; this gene acts during the development of the pancreas in the embryonic stage and is a possible mediator of the regeneration of β cells in the adult pancreas. Thus, it is hypothesized that elevated levels of mir-330-3p may inhibit pancreatic neogenesis through the down-regulation of AGTR2, causing a defect in the regeneration of the endocrine pancreas under high metabolic demand [50].

Additionally, in this systematic review, Zhu et al. [40] and Cao et al. [68] identified up-regulated mir-20a-5p, while Pheiffer et al. [62] found it down-regulated in GDM. Mir-20a-5p belongs to the mir-17-92 cluster and is associated with angiogenesis [62]. In GDM, the expression of angiogenic proteins is increased, such as Vascular Endothelial Growth Factor-A (VEGFA), Hypoxia-inducible factor 1 subunit alpha (HIF1A) [78], Phosphatase and Tensin homolog (PTEN) [79], and BCL2 apoptosis regulator (BCL2) [80]. This fact corroborates the regulatory effect of the decreased expression of mir-20a-5p found by Pheiffer et al. [62].

Zhu et al. [40] also associated mir-20a-5p with the insulin, MAPK, TGF-β, and mTOR signaling pathways. The PI3K/Akt pathway that regulates the cell cycle, glucose homeostasis, and insulin signaling, as well as the FoxO protein, which also regulates the insulin/PI3K/Akt pathway, were considered targets of mir-20a-5p. Thus, this miRNA becomes a possible biomarker of GDM, and the alteration of its expression can interfere with these pathways, generating hyperglycemia observed in GDM patients [62].

MAPK signaling pathway plays a role in developing vascular lesions, such as diabetes [81], and its abnormal signaling has been identified in pregnancy complications [40]. In addition, the TGF-β signaling pathway has been associated with preeclampsia [82]. In contrast, the mTOR signaling pathway controls energy balance [83] and blocking these pathways may contribute to the development of GDM [40].

Finally, mir-222-3p was identified by Tagoma et al. [72] and Filardi et al. [51] found it up-regulated, while Oostdam et al. [70] and Pheiffer et al. [62] found it down-regulated in GDM. Mir-222-3p belongs to the mir-222 cluster, is abundant in plasma during 24–28 weeks of gestation, and is a placental miRNA that acts on the proliferation of endometrial stromal cells [51,84], regulating the expression of estrogen receptor-α (ER-α) in estrogen-induced in-

sulin resistance in GDM [55,62], and is strongly linked to glucose metabolism in pregnancy, profoundly impacting in the weight birth [51].

Furthermore, high levels of this miRNA were found in the adipose tissue of GDM patients, negatively correlated with the levels of ER-α and glucose transporter type 4 (GLUT4) [55]. Women with GDM have higher levels of estradiol when compared to healthy women, and the estradiol and ER-α act on the GLUT4, becoming critical regulators of obesity and insulin resistance [51].

Due to the relation between miRNAs and the regulation of gene expression, information about the tissue origin of malignant cells could be generated. Since their initial discovery, it has been clear that miRNAs are expressed in a variety of cell types and that their expression patterns are tissue-specific and thus could have great diagnostic importance and prognostic value [85].

The placenta produces several miRNAs expressed specifically by placental cells. They can be dysregulated in the plasm and placenta of women with GDM, being also associated with pregnancy and birth-related outcomes. Detection of placental miRNAs in placental cells and circulation contributes to the understanding of the molecular pathways and intracellular signalization and their influences on GDM genetic background [86]. Therefore, identifying possible GDM biomarkers has a significant clinical value in developing diagnostic strategies and possibly preventing obstetric and maternal–fetal complications [7]. However, studies on early recognition, diagnostic criteria, possible biomarkers, and therapeutic targets for GDM show controversial results, mainly due to differences in ethnic, geographic, genetic, and environmental factors and diagnostic criteria [7,87,88].

