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DANIELLE CRISTINA HONÓRIO FRANÇA

Serotonina, melatonina e citocinas inflamatórias como potenciais mediadores na interação Diabetes Mellitus Gestacional e Incontinência Urinária Específica da Gestação

Tese apresentada à Faculdade de Medicina, Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Botucatu, como parte dos requisitos para obtenção do título de Doutora em Tocoginecologia.

Orientadora: Profª. Emérita Marilza Vieira Cunha Rudge

Botucatu

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Comissão Examinadora:

_____

Prof.ª Emérita Dra. Marilza Vieira Cunha Rudge

Faculdade de Medicina de Botucatu - UNESP

_____

Prof.ª Titular Dra. Iracema Mattos Paranhos Calderon

Faculdade de Medicina de Botucatu – UNESP

_____

Prof.ª Dra.Angélica Mércia Pascon Barbosa

Faculdade de Medicina de Botucatu - UNESP

_____

Prof.ª Dra. Danny Laura Gomes Fagundes-Triches

Universidade Federal de Mato Grosso – UFMT

_____

Prof. Dr. Belmiro Gonçalves Pereira

Universidade Estadual de Campinas – UNICAMP

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Epígrafe

“Se você está andando no caminho certo e disposto a continuar caminhando, eventualmente você vai progredir.”

Barack Obama

Lista de Abreviações

Português e Inglês

Lista de Abreviações em Português

5-HT	Serotonina (5-hidroxitriptamina)
APO	Desfechos perinatais adversos
ARD	Diástase dos músculos retos abdominais
ASC	Proteína associada à apoptose contendo CARD
C3	Componente 3 do sistema complemento
C4	Componente 4 do sistema complemento
CBA	Cytometric Bead Array
TCD4+	Linfócitos T CD4
CNPs	Polimorfismos de número de cópias
CV	Coeficiente de variação
DAMPs	Padrões moleculares associados a dano
DM	Diabetes mellitus
DMG	Diabetes mellitus gestacional
DMG-C	Diabetes mellitus gestacional continente
DMG-I	Diabetes mellitus gestacional incontinente
ELISA	Ensaio imunoenzimático
FACS	Citometria de fluxo (Fluorescence-Activated Cell Sorting)
FOXP3	Fator de transcrição forkhead box P3
FTO	Gene associado à obesidade e massa corporal
GAG	Ganho de peso gestacional
GTT	Teste Oral de Tolerância à Glicose

HbA1c	Hemoglobina glicada
HPA	Eixo hipotálamo-hipófise-adrenal
ICIQ-SF	Questionário Internacional de Incontinência Urinária – versão curta
IDO	Indolamina-2,3-dioxigenase
IFN-γ	Interferon gama
IgA	Imunoglobulina A
IgG	Imunoglobulina G
IgM	Imunoglobulina M
IKK	Quinase IκB
IL	Interleucina
IL-1β	Interleucina 1 beta
IL-2	Interleucina 2
IL-4	Interleucina 4
IL-6	Interleucina 6
IL-7	Interleucina 7
IL-8	Interleucina 8
IL-10	Interleucina 10
IL-12	Interleucina 12
IL-13	Interleucina 13
IL-17	Interleucina 17
INSR	Receptor de insulina
IMC	Índice de Massa Corporal

IU	Incontinência Urinária
IUE	Incontinência Urinária de Esforço
IUEG	Incontinência Urinária Específica da Gestação
IUU	Incontinência Urinária de Urgência
JNK	Quinase N-terminal de c-Jun
MC4R	Receptor melanocortina-4
miRNAs	MicroRNAs
MLT	Melatonina
MT1	Receptor de melatonina tipo 1
MT2	Receptor de melatonina tipo 2
MRA	Músculo Reto abdominal
MTNR1B	Gene do receptor de melatonina 1B
mRNA	RNA mensageiro
NG-C	Normoglicêmica continente
NG-I	Normoglicêmica incontinente
NK	Células natural killer
NLRs	Receptores do tipo NOD
NLRP3	Receptor NOD-like com domínio de pirina 3
OGTT	Teste oral de tolerância à glicose
O2–	Radical superóxido
PAMPs	Padrões moleculares associados a patógenos
PFM	Músculos do assoalho pélvico
PRRs	Receptores de reconhecimento padrão

ROS	Espécies reativas de oxigênio
SOD	Superóxido dismutase
SNC	Sistema Nervoso Central
SNPs	Polimorfismos de nucleotídeo único
SUI	Incontinência urinária de esforço
sMT6a	6-sulfatoximelatonina
T2DM	Diabetes mellitus tipo 2
TCF7L2	Fator de transcrição 7 tipo 2
TGF-β	Fator de crescimento transformador beta
Th1	Linfócitos T helper 1
Th2	Linfócitos T helper 2
Th17	Linfócitos T helper 17
Treg	Células T reguladoras
TNF-α	Fator de necrose tumoral alfa
TRP	Triptofano

Lista de Abreviações em Inglês

5-HT	Serotonin (5-hydroxytryptamine)
APO	Adverse perinatal outcomes
ARD	Abdominal rectus diastasis
ASC	Apoptosis-associated speck-like protein containing a CARD
BMI	Body mass index
C3	Complement component 3
C4	Complement component 4
CBA	Cytometric Bead Array
CD4+	CD4 T lymphocytes
CNS	Central nervous system
CNPs	Copy number polymorphisms
CV	Coefficient of variation
DAMPs	Damage-associated molecular patterns
DM	Diabetes mellitus
GDM	Gestational diabetes mellitus
GDM-C	Gestational diabetes mellitus continent
GDM-I	Gestational diabetes mellitus incontinent
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescence-activated cell sorting
FOXP3	Forkhead P3 transcription factor
FTO	Fat mass and obesity-associated gene

GWG	Gestational weight gain
GTT	Glucose Tolerant Test
HbA1c	Glycated hemoglobin
HPA axis	Hypothalamic-pituitary-adrenal axis
ICIQ-SF	International Consultation on Incontinence Questionnaire–Short Form
IDO	Indoleamine-2,3-dioxygenase
IFN-γ	Interferon-gamma
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IKK	IκB kinase
IL	Interleukin
IL-1β	Interleukin-1 beta
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-7	Interleukin-7
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-13	Interleukin-13
IL-17	Interleukin-17

INSR	Insulin receptor
JNK	c-Jun N-terminal kinase
MC4R	Melanocortin 4 receptor
miRNAs	MicroRNAs
MLT	Melatonin
MT1	Melatonin receptor type 1
MT2	Melatonin receptor type 2
MTNR1B	Melatonin receptor 1B gene
mRNA	Messenger RNA
NG-C	Normoglycemic continent
NG-I	Normoglycemic incontinent
NK cells	Natural killer cells
NLRs	NOD-like receptors
NLRP3	NOD-like receptor family pyrin domain containing 3
OGTT	Oral glucose tolerance test
O2–	Superoxide anion
PAMPs	Pathogen-associated molecular patterns
PFM	Pelvic floor muscles
PRRs	Pattern recognition receptors
PSUI	Pregnancy-specific urinary incontinence
RAM	Rectus abdominis muscle
ROS	Reactive oxygen species
SNPs	Single nucleotide polymorphisms

SOD	Superoxide dismutase
SUI	Stress urinary incontinence
sMT6a	6-sulfatoximelatonin
T2DM	Type 2 diabetes mellitus
TCF7L2	Transcription factor 7-like 2
TGF-β	Transforming growth factor beta
Th1	T-helper 1 cells
Th2	T-helper 2 cells
Th17	T-helper 17 cells
Treg	Regulatory T cells
TNF-α	Tumor necrosis factor-alpha
TRP	Tryptophan
UI	Urinary incontinence
UUI	Urge urinary incontinence

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Seção 1
Trajetória Acadêmica

Desde criança cresci vendo meus pais fazerem ciência, então esse era meu sonho também. Dos três aos cinco anos de idade participei de um projeto para crianças aprenderem sobre ciência, chamado “Cientista Mirim” e desde então defini que esse seria meu futuro profissional: ser cientista.

Quando estava no ensino médio estudei em uma escola técnica federal, onde comecei a produzir trabalhos científicos. No segundo ano do ensino médio conquistei minha primeira menção honrosa em um trabalho sobre filosofia da ciência. Isso me motivou a continuar estudando.

No último ano da escola eu queria realizar todos os cursos possíveis, pois amava me comunicar, fazer pesquisas, aprender. Foi então que decidi fazer duas graduações ao mesmo tempo: Medicina e Gerontologia. Cursei então Gerontologia no Claretiano e cursei Medicina na Universidade Estadual de Mato Grosso, tendo sido aprovada direto do terceiro ano do ensino médio.

Após esse grande desafio, terminei o curso de Gerontologia ainda na metade da jornada da Medicina. Fui então convidada pelo Claretiano à oportunidade de aproveitar algumas matérias da Gerontologia e continuar estudando no curso de Licenciatura em Biologia. Dessa forma, ingressei na Biologia e continuei me aperfeiçoando na área das ciências biológicas e da saúde.

Em 2020, o ano da pandemia, quando o mundo se fechou no isolamento social, as aulas da medicina foram suspensas. Vi então no tempo livre, a oportunidade de realizar um estágio no Laboratório de Imunologia Materno Infantil da UFMT. Foi lá nas práticas do laboratório que optei por ingressar no Mestrado, tendo sido então aprovada em 2021 em primeiro lugar no Mestrado em Imunologia e Parasitologia Básicas e Aplicadas da Universidade Federal de Mato Grosso – UFMT.

Cursei o mestrado com o prazo mínimo necessário para concluir (um ano) e terminei o curso de Biologia ao mesmo tempo, após isso optei por prestar o Doutorado na área que sempre quis seguir profissionalmente: Saúde da Mulher.

Então, iniciei o curso de Doutorado em Tocoginecologia na UNESP em 2022, sempre tendo sido meu maior sonho estudar nessa instituição, visto que parte da minha família é de Botucatu-SP e a UNESP foi aonde meus pais se conheceram, pois estudaram juntos na mesma sala na época da faculdade.

Terminei o Curso de Medicina nessa mesma época e fiz uma pós-graduação *lato sensu* em Ginecologia e Obstetrícia pela FGMed, além de me pós-graduar em Docência no Ensino Superior e em Citologia Oncótica pela UniBF, visando me aperfeiçoar na área que sou apaixonada e pretendo sempre atuar na prática-médica.

No meu Doutorado tive a oportunidade de ser orientada pela médica obstetra que mais admiro: a Prof.a Emérita Marilza Vieira Cunha Rudge, e tive apoio da CAPES com bolsa durante o doutorado: CAPES-DS Processo 88887.687380/2022-00 (01/04/2022-28/02/2023) e CAPES-PROEX Processo 88887.817628/2023-00 (01/03/2023-28/022026) ficando sempre muito feliz e gratificada, pois sem o apoio financeiro eu não poderia ter estudado tanto.

Esses quatro anos de Doutorado foram intensos, cresci imensamente na carreira acadêmica e profissional com publicações, apresentações em eventos científicos, experimentos e aulas, como exemplo no estágio docência realizado com a professora Dra. Ana Margarida Theodoro Caminhas, no qual aprendi muito sobre habilidades de comunicação com discentes e vi que desejo continuar na prática docente no futuro.

Agora concluo com grande alegria essa etapa da minha vida profissional, tendo dois artigos da minha pesquisa já publicados em revistas de alto impacto e tendo mais um para ser submetido. Sigo agora com o sonho de continuar me aperfeiçoando e estudando, sempre aberta às novas oportunidades e possibilidades científicas e acadêmicas. Fico muito honrada e feliz de poder dizer que toda minha trajetória acadêmica me formou como pessoa e profissional e sempre seguirei dando o meu melhor para todas minhas pacientes.

Seção 2
Contextualização

Serotonina, melatonina e citocinas inflamatórias como potenciais mediadores na interação Diabetes Mellitus Gestacional e Incontinência Urinária Específica da Gestação

O Diabetes Mellitus Gestacional (DMG) é a patologia que cursa com intolerância à glicose que se inicia durante o período gestacional. A incontinência urinária (UI) é a perda involuntária de urina, que pode acontecer por esforço (IUE) ou por urgência (IUU). Essas patologias são duas entidades clínicas com importante impacto social e econômico, associadas a significativos custos diretos e indiretos de saúde pública (1,2).

Mulheres com DMG apresentam risco aumentado de Incontinência Urinária Específica da Gestação (IUEG), ou seja, aquela que se inicia durante a gestação (3). No entanto, a associação direta entre DMG e IUEG permanece pouco estudada.

Em pesquisa transversal (4), foi examinada a prevalência de IUEG entre mulheres que tinham DMG, contando com a participação de 228 mulheres, onde foi descoberto que 49% das mulheres apresentavam perda involuntária de urina na gestação pelo menos uma vez por semana, e 50% das mulheres relataram desenvolvimento da IU em até 5 anos após o parto. Alta prevalência de obesidade pré-gestacional pode ter contribuído para a elevada taxa de IUEG durante a gravidez e após o parto, pois estava presente em 42% das mulheres durante a gravidez e em 46% após o parto. Sua conclusão foi que a IUEG era comum entre mulheres com DMG. Barbosa et al. (5) e Chuang et al. (6) observaram, de forma independente e concomitante, que o DMG é fator de risco independente para IU pós-parto. Além disso, descobriram que mulheres com DMG tem sintomas de IUEG que são fator de risco para IU 2 anos após o parto em comparação com mulheres sem DMG. Concluíram que o DMG é fator de risco independente para IU pós-parto com impacto significativo na qualidade de vida dessas mulheres (4, 5,6). Esse impacto na qualidade de vida foi confirmado por Piculo et al em 2020 (7, 8). E outros resultados mostraram o impacto do DMG nos músculos envolvidos na continência urinária em ratas diabéticas prenhes, relacionados à IUE principalmente (7, 9-12). Também foi

mostrado que mulheres com histórias recentes de DMG são mais jovens e, apresentam alta prevalência de IU (4, 13-14).

A fisiopatologia do DMG é explicada pela elevação de hormônios contrarreguladores da insulina, pelo estresse fisiológico imposto pela gravidez e por outros fatores genéticos ou ambientais (15,16). A gestação representa oportunidade única para a mulher, sendo necessário reduzir essas complicações não só durante este período, mas também na vida futura (17, 18).

A manutenção da continência urinária requer interações específicas entre várias células e mediadores, mas poucos estudos investigaram o papel que esses fatores podem desempenhar na IU associada à hiperglicemia. O meio ambiente hiperglicêmico durante a gestação gera ambiente pró-inflamatório, despertando dessa forma o interesse da comunidade científica para estudos de proteínas pró e antiinflamatórias, como biomarcadores para auxiliar na rotina laboratorial e no acompanhamento de pacientes com diabetes (17-20).

Em gestantes com diabetes gestacional ou com diabetes mellitus tipo 2, foram relatadas alterações nos percentuais, na função e nos receptores celulares, bem como em fatores solúveis no soro, como proteínas do sistema complemento, anticorpos e citocinas (21- 26). As citocinas atuam como fatores de ativação, proliferação, diferenciação e regulação celular, e são capazes de atuar como mediadores de respostas inflamatórias. São produzidas por vários tipos celulares, sendo os linfócitos TCD4+ as células que secretam a maioria das citocinas responsáveis pelos processos imunológicos. Esses mediadores podem ter ações pró-inflamatória (Th1) ou antiinflamatória (Th2), de acordo com o microambiente onde estão exercendo suas funções. Dentre as citocinas consideradas próinflamatórias estão as interleucinas (IL) IL-1, IL-2, IL- 6, IL-7, IL-8, IL-17, IFN- (interferon-gama) e TNF- (fator de necrose tumoral-alfa) e, entre as antiinflamatórias,IL-4, IL-10, IL-13 e TGF- (fator transformador de crescimento) (27-29). Sabe-se que no cérebro e na medula espinhal, citocinas próinflamatórias influenciam a regulação das vias de micção e afetam

perifericamente a função da bexiga, causando diretamente hipertrofia do músculo detrusor e hipersensibilidade do nervo aferente (18).

Vários mecanismos foram propostos para explicar a influência de mediadores inflamatórios no sistema urinário, dentre essas, a ação de citocinas no Sistema Nervoso Central (SNC), nas vias serotonérgicas centrais, na supraregulação da via hipotálamo-hipófise-adrenal (HHA) e na redução da atividade parassimpática (30). No SNC as citocinas pró-inflamatórias atuam na ativação da enzima indolamina-2,3-dioxigenase (IDO). Essa enzima é responsável pela degradação do triptofano (TRP), que é precursor do neurotransmissor serotonina (31,32). A serotonina ou 5- hidroxitriptamina (5-HT) é uma indolamina, derivada da hidroxilação e descarboxilação do aminoácido L-triptofano. Tem sido associada a ótimas respostas neurológicas, fisiológicas (33) e imunomodulatórias (34,35). Esse neurotransmissor desempenha papel importante, mas complexo na micção com ações no cérebro, na medula espinhal e na própria bexiga (36). Na literatura, estudos envolvendo citocinas inflamatórias e 5-HT em gestantes com DMG e IU ainda permanecem a ser elucidados.

O estresse oxidativo e a inflamação são características-chave na iniciação, progressão e manifestações clínicas de distúrbios do músculo detrusor, como a IUU (37). A melatonina (N-acetil-5-metoxitriptamina), cujo precursor é a serotonina, é produzida pela glândula pineal. Este hormônio tem ações antitumoral (38-40), pró e anti-inflamatórias (41-44). Na urina seu principal metabólito excretado é 6-sulfatoximelatonina (sMT6a), que sofre variação dependente do sistema nervoso (45). A melatonina apresenta efeitos protetores na disfunção do músculo liso. Estudos sugerem que o hormônio diminui o tônus vascular, por meio da redução de contração adrenérgica e o aumento do relaxamento induzido pela acetilcolina, atenua o tônus simpático por ativação direta de receptores de melatonina, eliminando radicais livres e protegendo contra a inflamação e adeficiência induzida por inflamação da função neuromuscular (37, 46-49). A melatonina é capaz de modular a contratilidade prejudicada do músculo detrusor por meio da normalização de contração, da polaridade mitocondrial, da função neuromuscular e do estresse oxidativo, o que sugere os efeitos da melatonina contra as alterações

cistométricas (49-51).Alterações na melatonina têm sido relacionadas a distúrbios na homeostase da glicose e de lipídios, aumento ganho de peso e massa de gordura corporal (52-54). Os níveis de melatonina são menores em gestantes diabéticas (54, 55). Este hormônio em gestantes normoglicêmicas tem efeito pro-oxidativo e induz aumento de espécies reativas do oxigênio (42). Em condições de hiperglicemia acredita-se que, provavelmente, os efeitos deste hormônio são limitados a processos anti-inflamatórios, com diminuição de espécies reativas do oxigênio (55).

Nenhum estudo anterior aos publicados por meio desta pesquisa (artigo 1 e 2), haviam antes relatado sobre o papel da melatonina em mulheres com DMG e IUEG. Estudos sobre o papel da melatonina e de seu precursor 5-HT em mulheres com DMG e IUEG, bem como as possíveis correlações com citocinas inflamatórias no sangue e urina dessas gestantes, podem elucidar se o trato urinário pode ser afetado por alterações de níveis séricos de hormônios e citocinas. Devido à função da melatonina em melhorar alterações metabólicas associadas à diabetes e à IU foi proposto a possibilidade do uso desse hormônio em gestantes com DMG.

Assim, tornou-se necessária mais pesquisa envolvendo esses mediadores inflamatórios e hormonais para melhor compreensão da IUEG associada ao DMG, visando estabelecer estratégias que melhorem os parâmetros maternos. Sendo possível que os padrões imunológicos e hormonais associados a alterações no metabolismo materno possam influenciar, via sangue e urina, contribuindo para maior susceptibilidade a disfunções do trato urinário. As mudanças expressivas na compreensão dos riscos e da patogênese de doenças crônicas com inflamação subclínica, constituem uma crescente causa da morbimortalidade. Os fatores de risco para o DMG e IUEG estimulam investigações capazes de entender a fisiopatologia comum entre elas, na busca do entendimento dos mecanismos neuroimunoendócrino.

Os resultados obtidos na tese estão apresentados em 3 artigos publicados:

Artigo 1 - França DCH, França EL, Sobrevia L, Barbosa AMP, Honorio-França AC, Rudge MVC; Diamater Study Group. Integration of nutrigenomics, melatonin, serotonin and inflammatory cytokines in the pathophysiology of pregnancy-specific urinary incontinence in women with gestational diabetes mellitus. Biochim Biophys Acta Mol Basis Dis. 2023 Aug;1869(6):166737. doi:10.1016/j.bbadis.2023.166737.

Artigo 2 - França DCH, Honorio-França AC, Silva KMR, Alves FCB, Bueno G, Costa SMB, Cotrim ACM, Barbosa AMP, França EL, Rudge MVC, et al. Serotonin and interleukin 10 can influence the blood and urine viscosity in gestational diabetes mellitus and pregnancy-specific urinary incontinence. Int J Mol Sci. 2023;24(24):17125. doi:10.3390/ijms242417125.

Artigo 3 - França DCH, França EL, Honório-França AC, Silva KMR, Queiroz AAd, Morais TC, França ECH, de Carvalho CNF, Fagundes-Triches DLG, Barbosa AMP, et al. Melatonin and inflammatory cytokines as modulators of the interaction between gestational diabetes mellitus and pregnancy-specific urinary incontinence. Metabolites. 2025;15:699. doi:10.3390/metabo15110699.

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Seção 3
Resumo da Tese

França, D. C. H. Serotonina, melatonina e citocinas inflamatórias como potenciais mediadores na interação Diabetes Mellitus Gestacional e Incontinência Urinária Específica da Gestação. 2025. Tese de Doutorado – Faculdade de Medicina de Botucatu (FMB), Universidade Estadual Paulista (UNESP), Brasil.

Introdução: O diabetes mellitus gestacional (DMG) e a incontinência urinária específica da gestação (IUEG) representam condições clínicas prevalentes com impacto direto na qualidade de vida e nos custos de saúde pública. Diversos estudos têm apontado a associação entre o DMG e a ocorrência de IUEG, sugerindo que a hiperglicemia materna pode comprometer o funcionamento do músculo detrusor. Essa alteração é potencializada por um ambiente pró-inflamatório decorrente do desequilíbrio entre citocinas inflamatórias e hormônios reguladores, como a serotonina e a melatonina. Contudo, ainda há lacunas na literatura quanto à compreensão dos mecanismos neuroimunoendócrinos envolvidos nesse processo fisiopatológico.

Justificativa: A identificação de biomarcadores hormonais e inflamatórios que possam predizer ou explicar a IUEG associada ao DMG pode auxiliar na prevenção e manejo precoce dessa condição. A literatura aponta que alterações nos níveis de melatonina e serotonina, bem como nos seus receptores e nas citocinas inflamatórias, podem desempenhar papel central na fisiopatologia do DMG e levar à uma maior predisposição à incontinência urinária. Portanto, investigar essas moléculas pode oferecer suporte ao desenvolvimento de estratégias nutricionais, farmacológicas ou hormonais voltadas à saúde materna.

Objetivos: Artigo 1) O artigo teve como objetivo integrar os conhecimentos sobre nutrigenômica, melatonina, serotonina e citocinas inflamatórias na compreensão da fisiopatologia da IUEG em mulheres com DMG. Ele buscou evidenciar como alterações no metabolismo materno induzidas pela hiperglicemia podem criar um ambiente inflamatório que afeta hormônios e sistemas musculares, favorecendo o desenvolvimento da IUEG.

Artigo 2) Avaliar os níveis de serotonina e citocina IL-10 no plasma e na urina de mulheres grávidas, entre 24 e 28 semanas de gestação, classificadas quanto à presença de DMG e IUEG, além de examinar como esses biomarcadores influenciam a viscosidade do sangue e da urina, com e sem adição exógena de serotonina e IL-10.

Artigo 3) O artigo tem como objetivo investigar o papel da melatonina, seus receptores (MT1 e MT2) e citocinas inflamatórias na interação entre DMG e a IUEG. A hipótese central foi que alterações nos níveis hormonais e imunológicos estariam associadas ao desenvolvimento de IUEG em mulheres com DMG, influenciando a função do trato urinário e a atividade muscular lisa por meio de mecanismos inflamatórios e hormonais.

Métodos: Artigo 1) Trata-se de uma revisão da literatura que reúne achados clínicos, epidemiológicos, experimentais e moleculares. O estudo integra informações de áreas como nutrigenômica, epigenética, endocrinologia, imunologia e fisiologia muscular para compreender os mecanismos interligados entre nutrientes, expressão gênica, inflamação e regulação hormonal, particularmente da melatonina e serotonina, no contexto do DMG e IUEG. A abordagem incluiu dados de estudos com humanos e modelos animais.

Artigo 2) Foram coletadas amostras de sangue e urina de gestantes com e sem diabetes gestacional e incontinência urinária específica da gestação, e avaliadas quanto aos níveis de serotonina e IL-10 e à viscosidade após adição exógena dessas substâncias.

Artigo 3) Trata-se de um estudo com gestantes divididas em quatro grupos com base na presença ou ausência de DMG e IUEG. As participantes responderam a questionários validados sobre incontinência urinária, e amostras de sangue e urina foram coletadas para análise dos níveis de melatonina, sulfato de melatonina, receptores de melatonina (MT1 e MT2) e citocinas inflamatórias (IL-1β, IL-6, IL-8, IL-10, IL-12, TNF-α). As análises foram realizadas por ELISA e citometria de fluxo, com estatísticas aplicadas para comparação entre os grupos.

Resultados: Artigo 1) A revisão demonstrou que em gestantes, o DMG leva à inflamação sistêmica com aumento de citocinas inflamatórias, alteração na produção de melatonina e serotonina, o que leva à consequente disfunção do músculo detrusor. Essas alterações contribuem para a IUEG. Além disso, a modulação nutricional pode interferir na expressão gênica e na resposta imunológica, apontando para possíveis estratégias preventivas e terapêuticas baseadas em alimentação funcional e controle glicêmico adequado.

Artigo 2) Os níveis de serotonina no plasma diminuíram, enquanto os níveis de serotonina na urina aumentaram nos grupos normoglicêmico com incontinência (NG-I), diabetes gestacional e continência (DMG-C) e diabetes gestacional com incontinência (DMG-I), em comparação ao grupo controle (NG-C). A IL-10 no plasma diminuiu no grupo DMG-I e foi mais elevada na urina nos grupos NG-I e DMG-I. A viscosidade do sangue foi maior, independentemente da incontinência urinária, nos grupos com DMG. O sangue e a urina, na presença de IL-10, apresentaram viscosidade semelhante em todos os grupos estudados

Artigo 3) Os resultados mostraram que gestantes com DMG e IUEG apresentaram níveis mais baixos de melatonina e IL-10, e níveis mais altos de MT1, MT2, IL-1β, IL-8 e TNF-α no sangue, indicando um ambiente inflamatório. A melatonina-sulfato (6-sulfaxotimelatonina) também foi reduzida nesse grupo. Correlações foram encontradas entre melatonina e IL-10 (diretamente proporcional) e TNF-α (inversamente proporcional), sugerindo que a redução de melatonina e o desequilíbrio imunológico podem contribuir para a patogênese da IUEG em gestantes com DMG.

Conclusão: Os dados indicam que a serotonina, a melatonina e as citocinas inflamatórias são mediadores na interação diabetes mellitus gestacional e incontinência urinária específica da gestação. A IUEG em gestantes com DMG está associada a um perfil inflamatório caracterizado por redução de citocinas anti-inflamatórias e aumento das pró-inflamatórias, além de desequilíbrios nos níveis séricos e urinários desses hormonios e seus receptores. A melatonina e a serotonina surgem, dessa forma, como potenciais biomarcadores e possível futuro alvo terapêutico para essa condição, dada sua relação com estresse oxidativo, modulação do sistema imune e ação no músculo detrusor.

Palavras-chave: Diabetes Gestacional; Incontinência Urinária; Citocinas; Melatonina; Serotonina; Hormonios.