5. Conclusions

A miRNA can regulate up to 200 mRNAs, indicating that miRNAs can individually regulate many different biological processes [52]. Thus, it is suggested that miRNAs be used as biomarkers in miRNA panels or risk assessment algorithms and not as individual biomarkers for diagnosing GDM [62]. In this systematic review, we found 55 articles that analyzed the alteration of miRNAs in GDM, identifying 82 altered miRNAs. The expression of these miRNAs varied depending on the type of sample used and the different gestational ages, which may explain the differences in the results. Additionally, these miRNAs were associated with several mechanisms, such as cell cycle homeostasis, growth, and proliferation of pancreatic β cells, glucose uptake and metabolism, insulin secretion, and resistance. Certainly, miRNAs are potential biomarkers for the early diagnosis of GDM. However, more research is needed to elucidate their diagnostic and predictive value and their pathogenic mechanisms in pregnancy.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jpm13071126/s1, File S1. Protocol registered in the International Prospective Register of Systematic Reviews (PROSPERO) on 16 November 2021, under number CRD42021291791.

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ANEXO B: PROSPERO

PROSPERO
International prospective register of systematic reviews

Evaluate which MicroRNAs are associated with the pathological mechanisms of Gestational Diabetes Mellitus (GDM), analyzing patients worldwide.

Pedro Silva, Angela Silva, Rodrigo Santos

Review methods were amended after registration. Please see the revision notes and previous versions for detail.

Citation

Pedro Silva, Angela Silva, Rodrigo Santos. Evaluate which MicroRNAs are associated with the pathological mechanisms of Gestational Diabetes Mellitus (GDM), analyzing patients worldwide.. PROSPERO 2021 CRD42021291791 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021291791

Review question

Evaluate which MicroRNAs are associated with the pathological mechanisms of Gestational Diabetes Mellitus (GDM), analyzing patients worldwide.

Searches

The following electronic bibliographic databases were accessed: Web of Science (https://www.periodicos.capes.gov.br/?option=com_pcollection&mn=70&smn=79&cid=81), PubMed (https://www.ncbi.nlm.nih.gov/PubMed/) and BVS Regional Portal (https://bvsalud.org/). A systematic literature search was performed to identify studies that evaluated which MicroRNAs are associated with GDM. The Web of Science, PubMed and BVS Regional Portal databases will be search from December 2021.

The studies were restricted to English, Portuguese and Spanish. Searches will be repeated before final statistics and new studies retrieved for inclusion.

Types of study to be included

It includes case-control, cohort, clinical case, cross-sectional, bibliographic reviews and systematic review studies to assess which microRNAs are associated with GDM.

Condition or domain being studied

Pregnancy changes the metabolism of carbohydrates, lipids and amino acids to create balance between fetal and maternal needs. During this period, there is an increase in insulin secretion and peripheral insulin resistance, a consequence of the secretion of “diabetogenic” hormones (placental lactogenic hormone, growth hormone and cortisol). When this compensatory mechanism is not enough to overcome insulin resistance, gestational diabetes mellitus (GDM) occurs.

Gestational diabetes can cause complications during pregnancy: fetal anomalies, polyhydramnios, fetal polycythemia / hypoxia, fetal death, neonatal hyperbilirubinemia, hypocalcemia and hypomagnesemia. Complications can be observed in childbirth, as premature birth, changes in the mode of delivery, shoulder dystocia or birth trauma.

MicroRNAs are small endogenous non-coding ribonucleic acids. They persist in several groups of eukaryotes and play critical roles during development and cell homeostasis. They are 19-25 nucleotides in length and regulate the translation of target messenger RNA. MicroRNAs can inhibit its translation, stabilize or induct its degradation. They regulate gene expression and are involved in controlling cell functions (differentiation, proliferation, apoptosis, metabolism). They are also involved in angiogenesis, oncogenesis and cardiovascular functions.

Therefore, the aim of this review was to assess the importance of microRNAs in development of GDM, in addition to their main target genes.

Participants/population

Woman worldwide of any ethnicity in any country in which a diagnosis of gestational diabetes mellitus has been received during a pregnancy (according to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) and American Diabetes Association (ADA) recommended by the Word Health Organization (WHO), ie participants that make up the case groups.

Intervention(s), exposure(s)

Exposure of interest are patients with confirmed diagnosis of GDM.