Thesis Abstract

França, D. C. H. Serotonin, melatonin and inflammatory cytokines as potential mediators in the interaction between Gestational Diabetes Mellitus and Pregnancy-Specific Urinary Incontinence. 2025. Doctorate Thesis- Botucatu Medical School (FMB), São Paulo State University (UNESP), Brazil.

Introduction: Gestational diabetes mellitus (GDM) and pregnancy-specific urinary incontinence (PSUI) represent prevalent clinical conditions with a direct impact on quality of life and public health costs. Several studies have pointed to the association between GDM and the occurrence of PSUI, suggesting that maternal hyperglycemia may compromise the functioning of the detrusor muscle. This alteration is potentiated by a pro-inflammatory environment resulting from an imbalance between inflammatory cytokines and regulatory hormones, such as serotonin and melatonin. However, there are still gaps in the literature regarding the understanding of the neuroimmunoendocrine mechanisms involved in this pathophysiological process.

Justification: The identification of hormonal and inflammatory biomarkers that can predict or explain PSUI associated with GDM may aid in the prevention and early management of this condition. Literature suggests that alterations in melatonin and serotonin levels, as well as in their receptors and inflammatory cytokines, may play a central role in the pathophysiology of GDM and lead to a greater predisposition to urinary incontinence. Therefore, investigating these molecules may support the development of nutritional, pharmacological, or hormonal strategies aimed at maternal health.

Objectives: Article 1) The article aimed to integrate knowledge about nutrigenomics, melatonin, serotonin, and inflammatory cytokines in understanding the pathophysiology of PSUI in women with GDM. It sought to highlight how alterations in maternal metabolism induced by hyperglycemia can

create an inflammatory environment that affects hormones and muscle systems, favoring the development of PSUI.

Article 2) To evaluate the levels of serotonin and IL-10 cytokine in the plasma and urine of pregnant women, between 24 and 28 weeks of gestation, classified according to the presence of GDM and PSUI, in addition to examining how these biomarkers influence blood and urine viscosity, with and without exogenous addition of serotonin and IL-10.

Article 3) The article aims to investigate the role of melatonin, its receptors (MT1 and MT2) and inflammatory cytokines in the interaction between GDM and PSUI. The central hypothesis was that alterations in hormonal and immunological levels would be associated with the development of PSUI in women with GDM, influencing urinary tract function and smooth muscle activity through inflammatory and hormonal mechanisms.

Methods: Article 1) This is a literature review that brings together clinical, epidemiological, experimental and molecular findings. The study integrates information from areas such as nutrigenomics, epigenetics, endocrinology, immunology, and muscle physiology to understand the interconnected mechanisms between nutrients, gene expression, inflammation, and hormonal regulation, particularly of melatonin and serotonin, in the context of GDM and PSUI. The approach included data from studies with humans and animal models.

Article 2) Blood and urine samples were collected from pregnant women with and without GDM and pregnancy-specific urinary incontinence, and evaluated for serotonin and IL-10 levels and viscosity after exogenous addition of these substances.

Article 3) This is a study with pregnant women divided into four groups based on the presence or absence of GDM and PSUI. Participants answered validated questionnaires about urinary incontinence, and blood and urine samples were collected for analysis of melatonin levels, melatonin sulfate, melatonin receptors (MT1 and MT2), and inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12,

TNF-α). Analyses were performed by ELISA and flow cytometry, with statistics applied for comparison between groups.

Results: Article 1) The review demonstrated that in pregnant women, GDM leads to systemic inflammation with increased inflammatory cytokines, altered melatonin and serotonin production, which leads to consequent detrusor muscle dysfunction. These alterations contribute to PSUI. In addition, nutritional modulation can interfere with gene expression and immune response, pointing to possible preventive and therapeutic strategies based on functional nutrition and adequate glycemic control.

Article 2) Plasma serotonin levels decreased, while urinary serotonin levels increased in the normoglycemic incontinence (NG-I), gestational diabetes and continence (GDM-C), and gestational diabetes incontinence (GDM-I) groups, compared to the control group (NG-C). Plasma IL-10 decreased in the GDM-I group and was higher in urine in the NG-I and GDM-I groups. Blood viscosity was higher, regardless of urinary incontinence, in the GDM groups. Blood and urine, in the presence of IL-10, showed similar viscosity in all groups studied.

Article 3) Results showed that pregnant women with GDM and PSUI had lower blood levels of melatonin and IL-10, and higher levels of IL-1β, IL-8, and TNF-α, indicating an inflammatory environment. Urinary melatonin-sulfate (6-sulfaxothymelatonin) was also reduced in this group. Correlations were found between melatonin and IL-10 (directly proportional) and TNF-α (inversely proportional), suggesting that reduced melatonin and immune imbalance may contribute to the pathogenesis of PSUI in pregnant women with GDM.

Conclusion: The data indicate that serotonin, melatonin, and inflammatory cytokines are mediators in the interaction between gestational diabetes mellitus and pregnancy-specific urinary incontinence. Urinary incontinence in pregnant women with gestational diabetes mellitus (GDM) is associated with an inflammatory profile characterized by a reduction in anti-inflammatory cytokines and an increase in pro-inflammatory cytokines, as well as imbalances in blood

and urinary levels of these hormones and their receptors. Melatonin and serotonin thus emerge as potential biomarkers and possible future therapeutic targets for this condition, given their relationship with oxidative stress, modulation of the immune system, and action on the detrusor muscle.

Keywords: Gestational Diabetes; Urinary Incontinence; Cytokines; Melatonin; Serotonin; Hormones.

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Review

Integration of nutrigenomics, melatonin, serotonin and inflammatory cytokines in the pathophysiology of pregnancy-specific urinary incontinence in women with gestational diabetes mellitus

Danielle Cristina Honorio França [a,*], Eduardo Luzía França [b], Luis Sobrevia [a,c,d,e,f,g], Angélica Mércia Pascon Barbosa [a,h], Adenilda Cristina Honorio-França [b], Marilza Vieira Cunha Rudge [a,*], Diamater Study Group [1]

[a] Department of Gynecology and Obstetrics, Botucatu Medical School (FMB), São Paulo State University (UNESP), Botucatu 18618-687, Brazil
[b] Institute of Biological and Health Science, Federal University of Mato Grosso (UFMT), Barra do Garças 78605-091, Brazil
[c] Cellular and Molecular Physiology Laboratory (CMPL), Department of Obstetrics, Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago 8330024, Chile
[d] Department of Physiology, Faculty of Pharmacy, Universidad de Sevilla, E-41012 Seville, Spain
[e] Faculty of Medicine and Biomedical Sciences, University of Queensland, Herston, QLD 4029, Australia
[f] Department of Pathology and Medical Biology, University of Groningen, 9713GZ Groningen, the Netherlands
[g] Tecnologico de Monterrey, Eutra, The Institute for Obesity Research (IOR), School of Medicine and Health Sciences, Monterrey 64710, Mexico
[h] Department of Physiotherapy and Occupational Therapy, School of Philosophy and Sciences, São Paulo State University (UNESP), Marilia 17525-900, Brazil

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ABSTRACT

Gestational diabetes mellitus is an important public health problem and has been associated with the development of pregnancy-specific urinary incontinence. The interaction is related to hyperglycemia, and inflammatory and hormonal patterns, which favor functional alterations in different organs and systems. Several genes associated with human diseases have been identified and partially characterized. Most of these genes are known to cause monogenic diseases. However, about 3 % of diseases do not fit the monogenic theory due to the complex interactions between multiple genes and environmental factors, as in chronic metabolic diseases such as diabetes. The nutritional, immunological, and hormonal patterns associated with changes in maternal metabolism may influence and contribute to greater susceptibility to urinary tract disorders. However, early systematic reviews have not yielded consistent findings for these associations. This literature review summarizes important new findings from integrating nutrigenomics, hormones, and cytokines in women with Gestational diabetes mellitus and pregnancy-specific urinary incontinence. Changes in maternal metabolism due to hyperglycemia can generate an inflammatory environment with increased inflammatory cytokines. This environment modulated by inflammation can alter tryptophan uptake through food and thus influence the production of serotonin and melatonin. As these hormones seem to have protective effects against smooth muscle dysfunction and to restore the impaired contractility of the detrusor muscle, it is assumed that these changes may favor the onset of urinary incontinence specific to pregnancy.

1. Nutrigenomic and intrinsic factors

Nutritional research has long sought knowledge about nutrients to meet the body's essential needs. However, in recent years a new strand has emerged to seek alternatives to improve the health of individuals through food. Thus, modern nutritional research aims at health promotion, performance improvement, and disease prevention. Thus came the concept of the development of nutritionally enriched or functional foods, which are part of the diet and contains, in addition to essential nutrients, bioactive components capable of reducing the risk of diseases.

* Corresponding authors.
E-mail addresses: daniellechfranca@gmail.com (D.C.H. França), eduardo.franca@ufmt.br (E.L. França), lsobrevia@uc.cl (L. Sobrevia), adenilda.franca@ufmt.br (A.C. Honorio-França), marilzarudge@gmail.com (M.V.C. Rudge), diamaterstudy@gmail.com (Diamater Study Group).
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Reported healthy millennial dietary practices have led to the concept of functional foods capable of preventing or treating some diseases [1]. Therefore, identifying biologically active molecules is important in this new vision of nutritional research to understand these prevention and protection mechanisms. These molecules' actions comprise an antioxidant, hemodynamic, modulation effects endocrine metabolism, hormonal control, immunological functions, and inflammatory processes [2].

Reddy et al. [3] define health and disease through the "molecular fingerprint," the hierarchical information around the DNA-RNA-protein-metabolite. This state does not represent the same thing for everyone but depends on the time, place, social class, environment, and diet. So likewise, some studies have shown that the use of diet to prevent and treat disease may differ between individuals, types of foods, and diseases. Furthermore, the disease development mechanisms may involve genetic susceptibility factors, such as variant genes and polymorphisms [4–6].

Thus, several works report the manifestation of individual genetic traits such as single nucleotide polymorphisms (SNPs), copy number polymorphisms (CNPs) and epigenetic phenomena, diet, and the appearance of some diseases [7–10]. Jaenisch et al. [9] describe that epigenetic mechanisms make the organism respond to gene expression changes by environmental interference. Corella et al. [10] report that multiple genes are involved in the health and disease process and that complex interactions occur between genetic and environmental factors. The genomic diversity of affected individuals, absorption and metabolism of nutrients, differences in dietary habits, availability and choices of nutrients are linked by cultural, geographic, economic, and gustatory perceptions and malnutrition [11,12]. Thus, to define a "healthy" or "diseased" phenotype, nutrigenomics addresses the effects of diet on gene transcription, protein expression, and metabolism [10,11] through the integration of genomics (gene analysis), transcriptomics (gene expression analysis), proteomics (global protein analysis) and metabolomics (metabolite profiling) to define a "healthy" or "diseased" phenotype.

Nutritional genomics is based on the premises that diet can alter gene expression; may be a risk factor for diseases, whereas some diet-regulated genes may affect the incidence, onset, and progression of chronic diseases [13], that the degree of impact of the diet on the health/disease balance may depend on the genetic composition of the individual and that dietary intervention according to nutritional status and individual nutritional needs and genotype can prevent chronic diseases [14]. Voruganti [15] linked bioactive foods and nutraceuticals as beneficial to cardiovascular health. Baldassare et al. [16] suggest that supplement use may reduce the development of diabetes and control the future onset of cardiovascular disease.

Advances and precision in genomic sequencing and their respective transcriptomes and high-precision long-reading sequencing revealed genomic variation throughout the human genome, contributing to new perspectives in understanding related to gene expression and the health process and disease [17]. Furthermore, genome-wide analysis (transcriptomic) can provide a comprehensive view of all active genes at a specific time and sample and thus reveal regulatory networks through proteomic and metabolomic markers from a broader biological perspective [11].

Several genes associated with human diseases have been identified and partially characterized. Most of these genes are known to cause monogenic diseases [18]. Among these diseases, galactosemia and phenylketonuria are genetic traits linked to nutrition [19,20]. Both diseases are easy to identify and can be treated through habit changes.

However, some diseases do not fit the monogenic theory due to the complex interactions between multiple genes and environmental factors, as in chronic metabolic diseases [13]. In this way, it becomes more difficult to establish a connection between genetic factors and nutritional aspects.

Excessive energy consumption, different types of fats, proteins, carbohydrates, and micronutrient deficiencies have been associated with obesity and diabetes. Rana et al. [21] showed that dietary factors are crucial in developing chronic diseases such as obesity and diabetes mellitus. Helm et al. [22] report that few studies have evaluated the impacts of nutrition and clinical outcomes in individuals with Gestational Diabetes Mellitus (GDM) and suggest that nutritional education in women with gestational diabetes can control hyperglycemia and adverse complications. In a meta-analysis study, Zhang et al. [23] suggest that dietary supplements may improve maternal metabolism and pregnancy outcomes in women with Gestational Diabetes Mellitus. Also, Vitacolonna et al. [24] related that nutritional, medical therapy, and lifestyle intervention are essential for preventing and treating GDM.

Strategies to identify susceptibility genes for obesity and GDM have been proposed, including positional cloning and microarray-based transcriptomic profiling, combining this profiling with critical genetic analysis [25].

In animal models, transcriptomic studies of animal insulin resistance states have been reported [26,27]. In genetically modified mice, disorders of fatty acid metabolism, nutrigenomics for insulin resistance, and diabetes mellitus were studied [27]. Furthermore, human and animal studies have shown insulin negatively correlated with INSR mRNA in skeletal muscle [28]. The profile of gene expression in skeletal muscle tissues of obese individuals, sensitive or resistant to insulin [27] or with type 2 diabetes mellitus [29], and potential regulatory genes in individuals with insulin resistance [30,31] were also addressed. Samra et al. [32] suggest that genetic variants and miRNAs may be potential biomarkers for GDM and the combination of molecular markers with serum proteins to predict GDM.

Also, analyses of integrative bioinformatics associated with analyses of gene pathways and networks, identification of transcription factors, and correlation of gene expression profiles associated with diseases and drug intake revealed that these genes are involved in metabolism or cell signaling [33] and suggest that nutritional therapy, together with appropriate pharmacological treatment, may be important in the control of DM2 and GDM, aiming to improve glycemic control.

Despite many studies related to the intrinsic mechanisms that involve these metabolic diseases, there are still several gaps that need to be explained in an attempt to develop effective protocols through a dietary intervention aimed at meeting individual nutritional needs, to improve the expression of certain genes or even gene suppression to prevent the establishment of phenotypes capable of associating with chronic and metabolic diseases.

2. Nutrigenomic in metabolic diseases

The development of effective strategies to combat metabolic diseases is highly complex due to the wide etiology involving these diseases, such as genetic, physiological, and socioeconomic [34,35], and environmental factors, such as unbalanced nutrition and sedentary lifestyle, over the years [36–39].

Nutrigenomics focused on the appropriate nutritional aspect is relevant, especially in the current social context where the consumption of foods characterized by a lipid and glucose caloric intake has increasingly replaced a food base on waxes, vegetables, and foods with fewer sugars, trans and saturated fats [40]. Mondal and Panda [41] alert the need for research linking nutrigenomics to curing diseases caused by a deficiency in nutritious foods. Macronutrient deficiency has been linked to DNA damage and hormonal changes. The serotonin transporter gene SLC6A4 plays a significant role in eating disorders and chronic diseases [42]. Tryptophan deficiency has been associated with changes in the concentration of the hormone serotonin and its precursor melatonin [43]. Melatonin-rich diets associated with chronotherapy have been proposed to prevent or treat metabolic disorders [44].

High serum glucose and cholesterol levels promoted by inadequate nutrition represent an important cause of morbidity and mortality due to the higher risk of chronic diseases, such as cardiovascular diseases and

diabetes mellitus [40,45]. Thus, nutrigenomics becomes important in clinical practice, in the prevention and treatment of metabolic diseases [46,47], mainly because, in the long term, it has an individual and personalized Omics integration function of nutrition, aiming at improvements in the human microbiome [48,49].

The increase in metabolic diseases in adults, especially in women, may impact the health indices of the future generation. Furthermore, due to the rapidly increasing prevalence of these diseases in women during pregnancy worldwide, the short- and long-term adverse consequences in future generations pose a major risk to public health in the 21st century [50]. Therefore, dietary and lifestyle interventions in obese or diabetic women have suggested beneficial effects [50,51].

Maternal nutritional status before pregnancy can have consequences for the child [52], with an increased risk of developing metabolic disorders, such as diabetes [53]. However, the impact of diet on health conditions can manifest itself in almost any stage of life: even the early influence on the form of pre, *peri*, and postnatal nutrition can interfere with metabolic programming with reflexes in adulthood [52–56].

Diabetes mellitus (DM), which is a metabolic syndrome of multiple etiologies with changes in glucose metabolism, evidenced by hyperglycemia, resulting from defects in insulin action, secretion, or both [57], is a disease that causes impact not only due to its clinical condition related to hyperglycemia but mainly due to functional changes that occur in different organs and systems, resulting from the chronic metabolic control of protein and lipid metabolism [58].

Studies have related the predisposition of type 2 diabetes mellitus (T2DM), before pregnancy, in obese patients [55] and the increase in gestational diabetes in obese women [59–61] since many factors link these two diseases. Furthermore, gestational diabetes and the associated perinatal complications are also important public health problems, with the rates increasing [62]. Among these factors not yet fully discovered impacting obesity and diabetes is the inflammatory microenvironment, and studies in this field are very interesting and may bring new perspectives [63].

The inflammatory process in obesity occurs due to chronic excess nutrients that induce insulin resistance and glucose metabolism impairment. Several hypotheses corroborate the association between diabetes and obesity. Among these, intracellular lipids can serve as lipotoxic substances directly inhibiting muscle insulin activity. In addition, the liver [64], inflammatory cytokines, and chemokines contribute to T2DM [65,66]. The activation of key inflammatory mediators, such as c-Jun N terminal kinase (JNK) and IkB kinase (IKK), also contribute to insulin resistance associated with obesity and changes in glucose metabolism [67].

The reaction of inflammation due to obesity and hyperglycemia may be one of the main events leading to complications in the future. Furthermore, it is known that diabetes can impact pre- and postnatal life, and the initial diet is essential to reduce metabolic diseases. Thus, during pregnancy, especially in women with metabolic diseases, it is necessary to reduce complications during this period and in the future [68].

Studies show that nutrition in early life is crucial and may be important for establishing future diseases, including metabolic diseases such as obesity and diabetes. Many works report that colostrum and human milk, as the first natural food to be offered in the postnatal period, are important functional foods that can function as a protective factor against various diseases over a long time. Furthermore, this initial imprint through human milk can modulate some body systems, including immune, neurological, and endocrine systems, causing potential long-term health effects, thus highlighting the importance of human milk as a major influence on the correct development of modification of disease susceptibility [69].

It is widely disseminated in the scientific literature that human milk protects against obesity at a later stage, playing an important role in the process of "metabolic programming" in early life [70]. Among the possible mechanisms related to this protection, it is suggested that this functional food acts against rapid weight gain in the postnatal period and consequently for obesity at a later stage of life, promoting a better development of self-regulation of food intake and bioactive substances of breast milk participating in the process of "metabolic programming," changing, for example, the number and/or size of adipose cells or inducing the phenomenon of metabolic differentiation [71].

Human milk contains a balanced content of macro and micronutrients essential for child growth and development, and its nutritional importance has been widely described. It is known that the composition of this secretion undergoes inter- and intra-individual variations. These variations in breast milk can be affected by factors such as maternal diet, nutritional status, smoking, childbirth, period of the day [72–74], and metabolic diseases [70,71]. Babies born and breastfed by women with high pre-gestational Body Mass Index (BMI) may be heavier and predisposed to develop obesity and related disorders in adulthood [74]. Fujimori et al. [75] showed that human milk of obese mothers had higher glucose ($p = 0.002$), triglycerides ($p = 0.008$), and cholesterol ($p = 0.001$) concentrations. Morceli et al. [76] verified in human colostrum of diabetic mothers, higher glucose ($p = 0.0008$), lipase levels ($p = 0.04504$), and lower fat content ($p = 0.0249$). However, there are still gaps in the effects of maternal weight on the glucose content of breast milk, its impact on weight gain and infant metabolism, and the risk of developing metabolic diseases.

Human milk has also been shown to reduce the incidence of acute illnesses and decrease the risk of many chronic diseases. However, studies on the long-term effects of this functional food have shown that it affects blood pressure, obesity/overweight, and diabetes [77]. The absence of breastfeeding or inadequately is recognized as a primary contributing factor to the future development of diabetes. Adequate breastfeeding is associated with a 24 % reduction in the risk of diabetes in childhood, and breastfeeding time is directly related to the risk of diabetes [78–80]. Thus, nutritional therapy and adequate glycemic control are important in diabetic pregnant women, aiming to prevent or delay complications both maternal and for their children.

Clinical, epidemiological, and experimental studies suggest that diet is implicated in the etiopathogenesis of diabetes [81]. In women with gestational diabetes, breastfeeding exclusively for at least six months postpartum decreases the risk of developing type 2 DM. In addition, breastfeeding is protective against the risk of the newborn developing obesity during infancy [77].

This functional food also contains several bioactive components, including hormones such as melatonin and serotonin. Melatonin in human milk exhibits circadian variation, with the highest concentrations observed at night, while serotonin is highest during the day. The circadian variation of melatonin suggests a physiological profile and helps in the synchronization between mother and child [72]. Nutritional and immunological components in human milk also show circadian variation and can be altered due to hyperglycemia [72,76,82–84]. Fig. 1 shows the main nutritional, hormonal, and immunological components in human milk that show circadian variation or present changes in the function of hyperglycemia.

Given the complexity of the bioactive components present in human milk and their actions for both the mother and her children, as well as their ability to act without causing inflammatory processes [85], it highlights the need to study the effects in the medium and long-term effects of hormonal, immunological, and nutritional interactions [69] and their possible effects on metabolic and immune programming.

3. Gestational diabetes mellitus: the impact of nutrigenomic

During lifelong development, there are several important key periods: conception, pregnancy, childhood, and puberty. Thus, exposure scans to intrinsic or extrinsic factors can protect or predispose individuals to developing metabolic diseases. According to the integrative theory of the "Origins of Health Development and Disease," the origin of the diseases, such as cancer, cardiovascular diseases, neuropsychiatric,

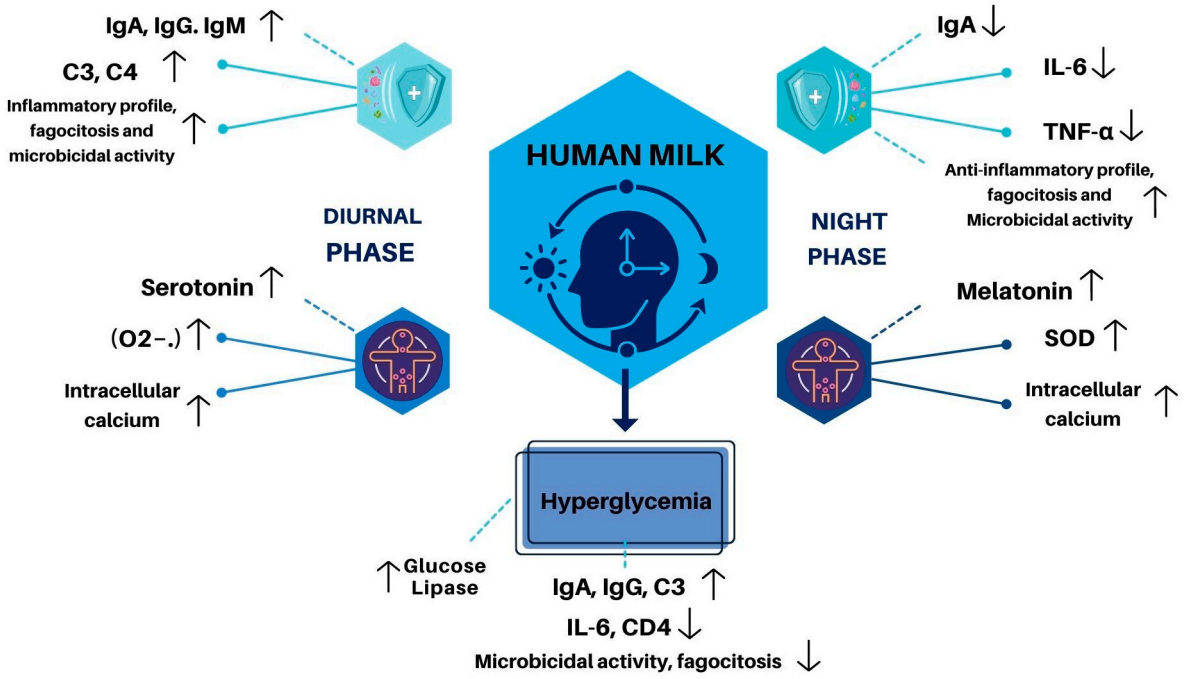


Fig. 1. The nutritional, hormonal, and immunological components in human milk present circadian fluctuations and changes in the function of hyperglycemia. Immunoglobulin A (IgA); immunoglobulins G (IgG); immunoglobulins M (IgM), complement 3 (C3) and complement (C4), superoxide anion ($O_2^{-\bullet}$) interleukin 6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α), Superoxide dismutase (SOD), CD4 T lymphocytes (CD4 cells).

obesity, and diabetes, is related to early exposure to the fetal microenvironment [86].

Maternal metabolic changes and the impact of metabolism on the next generation have aroused much interest in the scientific community. Gestational Diabetes Mellitus is a metabolic disorder that begins or is diagnosed during pregnancy. GDM can be considered any glucose intolerance of varying magnitude during pregnancy. Elevation of insulin counter-regulatory hormones explains its pathophysiology during pregnancy, adding to the physiological stress of this period and previous determinants of the pregnant woman, such as environmental and genetic factors [87–89]. This disease is a notable example that fits with fetal metabolic programming and hypotheses of the developmental origins of health and disease, as fetal exposure to diabetes and diabetes-related metabolic disorders can alter the functional development of organs and, thus, potentially increase the infant mortality rate [90,91].

GDM is one of the most common complications in pregnancy and exhibits prevalence in many countries, ranging from 3 % to 28 %, depending on the population studied and the applicable diagnostic criteria [92–95], and usually regresses after childbirth [96]. In addition, GDM is associated with an increased risk of maternal complications. The consequence for the newborn with metabolic alteration, steatosis, overweight, epigenetic programming, and less access to breast milk may contribute to future diseases since children of mothers with GDM are at higher risk of developing DM2 later in life [97–100].

GDM also is a state of low-grade systemic inflammation [101], capable of influencing several systems, including the neurological and immune systems. Thus, nutritional care with adequate glycemic control in pregnant women with GDM is essential to minimize such changes [102–104]. Also, during pregnancy, adequate nutritional status, resulting from the association of a balanced diet and weight gain within normal ranges, contributes to the reduction of maternal and child morbidity and mortality since GDM represents one of the situations of high gestational risk [105].

Pregnancy promotes changes in diet, insulin sensitivity, and hormone production, which results in changes capable of causing reflexes in carbohydrate metabolism and insulin needs [105]. In the state of diabetes or hyperglycemic conditions, the deficiency in the production or action of insulin leads to changes in the metabolism of carbohydrates, lipids, and proteins, with a significant impact on pregnancy and the intrauterine environment, especially when treated inadequately [106,107].

It is worth mentioning the role of nutrigenomic in searching for gene-diet associations to find associations between genes, foods, and outcomes, such as intermediate risk factors or disease. Long-term nutrigenomics is to improve the health of individuals and thus prevent diseases, but research in nutritional genomics often links aberrant diet-influenced phenotypes to one or more genes [108].

Dietary factors are important contributors to chronic diseases, and poor eating habits are recognized as one of the main determinants of risks for metabolic diseases, including diabetes mellitus [109,110]. The study, evaluating the clinical aspect and role of new nutrigenetic markers and traditional parameters, followed 29 women with a history of GDM for three years postpartum to predict early subclinical atherosclerosis and identified nine single nucleotide polymorphisms (SNPs) from nine genes related to nutrition and metabolism [111]. Another study evaluating clinical, nutritional, and metabolic aspects of women with GDM in association with SNPs showed a strong association between the rs7903146 variant of the TCF7L2 gene and the risk of developing GDM (OR: 2.56; 95 % CI: 1.24–5.29) and positive correlation between lipid parameters and polymorphisms of PPARG2 ($p = 0.03$), APOA5 ($p = 0.02$), MC4R ($p = 0.03$), LDLR ($p = 0.04$) and FTO ($p = 0.03$). They also showed an association between pre-gestational BMI ($p = 0.02$) and the rs17782313 variant, close to the MC4R gene, in women with GDM. The authors concluded that to develop alternatives to prevent GDM must be proposed that associate nutritional parameters and genetic variants involved in maternal nutrition and metabolism [88]. Some genes have also been associated with the pathophysiology of GDM. Yahaya et al. [112] identified 83 genes associated with GDM, with the TCF7L2, MTNR1B, CDKAL1, IRS1, and KCNQ1 genes having the highest prevalence. The GCK, KCNJ11, IGF2BP2, and MTNR1B genes have also been associated with GDM. Interestingly, these genes also share T2DM risk [6,113–115] and insulin resistance and defects in insulin secretion that

are common features in the pathogenesis of both clinical conditions.

Galaleti et al. [116], evaluating the gene expression profile of whole blood cells in pregnant women, showed that the screening profile for gestational diabetes could be separated into two populations. Genetic separation can influence the best diagnostic method of screening for gestational diabetes. Wang et al. [117] analyzed the association of some important genes in the placental tissue of women with GDM. They identified the expression of 238 different genes, of which 76 were down-regulated and 162 up-regulated. The genes for chemokines CXCL9 and CXCL10, protein tyrosine phosphatase, C-type receptor (PTPRC), and human leukocyte antigen (HLA) have also been identified as central genes associated with immune responses, as well as thioredoxin and miR-2 binding proteins223-3p, miR-520 to GDM pathogenesis.

In patients with GDM, changes in DNA methylation, microRNA gene silencing, and histone were also observed [118]. Kwak et al. [119] compared 1399 women with GDM and 2025 non-diabetic women across 2.19 million genotyped or imputed markers and managed to select 11 loci for genotyping in samples of 931 with GDM and 783 non-diabetics. Ren et al. [120] evaluated MTNR1B and the association with diabetes-related features in 1383 point subjects and followed 374 subjects for 3 to 5 years. Finally, Dalfrà et al. [121] reported that genetic, epigenetic, and environmental factors might be determinants for the onset of GDM and suggest similarities between susceptibility genes to DM2 and GDM.

Although dietary factors and poor eating habits contribute to impact the incidence of chronic diseases and are recognized as one of the main determinants of metabolic disease risk [109], one must consider the complexity of analyzing multiple genes in multiple metabolic pathways involved in GDM pathogenesis., which makes it difficult to interpret genetic tests linked to knowledge of nutrients and specific diets.

Integrated care for pregnant women diagnosed with GDM is essential to reduce the possible complications resulting from the disease. It is also important to use postpartum measures to encourage breastfeeding, as this food is crucial for the metabolism of these children in adulthood [122,123].

4. Gestational diabetes mellitus with pregnancy-specific urinary incontinence associated

Individuals' genetics inherited from their ancestors may be responsible for variations in responses to food and susceptibility to chronic diseases [13]. Diabetes associated with maternal malnutrition, eating disorders, and complications such as immunological changes or kidney disease can impact maternal health and future generations [124,125]. Also, proper nutrition and lifestyle are part of the prevention and treatment strategy for metabolic diseases and urinary incontinence, especially during pregnancy [47].

In this way, GDM has been related to the increased risk for urinary incontinence [126], having an important social [127] and economic impact, with the high direct and indirect cost of public health due to the association of the two pathologies [128,129].

Involuntary urine loss, also called urinary incontinence (UI), affects many women's quality of life in every biopsychosocial aspect [130]. Physically, it is directly linked to decreased functionality, muscle and neural control of the pelvic region, and changes in the storage capacity and control of the bladder and urinary sphincter [131]. In addition, several factors can contribute to UI, including age, multiparity, obesity, history of pelvic surgery, smoking, and chronic disease such as diabetes [132].

Urinary incontinence has been studied in the gestational period [133,134], where physiological changes in pregnancy make these women more vulnerable to urinary incontinence, such as changes in progesterone levels, increased intra-abdominal pressure and changes in the female pelvic muscles [135]. When urinary incontinence occurs for the first time in the gestational period, it is called Pregnancy-Specific Urinary Incontinence (PSUI) and may persist in the postpartum period [136].

One in three women is diagnosed with urinary incontinence [137], increasing the prevalence in women when associated with pregnancy, evolving increasingly according to gestational trimesters, with pregnancy being a risk factor for UI even in nulliparas [132].

Urinary incontinence can be classified according to the predominant type of characteristic, the most common being stress and urge incontinence, which can be present simultaneously in a mixed way [138]. Urinary incontinence, in general, can occur in drops, jets, or a large amount of urinary loss. Urge urinary incontinence (UUI) is the sudden need to urinate, culminating in urine loss [138]. Stress urinary incontinence (SUI) is very common in pregnancy [134,138] due to increased emesis requiring muscle exertion. It is important to highlight that studies have shown a proportional relationship between increased nausea and vomiting and pelvic pain [139].

Due to fetal growth and changes in uterine size, in the last two gestational trimesters and postpartum, there are changes in the pelvic floor muscles and musculoskeletal anatomical changes that can facilitate urinary incontinence, especially when delivery progresses to the vaginal route [140–142]. Studies have also shown that vaginal deliveries using the episiotomy technique increase the risk of postpartum urinary incontinence [143]. Conversely, pregnant women submitted to cesarean section have no effects of harm or protection for the risk of postpartum UI [144,145].

Pelvic floor muscles (PFM) have several urogenital functions, such as facilitating bladder emptying, assisting in the genesis of pleasure and orgasm, and sustaining the pelvic organs and the lower part of the abdominal muscles [146].

In pregnancy, associated with decreased strength of the musculature and pelvic floor ligaments [147], it is possible to perceive increased mobility of the urethra canal, which can lead the urethra to be located below this musculature and, consequently, increase the pressure on the bladder neck, thus causing the loss of control of the urinary sphincter and involuntary extravasation of urine [140].

The rectoabdominal muscles, during pregnancy, may also be affected, suffering from the process of distancing the alba line called muscular diastasis. Some studies have shown that abdominal rectus diastasis (ARD) may predict urinary incontinence during pregnancy [148,149]. It is worth noting that the strength of this muscle group also decreases as pregnancy progresses [149]. Therefore, rectoabdominal muscle (RAM) and pelvic floor muscle (PFM) dysfunctions may be associated with the development of pregnancy-specific urinary incontinence [150].

To prevent urinary incontinence during pregnancy, pelvic muscle training can be performed even postpartum [151]. In addition, nutrigenomic aspects may also be related to the presence or absence of urinary incontinence in women. For example, decreased obesity, water intake, and adequate nutrition are suggested factors for improving the strength of pelvic floor muscles [152].

In patients with high blood glucose levels, there is an increase in symptoms of urinary exertion [153]. In addition, in cases of metabolic syndrome, glucose metabolism can lead to the relaxation of pelvic floor muscles, especially when associated with oxidative stress [154]. Thus, metabolic diseases could directly contribute as a predisposition factor for developing urinary incontinence [153].

In maternal hyperglycemia, the reduction of the normal action capacity of the hormone insulin promotes harmful effects on cellular metabolism, which can also be reflected in the muscles that can suffer consequent pelvic muscle atrophy, which facilitates the predisposition to muscle weakening and UI [155–157].

The prevalence of Pregnancy-Specific Urinary Incontinence (PSUI) among women who had GDM was previously analyzed in the literature. In a cross-sectional study, Kim et al. [158] examined the prevalence of PSUI in 228 women who had GDM and reported that 49 % of women had incontinence during weekly or more pregnancy, and 50 % reported UI within five years after childbirth. A high prevalence of obesity may have contributed to the high rate of PSUI during pregnancy and after delivery,

as it was present in 42 % of women during pregnancy and 46 % after delivery. The authors concluded that PSUI was common among women with GDM. Barbosa et al. [159] and Chuang et al. [160] independently and concomitantly observed that GDM is an independent risk factor for postpartum UI.

Furthermore, they found that women with GDM have symptoms of UEG that are a risk factor for UI two years postpartum compared to women without GDM. They concluded that GDM is an independent risk factor for postpartum UI with a significant impact on the quality of life of these women [158–160]. Piculo et al. [161] confirmed this impact on quality of life. In addition, other research has shown the impact of diabetes on muscles involved in urinary continence [162–165]. A comprehensive analysis in women with altered blood glucose levels revealed the existence of a triad characterized by GDM, PSUI, and myopathy of the rectus abdominis and pelvic floor muscles in predicting UI 6–12 months postpartum (long-UI) [166].

UI, when identified during pregnancy, besides being a health problem for women, is also a problem for offspring since enuresis has been identified in children of mothers who had urinary incontinence [167]. Enuresis is reported as a polyuria associated with changes in detrusor muscle activity [168] that leads to an inability to control the urinary sphincter. Furthermore, in children, enuresis is a symptom that has also been identified in some cases of diabetes, being an important indicator of renal involvement [169]. Thus, urinary incontinence during pregnancy is a problem that can directly affect the quality of life of the maternal-fetal binomial.

In addition, in a consensual manner, the presence of a maternal hyperglycemic environment has also been related to the increased risk of adverse effects (APO) in the perinatal period [168] in the short and long term [170] for both mother and conceptus [171].

A better understanding of GDM and the pathophysiology of pregnancy-specific urinary incontinence is essential for managing and preventing these two associated pathologies. Adequate and balanced glycemic control remains the cornerstone of prevention and defining education strategies on exercise and dietary management to improve glycemic levels.

5. Influence of immunological factors in gestational diabetes mellitus and pregnancy-specific urinary incontinence

Interconnected systems have been designed in nutritional immunology through modeling and computer methodologies combined with pre-clinical and clinical studies. These studies seek the integration of the immunological system of nutrition, the microbiome, metabolic alterations, and genetic predisposition to delineate the aspects involved in the health and disease process and predict the effects of nutrition-based interventions. This approach of integrated nutritional intervention with omics and modeling became an alternative to simulate pathophysiological mechanisms and immunological changes in organisms after nutrient intake [172].

Several dietary nutrients have functions of maintaining or improving immune activity through inhibition of pro-inflammatory mediators, promotion of anti-inflammatory action, modulation of cell-mediated immunity, alteration of antigen-presenting cell functions, and communication between the immune, neuronal, and endocrine systems [173]. In pregnant women with GDM, nutritional care for adequate glycemic control contributes to a good obstetric result. Several macronutrients and minerals have been associated with the immune system and changes in glucose metabolism. There is also evidence that reduction in some minerals, including zinc, occurs in insulin reduction, decreased insulin sensitivity, and increased inflammatory biomarkers, including cytokines and C-reactive protein [174].

Diabetes is related to a higher incidence and severity of infectious diseases [175,176]. Hyperglycemia caused by the disease seems to be responsible for this lower immunological response due to the generation of a pro-inflammatory environment capable of influencing the development of the fetus [103,177] and altering the metabolic and immunological programming of the conceptus [178–180] which can result in chronic metabolic diseases in adulthood, probably due to a differentiated schedule in the uterus and effects on fetal tissues [181].

In women with GDM, there is an increase in specific inflammatory markers [182]. The reaction of inflammation due to hyperglycemia and the immunological response may be the main events that lead to maternal complications, including changes in metabolism, increased susceptibility to infections, decreased fertility and spontaneous miscarriages, and immunological alterations [183,185].

The main immunological changes reported are changes in the percentages and function of cell types and soluble factors present in serum, such as cytokines, complement system proteins, and antibodies [102,185,186]. In addition, some studies also indicate that the association between diabetes and obesity occurs in alterations in the concentration of cytokines in T lymphocyte phenotypes [102,184,187] and monocytes/macrophages [186,188].

Infiltration of immune cells in adipose tissue, especially cell subtypes with distinct phenotypes and effector functions, has been reported [58]. In addition, macrophages play an important role during pregnancy due to their plasticity characteristic concerning polarization, in which these cells differ from specific functions and phenotypes [67].

According to their activation status, macrophages have been divided into two phenotypes, M1 and M2 [189]. M1 macrophages show increased microbicidal activity, secrete inflammatory cytokines such as IL-1β, IL-6, and TNF-α, and low levels of anti-inflammatory cytokines [190]. In contrast, M2 macrophages are anti-inflammatory cells and secrete cytokines such as IL-10 and IL-1 receptor antagonists [191]. In diabetes, macrophages play an important role in the development and progression of the disease by the secretion of cytokines by activating regulators such as the inflammasome [192]. Inflammation is a central component of the innate immune system capable of protecting from infections by pathogens, metabolism syndrome, oxidative stress, and the growth of tumors. However, excessive immunological responses mediated by inflammasomes may promote the pathogenesis of various metabolic diseases [193].

Inflammasomes are cytosolic complexes that have a high molecular weight. Its formation occurs after NOD receptors (NLRs) are activated through homotypic interactions with adaptor and effector molecules ASC and procaspase-1, respectively. There are many inflammasomes, and each is influenced by a unique response of pattern recognition receptors (PRRs) that can bind to conserved molecules typically associated with pathogens (PAMPs) or molecules associated with host cell damage (DAMPs) [194,195]. Among the NLRs receptors associated with the inflammasome assembly, NLRP3 seems physiologically important. It cleaves procaspase-1 via ASC and can be activated by endogenous and exogenous agonists [196].

Under hyperglycemic conditions, macrophages secrete cytokines by activating NLRP3 [192]. The expression of inflammasome components in adipose tissue has been attributed primarily to macrophage infiltration, and studies have suggested a possible role of the inflammasome in human insulin resistance. A positive correlation was observed between NLRP3 expression in macrophages and fasting glucose levels after 24 in patients undergoing an intervention program for weight loss. In addition, studies report a higher concentration of macrophages and expression of the NLRP3 inflammasome in individuals with decreased glucose tolerance or diabetes [195].

Close coordination between immune cells and cytokine production is required during pregnancy. These proteins can alter cell function and gene expression since they act as mediators of cell activation, proliferation, differentiation, and regulation and act in inflammatory responses. Several metabolic diseases, including GDM, are associated with the altered production of cytokines [102,103,197]. Despite being produced by various cell types, TCD4+ lymphocytes secrete most cytokines responsible for immunological processes. Cytokines considered pro-inflammatory include IL-1 interleukins (IL), IL-2, IL-6, IL-7, IL-8, IL-

17, IFN-γ (interferon-gamma), and TNF-α (tumor necrosis factor-alpha) and, among the anti-inflammatory factors, IL-4, IL-10, IL-13 and TGF-β (growth transforming factor β) [198–200].

According to the microenvironment where they are performing their functions and the cytokine production bases, the T cells were classified into Th1 (producing mainly IFN-γ), Th2 (producing mainly IL-4 and IL-13), Th17 (producing mainly IL-17) and Treg (characterized by the expression of Foxp3 and secretion of IL-10). Th1 and Th17 cells promote pro-inflammatory responses by stimulating M1 macrophages, while Th2 and Treg correlate with anti-inflammatory responses [201–203]. Fagundes et al. [102], studying women with GDM and altered BMI, found that maternal hyperglycemia modifies the phenotypes of T cells and cytokines profile in maternal and cord blood. Hara et al. [103] evaluated women with GDM, showed changes in the expression of NK cells in blood and placenta, lower IL-2 concentrations and higher IFN-γ levels in maternal and cord blood and higher levels of IL-8 in placental, and suggested that changes occur in the cells and cytokines that generate an inflammatory environment evidenced by the higher concentration of cytokines with inflammatory function and cells expressing CD16+.

Responses characterized as Th17 and T regulatory (Treg) have been pointed out as relevant factors in pregnancy [204,205]. Diabetic pregnant women have a higher concentration of IL-17 in the blood [186], and this cytokine seems to play an important immunomodulatory role in monocytes/macrophages [83]. Similarly, Treg cells have the CD4+CD25+FOXP3+ phenotype and include type 1 regulatory T cells, which secrete high amounts of IL-10 and Th3 cells, which produce TGF-β [184,206]. Queiroz et al. [184] analyzed in the placenta, maternal and cord blood of diabetic pregnant women the regulatory T cells and the expression of Fas in T cells. They suggested hyperglycemia alters Treg cells and Fas expression in memory T cells. Lobo et al. [207] observed in patients with GMD that cells produce TNF-α and IFN-γ but not IL-10, which may explain their role in the pathophysiology of diabetes. Zhang et al. [208] showed that women with GDM exhibit higher levels of Tregs of memory, suggesting that the cellular function of pregnancy memory may be related to immunity and metabolism.

Effects of cytokines on gene expression and cellular stress response have been reported to cause epigenetic impact with consequences on placental development, fetal growth, metabolic phenotype, and long-term health in children of diabetic mothers. In addition, some inflammatory cytokines, such as TNF, have been reported as embryotoxic cytokines and are elevated in plasma and reproductive tract tissues in inflammatory diseases and diabetes [209].

In pregnant women, especially those with GDM, the maintenance of urinary continence requires specific interactions between several cells and mediators [210]. For example, the bladder is an organ affected by hyperglycemia that demonstrates dysuria, frequency, urgency, incomplete bladder emptying, nocturia, stress incontinence, and recurrent cystitis, with a strong link between diabetic and neuronal changes [211]. In addition, pro-inflammatory cytokines influence the regulation of urination pathways in the brain and spinal cord and peripherally affect bladder function, directly causing hypertrophy of the detrusor muscle and hypersensitivity of the afferent nerve [212]. Several mechanisms have been proposed to explain the influence of inflammatory mediators on the urinary system, including their actions at the level of the central nervous system [87], on the central serotonergic pathways, on the supraregulation of the hypothalamus-pituitary-adrenal (HPA) axis and in the reduction of parasympathetic activity [213].

6. Serotonin and melatonin as immunomodulatory hormones in GDM and PSUI

The rise of polygenic diseases such as diabetes has been linked more predominantly to lifestyle than to changes in the nuclear genome sequence. However, evidence supporting the dissemination of genomic information for nutrigenomic purposes remains sparse [214], especially when associating two pathologies such as GDM and PSUI. Therefore, additional knowledge about neuroimmunoendocrine interactions involved in both pathologies is needed.

The neuroendocrine system is an important part of the system, massively interacting with multistage networks that define health and well-being. The circadian rhythm cyclically regulates many hormones that modulate insulin and glucose secretion. Since glucose can activate the circadian gene expression of Per1, Per2, and Bmal1, metabolism and circadian rhythms are tightly intertwined through the modulation of the expression and behavior of these genes [214,215].

In the Central Nervous System, pro-inflammatory cytokines activate the enzyme indolamine-2,3-dioxygenase (IDO). This enzyme is responsible for the degradation of tryptophan (TRP), which is the precursor of the neurotransmitter serotonin [216,217]. In addition, these proteins promote the generation of neurotoxins that contribute to the apoptosis of neurons [218,219].

Serotonin (5-hydroxytryptamine-5-HT) is an indolamine derived from the amino acid L-tryptophan. It has been associated with excellent neurological, physiological, and immunomodulatory responses [218]. Among the actions of serotonin, its role in regulating appetite and metabolism in inflammatory diseases is highlighted [219,220].

Some authors suggest that serotonin plays a neuroprotective role in chronic metabolic diseases due to its ability to modulate the production of inflammatory cytokines [219]. In addition, consuming food enriched with tryptophan increases urinary serotonin metabolites and melatonin [220,221]. This neurotransmitter plays an important but complex role in urination with actions in the brain, spinal cord, and bladder [222], suggesting that this hormone may play an important role in the pathogenesis of GDM associated with PSUI.

The consumption of foods rich in tryptophan also influences the production of melatonin [223]. Melatonin, produced by the pineal gland, is synchronized by light/dark circadian stimulus through the retinohypothalamic-pineal tract synthesized from serotonin, coming from the essential amino acid tryptophan, which has pro and anti-inflammatory action [82,224–227]. It has several functions, including regulation of circadian rhythms [228,230], pro and anti-inflammatory actions [82,225–228], and antitumor effects [229–231]. In addition, this hormone has an important immunomodulatory role in controlling metabolic diseases. In women with GDM, studies report that inflammatory cells and cytokines released by T cells are responsible for most changes in the microenvironment, both maternal and fetal [102,103]. Adipose tissue cells also participate significantly, releasing hormones such as leptin, adiponectin, and resistin, whose alterations may contribute to part of this inflammatory process [72,188,232]. In the microenvironment, there is an interaction between these cells and bioactive mediators, including hormones and cytokines, released by these cells. The hormone serotonin and melatonin are mediators capable of acting directly on these cells [173,218,233,234].

Alterations in melatonin have been linked to disturbances in glucose and lipid homeostasis [213,216,217]. Melatonin acts on adipose tissue by increasing the lipogenic response and decreasing lipolytic activity [235,236]. This hormone can limit weight gain, regulate lipid metabolism, and prevent side effects on glucose homeostasis, such as decreased insulin sensitivity [236,237].

In addition, melatonin levels are altered in diabetic pregnant women [76,84,233]. Honorio-França et al. [82] showed that melatonin in normoglycemic pregnant women has a pro-oxidative effect and induces an increase in reactive oxygen species. Morceli et al. [76] suggest that in hyperglycemia conditions, this hormone's effects are likely limited to anti-inflammatory processes, with a decrease in reactive oxygen species. Louis et al. [84] evaluated the blood and placenta of mothers with hyperglycemia. They showed alteration in oxidative processes and the mechanisms of apoptosis, especially in the vascularized layers of the placenta, and the melatonin hormone was able to modulate these alterations. They showed the importance of the melatonin hormone in controlling oxidative stress and apoptosis in the maternal blood and controlling apoptosis in trophoblastic cells.

Oxidative stress and inflammation are key features in the initiation, progression, and clinical manifestations of smooth muscle disorders [238]. Melatonin (*N*-acetyl-5-methoxytryptamine), in the urine, is excreted with metabolite 6-sulfatoxymelatonin (sMT6a), which varies depending on the nervous system [239].

Melatonin has protective effects on smooth muscle dysfunction. Studies suggest that the hormone decreases vascular tone through the reduction of adrenergic contraction and the increase in relaxation induced by acetylcholine, attenuating sympathetic tone by directly activating melatonin receptors, eliminating free radicals, and protecting against inflammation and induced deficiency by inflammation of neuromuscular function [238,240–243]. Melatonin can also restore impaired detrusor muscle contractility through normalization of contraction, mitochondrial polarity, neuromuscular function, and oxidative stress, suggesting the effects of melatonin against cystometric changes [85,243–245]. Thus, the hormones serotonin and melatonin are mediators capable of acting directly on these cells. In the case of melatonin, despite being mostly synthesized by the pineal gland, this hormone can also be released by macrophages, which could be directly linked in the case of inflammation. Also, changes in the concentration of this hormone can directly influence the microenvironment because this hormone has the classic anti-inflammatory action. In the case of serotonin, as it is a hormone capable of modulating inflammatory cytokines, alterations of serotonin in the microenvironment can affect through the reduction or increase of these mediators. In this way, both hormones can influence or suffer action directly in the microenvironment in the modulation of inflammation.

Thus, epigenetic and nutritious factors associated with changes in maternal metabolism caused by hyperglycemia in women with GDM can generate an inflammatory environment with increased inflammatory cytokines. This environment, altered by inflammation, can alter the concentration of serotonin and melatonin. As these hormones seem to act as protective effects against smooth muscle dysfunction and restore the impaired contractility of the detrusor muscle, it is assumed that these changes may favor the onset of urinary incontinence specific to pregnancy. Fig. 2 summarizes the main points of the interactions between nutrigenomics, hormones, and inflammatory cytokines that may be favorited the pathogenesis of GDM and PSUI.

Understanding the influence of functional foods on nutrigenomics, nutritional immunology, and chrononutrition can be strategies to reduce maternal complications pre, during, and post-pregnancy and correct possible changes in metabolic and immunological programming in future generations. Fig. 3 summarizes the main factors associated with nutrigenomics and neuroimmunoendocrine modulation.

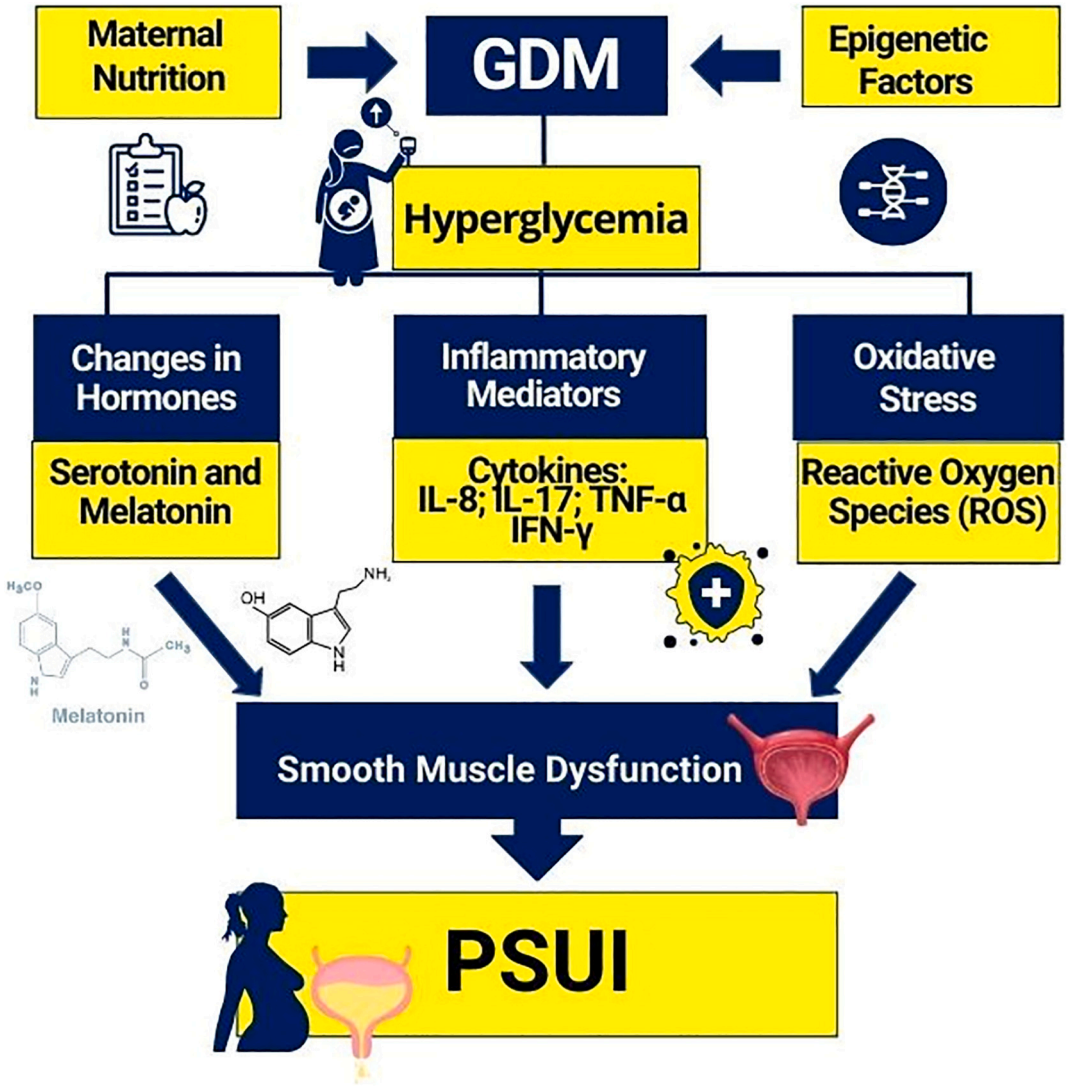


Fig. 2. Interactions between nutrigenomic (maternal nutrition and epigenetic factors) in the pathogenesis of Gestational Diabetes Mellitus (GDM) and Pregnancy-Specific Urinary Incontinence (PSUI). Interleukin 8 (IL-8), interleukin 17 (IL-17), Tumor Necrosis Factor-alpha (TNF-α), Interferon-gamma (IFN-γ). Reactive Oxygen Species (ROS).