Comparator(s)/control

The control group will not be parsed. The study relates the association of microRNAs in the pathophysiology of GDM, thus considering only the participants of the case groups.

Context

Free texts, language and publication data (Portuguese, English and Spanish, 10 years, respectively)

Main outcome(s)

We will identify the microRNAs involved in GDM in order to elucidate the pathophysiological mechanisms involved in the disease. Thus, reinforcing the role of microRNAs as promising biomarkers in molecular diagnosis and therapeutic responses.

Measures of effect

Data extraction will be performed with the identification of microRNAs, taking into account the mechanisms of which are associated, the direction of their regulation and in which tissue they were collected. Disregarding data related to animal models.

Additional outcome(s)

MicroRNAs can be obtained by different types of body fluids and thus provide more information about the pathophysiological factors associated with greater accuracy and diagnosis of GDM.

Measures of effect

Not applicable

Data extraction (selection and coding)

The title and summary of articles retrieved during the initial search will be independently reviewed by the researchers (R.S.S. and A.A.S.R) using a systematic strategy based on defined inclusion criteria. Any differences of opinion among reviewers regarding the inclusion or exclusion of an article will be discussed until consensus is reached.

Abstracts will be analyzed based on the following criteria:

(1) microRNA analysis in patients with Gestational Diabetes Mellitus.
(2) clinical trial studies, cross-sectional studies, systematic review, case-control study;
(3) publication in the last 5 years. No restrictions were imposed on the minimum sample size.
Non-data-based articles (books, theoretical articles and minor reviews) will be excluded.

All identified studies will be analyzed and duplicates removed. The PECO (Population with the problem: Woman worldwide with at least one pregnancy; Exposure: gestational diabetes mellitus; Comparator: patients which a diagnosis of gestational diabetes mellitus has been received during a pregnancy; Outcome: association of microRNAs with gestational diabetes mellitus; question of the study characterizes the search strategy. However, there are essential items that should be present in all reviews such as the clear definition of the research questions: Evaluate which MicroRNAs are associated with Gestational Diabetes Mellitus in patients worldwide.

Risk of bias (quality) assessment

The selected articles will be analyzed by two independent reviewers of the systematic review instrument (The Joanna Briggs Institute), for inclusion of each study, according to their quality. JBI's critical assessment tools assist in assessing the reliability, relevance and results of published works. Taking into consideration the type of study that will be approached, which may be analytical, case-control, cohort study, case reports, studies of systematic reviews and among others

Strategy for data synthesis

We will provide a summary of the studies included in the findings, structured around the type of pathology addressed and the type of biomarker used. We will discuss which microRNAs are associated with GDM by analyzing the relationship of pathophysiologies mechanisms.

Analysis of subgroups or subsets

We can consider an analysis based on the PECO elements and the study design characteristics used by the authors.

Contact details for further information

Pedro Silva
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Organisational affiliation of the review

Federal University of Goiás

Review team members and their organisational affiliations

Mr Pedro Silva. Federal University of Goiás
Angela Silva. Federal University of Goiás
Rodrigo Santos. Federal University of Goiás

Type and method of review

Epidemiologic, Systematic review

Anticipated or actual start date

01 December 2021

Anticipated completion date [1 change]

31 December 2022

Funding sources/sponsors

This work was supported by the personal resources from the research coordinators (Angela Adamski da Silva Reis, Ph.D. and Rodrigo da Silva Santos, Ph.D.)

Conflicts of interest

Language

English, Portuguese-Brazil, Spanish

Country

Brazil

Stage of review

Review Ongoing

Subject index terms status

Subject indexing assigned by CRD

Subject index terms

Diabetes, Gestational; Female; Humans; MicroRNAs; Pregnancy

Date of registration in PROSPERO

17 December 2021

Date of first submission

16 November 2021

Stage of review at time of this submission

The review has not started

Stage	Started	Completed
Preliminary searches	No	No
Piloting of the study selection process	No	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Revision note

New uptodate for the end date

The record owner confirms that the information they have supplied for this submission is accurate and complete and they understand that deliberate provision of inaccurate information or omission of data may

be construed as scientific misconduct.