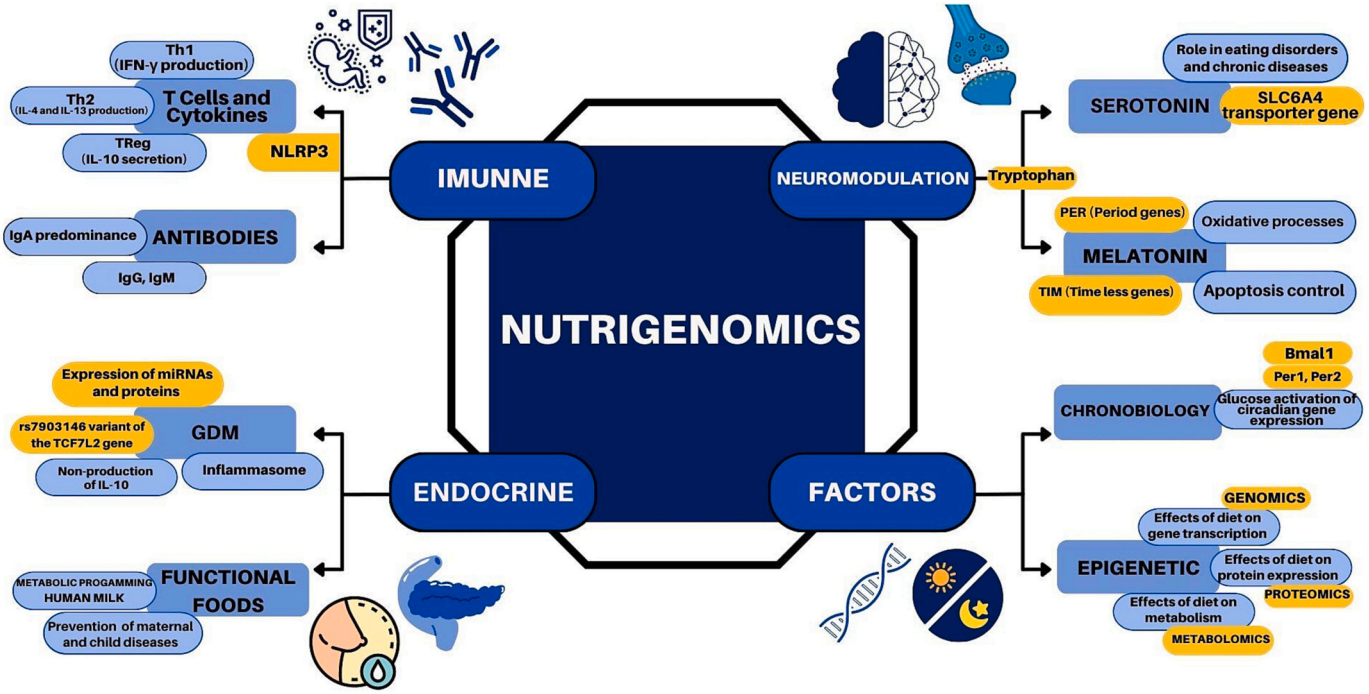


Fig. 3. Schematic pathways of the nutrigenomics acts with neuroimmunoendocrine modulation in the pathogenesis of Gestational Diabetes Mellitus (GDM). Epigenetic factors deserve attention due to the action of genes in all pathways. Mainly important are their correlations with metabolomics in the pathogenesis of GDM. Neuromodulation and chronobiology are associated, as tryptophan triggers the release of the hormone serotonin and melatonin, related to the genes SLC6A4 (serotonin transporter gene) and PER (periodic genes) and TIM (timeless genes) related to melatonin. Immunomodulation aimed at the predominance of Immunoglobulin A (IgA), presence of immunoglobulins G (IgG) and M (IgM) as antibodies, in addition to the NLRP3 gene being important in the release of lymphocytes by macrophages in the actions of T helper 1(Th1) responses producing gamma interferon, T helper 2 (Th2) producing Interleukin 4 (IL-4) and 13 (IL-13), and the regulatory T Lymphocyte in the secretion of interleukin 10 (IL-10). In addition, the expression of miRNAs and proteins and the rs7903146 variant of the TCF7L2 gene are important in the genesis of gestational diabetes.

7. Conclusion

Despite great advances in medicine, including improvements in detection, diagnosis, and treatment methods, GDM and PSUI are pathologies associated with increased financial costs in public health since women with GDM are usually young and have a high prevalence of PSUI. Significant changes in understanding the risks and pathogenesis of chronic diseases with subclinical inflammation are a growing cause of morbidity and mortality. There are still many gaps about the common risk factors involved in the pathophysiology of GDM and PSUI. Further studies are needed to understand the interactions between neuroimmunoendocrine mechanisms and nutrigenomics.

Considering that pro-inflammatory cytokines influence the regulation of urination pathways and peripherally affect bladder function and that one of the pathways of action of cytokines in the central nervous system involves the activation of the enzyme indolamine-2,3-dioxygenase (IDO), an enzyme responsible for the degradation of tryptophan, the precursor of the serotonin neurotransmitter, and that this hormone is a precursor of melatonin, changes in melatonin in hyperglycemic mothers may be associated with an increase in inflammatory cytokines and these changes may contribute to the increase in PSUI in mothers with GDM via the central nervous system (CNS).

Future studies on the role of melatonin and its 5-HT precursor in women with GDM and PSUI, as well as possible correlations with inflammatory cytokines in these pregnant women, may contribute to a better understanding of these two associated pathologies and whether the urinary tract can be affected by changes in serum levels of hormones and cytokines. In addition, due to the role of melatonin in improving metabolic changes associated with diabetes and urinary dysfunction, this hormone may represent an alternative for future therapies in pregnancies with GDM and PSUI.

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Declaration of competing interest

I declare that I have no personal or pecuniary interest, direct or indirect, in any matter that raises or may conflict with my duties at the Institution.

Data availability

No data was used for the research described in the article.

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Artigo 2

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Article

Serotonin and Interleukin 10 Can Influence the Blood and Urine Viscosity in Gestational Diabetes Mellitus and Pregnancy-Specific Urinary Incontinence

Danielle Cristina Honório França [1], Adenilda Cristina Honorio-França [2,*], Kênia Maria Rezende Silva [2], Fernanda Cristina Bérgamo Alves [1], Gabriela Bueno [1], Sarah Maria Barneze Costa [1], Aron Carlos de Melo Cotrim [2], Angélica Mércia Pascon Barbosa [1,3], Eduardo Luzía França [2], Marilza Vieira Cunha Rudge [1,*] and The Diamater Study Group [1,†]

[1] Department of Gynecology and Obstetrics, Botucatu Medical School, São Paulo State University, Botucatu 05508-070, SP, Brazil; daniellechfranca@gmail.com (D.C.H.F.); fernandacbalves15@gmail.com (F.C.B.A.); gabriela.bueno24@unesp.br (G.B.); sarah.barneze@unesp.br (S.M.B.C.); angelicapascon@gmail.com (A.M.P.B.); diamaterstudy@gmail.com (The Diamater Study Group)
[2] Biological and Health Sciences Institute, Federal University of Mato Grosso, Barra do Garças 78605-091, MT, Brazil; keniarezende1@gmail.com (K.M.R.S.); aroncarlosbg@gmail.com (A.C.d.M.C.); eduardo.franca@ufmt.br (E.L.F.)
[3] Department of Physiotherapy and Occupational Therapy, School of Philosophy and Sciences, São Paulo State University, Marilia 17525-900, SP, Brazil
* Correspondence: adenilda.franca@ufmt.br (A.C.H.-F.); m.rudge@unesp.br (M.V.C.R.)
† Membership of The Diamater Study Group is provided in the Acknowledgments.

Abstract: Serotonin and interleukin 10 (IL-10) may play a role in gestational diabetes mellitus. Hyperglycemic environment, the detrusor musculature of the bladder and pelvic floor muscles may become damaged, leading to urination problems and urine viscosity in pregnant women with gestational diabetes mellitus and pregnancy-specific urinary incontinence. Urine and blood samples were collected from pregnant women between 24 and 28 weeks of gestation. The serotonin concentration and cytokine IL-10 levels were evaluated in plasma and urine. In the total blood and urine, the viscosity was evaluated in the presence and absence of exogenous serotonin and IL-10. The plasma serotonin levels decreased, while the urine serotonin levels increased in the normoglycemic incontinent (NG-I), hyperglycemic continent (GDM-C), and hyperglycemic incontinent (GDM-I) groups. The IL-10 in the plasma decreased in the GDM-I group and was higher in the urine in the NG-I and GDM-I groups. The blood viscosity was higher, independently of urinary incontinence, in the GDM groups. The serotonin increased the blood viscosity from women with GDM-C and urine in the NG-I, GDM-C, and GDM-I groups. Blood and urine in the presence of IL-10 showed a similar viscosity in all groups studied. Also, no difference was observed in the viscosity in either the blood or urine when in the presence of serotonin and IL-10. These findings suggest that serotonin and IL-10 have the potential to reduce blood viscosity in pregnant women with gestational diabetes and specific urinary incontinence, maintaining values similar to those in normoglycemic women's blood.

Keywords: hormone; immunomodulation; cytokines; rheology; involuntary loss of urine

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1. Introduction

Gestational diabetes mellitus (GDM) is an important metabolic disease with increased insulin counter-regulatory hormones generating glucose intolerance, an inflammatory environment, abnormalities in oxidative stress, and altering the profile of released cytokines and other hormones [1,2].

In maternal hyperglycemia, the effects on cellular metabolism can affect systems and tissues, like the pelvic floor muscles (PFMs) and the urinary system, with pre-disposition to muscle weakening, atrophy, and pregnancy-specific urinary incontinence (PSUI) [3].

In the central nervous system, cytokines influence the urination pathways. In the peripheral nervous system, cytokines act on the sensitivity of the afferent nerve, the bladder, and the detrusor muscle tonus [3,4]. IL-10 (interleukin 10) is a cytokine with an autoregulatory and inhibitory role in producing inflammatory cytokines, promoting a suppressive effect on inflammatory processes [5]. This cytokine is present at lower concentrations in certain urogynecological pathologies [5] and pregnant women with gestational diabetes mellitus [6].

Cytokines can influence the central serotonergic pathways in the urinary system [7,8]. The serotonergic contraction of the bladder is altered in rats with an overactive bladder model [9]. Serotonergic paraneurons express serotonin, a hormone derived from tryptophan that can be associated with immunomodulatory responses, alterations in mechanisms of disorders, and the control of the appetite [3,7,10,11].

Research has indicated that serotonin may impact bladder activity in cases of pathological conditions, which is primarily influenced by inflammatory agents [12]. Urethral paraneurons produce and release serotonin, which is believed to be one of the hormones that can cause bladder contractions [13].

This hormone can regulate the production and release of pro-inflammatory cytokines, acting directly in the pathways responsible for changes in the maternal–fetal hyperglycemic environment [3]. Additionally, IL-10 can regulate serotonin transporter activity and serotonergic responses [14].

Dysfunction in the lower urinary tract is presented as the most common complication of diabetes mellitus, highlighting even more the interaction between GDM and PSUI [15]. The SLC6A4 gene, responsible for serotonin transportation, is linked to metabolic diseases, and gestational diabetes mellitus causes immunological changes in cytokine expression [1,16,17]. However, the influence of IL-10 and serotonin on pregnancy-specific urinary incontinence in pregnant women with gestational diabetes mellitus is not fully understood.

Endothelial dysfunction and vessel wall damage are common among diabetic patients [18]. Studies have found that hyperglycemia, cytokines, and serotonin can alter blood viscosity [19–22]. Serotonin has been found to reduce erythrocyte deformability and increase leukocyte adhesion, which can lead to sustained microcirculatory deficiencies and have a significant impact on the course of the disease [23,24]. The serotonergic pathway has been linked to urinary incontinence [25] as well as the development of gestational diabetes mellitus (GDM) [26]. Also, IL-10 appears to play an important role in this process by regulating serotonin activity and the production of serotonin reuptake receptors [14].

No study has yet linked hormonal factors and cytokines' effects on blood viscosity in women with GDM and pregnancy-specific urinary incontinence. Thus, this study evaluated the influence of serotonin and IL-10 on blood and urine viscosity in pregnant women with gestational diabetes mellitus and pregnancy-specific urinary incontinence.

2. Results

The clinical information of the pregnant patients is presented in Table 1. Pregnant patients with diabetes independently of urinary incontinence were older than those in the NG-C and NG-I groups. In addition, the GDM-C and GDM-I groups had a higher percentage of overweight pregnant women and glycemic index ($p < 0.05$). However, no significant difference in urine density was observed between the groups (Table 1).

In women with gestational diabetes mellitus and urinary incontinence, there was a decrease in hemoglobin and hematocrit levels. The erythrocytes and lymphocytes increased, whereas the neutrophils decreased in the blood from women of the GDM-C group. No difference between the groups was observed in the leukocytes, monocytes, eosinophils, or platelets (Table 2).

Table 1. Clinical data on pregnant women divided into four groups based on their glycemic levels and urinary continence: normoglycemic continent (NG-C), normoglycemic with pregnancy-specific urinary incontinence (NG-I), gestational diabetes mellitus continent (GDM-C), and gestational diabetes mellitus with pregnancy-specific urinary incontinence (GDM-I).

Groups	NG-C (N = 28)	NG-UI (N = 28)	GDM-C (N = 20)	GDM-I (N = 21)
Age	25.14 ± 4.68	26.53 ± 6.91	30.40 ± 6.42	30.47 ± 6.56
BMI categories (%):				
Normal weight	58.8	51.7	38.6	36.7
Overweight	41.2	48.3	61.4	63.3
Obesity	0	0	0	0
Glucose level (mg/dL)	75.28 ± 6.87	75.18 ± 7.37	92.12 ± 9.88 *	90.32 ± 12.18 *#
Urine Density	1.01 ± 0.011	1.01 ± 0.008	1.01 ± 0.006	1.01 ± 0.006

Note: BMI: body mass index categories (normal weight: 18.5 and 24.9 kg/m^2, overweight: 25 and 29.9 kg/m^2, and obesity: over 30 kg/m^2); data correspond to the median and standard deviation of the pregnant women. $p < 0.05$. * difference between the control group (NG-C) and the NG-I, GDM-C, and GDM-I groups; # difference between NG-I and GDM-I groups.

Table 2. Hematological values in the blood from women were divided into groups based on their glycemic levels and urinary continence: normoglycemic continent (NG-C), normoglycemic with urinary incontinence (NG-I), gestational diabetes mellitus continent (GDM-C), and gestational diabetes mellitus with urinary incontinence (GDM-I).

Parameters	NG-C	NG-I	GDM-C	GDM-I	*p*
Erythrocytes (10^6/µL)	4.10 ± 0.37	4.00 ± 0.41	4.37 ± 0.33 *	4.00 ± 0.43 #	0.0361
Hemoglobin (g/dL)	12.57 ± 0.88	12.37 ± 1.06	13.06 ± 1.04	11.91 ± 1.30 *#	0.0242
Hematocrit (%)	38.56 ± 2.32	38.18 ± 3.08	39.11 ± 2.91	35.87 ± 3.52 *#	0.0239
Leukocytes (10^3/µL)	9.68 ± 2.15	9.26 ± 2.66	9.12 ± 1.66	10.12 ± 2.60	0.5677
Neutrophils (%)	69.33 ± 5.77	69.08 ± 5.54	57.56 ± 20.01 *	68.88 ± 7.17	0.0019
Lymphocytes (%)	21.30 ± 4.92	21.91 ± 4.39	31.63 ± 17.09 *	21.30 ± 5.12	0.0011
Monocytes (%)	7.48 ± 1.51	7.22 ± 1.55	8.56 ± 2.59	7.52 ± 2.50	0.1236
Eosinophils (%)	1.80 ± 0.96	1.84 ± 1.38	1.98 ± 1.12	2.17 ± 2.71	0.2317
Platelets (10^3 µL)	251.32 ± 57.92	232.25 ± 60.00	284.84 ± 51.89	250.67 ± 73.97	0.1017

Data correspond to the mean and standard deviation of the pregnant women. $p < 0.05$. * difference between the control group (NG-C) and the NG-I, GDM-C, and GDM-I groups; # difference between GDM-C and GDM-I groups.

The serotonin levels in plasma and urine are shown in Figure 1. In the plasma, there was a decrease in the serotonin concentrations in the NG-I, GDM-C, and GDM-I groups (Figure 1A). The serotonin levels were higher in the urine in the NG-I group and lower in the GDM-I group. The highest serotonin levels in urine were observed in the NG-I group (Figure 1B). The plasma/urine serotonin ratio was lower in the NG-I and GDM-I groups (Figure 1C).

The IL-10 levels in the plasma and urine are shown in Figure 2. In the plasma, there was a decrease in the IL-10 concentrations in the GDM-I (Figure 1A). The Il-10 levels were higher in the urine in the NG-I and GDM-I groups (Figure 1B). No difference was observed in the plasma/urine IL-10 ratio in the groups studied (Figure 1C).

The Pearson test correlated the serotonin and Il-10 levels in the plasma and urine (Table 3). The GDM-C group, in particular, exhibited a negative correlation of serotonin levels between plasma and urine. No correlation ($p > 0.05$) was found with serotonin levels in the NG-C, NG-I, or GDM-I groups. The IL-10 levels showed a negative correlation between plasma and urine in the NG-C group and a positive correlation in the NG-I group. No correlation ($p > 0.05$) was found with IL-10 levels in the hyperglycemic groups. There was a positive correlation between serotonin and IL-10 in the urine of the pregnant patients in the GDM-I group. No correlation ($p > 0.05$) was found between the serotonin and IL-10 levels in the plasma (Table 3).

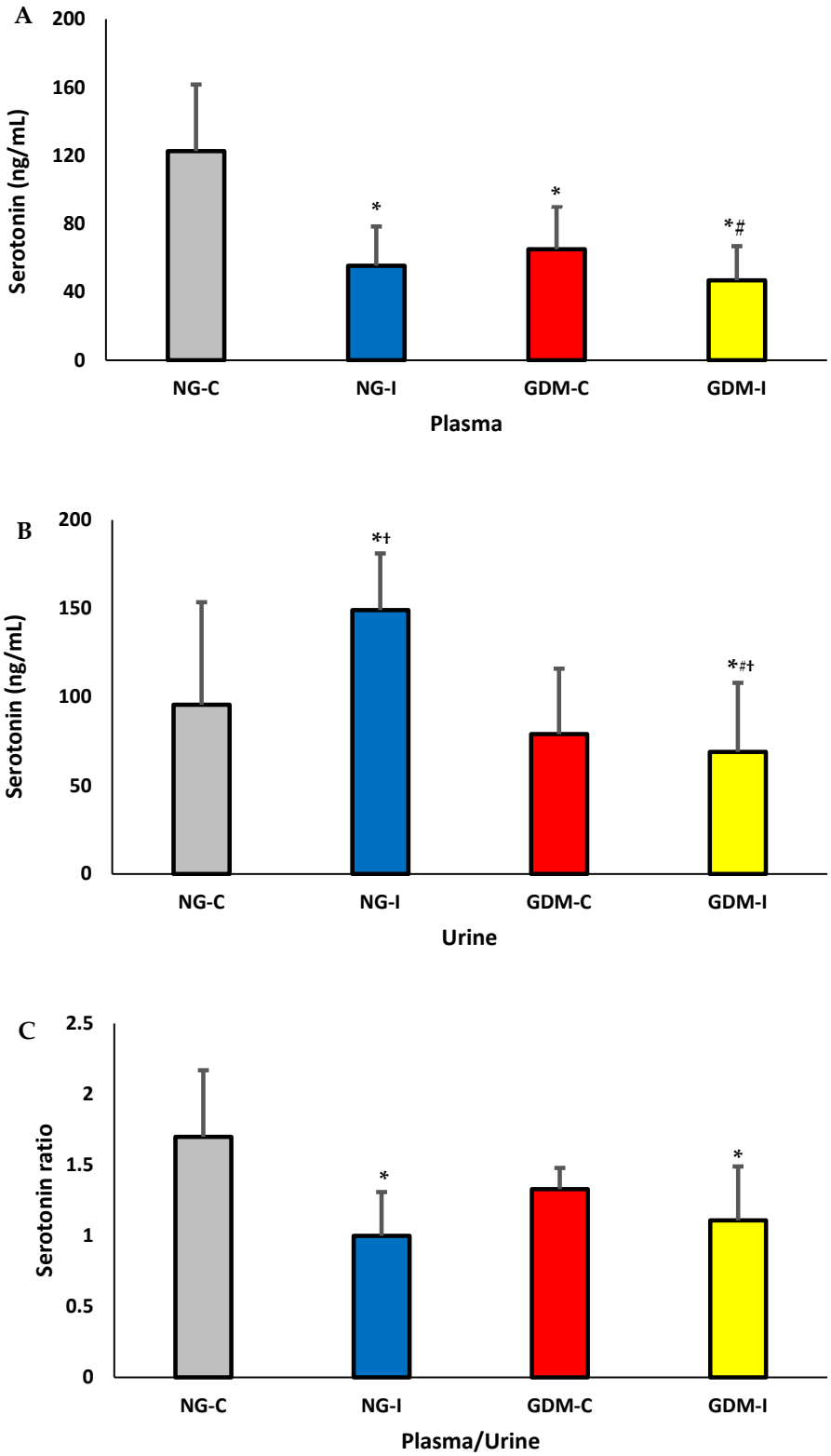


Figure 1. Serotonin levels in plasma (**A**), urine (**B**), and plasma/urine serotonin ratio (**C**) from pregnant patients with gestational diabetes mellitus (GDM) with or without urinary incontinence. NG-C: normoglycemic continent, NG-I: normoglycemic with urinary incontinence, GDM-C: gestational diabetes mellitus continent, and GDM-I: gestational diabetes mellitus with urinary incontinence. $p < 0.05$. * difference between the control group (NG-C) and the NG-I, GDM-C, and GDM-I groups; # difference between NG-I and GDM-I groups; † difference between plasma and urine, considering the same groups.

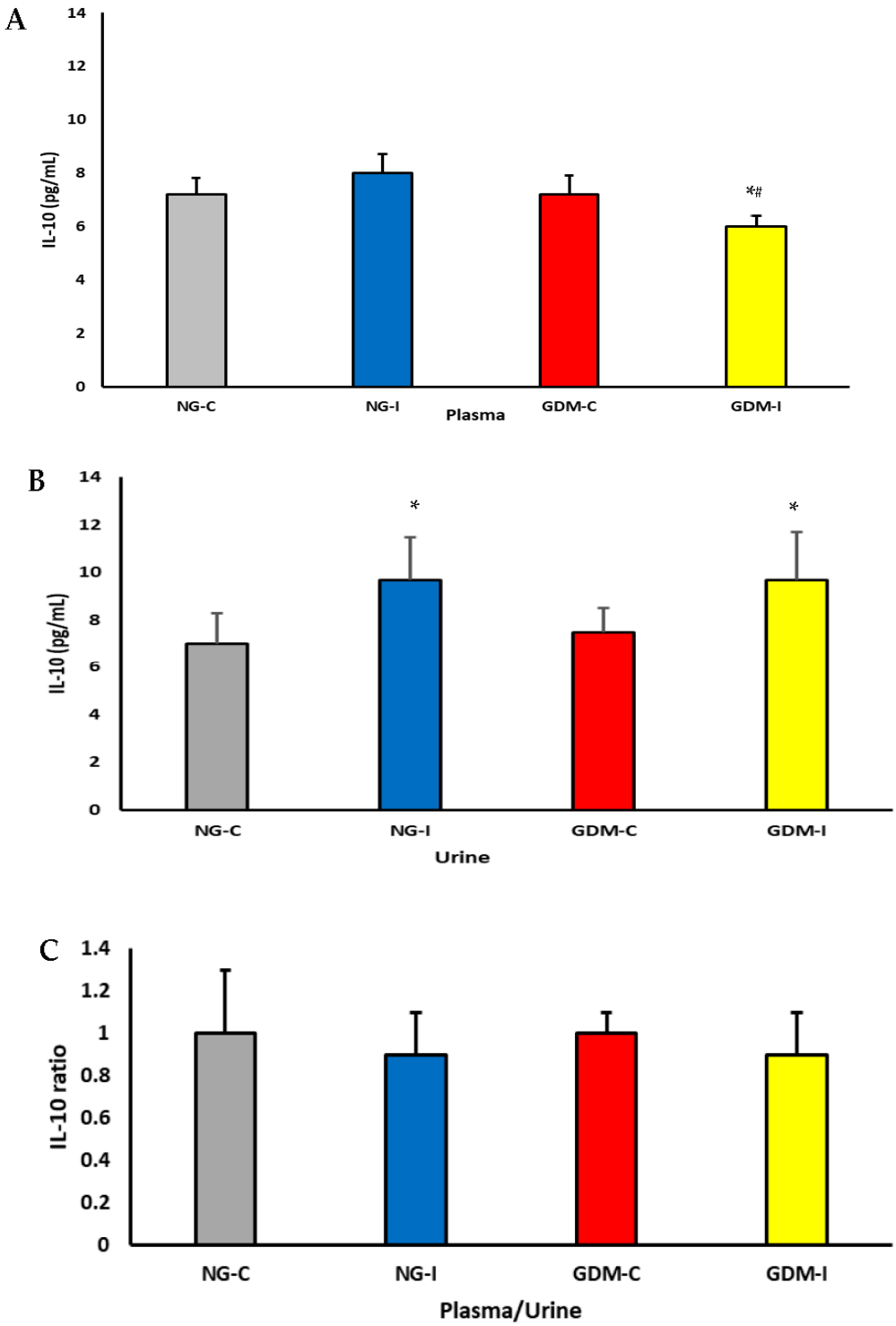


Figure 2. IL-10 cytokine levels (pg/mL) in plasma (**A**), urine (**B**), and plasma/urine serotonin ratio (**C**) from pregnant patients with gestational diabetes mellitus (GDM) with or without urinary incontinence. NG-C: normoglycemic continent, NG-I: normoglycemic with urinary incontinence, GDM-C: gestational diabetes mellitus continent, and GDM-I: gestational diabetes mellitus with urinary incontinence. $p < 0.05$. * difference between the control group (NG-C) and the NG-I, GDM-C, and GDM-I groups; # difference between NG-I and GDM-I groups.

Table 3. Pearson's linear correlations and values of linear coefficients (r) and statistical significance (* *p*) of serotonin (ng/mL) and IL-10 (pg/mL) between plasma and urine and between serotonin (pg/mL) and IL-10 (pg/mL) in the plasma and urine in the groups. NGC: normoglycemic continent; NGI: normoglycemic incontinent; GDM-C: gestational diabetes mellitus continent; GDM-I: gestational diabetes mellitus incontinent.

Groups	NG-C	NG-I	GDM-C	GDM-I
		Plasma/Urine		
Serotonin	r = −0.1617 *p* = 0.7291	r = −0.4782 *p* = 0.2777	r = −0.8138 * *p* = 0.0487	r = 0.1442 *p* = 0.7578
IL-10	r = −0.7273 * *p* = 0.0408	r = 0.8413 * *p* = 0.0486	r = 0.3988 *p* = 0.3277	r = 0.2742 *p* = 0.5110
		Serotonin/IL-10 cytokine		
Plasma	r = −0.4946 *p* = 0.2127	r = −0.2316 *p* = 0.5810	r = 0.4427 *p* = 0.2720	r = −0.1846 *p* = 0.6616
Urine	r = 0.1782 *p* = 0.6728	r = −0.3080 *p* = 0.4580	r = 0.0706 *p* = 0.8680	r = 0.9379 * *p* = 0.0331

Figure 3 shows the blood and urine viscosity. The blood viscosity was higher in the GDM-C and GDM-I groups compared to the NG groups (Figure 3). No difference was observed in the urine viscosity between the groups.

The Pearson test correlated the serotonin, viscosity, and IL-10 and serotonin in blood and urine (Table 4). The GDM-I group, in particular, exhibited a negative correlation between serotonin levels and viscosity in the blood. IL-10 correlated inversely with viscosity in the blood of pregnant NG-I and GDM-I patients. In urine, the serotonin positively correlated with viscosity in the GDM-C group. No correlation ($p > 0.05$) was found with IL-10 levels and viscosity in urine (Table 3).

Table 4. Pearson's linear correlations and values of linear coefficients (r) and statistical significance (* *p*) of the viscosity (mPa) between serotonin (pg/mL) and IL-10 (pg/mL) in the blood and urine of women from the groups. NG-C: normoglycemic continent; NG-I: normoglycemic incontinent; GDM-C: gestational diabetes mellitus continent; GDM-I: gestational diabetes mellitus incontinent.

	Viscosity	NG-C	NG-I	GDM-C	GDM-I
Blood	Serotonin	r = 0.5933 *p* = 0.1210	r = −0.4010 *p* = 0.3248	r = −0.4620 *p* = 0.2490	r = −0.7082 * *p* = 0.0493
	IL-10	r = 0.4225 *p* = 0.2970	r = −0.7775 * *p* = 0.0231	r = 0.6091 *p* = 0.1089	r = −0.8069 * *p* = 0.0155
Urine	Serotonin	r = 0.3562 *p* = 0.3865	r = −0.0682 *p* = 0.8724	r = 0.7711 * *p* = 0.0250	r = −0.5800 *p* = 0.1317
	IL-10	r = 0.2602 *p* = 0.5337	r = 0.2505 *p* = 0.5496	r = −0.3784 *p* = 0.3553	r = −0.1908 *p* = 0.6513

The serotonin increased the blood viscosity in the blood of women with GDM-C and urine in the NG-I, GDM-C, and GDM-I groups (Figure 4). Blood and urine in the presence of IL-10 showed a similar viscosity in all groups studied (Figure 5). Also, no difference was observed in the viscosity in either blood or urine when in the presence of serotonin and IL-10 (Figure 6).

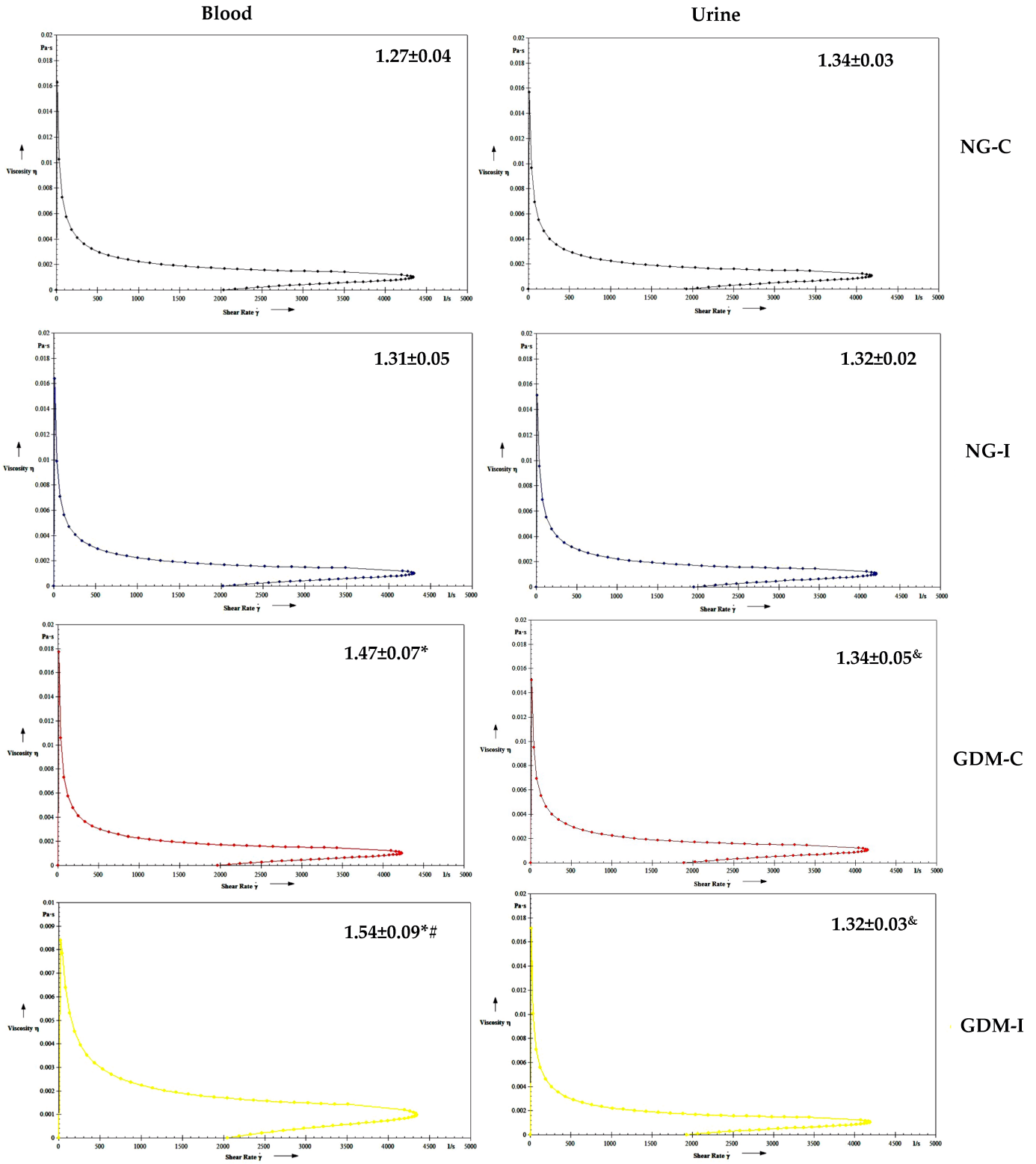


Figure 3. Blood and urine viscosity (mPa) in the groups. NGC: normoglycemic continent; NGI: normoglycemic incontinent; GDM-C: gestational diabetes mellitus continent; GDM-I: gestational diabetes mellitus incontinent. * difference between the control group (NG-C) and the NG-I, GDM-C, and GDM-I groups; # difference between NG-I and GDM-I groups; & difference between plasma and urine, considering the same groups.

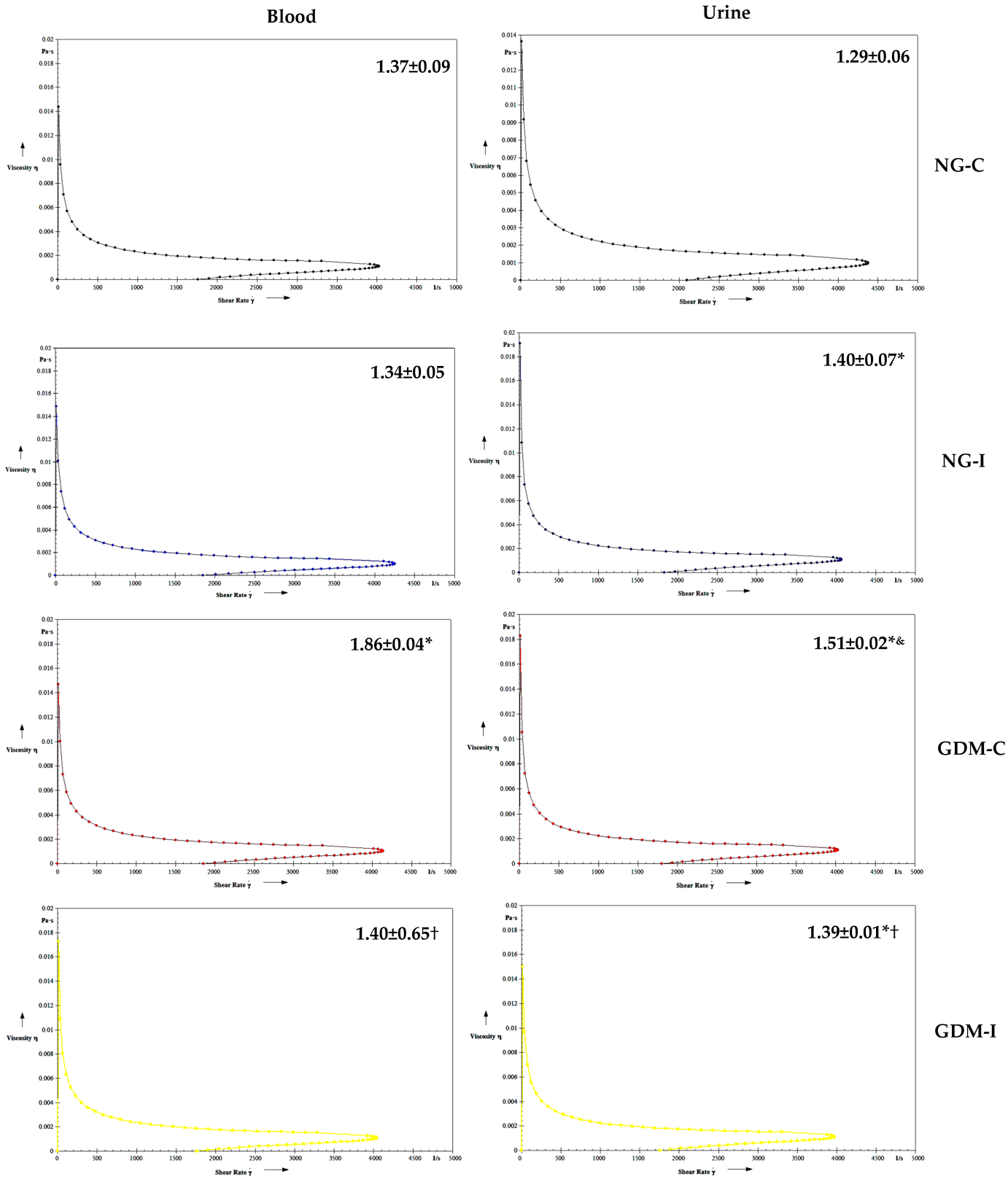


Figure 4. Blood and urine viscosity (mPa) treated with serotonin (100 ng/mL) in the groups. NGC: normoglycemic continent; NGI: normoglycemic incontinent; GDM-C: gestational diabetes mellitus continent; GDM-I: gestational diabetes mellitus incontinent. * difference between the control group (NG-C) and the NG-I, GDM-C, and GDM-I groups; † difference between GDM-C and GDM-I groups, & difference between plasma and urine, considering the same groups.

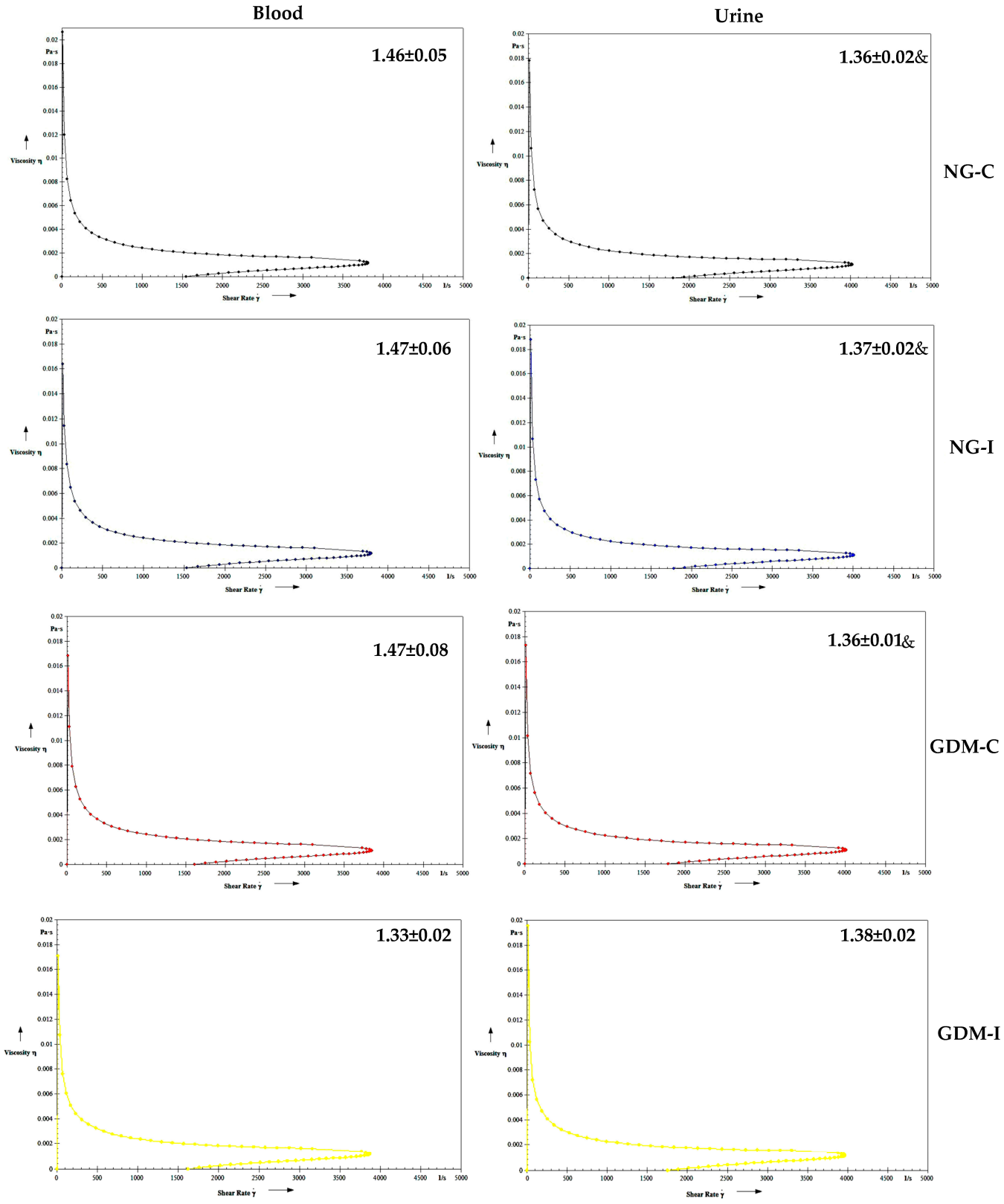


Figure 5. Blood and urine viscosity (mPa) treated with IL-10 cytokines (100 ng/mL) in the groups. NGC: normoglycemic continent; NGI: normoglycemic incontinent; GDM-C: gestational diabetes mellitus continent; GDM-I: gestational diabetes mellitus incontinent. & difference between plasma and urine, considering the same groups.

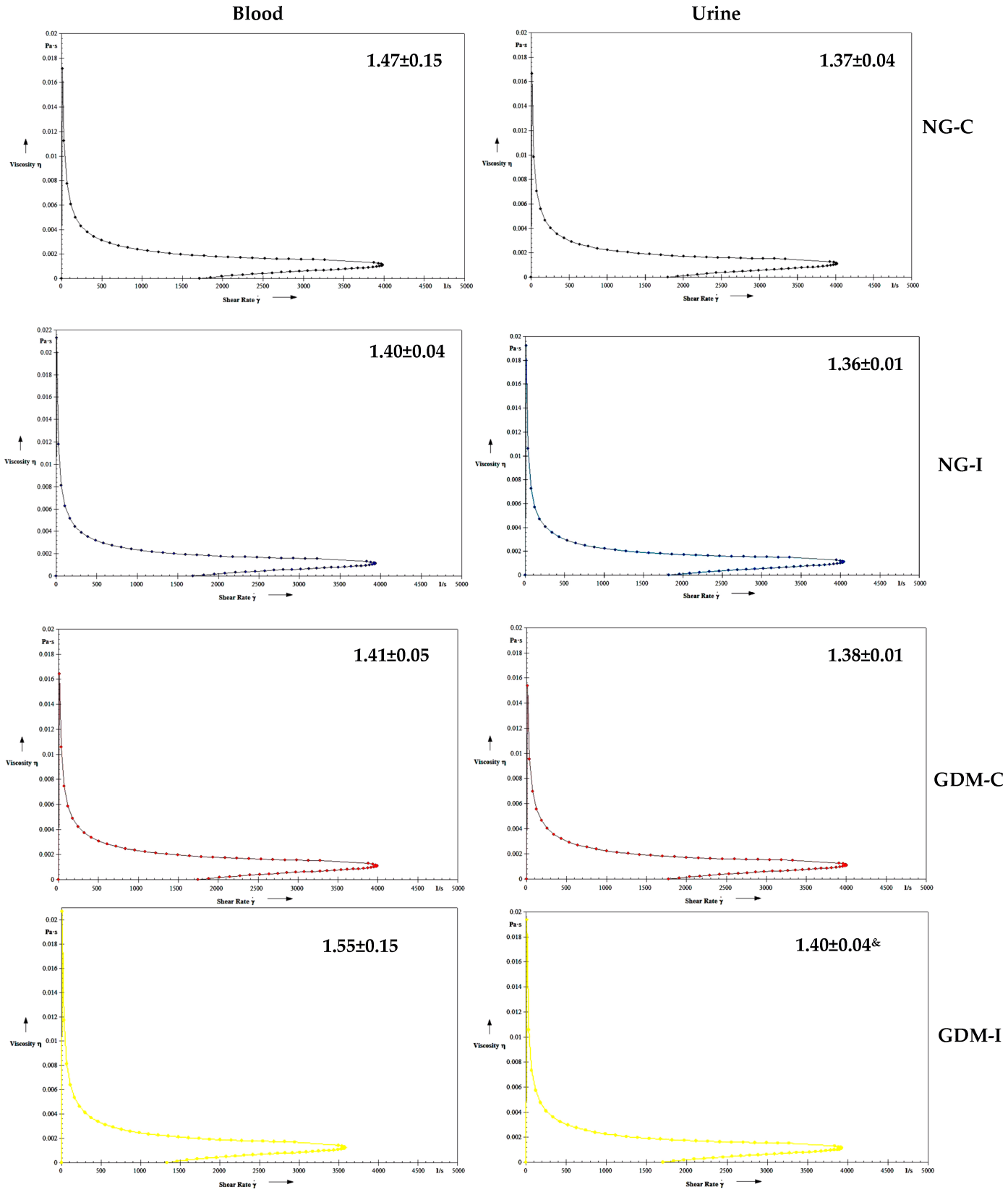


Figure 6. Blood and urine viscosity (mPa) treated with serotonin and IL-10 cytokines (100 ng/mL) in the groups. NGC: normoglycemic continent; NGI: normoglycemic incontinent; GDM-C: gestational diabetes mellitus continent; GDM-I: gestational diabetes mellitus incontinent. & difference between plasma and urine, considering the same groups.

The viscosity showed a positive correlation with hematocrit (r = 0.7464; $p = 0.0337$) and a negative correlation with plaquettes (r = −0.8599; $p = 0.0062$) in the NG-I group. No correlation ($p > 0.05$) was found with viscosity or other blood parameters (Table 4).

3. Discussion

Diabetes can impact various systems in the body, such as the nervous and immune systems. In pregnant women with gestational diabetes mellitus, hyperglycemia can cause changes in cellular metabolism, muscle alterations in the pelvic and abdominal regions, and pregnancy-specific urinary incontinence [27,28]. In this work, women with GDM and PSUI showed a reduction in hemoglobin, hematocrit (Table 2), serotonin hormone (Figure 1), and anti-inflammatory cytokine interleukin 10 (IL-10) (Figure 2), and these alterations were reflected in blood viscosity (Figure 3).

Various risk factors can influence hyperglycemia in gestation, including advanced maternal age and increased body mass index (BMI). The risk of developing gestational diabetes mellitus (GDM) is higher in pregnant women with a higher BMI, which may be related to changes in their lipid metabolism [29,30]. In this study, women with gestational diabetes mellitus had higher glucose levels, regardless of urinary incontinence.

Diabetes can affect the entire body, causing micro and macro blood circulation changes. Pregnant women with gestational diabetes mellitus may experience hematological alterations [31]. Here, hyperglycemia is associated with an increase in erythrocytes and lymphocytes. Studies have shown that increased hemoglobin, platelets, and lymphocytes are linked to a higher risk of developing gestational diabetes mellitus (GDM) [32]. These changes in hematological parameters can lead to a chronic inflammatory environment, which can cause insulin resistance, impair the function of β cells, and affect insulin [32,33]. Interestingly, women with GDM and urinary incontinence present a decrease in hemoglobin and hematocrit levels, suggesting that the reduction in hematological parameters in women with GDM-I may be due to hyperglycemia associated with the involuntary loss of urine.

During pregnancy, hormonal factors are crucial, particularly when linked to hyperglycemia. Cystometric changes alter the neuromuscular function and smooth muscle cell action [34]. Serotonin receptor agonists have been trained in treating lower urinary tract dysfunctions associated with diabetes, and studies in diabetic rats show improved voiding efficiency and sphincter activity, pointing out that serotonin may have protective effects on smooth muscle disorders [35].

Studies conducted on pregnant women with gestational diabetes mellitus have shown decreased hormone serotonin [36]. In this study, regardless of urinary incontinence, there was a decrease in the serotonin concentrations in plasma and urine in women with GDM. However, there were lower serotonin levels in the plasma in the group of normoglycemic pregnant women who also had urinary incontinence. At the same time, the concentration of this hormone in the urine was high. The lower blood/urine ratio of the hormone confirmed this in both groups with urinary incontinence. These findings support the notion that hyperglycemia associated with involuntary urine loss can affect serotonergic pathways.

Urinary incontinence can be influenced by genetic factors and the serotonergic pathway [25]. Neurochemistry studies reveal that sphincter motor neurons have high concentrations of neurotransmitters and receptors [37]. The regulation of bladder function is influenced by central serotonergic modulation, and neurotransmitter changes can affect urination and may explain the association with urinary incontinence [38]. Reduced serotonin function in the brain and spinal cord can lead to a decrease in bladder activity [37]. Research on rodents has shown that decreasing serotonin in the brain can cause changes in urinary frequency, which can be corrected with serotonin reuptake inhibitors [39]. This provides further evidence that abnormal serotonin functions can be associated with urinary incontinence [40].

Serotonergic modulation is essential in controlling continence by acting along the neural, medullary, and supramedullary routes. It also affects the central serotonergic pathways in the tissue environment of the detrusor smooth muscle, the skeletal muscle of

the external urethral sphincter, and the pelvic floor [41]. Changes in serotonin excretion and reduced plasma serotonin levels can lead to glucose intolerance and increase the risk of gestational diabetes [42]. Here, in women with gestational diabetes, the serotonin levels in the plasma are inversely proportional to those found in urine. It is noteworthy that serotonin signaling in pancreatic islets plays a crucial role during pregnancy [43].

There is a lack of information on serotonin metabolism in mothers with GDM due to the complex nature of this process and its relevance to glucose regulation during pregnancy [26]. Serotonin is mainly known for its effects on the central nervous system and critical role in regulating physiological processes [44]. It is co-secreted with insulin in a glucose-dependent manner [45] and is involved in physiological pathways and adaptive processes during pregnancy [46]. Research using metabolic markers in urine and plasma has demonstrated that serotonin plays a role in the onset of GDM [26,47] and that the serotonergic pathway contributes to urinary incontinence [25], which can lead to a homeostatic imbalance. Therefore, maintaining physiological levels of both serotonin and IL-10 is crucial in women with GDM urinary incontinence.

Gestational diabetes mellitus is characterized by an inflammatory state in which cytokines play an important role in the genesis and control of inflammation processes [48]. Insulin resistance can also result from the cytokines, which may be the molecular basis of the inflammation in the disease [49]. Serotonin can stimulate the release and production of pro-inflammatory cytokines, but the essential anti-inflammatory cytokine, IL-10, can modulate serotonin by regulating the activity of serotonin transporters, the production of serotonin reuptake receptors, and serotonergic responses [14].

Maintaining a balance between pro-inflammatory and anti-inflammatory cytokines is crucial for a successful pregnancy, and the immune system plays a significant role in this process [1,50]. During pregnancy, an anti-inflammatory status dominates, which is essential for the natural progression of pregnancy, especially in the case of diabetes [51,52]. However, certain factors, like metabolic diseases, may predispose individuals to decreased levels of anti-inflammatory cytokines [51]. A lower level of the anti-inflammatory IL-10 in the blood has been linked to insulin resistance, metabolic syndrome, and type II diabetes [53]. Pregnancy can affect the immune system [52], so decreasing anti-inflammatory factors could result in disorders such as GDM.

The current study has demonstrated that the cytokine IL-10 was reduced in plasma and increased the excretion in the urine of pregnant women with gestational diabetes and urinary incontinence. Interestingly, the IL-10 levels increased in the urine of pregnant women with normoglycemic urinary incontinence and showed a positive correlation between plasma and urine in this group and a positive correlation between serotonin and IL-10 in the urine of pregnant patients with gestational diabetes and urinary incontinence. These findings indicate that hyperglycemia, especially when combined with involuntary loss of urine, can decrease the production of IL-10 in the body. This decrease in IL-10 levels may be associated with reduced serotonin levels and greater excretion of the cytokines in the urine, leading to an imbalance in anti-inflammatory mechanisms and promoting inflammation.

The pathogenesis of gestational diabetes may be linked to the hematological changes and increased viscosity caused by chronic inflammation. Abnormalities in hematological cells may reflect maternal immune dysregulation and viscosity, playing a role in GDM pathophysiology [32]. In this study, the blood viscosity increased in both gestational diabetes groups (GDM-C and GDM-I), suggesting that hormones and cytokines may affect blood rheological parameters in patients with gestational diabetes and urinary incontinence.

Rheological parameters are used in cardiovascular research to detect patients prone to cardiovascular disease and to monitor women at risk of pre-eclampsia [54]. The typical inflammatory process of diabetes can alter blood flow, which may cause microcirculation and blood viscosity changes, which are crucial for maintaining the structural integrity and non-deformation of cells [55].

Studies have reported changes in the structure of red blood cells and increased blood viscosity in diabetic patients [20]. Furthermore, the use of immunomodulatory agents has been linked to the recovery of cell structure and function [21,50] and an improvement in blood viscosity [20], which may lead to better flow characteristics.

The use of immunomodulators during pregnancy has gained global interest. Current evidence mainly reports outcomes related to the newborn and the potential effect on the ontogeny of the immune system [56]. Previous studies have reported the effects of cytokines [21,57] as immunomodulators capable of acting on blood viscosity. However, this study is the first to describe changes in viscosity and the potential use of immunomodulators to restore viscosity in pregnant women with GDM and urinary incontinence. Yet there remains a dilemma regarding prioritizing the risk of continuing the medication during pregnancy to the detriment of the risk of maternal disease action.

In some cases, there is a recommendation to interrupt biological therapies at the beginning of pregnancy, except in cases where the potential benefits for the mother outweigh the potential risk for the fetus [58]. In this sense, future research should address gaps in the knowledge about the use of immunomodulators during pregnancy, especially in pregnant women with gestational diabetes and urinary incontinence. This would help to evaluate the impact of molecule biological therapies on fetal/newborn organogenesis and the effect of concomitant immunomodulators on maternal disease action [58,59].

Considering that serotonin and IL-10 levels are altered in pregnant women with gestational diabetes and urinary incontinence, and there was a positive correlation between viscosity and serotonin and between viscosity and IL-10 in the blood of these pregnant patients, we aimed to verify the possible effects of this hormone and cytokine on viscosity. The treatment with IL-10 or combined IL-10–serotonin was associated with reduced viscosity. The clinical potential for using cytokines [21,60,61] has been reported for various diseases.