The record owner confirms that they will update the status of the review when it is completed and will add publication details in due course.

Versions

17 December 2021
17 December 2021
28 July 2022

ANEXO C: PRISMA 2020 CHECKLIST

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	
Study characteristics	17	Cite each included study and present its characteristics.	
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	
	23b	Discuss any limitations of the evidence included in the review.	
	23c	Discuss any limitations of the review processes used.	
	23d	Discuss implications of the results for practice, policy, and future research.	
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	
Competing interests	26	Declare any competing interests of review authors.	
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

ANEXO D: JBI CRITICAL APPRAISAL CHECKLIST

JBI CRITICAL APPRAISAL CHECKLIST FOR CASE CONTROL STUDIES

Reviewer_____Date_____

Author_____Year_____ Record Number_____

	Yes	No	Unclear	Not applicable
1. Were the groups comparable other than the presence of disease in cases or the absence of disease in controls?	☐	☐	☐	☐
2. Were cases and controls matched appropriately?	☐	☐	☐	☐
3. Were the same criteria used for identification of cases and controls?	☐	☐	☐	☐
4. Was exposure measured in a standard, valid and reliable way?	☐	☐	☐	☐
5. Was exposure measured in the same way for cases and controls?	☐	☐	☐	☐
6. Were confounding factors identified?	☐	☐	☐	☐
7. Were strategies to deal with confounding factors stated?	☐	☐	☐	☐
8. Were outcomes assessed in a standard, valid and reliable way for cases and controls?	☐	☐	☐	☐
9. Was the exposure period of interest long enough to be meaningful?	☐	☐	☐	☐
10. Was appropriate statistical analysis used?	☐	☐	☐	☐

Overall appraisal: Include ☐ Exclude ☐ Seek further info ☐

Comments (Including reason for exclusion)

_____

_____

_____

JBI CRITICAL APPRAISAL CHECKLIST FOR COHORT STUDIES

Reviewer_____Date_____

Author_____Year_____ Record Number_____

	Yes	No	Unclear	Not applicable
1. Were the two groups similar and recruited from the same population?	☐	☐	☐	☐
2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?	☐	☐	☐	☐
3. Was the exposure measured in a valid and reliable way?	☐	☐	☐	☐
4. Were confounding factors identified?	☐	☐	☐	☐
5. Were strategies to deal with confounding factors stated?	☐	☐	☐	☐
6. Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	☐	☐	☐	☐
7. Were the outcomes measured in a valid and reliable way?	☐	☐	☐	☐
8. Was the follow up time reported and sufficient to be long enough for outcomes to occur?	☐	☐	☐	☐
9. Was follow up complete, and if not, were the reasons to loss to follow up described and explored?	☐	☐	☐	☐
10. Were strategies to address incomplete follow up utilized?	☐	☐	☐	☐
11. Was appropriate statistical analysis used?	☐	☐	☐	☐

Overall appraisal: Include ☐ Exclude ☐ Seek further info ☐

Comments (Including reason for exclusion)

JBI CRITICAL APPRAISAL CHECKLIST FOR ANALYTICAL CROSS SECTIONAL STUDIES

Reviewer_____Date_____

Author_____Year_____ Record Number_____

	Yes	No	Unclear	Not applicable
1. Were the criteria for inclusion in the sample clearly defined?	☐	☐	☐	☐
2. Were the study subjects and the setting described in detail?	☐	☐	☐	☐
3. Was the exposure measured in a valid and reliable way?	☐	☐	☐	☐
4. Were objective, standard criteria used for measurement of the condition?	☐	☐	☐	☐
5. Were confounding factors identified?	☐	☐	☐	☐
6. Were strategies to deal with confounding factors stated?	☐	☐	☐	☐
7. Were the outcomes measured in a valid and reliable way?	☐	☐	☐	☐
8. Was appropriate statistical analysis used?	☐	☐	☐	☐

Overall appraisal: Include ☐ Exclude ☐ Seek further info ☐

Comments (Including reason for exclusion)