The biological significance of differences in IL-10 and serotonin levels and the restoration of viscosity associated with two agents in the blood from mothers with gestational diabetes and urinary incontinence noted in our study indicates the possible use of this cytokine as a potential immunomodulatory agent. Further studies are needed to verify the mechanisms involved in changes in serotonin and IL-10 levels in blood and urine and the modulatory role of these bioactive components in gestational diabetes associated with pregnancy-related urinary incontinence.

It is important to note that the data collected in this study only pertain to a specific period and do not account for the severity or persistence of urinary incontinence during and after pregnancy. This limitation highlights the need for further investigation into the effects of gestational diabetes mellitus (GDM) on urinary continence, as well as the impact of other factors during pregnancy. Follow-up studies with pregnant women suffering from GDM are necessary to evaluate hyperglycemia and urinary incontinence with a focus on other contributing factors.

In summary, this study provides evidence of the complexity and relevance of neuroimmunoendocrine interactions in pregnant women. Metabolic changes and excess circulating glucose in pregnant women with gestational diabetes and urinary incontinence can lead to serious complications since pregnant women present changes in serotonin and IL-10 cytokine levels, as well as in hematological and rheological parameters, and monitoring and controlling glucose levels are crucial to preventing adverse outcomes. In gestational diabetes, increased blood viscosity can cause hypertensive peaks that disrupt fetal balance in the intrauterine cavity. High blood viscosity and hyperglycemia can also alter the feto-placental unit, resulting in complications related to the loss of gestational balance [62–64]. Early diagnosis and treatment can prevent progression in patients with GDM-I. Assessing blood and urinary viscosity in pregnant patients can be an easy, quick, and economically viable means of early analysis of the association between GDM-I and high blood viscosity.

Furthermore, in the clinical monitoring of pregnant women postpartum, it can also be used to predict long-term urinary incontinence, evaluating at 6 and 12 months after

birth. It would then be a viable means of monitoring these postpartum women. In this way, possible therapeutic interventions and monitoring in pregnancies planned using these parameters can be started early and after pregnancy.

4. Materials and Methods

4.1. Study Design and Participants

A cross-sectional study was conducted in the Perinatal Diabetes Research Center, Botucatu, São Paulo, Brazil. Out of the 114 pregnant patients who were examined, the inclusion criteria were being pregnant with a single live fetus, undergoing the oral glucose tolerance test between the 24th and 28th week of pregnancy, being at least 18 years old and at most 40 years old, signing the informed consent form, not taking antidepressants, and not having autoimmune diseases. In total, 17 pregnant patients with pre-existing conditions such as type 1 or type 2 diabetes, obesity, autoimmune diseases, fetal malformations, twin pregnancies, aged under 18 or over 40, or who used antidepressants were excluded from the study.

Fasting plasma glucose was determined in the first trimester of pregnancy. The pregnant women underwent a 75 g oral glucose tolerance test (OGTT-75g) between the 24th and 28th week of pregnancy. The OGTT-75g was altered if any of the following plasma glucose values were met or exceeded: fasting glycemia of 92 mg/dL, post-load 1 h glucose of 180 mg/dL, and post-load 2 h glucose of 153 mg/dL [65]. The pregnant women also completed the International Consultation on Incontinence Questionnaire Short Form (ICIQ-SF) [66], which is a validated tool that assesses the impact of urinary incontinence (UI) on a person's quality of life. The questionnaire helps to classify urinary loss and the severity index of UI (ISI) [67] and is also validated as a quantitative and semi-objective measure of the severity of UI. Based on the results of the OGTT-75g and the ICIQ-SF questionnaire, the pregnant women were divided into four groups according to their blood glucose results during the oral glucose tolerance test and urinary continence: normoglycemic continent (NG-C), normoglycemic with pregnancy-specific urinary incontinence (NG-I), gestational diabetes mellitus continent (GDM-C), and gestational diabetes mellitus with pregnancy-specific urinary incontinence (GDM-I). Eligible participants voluntarily agreed to participate in the study. The sample consisted of 28 participants in group NG-C, 28 in group NG-I, 20 in group GDM-C, and 21 in group GDM-I, totaling 97 participants. The scheme for obtaining samples and experimental design is described in Figure 7. All patients signed the Informed Consent Form.

4.2. Blood and Plasma Separation

Blood samples were collected in tubes containing EDTA via venipuncture (approximately 5 mL) from pregnant women who attended the Perinatal Diabetes Research Central at the Botucatu Medical School (FMB-UNESP) during the project development period. A part of the blood samples was centrifuged at room temperature for 15 min at 160× *g* to obtain the plasma and stored at −80 °C for posterior IL-10 and serotonin assays. Another part of the total blood samples was reserved for the hematological and hemorheology analyses.

4.3. Urine Separation

The participants collected urine samples from the medium flow using specific urine collection bottles and following instructions for cleanliness. The samples were centrifuged at 160× *g* for 15 min, and then separated and stored at −80 °C for further analysis.

4.4. Plasma Glucose Determination

Fasting plasma glucose was determined in the first trimester of pregnancy. Samples of 20 µL plasma, the standard of 100 mg/dL (BioTécnica®, Ref 10.008.00, Varginha, Brazil), were placed in 2.0 mL phosphate-buffered solution (0.05 M, pH 7.45, with amino antipyrine 0.03 mM, 15 mM sodium p-hydroxybenzoate, 12 kU/L glucose oxidase and 0.8 kU/L

peroxidase). The suspensions were mixed and incubated for 5 min at 37 °C. The reactions were read on a spectrophotometer at 510 nm.

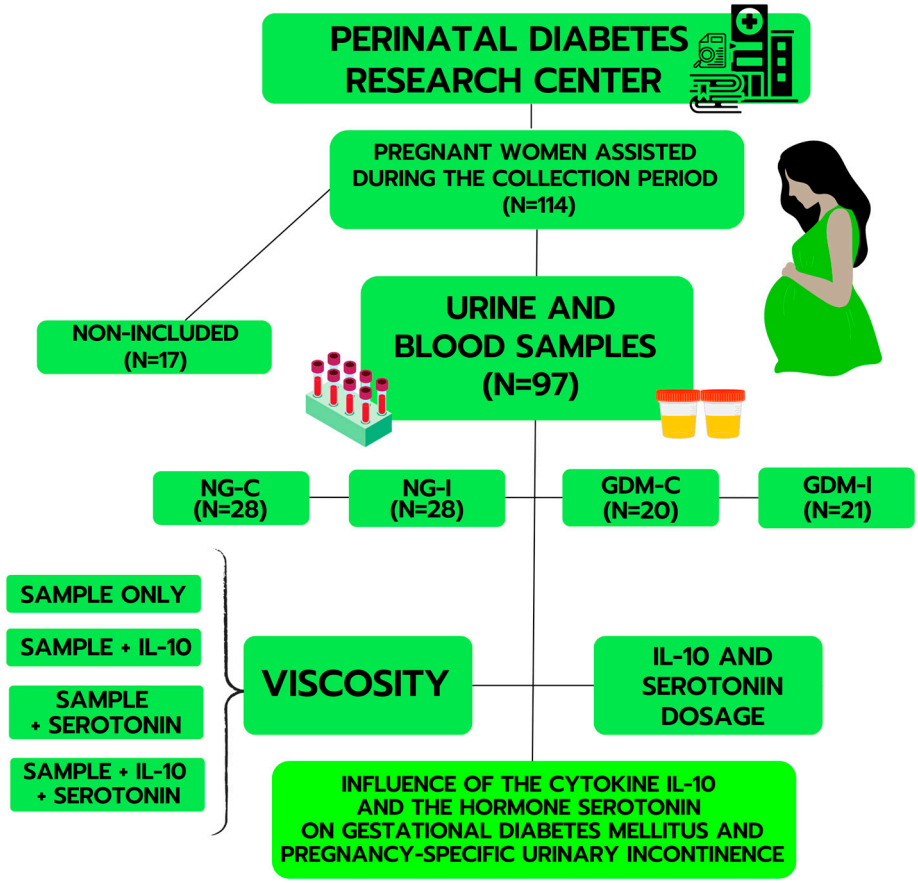


Figure 7. Representative scheme for obtaining samples and experimental design.

4.5. Hematological Parameters

The total blood samples with EDTA from the pregnant women were sent for laboratory analysis to obtain hematological data such as erythrogram, leukogram, and platelet count using an automated device (Sysmex KX-21N™, Kōbe, Hyōgo, Japan) [68].

4.6. Serotonin Determination

The quantitative determination of serotonin hormone concentration in human plasma and urine was performed via enzyme immunoassay in ELISA microplates with the following characteristics: the lower detection limit was 3.97 ng/mL, and the intra-assay and inter-assay coefficients of variation (%) were >10% and >12%, respectively. According to the manufacturer's instructions, the serotonin present in the plasma was determined using an ELISA kit from Cloud-Clone Corp. (Katy, TX, USA). The reaction values were measured by absorbance in a spectrophotometer with a 450 nm filter. The results were obtained through a standard curve and expressed in ng/mL.

4.7. IL-10 Cytokine Determination

The concentrations of cytokine IL-10 present in the serum and urine samples were evaluated using the "Cytometric Bead Array" Kit (CBA, BD Bioscience, San Jose, CA, USA). The bead with fluorescence intensity was conjugated with a specific capture antibody for cytokines, mixed to form the CBA, and read in the FL3 channel of the FACSCalibur flow cytometer (BD Biosciences®, San Jose, CA, USA). The bead populations were visualized according to their respective fluorescence intensities: from least bright to brightest. In the

CBA, the cytokine capture beads were mixed with the detection antibody conjugated to the fluorochrome PE and then incubated with the samples to form the "sandwich" assay. The acquisition tubes were prepared with 50 µL of the sample, 50 µL of bead mixture, and 50 µL of detection reagent (PE Detection Reagent/1 vial, 4 mL). The same procedure was performed to obtain the standard curve. The tubes were incubated for three hours at room temperature without light. The cytokine was analyzed using flow cytometry (FACSCalibur, BD Bioscience, San Jose, CA, USA). The results were generated in graphs and tables using the CellQuest Pro (BD)® software (BD Bioscience, San Jose, CA, USA), and the data were analyzed using the FCAP Array software v3.0 (BD Bioscience, San Jose, CA, USA).

4.8. Hemorheological Parameters

The total blood with EDTA from each woman was subjected to hemorheology analysis to obtain data on blood viscosity. A compact modular rheometer, Anton-Paar® Cone-Plate model—MCR 102 (Anton Paar® GmbH, Ostfildern, Germany), was used, and the graphs were obtained using the Rheoplus software v3.6.1 (Anton Paar, Ostfldern, Germany). For the hemorheology analysis, 750 µL of whole blood with EDTA was used, maintaining a temperature of 37 °C in the plate with 60 flow measurement points [20].

4.9. Treatment of Blood with IL-10 and Serotonin

To assess the effect of IL-10 and serotonin on blood viscosity, a 580 µL sample of blood was incubated with 20 µL of IL-10 (Sigma, St Louis, MO, USA; final concentration 100 pg/mL) and/or serotonin (Sigma, St. Louis, MO, USA; final concentration 100 pg/mL) for 1 h at 37 °C. The dose–response curve previously determined this concentration. The blood was used immediately for the rheological analysis.

4.10. Statistics

The statistical analyses were performed using the BioEstat 5.0 program—qualitative variables through relative and absolute frequency. Quantitative variables were described using their means and standard deviation, and data were analyzed for normal distribution using the Shapiro–Wilk test. The ANOVA test was used to compare mean values; the Tukey post-test was used for further group analysis. For the correlation analysis, the Pearson test was used. The adopted significance criterion was 5% ($p < 0.05$).

5. Conclusions

Based on the data from the present study, it can be concluded that pregnant women with gestational diabetes mellitus and pregnancy-specific urinary incontinence experience decreased hemoglobin, hematocrit, serotonin, and IL-10 in their blood. These changes increase blood viscosity, suggesting an interaction between hematological, hormonal, endocrine, and immunological components during the disease. Hyperglycemia due to gestational diabetes has been associated with lower plasma serotonin and IL-10 levels. This research highlights the potential of serotonin and IL-10 in reducing the blood viscosity of pregnant women with gestational diabetes and pregnancy-specific urinary incontinence and maintaining values similar to those found in the blood of normoglycemic women.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the São Paulo State University Research Ethics Committee, Brazil. The study received approval from the Institutional Review Board (CAAE: 57896522.0.0000.5411) on 9 May 2022.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement: The authors will make the data supporting this study's interpretations available if requested.

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Conflicts of Interest: The authors declare that the research was conducted without commercial or financial relationships with a potential conflict of interest.

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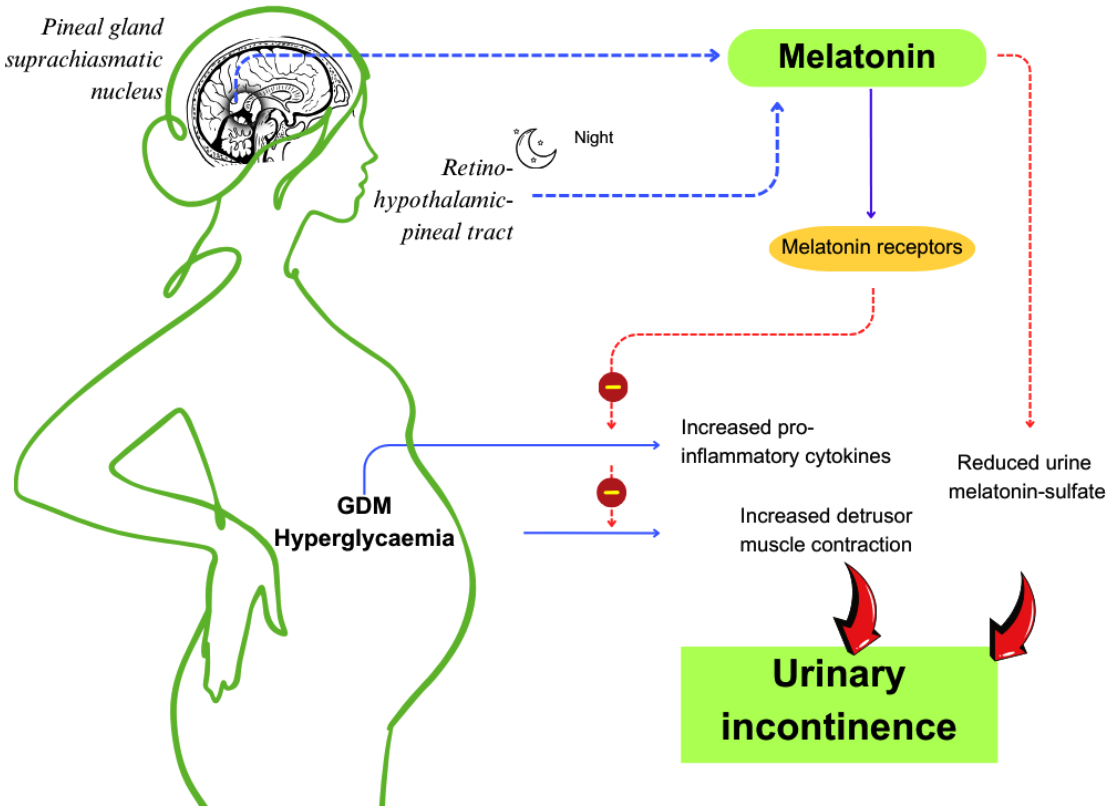
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Artigo 3



Graphical abstract

metabolites

MDPI

Article

Melatonin and Inflammatory Cytokines as Modulators of the Interaction Between Gestational Diabetes Mellitus and Pregnancy-Specific Urinary Incontinence

Danielle Cristina Honório França [1], Eduardo Luzia França [2], Adenilda Cristina Honório-França [2,*], Kênia Maria Rezende Silva [2], Adriele Ataídes de Queiroz [2], Tassiane Cristina Morais [3], Emanuelle Carolina Honorio França [2], Carolina Neiva Frota de Carvalho [1], Danny Laura Gomes Fagundes-Triches [2], Angélica Mércia Pascon Barbosa [1,4], Iracema de Mattos Paranhos Calderon [1], Luis Sobrevia [1,5,6,7] and Marilza Vieira Cunha Rudge [1,*]

1 Department of Gynecology and Obstetrics, Botucatu Medical School (FMB), São Paulo State University (UNESP), Botucatu 18618-687, Brazil; danielle.franca@unesp.br (D.C.H.F.); carolina.n.carvalho@unesp.br (C.N.F.d.C.)
2 Institute of Biological and Health Science, Federal University of Mato Grosso (UFMT), Barra do Garças 78605-091, Brazil; eduardo.franca@ufmt.br (E.L.F.)
3 School of Sciences, Santa Casa de Misericórdia de Vitória (EMESCAM), Vitória 29045-402, Brazil
4 Department of Physiotherapy and Occupational Therapy, School of Philosophy and Sciences, São Paulo State University (UNESP), Marilia 17525-900, Brazil
5 Cellular and Molecular Physiology Laboratory (CMPL), Department of Obstetrics, Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago 8330024, Chile
6 Department of Physiology, Faculty of Pharmacy, Universidad de Sevilla, E-41012 Seville, Spain
7 Faculty of Medicine and Biomedical Sciences, University of Queensland, Herston, QLD 4029, Australia
* Correspondence: adenilda.franca@ufmt.br (A.C.H.-F.); m.rudge@unesp.br or marilzarudge@gmail.com (M.V.C.R.)

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Abstract

Background: The pathogenesis of developing gestational diabetes mellitus (GDM) integrated with pregnancy-specific urinary incontinence (PSUI) may be related to immunological and hormonal factors. Inflammatory cytokines influence the function and regulation of the urinary tract, and changes in melatonin concentration are a predisposing factor for smooth muscle dysfunction and cystometric changes. Objective: This study examines the influence of melatonin, MT1 and MT2 receptors, and inflammatory cytokines in the blood and urine of pregnant women with GDM and PSUI. Methods: Two hundred sixty-nine pregnant women were approached during the diagnostic investigation of GDM and answered a specifically structured questionnaire about the involuntary loss of urine. According to these criteria, mothers were divided into four groups: continent normoglycemic (NG-C), incontinent normoglycemic (NG-I), continent GDM (GDM-C), and incontinent GDM (GDM-UI). Blood and urine samples were collected to determine the levels of melatonin, melatonin sulfate, melatonin receptors (MT1 and MT2), and inflammatory cytokines. Results: Blood level of melatonin and IL-10 was lower, but MT1, MT2, IL-1β, IL-8, and TNF-α were higher in GDM-UI compared with the NG-C group. The melatonin sulfate level was lower in the urine of the GDM-UI group compared with the NG-C group. Conclusions: Maternal hyperglycemia associated with urinary incontinence generates an inflammatory environment characterized by reduced melatonin and IL-10 and increased IL-1β, IL-8, and TNF-α in the blood of mothers with GDM with UI. This environmental condition may be involved in the pathogenesis of these pathologies.

Keywords: neuropharmacology; hormones; hyperglycemia; involuntary loss of urine; hormone receptor; immunomodulation; gestation; diabetes

1. Introduction

Pregnancy is associated with metabolic changes that involve the increased release of insulin counter-regulatory hormones, leading to insulin resistance and glucose intolerance, which can contribute to the development of Gestational Diabetes Mellitus (GDM) [1]. This condition impacts maternal and neonatal health worldwide, increases health care costs, and predisposes to maternal–fetal complications [2,3]. Women with GDM are at higher risk of developing pregnancy-specific urinary incontinence (UI) [4], a condition that significantly affects biopsychosocial aspects of pregnancy. Hyperglycemia has been associated with increased relaxation of pelvic floor muscles [5], while insulin resistance contributes to muscle atrophy and weakness, both of which predispose to urinary incontinence [6,7].

The hyperglycemic maternal microenvironment in GDM is also associated with inflammation, as it releases proinflammatory cytokines [8]. These molecules regulate inflammatory responses, act in the brain and spinal cord, influence micturition, and have peripheral effects on bladder function. Oxidative stress and inflammation are also key mediators in the onset and progression of smooth muscle dysfunctions [9].

Genetic and hormonal factors and maternal eating habits are also involved in GDM and PSUI. Systemic inflammation modulates the uptake of molecules and directly influences the production of hormones, such as melatonin [10,11]. Melatonin is the immunomodulatory agent mainly produced and secreted by the pineal gland during its nocturnal peak [11–13], being directly influenced by the light diffused through the retina to the hypothalamic suprachiasmatic nucleus in the Central Nervous System (CNS) [10].

Melatonin has been related to the regulation of the circadian cycle and rhythmically participates in the modulation of the secretion of insulin and glucose molecules [11], favors the differentiation of neurons, and has the anti-apoptotic and antioxidative potential [14,15], being excreted in the form of its metabolite melatonin–sulfate (6-Sulfatoxymelatonin) in the urine [16].

This molecule also exerts protective effects on smooth muscle physiology by restoring detrusor contractility, normalizing mitochondrial function, and modulating neuromuscular activity, thereby preventing cystometric changes and inflammation [15]. Its biological actions are mediated by MT1 and MT2 receptors, expressed not only in the CNS but also in peripheral tissues, which synchronize circadian rhythm and metabolic functions [17–19]. Imbalance in this signaling system is directly linked to the pathophysiology of diabetes and related complications [20].

An imbalance in the biological actions of this hormone is linked to the pathophysiology of metabolic diseases, including diabetes mellitus [20]. However, the roles of inflammatory regulatory cytokines and melatonin in maintaining urinary continence in pregnant women with GDM are still unclear. This study evaluated the levels of melatonin, MT1 and MT2 receptors, and inflammatory cytokines in the blood and urine of pregnant women with GDM and PSUI.

2. Materials and Methods

2.1. Study Population

This study is a cross-sectional observational study conducted at the Perinatal Diabetes Research Center at the Botucatu Medical School (FMB—UNESP, Brazil). Pregnant women who met the following inclusion criteria were included: age between 18 and 40 years,

GDM diagnosis, and a single pregnancy with a live fetus. Patients signed the Informed Consent Form. Pregnant women with urinary incontinence before pregnancy, Type 1 or Type 2 diabetes mellitus, pregnant women with polycystic ovary syndrome, or conception by assisted reproduction were excluded from the study. Multiple pregnancies, collagen diseases, autoimmune diseases, use of tricyclic antidepressants, and fetal malformations were also excluded from the study.

Two hundred sixty-nine pregnant women were approached during the GDM diagnostic investigation, which included the 75-g oral glucose tolerance test (OGTT), fasting glucose, and glycemic profile. The diagnosis of gestational diabetes mellitus (GDM) was established according to the American Diabetes Association [21] criteria, based on plasma glucose. The participants answered the International Consultation on Incontinence Questionnaire—Short Form (ICIQ-SF) [22], a questionnaire validated in Brazil that assesses the impact of UI on women's quality of life to classify urinary loss and determine the severity index of UI [23]. The questionnaire was validated as a quantitative and semi-objective measure of UI severity.

Following the selection criteria of the participants, 114 pregnant women were included in the study and classified into the following study groups: continent normoglycemic (NG-C, 29 patients), normoglycemic with pregnancy-specific urinary incontinence (NG-I, 31 patients), continent with GDM (GDM-C, 26 patients), and GDM with pregnancy-specific urinary incontinence (GDM-I, 28 patients). The scheme for obtaining samples and experimental design is described in Figure 1. All patients signed the Informed Consent Form.

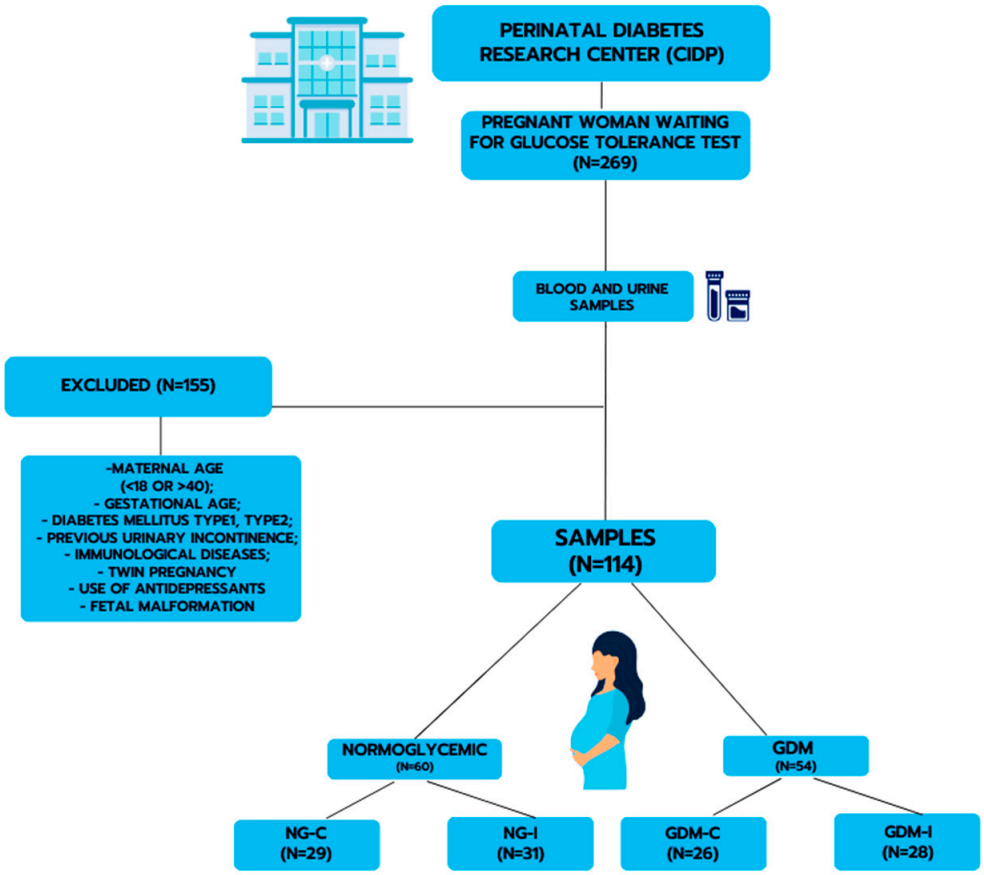


Figure 1. Representative scheme for obtaining samples and experimental design.

2.2. Blood and Urine Samples

Blood samples (8 mL) were collected in Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NJ, USA) without anticoagulant and centrifuged (160× *g*, 15 min, at room temperature). The serum was separated and stored at −80 °C for further analysis. The leukocytes were separated by density gradient and lysed with Triton X-100 (Sigma, St. Louis, MO, USA) for 5 min at room temperature. The lysate was centrifuged (1500× *g*, 10 min, 8 °C) to remove cellular debris.

Urine samples (15 mL) were collected in the morning, as the first voids of the day, to reduce circadian variability in melatonin metabolite levels. During a period of moderate urinary flow, samples were collected in sterile urine containers in accordance with aseptic collection guidelines. Subsequently, urine samples were centrifuged (160× *g*, 15 min, at room temperature), and supernatants were stored at −80 °C for later analysis.

2.3. Melatonin Determination

Quantitative determination of melatonin hormone concentration in human serum was performed by enzyme immunoassay on ELISA microplates with an ELISA kit (Imuno-Biological Laboratories-Melatonin-ELISA RE54021-IBL, Hamburg, Germany). The lower detection limit was 1.6 pg/mL, with intra-assay and inter-assay coefficients of variation (CV%) of 3.0% to 11.4% and 6.4% to 19.3%, respectively. The melatonin was extracted by affinity chromatography and concentrated in a vacuum centrifuge (500× *g*, 180 min, 4 °C). Reaction was measured by absorbance at 450 nm in a plate spectrophotometer (ThermoPlate TP-Reader, Thermo Fisher Scientific, Waltham, MA, USA). The results were calculated against a melatonin standard curve and expressed in picograms per milliliter (pg/mL).

2.4. Melatonin Receptors Determination

A sandwich enzyme immunoassay ELISA kit (Cloud Clone Corporation, Houston, TX, USA) was used for in vitro quantitative measurement of melatonin receptor 1A (MT1) and 1B (MT2) in lysed human blood cells according to the manufacturer's instructions. The lower detection limit was 156 pg/mL (0.156 ng/mL), and intra-assay and inter-assay CV% were <10% and <12%, respectively. Reaction was measured by absorbance at 450 nm in a plate spectrophotometer (Thermo Plate TP-Reader, Thermo Fisher Scientific, Waltham, MA, USA). The results were calculated against MT1 and MT2 standard curves and expressed as picograms per milliliter (pg/mL).

2.5. Urinary Melatonin–Sulfate Measurement

The concentration of melatonin–sulfate urine (sMT6a) was measured by enzyme immunoassay on microplates using an ELISA kit (Imuno-Biological Laboratories–Melatonin–sulfate Urine ELISA RE54031-IBL, Hamburg, Germany). The lower detection limit was 1.0 ng/mL, and the intra-assay and inter-assay coefficients of variation (CV%) were 5.2% to 12.2% and 5.1% to 14.9%, respectively. Reaction was measured by absorbance at 450 nm in a plate spectrophotometer (ThermoPlate TP-Reader, Thermo Fisher Scientific, Waltham, MA, USA). The results were calculated against a melatonin sulfate standard curve and expressed in nanograms per milliliter (ng/mL). For the melatonin/melatonin–sulfate ratio, the results of melatonin–sulfate were expressed in pg/mL.

2.6. Cytokines Determination

Cytokine concentrations in serum and urine samples were evaluated using the Cytometric Bead Array (CBA) Kit (BD Bioscience, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. The cytokines IL-1β, IL-6, IL-8, IL-10, IL-12, and TNF-α were analyzed using flow cytometry (FACS Calibur, BD Bioscience, USA). The results were

generated using the BD CellQuest Pro Software Acquisition (version 5.1, BD Biosciences, USA) and analyzed using the FCAP Array Software v3.0 (BD Biosciences, San Jose, CA, USA).

2.7. Statistics

Statistical analyses were performed using BioEstat® version 5.0 software (Mamirauá Institute, Belém, Brazil). Data were expressed as means ± standard deviations (SD). The sample size was estimated using the same software, assuming a statistical power of 80% ($\beta = 0.20$) and a significance level of 5% ($\alpha = 0.05$). The calculation was based on previous studies from our research group that evaluated similar immunometabolic parameters and indicated an expected difference of approximately 20% between groups.

The D'Agostino normality test was applied to assess data distribution. Two-way analysis of variance (ANOVA) followed by the Bonferroni post-test was used for group comparisons. Pearson's correlation test was applied to evaluate associations between cytokine levels, melatonin concentrations, and metabolic parameters. The adopted significance criterion was $p < 0.05$.

Additionally, Cohen's d was used to estimate the magnitude of intergroup differences, and results were interpreted according to conventional thresholds: d = 0.2 (small), d = 0.5 (medium), and d ≥ 0.8 (large).

3. Results

3.1. Study Groups

The clinical information of the mothers is given in Table 1. Mothers with GDM, regardless of the presence or absence of urinary incontinence, were older than those in the corresponding normoglycemic groups. The GDM groups had higher BMI and glycaemia ($p < 0.05$), and the GDM-UI group had a higher HbA1c level than all other groups. However, no significant difference in urine density was observed between the groups.

Table 1. Clinical data on pregnant women.

	NG-C (n = 29)	**NG-UI (n = 31)**	**GDM-C (n = 26)**	**GDM-UI (n = 28)**
Age	25.0 ± 4.6	27.1 ± 7.2	30.5 ± 6.1 *	30.9 ± 6.3 *†
BMI	29.0 ± 4.2	30.0 ± 5.5	34.7 ± 8.2 *	36.0 ± 7.1 *†
Glucose (mg/dL)	76.2 ± 6.9	76.2 ± 7.9	93.2 ± 10.6 *	92.0 ± 10.3 *†
HbA1c (%)	4.9 ± 0.3	5.0 ± 0.3	5.0 ± 0.4	5.2 ± 0.4 *†
Urinary density	1.0 ± 0.1	1.01 ± 0.1	1.0 ± 0.1	1.2 ± 0.1

Note: NG-C: normoglycemic continent, NG-UI: normoglycemic with urinary incontinence, GDM-C: gestational diabetes mellitus continent, and GDM-UI: gestational diabetes mellitus with urinary incontinence; BMI at 24 to 28 weeks of pregnancy; HbA1c, glycated hemoglobin; PSUI, pregnancy-specific urinary incontinence. The age, BMI, Glycemia, HbA1c, and urinary density are expressed as mean ± S.E.M. * $p < 0.05$ versus NG-C and NG-UI. † $p < 0.05$ versus NG-UI.

3.2. Blood Melatonin and Melatonin Receptors

The levels of melatonin, as well as those of the MT1 and MT2 receptors, were measured in blood samples from pregnant women. The serum level of melatonin was higher in GDM-C but lower in GDM-UI groups compared with NG-C or NG-UI groups (Figure 2A). The melatonin serum level was lower in the GDM-UI group compared with the GDM-C group.

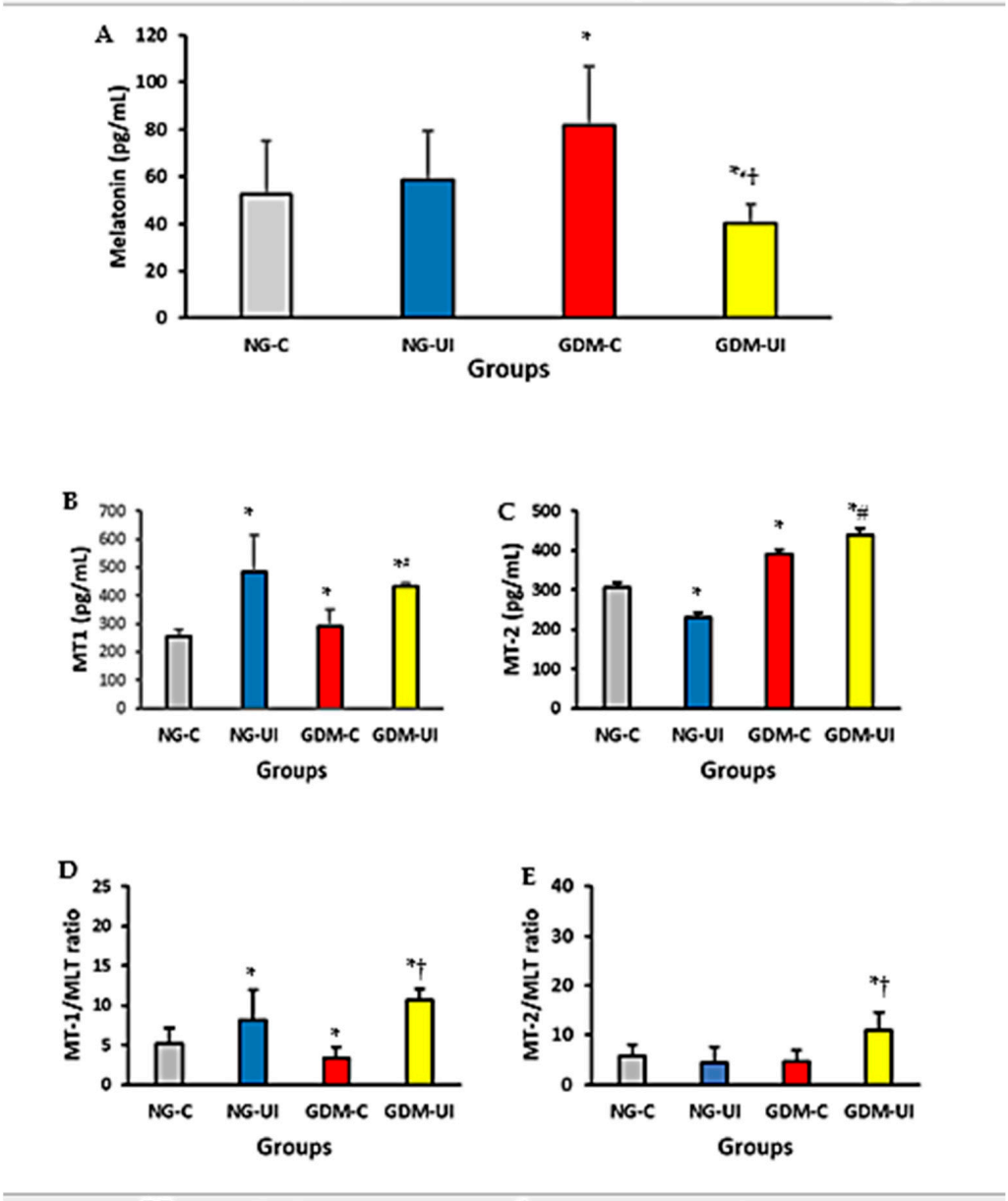


Figure 2. Blood levels of melatonin and its receptors in pregnant women. (**A**) Melatonin concentration; (**B**) MT1 receptor level; (**C**) MT2 receptor level; (**D**) MT1/melatonin (MT1/MLT) ratio; (**E**) MT2/melatonin (MT2/MLT) ratio. Measurements were performed in women with normoglycemia (NG) or gestational diabetes mellitus (GDM), without urinary incontinence (NG-C, GDM-C) or with urinary incontinence (NG-UI, GDM-UI). Data are presented as mean ± standard error of the mean (S.E.M.). Significant differences between normoglycemic and GDM groups were observed for melatonin ($p = 0.0268$), MT1 ($p = 0.0394$), MT2 ($p = 0.0205$), MLT/MT1 ($p = 0.0066$), and MLT/MT2 ($p = 0.0205$). Symbols of significance: * difference between the control group (NG-C) and the other groups (NG-UI, GDM-C, and GDM-UI); # difference between NG-UI and GDM-UI groups; †—difference between GDM-C and GDM-UI ($p < 0.05$ vs. GDM-C).

The MT1 receptor level in the blood of NG-UI and GDM-UI groups was higher than in NG-C and GDM-C groups (Figure 2B). The MT2 receptor level was higher in the blood of GDM-C and GDM-UI but lower in the NG-UI groups compared with the NG-C group (Figure 2C). Additionally, the MT2 receptor blood level in the NG-UI group was lower than in the GDM-C and GDM-UI groups.

The MT1/melatonin ratio was higher in NG-UI and GDM-UI but lower in GDM-C compared with NG-C groups (Figure 2D). The MT1/melatonin ratio in GDM-UI was also

higher than that of the GMD-C group. However, the MT2/melatonin ratio was higher in the GDM-UI group compared with all other groups in this study.

Serum melatonin and its MT1 and MT2 receptors demonstrated distinct modulation patterns between groups (Figure 3). Effect size analysis revealed that MLT concentrations were moderately increased in GDM-C (Cohen's d = −0.98 vs NGC) but reduced in GDM-UI (Cohen's d = 0.67 vs NG-C). For the receptor, the most pronounced difference was observed in MT1 (Cohen's d = −7.51, NG-C vs GDM-UI).



Figure 3. Heatmap of Effect Sizes (Cohen's d) for melatonin and its receptors. Heatmap representing the effect sizes (Cohen's d) of group comparisons for circulating and their receptors. Each square indicates the magnitude and direction of the difference between groups (NG-C, NG-UI, GDM-C, and GDM-UI). Lighter colors denote higher effect sizes, illustrating the most pronounced differences observed in MT1 and MLT/MT1 markers, particularly in the GDMI group.

3.3. Urine Melatonin–Sulfate Level

Melatonin–sulfate levels were measured in urine samples from pregnant women. Urinary melatonin–sulfate concentrations were lower in NG-UI, GDM-C, and GDM-UI groups compared with the normoglycemic continent group (NG-C) (Figure 4A). The blood melatonin-to-urine melatonin–sulfate ratio was higher in GDM-C compared with all other groups (Figure 4B). Conversely, the inverse ratio (urine melatonin–sulfate to blood melatonin) was lower in NG-UI and GDM-C and elevated in GDM-UI (Figure 4C).

Effect size analysis supported these findings, indicating a moderate difference between GDM-C and NG-C (Cohen's d = 0.63), a small effect for GDM-UI (d = 0.18), a large difference between NG-C and NG-UI (d = 0.82), and a moderate difference between NG-UI and GDM-UI (d = −0.66).

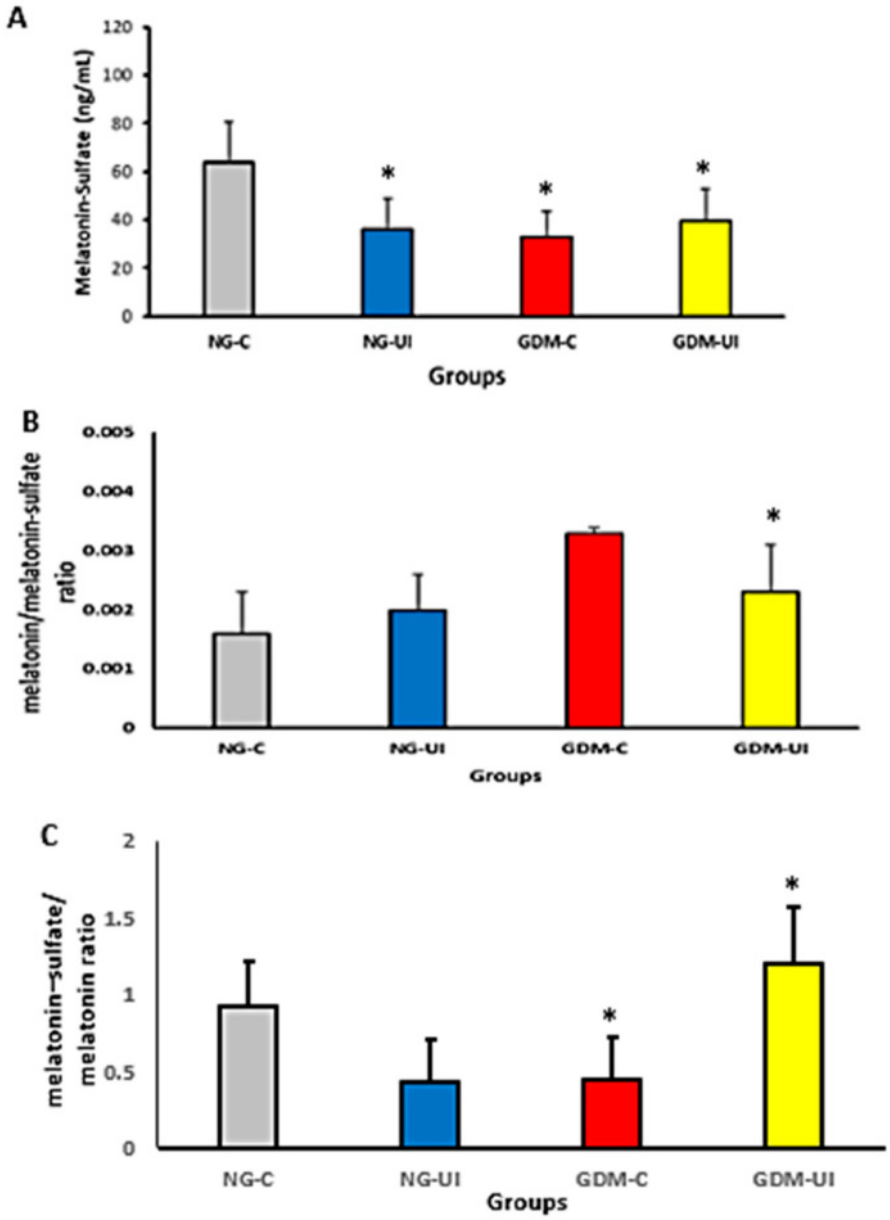


Figure 4. Dynamics of Urinary and Circulating Melatonin in Women with and without Gestational Diabetes Mellitus and Urinary Incontinence. (**A**) Urinary melatonin–sulfate concentration (MLT-S). (**B**) Serum melatonin/urinary melatonin–sulfate ratio (MLT/MLT-S) and (**C**) Urine melatonin–sulfate/melatonin serum ratio (MLT) by group, calculated from measured data. Groups are color-coded as NG-C (gray), NG-UI (blue), GDM-C (red), and GDM-UI (yellow). Measurements were performed in women with normoglycemia (NG) or gestational diabetes mellitus (GDM), without urinary incontinence (NG-C, GDM-C) or with urinary incontinence (NG-UI, GDM-UI). Data are expressed as mean ± standard error of the mean (S.E.M.). Significant differences between normoglycemic and GDM groups were observed for melatonin–sulfate ($p = 0.0093$), MLT serum/MLT urine ($p = 0.0153$), and MLT urine/MLT serum ($p = 0.0048$). Symbols of significance: * $p < 0.05$ vs. control group (NG-C).

3.4. Serum and Urine Cytokine Levels

The levels of IL-1β, IL-6, IL-8, IL-10, IL-12, and TNF-α cytokines were assessed in the serum and urine (Figures 5 and 6). The serum level of IL-1β was higher in GDM-C and GDM-IU compared with NG-C and NG-UI groups (Figure 5A). However, the level of this cytokine in the urine remained unchanged. The IL-1β level in serum was lower compared with urine only in the NG-C and NG-UI groups. The IL-6 levels were similar across all

experimental groups (Figure 5B). The serum level of IL-8 was higher in the NG-UI and GDM-UI groups than in the NG-C and GDM-C groups; however, the urine level of this cytokine was unchanged between the groups (Figure 5C). All IL-8 concentrations detected in the urine were higher than those in blood samples in all patient groups.

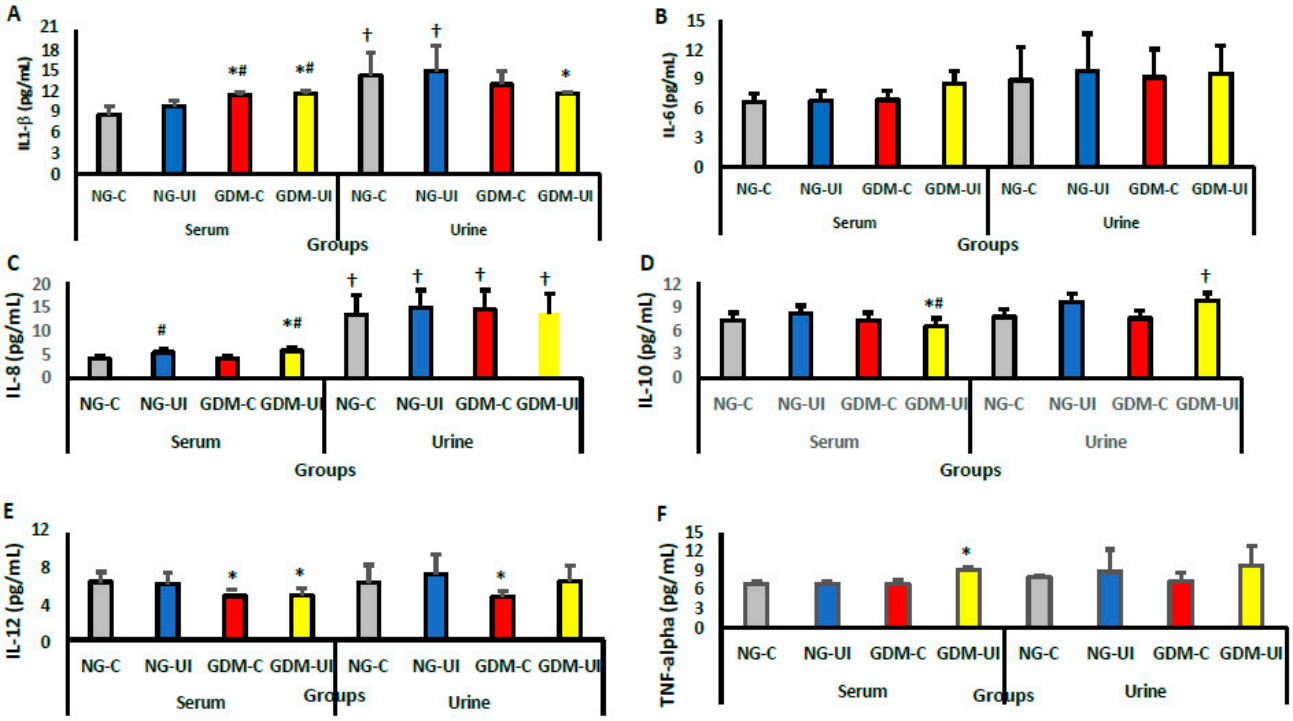


Figure 5. Cytokine concentrations (pg/mL) measured in serum and urine samples. (**A**) IL-1β; (**B**) IL-6; (**C**) IL-8; (**D**) IL-10; (**E**) IL-12; (**F**) TNF-α. Analyses were conducted in women with normoglycemia or gestational diabetes mellitus (GDM), classified as continent or with urinary incontinence (UI). Data are presented as mean ± standard error of the mean (S.E.M.). Group abbreviations: NG-C, normoglycemic continent; NG-UI, normoglycemic with urinary incontinence; GDM-C, GDM continent; GDM-UI, GDM with urinary incontinence. Significant differences with corresponding *p*-values were observed as follows: IL-1β ($p = 0.0010$), IL-6 ($p = 0.6241$), IL-8 ($p = 0.0484$), IL-10 ($p = 0.0120$), IL-12 ($p = 0.0229$), and TNF-α ($p < 0.0001$) between normoglycemic and GDM groups (*); IL-1β ($p = 0.0001$), IL-6 ($p = 0.3994$), IL-8 ($p = 0.0231$), IL-10 ($p = 0.0416$), IL-12 ($p = 0.5645$), and TNF-α ($p = 0.1181$) between continent and incontinent groups (#); and IL-1β ($p = 0.0001$), IL-6 ($p = 0.5888$), IL-8 ($p = 0.0308$), IL-10 ($p = 0.0450$), IL-12 ($p = 0.8397$), and TNF-α ($p = 0.3035$) between serum and urine samples (†). Symbols: * normoglycemic vs. GDM; # continent vs. incontinent; † serum vs. urine.

The serum level of IL-10 was lower in GDM-UI compared with all other groups in both serum and urine (Figure 5D). IL-12 serum levels in GDM-C and GDM-UI were lower than in the NG groups (Figure 5E). However, the level of TNF-α in serum was higher in GDM-UI than in all other groups, both in serum and in urine (Figure 5F).

The serum/urine ratio for IL-1β was lower in NG-C, and the ratio for IL-12 was higher in GDM-UI (Table 2). However, the serum-to-urine ratios for IL-6, IL-8, IL-10, and TNF-α were similar between the groups (Table 2). Changes in blood melatonin levels were positively correlated with IL-10 but negatively correlated with TNF-α levels (Table 3). No significant correlation was found between blood melatonin and the other cytokines. Additionally, changes in urinary melatonin–sulfate levels were positively correlated with IL-6 and IL-10 levels (Table 4). No significant correlation was found between urinary melatonin sulfate levels and the other cytokines.

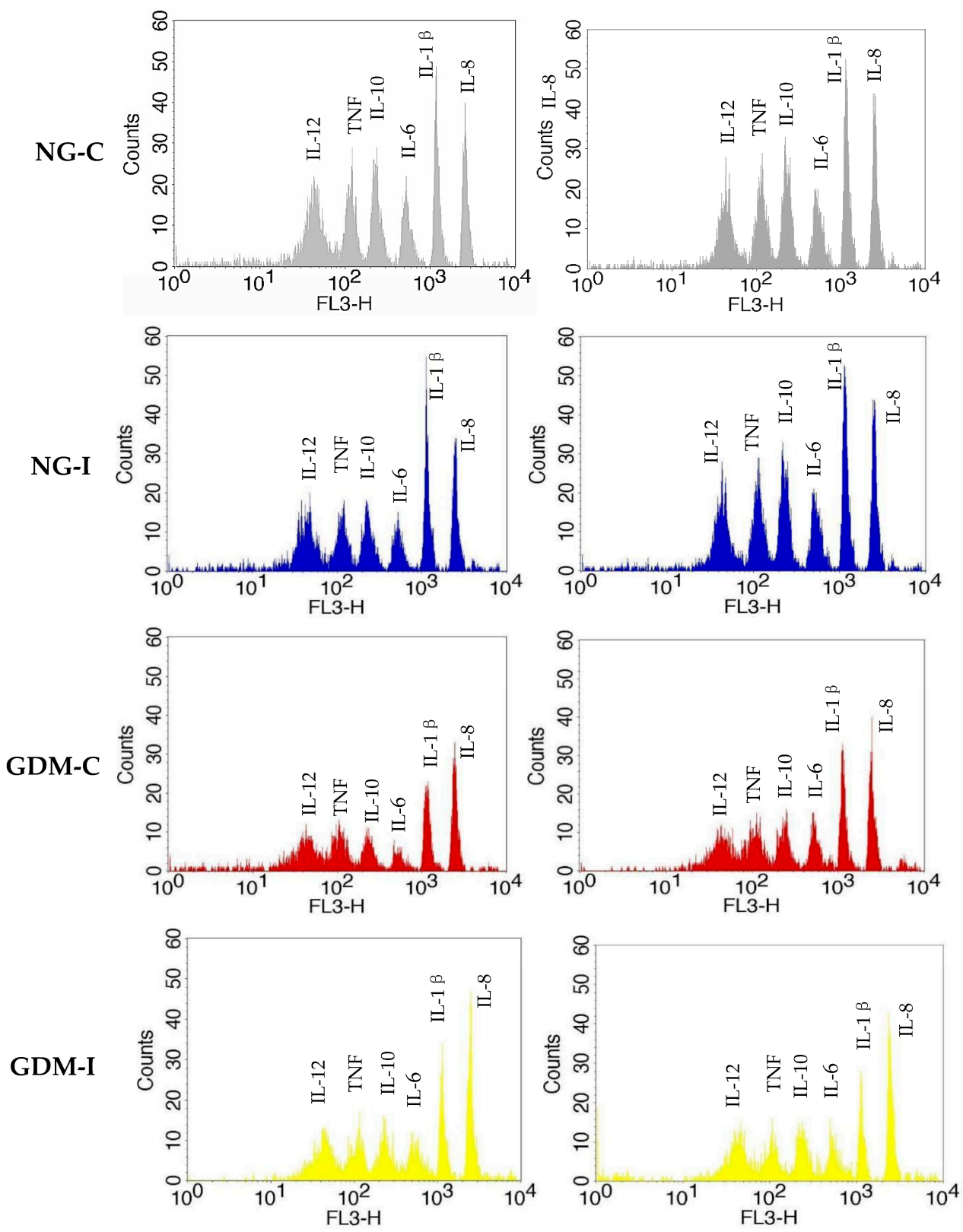


Figure 6. Fluorescence intensity of cytokines in serum and urine. Fluorescence analyses were performed using flow cytometry (FACSCalibur, Becton Dickinson, Franklin Lakes, New Jersey, USA). Cytokine levels were quantified as fluorescence intensity peaks (FL3 channel). Measurements were performed in women with normoglycemia or gestational diabetes mellitus (GDM), without urinary incontinence (NG-C, GDM-C) or with urinary incontinence (NG-UI, GDM-UI).

Table 2. Plasma/Urine cytokine ratio.

Cytokines	Blood/Urine Ratio				Statistical
	NG-C	NG-UI	GDM-C	GDM-UI	
IL-1 β	0.63 ± 0.14	0.88 ± 0.31 *	0.89 ± 0.10 *	1.06 ± 0.04 *	F = 17.6607; p = 0.0001
IL-6	0.86 ± 0.32	0.92 ± 0. 50	0.80 ± 0.30	0.86 ± 0.28	F = 0.1333; p = 0.9387
IL-8	0.39 ± 0.12	0.38 ± 0.11	0.37 ± 0.09	0.46 ± 0.22	F = 0.3809; p = 0.7704
IL-10	0.97 ± 0.27	0.90 ± 0.22	0.96 ± 0.15	0.87 ± 0.22	F = 0.4176; p = 0.7450
IL-12	1.01 ± 0.41	0.99 ± 0.34	0.94 ± 0.22	1.20 ± 0.15 *	F = 2.3077 p = 0.0482
TNF-α	0.86 ± 0.05	0.85 ± 0.18	0.98 ± 0.15	0.90 ± 0.33	F = 1.2564; p = 0.3088

Note: Results are expressed as the mean plasma/urine ratio. * Statistical difference between ND-C and the other groups.

Table 3. Correlation between plasma melatonin and cytokines.

Melatonin	NG-C	NG-UI	GDM-C	GDM-UI
IL-1 β	r = −0.4102 p = 0.3607	r = −0.6301 p = 0.1292	r = −0.6414 p = 0.1204	r = 0.2530 p = 0.5840
IL-6	r = 0.0710 p = 0.8797	r = 0.0690 p = 0.8831	r = −0.2908 p = 0.5269	r = 0.2798 p = 0.5433
IL-8	r = 0.4632 p = 0.2951	r = 0.2298 p = 0.6200	r = 0.2911 p = 0.5265	r = 0.0346 p = 0.9413
IL-10	r = 0.2824 p = 0.5394	r = −0.2685 p = 0.5605	r = 0.5486 p = 0.2021	r = 0.8980 * p = 0.0470
IL-12	r = −0.4874 p = 0.2672	r = −0.0890 p = 0.8496	r = 0.4474 p = 0.3141	r = −0.0698 p = 0.8818
TNF-α	r = 0.2669 p = 0.5628	r = 0.4940 p = 0.2598	r = 0.4033 p = 0.3696	r = −0.9310 * p = 0.0427

Note: * statistical difference; r = Pearson correlation coefficient.

Table 4. Correlation between urinary melatonin–sulfate and cytokines.

Melatonin–sulfate	NG-C	NG-UI	GDM-C	GDM-UI
IL-1 β	r = −0.1597 p = 0.7056	r = −0.1267 p = 0.7649	r = −0.2110 p = 0.6159	r = −0.4096 p = 0.3135
IL-6	r = −0.1461 p = 0.7298	r = 0.2588 p = 0.5360	r = 0.0018 p = 0.9966	r = 0.8984 * p = 0.0024
IL-8	r = 0.4240 p = 0.2941	r = 0.4627 p = 0.2482	r = −0.0991 p = 0.9829	r = 0.0278 p = 0.9480
IL-10	r = 0.3738 p = 0.3617	r = 0.1833 p = 0.6639	r = −0.6275 p = 0.0958	r = 0.7669 * p = 0.0263
IL-12	r = −0.0266 p = 0.9501	r = 0.1666 p = 0.8496	r = −0.2638 p = 0.5279	r = −0.4411 p = 0.2739
TNF-α	r = −0.2657 p = 0.5247	r = 0.0696 p = 0.6933	r = 0.2398 p = 0.5672	r = −0.5681 p = 0.1417

Note: * statistical difference; r = Pearson correlation coefficient.

4. Discussion

Gestational diabetes mellitus (GDM) is a pregnancy-related metabolic disorder characterized by hyperglycemia, which can compromise the structural integrity and contractile function of pelvic and abdominal muscles through mechanisms involving insulin resistance

and altered glucose metabolism. These alterations may lead to muscle relaxation, weakness, and atrophy, ultimately predisposing women to the development of pregnancy-specific urinary incontinence (PSUI) [4,6,24]. Since GDM is associated with increased release of proinflammatory cytokines [8], and systemic inflammation increases melatonin synthesis [25], this study aimed to determine whether women with GDM and PSUI showed altered blood and urine levels of melatonin and inflammatory cytokines. The findings suggest that women with GDM who develop PSUI show an altered pattern of cytokines in the blood and urine in which melatonin may play a role.

Various risk factors associated with GDM include pre-pregnancy body mass index (BMI) and gestational weight gain (GWG). The women with GDM included in this study showed pre-pregnancy BMI higher than in normal pregnancies, agreeing with other reports in patients with this disease [26–28]. Women in the GDM-UI group had the highest BMI between 24 and 28 weeks of gestation, supporting the possibility that these women may be more likely to develop GDM-associated urinary incontinence due to increased weight, compared with women with normal or overweight BMI [29].

Several hormonal factors play key roles in response to hyperglycemia in pregnancy. Pregnant women with type 2 diabetes mellitus (T2DM) showed elevated serum and colostrum levels of melatonin [12,30]. The latter might be a defense mechanism triggered in pregnant women with pre-established diabetes since melatonin acts as a protective factor against smooth muscle disorders [31]. To date, melatonin has been shown to restore the impaired contraction activity of the pelvic floor muscles in patients with urinary incontinence, attenuate sympathetic tone, improve neuromuscular function, protect against inflammation, and scavenge free radicals [15,32,33]. Our findings indicate that serum melatonin concentrations were higher in pregnant women with GDM and urinary continence (GDM-C) compared with those with GDM-UI. This elevation may represent a compensatory mechanism to maintain homeostasis in a hyperglycemic environment [28]. Conversely, women with GDM-UI exhibited reduced melatonin levels, suggesting a loss of this protective effect. Women with urinary incontinence, regardless of glycemic status, also showed lower urinary melatonin–sulfate concentrations, consistent with altered melatonin metabolism.

Changes in the pressure–volume dynamics of the bladder can disrupt neuro-muscular and smooth muscle regulation, leading to impaired detrusor contractility [33,34]. These abnormalities have been shown to improve following melatonin treatment, reinforcing the neuroendocrine role of melatonin in urinary control [35,36]. The hormone acts primarily through its receptors MT1 and MT2, whose deficiency has been associated with insulin resistance and reduced glycolytic activity, respectively [37,38]. Despite lower circulating melatonin in GDM-UI, MT1 receptor expression was increased in leukocytes, suggesting receptor upregulation as a compensatory response.

Regarding the MT2 receptor, there was an increase in both diabetic groups. This increase in receptors, associated with the lower melatonin concentration in GDM-UI pregnant women, demonstrates that, despite the availability of receptors to transmit neuroendocrine signals, the decrease in melatonin probably reflects difficulty in improving detrusor activity in pregnant women with GDM. In addition, the NG-UI group had lower levels of MT2, suggesting that urinary incontinence may predispose to the worsening of myoneural signaling involved in the hormone mechanism melatonin during pregnancy.

Effect size analysis corroborated these observations, revealing large differences between NG-C and NG-UI and moderate divergence between NG-UI and GDM-UI, consistent with a progressive disruption of the pineal–renal feedback axis. Taken together, these findings suggest that GDM-C retains circulating melatonin due to reduced renal elimination. In contrast, GDM-UI exhibits increased urinary loss despite lower serum levels, reflecting

inefficient renal conservation. Physiological pregnancy (NG-C) maintains a balanced melatonin profile, while urinary incontinence, particularly in the context of gestational diabetes, appears to alter melatonin homeostasis and receptor expression.

It is reported that mothers who experienced GDM have endocrine and immune system changes [15]. Melatonin is an immunomodulatory agent during pregnancy due to its antioxidant effects and its potential to regulate processes involved in human reproduction [39], primarily by modulating cytokine synthesis and release [40]. Proinflammatory cytokines, such as IL-1β, IL-6, IL-12, IL-8, and TNF-α, regulate micturition at the spinal cord and brain levels, leading to afferent nerve hypersensitivity and promoting hypertrophy of the bladder muscle [41]. In the present study, women in the GDM-UI group showed increased serum levels of IL-8 and TNF-α but reduced levels of the anti-inflammatory cytokine IL-10. These results suggest a proinflammatory profile that contributes to the pathophysiology of GDM associated with PSUI. However, TNF-α may be embryotoxic when increased in serum and tissue in diabetes mellitus [42]. Therefore, the increase in serum TNF-α in women with GDM-UI may be detrimental to the maternal-fetal binomial.

Interestingly, urinary cytokine profiles have been studied in other urinary tract disorders, such as bladder pain syndrome, as potential biomarkers of the pathology [43]. However, the involvement of cytokines in the etiology of GDM-associated urinary incontinence is unknown. The results show that melatonin and cytokine levels in urine and blood were correlated only for women with GDM-UI. The reduced melatonin levels observed in this group of pregnant women correlated positively with IL-10 and inversely with TNF-α, reinforcing the possibility that urinary incontinence may result from a defective biological effect of melatonin. Reduced urinary melatonin sulfate levels in women with GDM-UI showed a positive correlation with IL-10 and melatonin, suggesting that hyperglycemia and urinary incontinence alter endocrine and immune systems, creating an inflammatory environment in women with GDM and pregnancy-specific urinary incontinence.

A limitation of this study is its cross-sectional design, which does not allow for inferences about the temporal or mechanistic relationships between gestational diabetes mellitus, pregnancy-specific urinary incontinence, melatonin levels, and cytokine profiles. Furthermore, maternal age, body mass index (BMI), and gestational age differed between groups, which may have influenced cytokine and melatonin levels; consequently, only associations can be observed. Future longitudinal studies are needed to clarify the effects of these variables and the underlying mechanisms involved.

Despite these limitations, this study has several strengths. The assessment of melatonin levels, its receptors (MT1 and MT2), and inflammatory cytokines in serum and urine provides an integrative view of neuroimmunoendocrine interactions during pregnancy. Assessment of the systemic and excretory compartments identified potential regulatory patterns in melatonin metabolism associated with gestational diabetes and urinary incontinence. This comprehensive approach represents a methodological advantage and contributes new evidence supporting the involvement of the melatonin system in pregnancy-related metabolic and inflammatory conditions.

5. Conclusions

Maternal hyperglycemia associated with urinary incontinence creates an inflammatory environment, characterized by reduced melatonin and IL-10 and increased IL-1β, IL-8, and TNF-α in the plasma of mothers with gestational diabetes mellitus and incontinence-specific urinary tract issues. These changes act as modulators in the pathogenesis of both pathologies.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and adhered to all applicable human research procedures. No animals were utilized in the research. The Research Ethics Committee at São Paulo State University, Brazil, approved the study. The study received approval from the Research Ethics Committee (CAAE: 57896522.0.0000.5411) on 9 May 2022.

Informed Consent Statement: Written informed consent was obtained from all patients.

Data Availability Statement: The data supporting the findings of this study are available from the corresponding authors upon reasonable request.

Conflicts of Interest: The authors declare that the research was conducted without any commercial or financial relationships that could create conflicts of interest.

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Seção 5
Perspectivas Acadêmicas e Científicas

Perspectivas em curto prazo:

A continuação de publicações na área de ginecologia e obstetrícia, a fim de contribuir para a pesquisa em saúde da mulher no país. Além da divulgação da pesquisa em meios científicos, visando a maior propagação conhecimento.

Perspectivas em médio prazo:

Seguir dando continuidade na prática e pesquisas clínicas na área de saúde da mulher, por meio de um futuro projeto de pós-doutorado, objetivando a realização de mais trabalhos em maiores medidas de prevenção, promoção, tratamento e acompanhamento de gestantes com DMG e IUEG.

Perspectivas em longo prazo:

Lecionar e atender na área de saúde da mulher, com foco em gestantes e mulheres com incontinência urinária e diabetes mellitus, bem como a elaboração futura de guidelines para a população alvo.

Seção 6
Anexos

Anexo 1 - Termo de Consentimento Livre e Esclarecido da Pesquisa Clínica

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO – TCLE
(TERMINOLOGIA OBRIGATÓRIA EM ATENDIMENTO A RESOLUÇÃO 466/12-CNS-MS)

A sra. está sendo convidada a participar de uma pesquisa intitulada: **"Serotonina, melatonina e citocinas inflamatórias como potenciais mediadores na interação Diabetes Mellitus Gestacional e Incontinência Urinária Específica da Gestação"**, que visa o estudo da interação entre Diabetes Mellitus Gestacional e Incontinência Urinária Específica da Gestação do ponto de vista imunológico e hormonal. Alterações imunológicas e hormonais podem estar presentes no diabetes durante o período gestacional e podem gerar transformações na composição e funcionamento dos músculos do assoalho pélvico e abdominais, que por sua vez, não conseguem controlar a uretra e a bexiga de forma adequada, causando perda involuntária de urina (incontinência urinária). Pesquisas sobre a relação entre Diabetes Gestacional e Incontinência Urinária vem trazendo importantes conhecimentos sobre o assunto, entretanto, ainda existem lacunas a serem preenchidas para que se consolide o entendimento dos efeitos dessas patologias associadas.

Dessa forma, solicitamos autorização para aplicação de questionário relativo à perda involuntária de urina e também solicitamos autorização para coleta de aproximadamente 8ml de sangue e 15ml de urina, para a realização de testes imunológicos e hormonais. As amostras utilizadas neste estudo serão utilizadas em sua totalidade na pesquisa e não serão armazenadas em Biorrepositório.

O procedimento para a coleta desse material envolve riscos e desconfortos mínimos. A participante poderá se sentir levemente constrangida ao responder as perguntas do questionário sobre perda involuntária de urina. Além disso, poderá ter desconforto mínimo no momento da coleta de sangue e urina. Visando reduzir os riscos, a coleta será realizada por uma equipe de saúde capacitada. Para garantir e assegurar a privacidade dos dados pessoais das participantes da pesquisa, nenhuma informação que permita identificar a paciente será divulgada, garantindo o anonimato. Garantimos também, caso necessário, o direito à assistência integral e gratuita às participantes, devido a danos decorrentes da participação na pesquisa e pelo tempo que for necessário ou encaminhamento de assistência especializada. Além disso, lembramos que as participantes têm o direito de buscar indenização diante de eventuais danos decorrentes da pesquisa.

O seu benefício em participar deste estudo envolve o acesso ao diagnóstico das patologias e orientações adequadas fornecidas por uma equipe especializada na área. Além disso, a pesquisa poderá gerar maiores conhecimentos que podem contribuir para melhor qualidade de vida em futuras gestantes com diabetes mellitus gestacional e incontinência urinária específica da gravidez.

Fique ciente de que sua participação neste estudo é voluntária e que mesmo após ter dado seu consentimento para participar da pesquisa, você poderá retira-lo a qualquer momento, sem qualquer prejuízo na continuidade do seu tratamento. Este Termo de Consentimento Livre e Esclarecido será elaborado em 2 vias de igual teor, o qual 01 via será entregue a senhora devidamente rubricada, e a outra via será arquivada e mantida pelos pesquisadores por um período de 5 anos após o término da pesquisa. Qualquer dúvida adicional você poderá entrar em contrato com o Comitê de Ética em Pesquisa através dos telefones (14) 3880-1608 ou 3880-1609 que funciona de 2ª a 6ª feira das 8 às 11.30 e das 14 às 17 horas, na Chácara Butignolli s/nº em Rubião Júnior – Botucatu - São Paulo.

Após terem sido sanadas todas minhas dúvidas a respeito deste estudo, CONCORDO EM PARTICIPAR de forma voluntária, estando ciente que todos os meus dados estarão resguardados através do sigilo que os pesquisadores se comprometeram. Estou ciente que os resultados desse estudo poderão ser publicados em revistas científicas, sem, no entanto, que minha identidade seja revelada.

Eu:_____ RG:_____
Tel:(___)_____Cel:(__)_____End:_____
Concordo voluntariamente em participar dos procedimentos de avaliação e intervenção, os quais fui devidamente esclarecida.

Data:____/____/_____ _____
Assinatura da Participante

Pesquisadora Responsável:

_____
MSc. Danielle Cristina Honório França

A pesquisadora responsável e sua orientadora, Dra. Marilza Rudge, podem ser encontrados no Distrito Rubião Jr - s/n, Botucatu/SP, no telefone (14) 3815-1711 ou por e-mail: danielle.franca@unesp.br / marilzarudge@gmail.com.

Anexo 2 – Aprovação do CEP

UNESP -FACULDADE DE MEDICINA DE BOTUCATU

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Serotonina, melatonina e citocinas inflamatórias como potenciais mediadores na interação Diabetes Mellitus Gestacional e Incontinência Urinária Específica da Gestação

Pesquisador: DANIELLE CRISTINA HONORIO FRANCA

Área Temática:

Versão: 1

CAAE: 57896522.0.0000.5411

Instituição Proponente: Departamento de Ginecologia e Obstetrícia

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 5.395.627

Apresentação do Projeto:

As informações apresentadas nos campos "Apresentação do Projeto", "Objetivo da Pesquisa" e "Avaliação dos Riscos e Benefícios" foram retiradas do arquivo Informações Básicas da Pesquisa.

Estudo cujo objetivo é avaliar o papel da serotonina, melatonina e citocinas inflamatórias como potenciais mediadores na interação DMG e IUEG. Trata-se de um estudo Estudo observacional de corte transversal, que será desenvolvido no Centro de Investigação do Diabete Perinatal (CIDP) da FMB-Unesp com gestantes de 18-40 anos, diagnosticadas com DMG, pela realização do TOTG-75g e do perfil glicêmico. As gestantes sofrerão coleta de amostra de sangue e urina, alme de responder a um questionário estruturado específico e também à pergunta sobre perda involuntária de urina, onde a resposta afirmativa identificará a Incontinência Urinária Específica da Gravidez

Tamanho da Amostra: 64

Critérios de elegibilidade adequados.

Desfecho Primário:

Média glicêmica e presença ou ausência de Incontinência Urinária Especifíca da Gravidez.

INTRODUÇÃO:

Diabetes mellitus gestacional (DMG) e incontinência urinária (IU) são duas entidades clínicas com importante impacto social e econômico, associadas a significativos custos diretos e indiretos de saúde pública (1,2). Mulheres com DMG apresentam risco aumentado de IU (3). No entanto, a

Endereço: Chácara Butignolli , s/n
Bairro: Rubião Junior **CEP:** 18.618-970
UF: SP **Município:** BOTUCATU
Telefone: (14)3880-1609 **E-mail:** cep@fmb.unesp.br

associação direta entre DMG e Incontinência Urinária Específica da Gravidez (IUEG), ou seja,aquela que se inicia durante a gestação, permanece pouco estudada. Em pesquisa transversal (4), foi examinada a prevalência de IUEG entre mulheres que tinham DMG, contando com a participação de 228 mulheres, onde foi descoberto que 49% das mulheres tinham incontinência durante a gravidez semanalmente ou mais, e 50% das mulheres relataram IU dentro de 5 anos após o parto. Alta prevalência de obesidade pode ter contribuído para a elevada taxa de IUEG durante a gravidez e após o parto, pois estava presente em 42% dasmulheres durante a gravidez e em 46% após o parto. Sua conclusão foi que a IUEG era comum entre mulheres com DMG. Barbosa et al. (5) e Chuang et al. (6) observaram, de forma independente e concomitante, que o DMG é fator de risco independente para IU pós-parto. Além disso, descobriram que mulheres com DMG tem sintomas de IUEG que são fator de risco para IU 2 anos após o parto em comparação com mulheres sem DMG. Concluíram que o DMG é fator de risco independente para IU pós-parto com impacto significativo na qualidade de vida dessas mulheres (4, 5,6). Esse impacto na qualidade de vida foi confirmado por Piculo em 2020 (7, 8). Além disso outras pesquisas mostraram o impacto do DMG nos músculos envolvidos na continência urinária em ratas diabéticas prenhes (7, 9-12). Também foi mostrado que mulheres com histórias recentes de DMG são mais jovens e, apresentam alta prevalência de IU (4, 13-14). Em 2013, 382 milhões de pessoas tinham diabetes no mundo, com forte probabilidade de que aumente para 592 milhões em 2035. O Brasil, um país de baixa e média renda (LMIC), e um dos 10 principais em termos de número de pessoas diabéticas, com projeções de quase 19,2 milhões (15). A fisiopatologia do DMG é explicada pela elevação de hormônios contrarreguladores da insulina, pelo estresse fisiológico imposto pela gravidez e a fatores predeterminantes como genéticos ou ambientais (16). A gestação representa oportunidade única para a mulher, sendo necessário reduzir essas complicações não só durante este período, mas também na vida futura (17). A manutenção da continência urinária requer interações específicas entre várias células e mediadores (18), mas poucos estudos investigaram o papel que esses fatores podem desempenhar na IU associada à hiperglicemia. A hiperglicemia materna gera ambiente pró-inflamatório (19), despertando dessa forma o interesse da comunidade científica para estudos de proteínas pró e antiinflamatórias, como biomarcadores para auxiliar na rotina laboratorial e no acompanhamento de pacientes com diabetes (20). Em gestantes com diabetes foram relatadas alterações nos percentuais, na função e nos receptores celulares, bem como em fatores solúveis no soro, como proteínas do sistema complemento, anticorpos e citocinas (21- 26). As citocinas atuam como fatores de ativação, proliferação, diferenciação e regulação celular, e são capazes de atuar como mediadores de respostas inflamatórias. São produzidas por vários tipos

celulares, sendo os linfócitos TCD4+ as células que secretam a maioria das citocinas responsáveis pelos processos imunológicos. Esses mediadores podem ter ações pró-inflamatória (Th1) ou antiinflamatória (Th2), de acordo com o microambiente onde estão exercendo suas funções. Dentre as citocinas consideradas próinflamatórias estão as interleucinas (IL) IL-1, IL-2, IL- 6, IL-7, IL-8, IL-17, IFN- (interferon-gama) e TNF- (fator de necrose tumoral-alfa) e, entre as antiinflamatórias,IL-4, IL-10, IL-13 e TGF- (fator transformador de crescimento) (27-29). Sabe-se que no cérebro e na medula espinhal, citocinas próinflamatórias influenciam a regulação das vias de micção e afetam perifericamente a função da bexiga, causando diretamente hipertrofia do músculo detrusor e hipersensibilidade do nervo aferente (17). Vários mecanismos foram propostos para explicar a influência de mediadores inflamatórios no sistema urinário, dentre essas, a ação de citocinas no Sistema Nervoso Central (SNC), nas vias serotonérgicas centrais, na supraregulação da via hipotálamo-hipófise-adrenal (HHA) e na redução da atividade parassimpática (30).No SNC as citocinas pró-inflamatórias atuam na ativação da enzima indolamina-2,3-dioxigenase (IDO). Essa enzima é responsável pela degradação do triptofano (TRP), que é precursor do neurotransmissor serotonina (31,32). A serotonina ou 5- hidroxitriptamina (5-HT) é uma indolamina, derivada da hidroxilação e descarboxilação do aminoácido L-triptofano. Tem sido associada a ótimas respostas neurológicas, fisiológicas (33) e imunomodulatórias (34,35). Esse neurotransmissor desempenha papel importante, mas complexo na micção com ações no cérebro, na medula espinhal e na própria bexiga (36). Na literatura, estudos envolvendo citocinas inflamatórias e 5-HT em gestantes com DMG e IU ainda permanecem a ser elucidados. O estresse oxidativo e a inflamação são características-chave na iniciação, progressão e manifestações clínicas de distúrbios do músculo liso (37). A melatonina (N-acetil-5-metoxitriptamina), cujo precursor é a serotonina, é produzida pela glândula pineal. Este hormônio tem ações antitumoral (38-40), pró e anti-inflamatórias (41-44). Na urina seu principal metabólito excretado é 6-sulfatoximelatonina (sMT6a), que sofre variação dependente do sistema nervoso (45). A melatonina apresenta efeitos protetores na disfunção do músculo liso. Estudos sugerem que o hormônio diminui o tônus vascular, por meio da redução de contração adrenérgica e o aumento do relaxamento induzido pela acetilcolina, atenua o tônus simpático por ativação direta de receptores de melatonina, eliminando radicais livres e protegendo contra a inflamação e adeficiência induzida por inflamação da função neuromuscular (37, 46-49). A melatonina também é capaz de restaurar a contratilidade prejudicada do músculo detrusor por meio da normalização de contração, da polaridade mitocondrial, da função neuromuscular e do estresse oxidativo, o que sugere os efeitos da melatonina contra as alterações cistométricas (49-51).Alterações na melatonina têm sido

relacionadas a distúrbios na homeostase da glicose e de lipídios, aumento ganho de peso e massa de gordura corporal (52-54). Os níveis de melatonina são menores em gestantes diabéticas (54, 55). Este hormônio em gestantes normoglicêmicas tem efeito pro-oxidativo e induz aumento de espécies reativas do oxigênio (42). Em condições de hiperglicemia acredita-se que, provavelmente, os efeitos deste hormônio são limitados a processos anti-inflamatórios, com diminuição de espécies reativas do oxigênio (55). Nenhum estudo relatou sobre o papel da melatonina em mulheres com DMG e IUEG. Estudos sobre o papel da melatonina e de seu precursor 5-HT em mulheres com DMG e IUEG, bem como as possíveis correlações com citocinas inflamatórias no sangue e urina dessas gestantes, podem elucidar se o trato urinário pode ser afetado por alterações de níveis séricos de hormônios e citocinas. Devido a função da melatonina em melhorar alterações metabólicas associadas à diabetes e à disfunção urinária é possível que este hormônio possa ser utilizado em gestações com DMG. Assim, são necessários trabalhos envolvendo esses mediadores inflamatórios e hormonais para melhor compreensão da IUEG associada ao DMG, visando estabelecer estratégias que melhorem os parâmetros maternos. É possível que os padrões imunológicos e hormonais associados a alterações no metabolismo materno possam influenciar, via sangue e urina, contribuindo para maior susceptibilidade a disfunções do trato urinário. As mudanças expressivas na compreensão dos riscos e da patogênese de doenças crônicas com inflamação subclínica, constituem uma crescente causa da morbimortalidade. Os fatores de risco para o DMG e IU estimulam investigações capazes de entender a fisiopatologia comum entre elas, na busca do entendimento dos mecanismos neuroimunoendócrino.

Objetivo da Pesquisa:

Objetivo Primário: Objetivo Primário: Investigar o papel da serotonina, melatonina e citocinas inflamatórias como potenciais mediadores na interação DMG e IUEG.

Objetivo Secundário: 1. Comparar em gestantes com e sem DMG e com e sem IUEG as concentrações:(i) séricas e urinárias de 5-HT;(ii) séricas de melatonina;(iii) urinária de 6-sulfatoximelatonina (sMT6a); (iv) séricas e urinária de IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12-p70, IL-17A, IFN- e TNF-. 2. Avaliar a correlação entre as concentrações propostas (hormônios e citocinas) e a presença de DMG e IUEG.

Metodologia Proposta: Desenho de estudo: Estudo observacional de corte transversal, que será desenvolvido no Centro de Investigação do Diabete Perinatal (CIDP) da FMB-Unesp. Sujeitos e Grupos de Estudo: As gestantes serão selecionadas no momento da investigação diagnóstica do DMG, pela realização do TOTG-75g e do perfil glicêmico. Os resultados alterados desses dois

testes diferenciam duas condições de hiperglicemia na gestação – o DMG, sempre que o TOTG-75g estiver alterado, e a Hiperglicemia Gestacional Leve (HGL), quando apenas o PG estiver alterado (56). Para a composição dos grupos e análise dos resultados, as gestantes com hiperglicemia, independentemente se identificadas pelo TOTG-75g alterado ou pelo PG alterado, serão classificadas nos grupos "com DMG". As gestantes incluídas responderão a um questionário estruturado específico e também à pergunta sobre perda involuntária de urina, onde a resposta afirmativa identificará a IUEG. Considerando que a incidência de IU foi maior em mulheres DMG positivo (44,8%) em comparação com mulheres sem DMG (18,4%) (5), assumindo um nível de confiança bilateral de 95% e poder de 80%, será necessário a avaliação de 64 gestantes. De acordo com a presença ou não de DMG e de IUEG, as gestantes serão distribuídas em quatro grupos de estudo: (i) sem DMG e sem IUEG; (ii) sem DMG e com IUEG; (iii)com DMG e sem IUEG; (iv) com DMG e com IUEG. Critério de Inclusão: Serão incluídas gestantes que preencherem os seguintes critérios de inclusão: Idade entre 18 e 40 anos; com investigação diagnóstica de DMG; gestação única com feto vivo e que assinarem o Termo de Consentimento Livre e Esclarecido (TCLE). Critério de Exclusão: Serão excluídas as gestantes com incontinência urinária anterior à gestação; diabetes mellitus tipo 1 ou tipo 2, esclerose múltipla, doenças do colágeno, doenças autoimunes; gestação com malformações fetais diagnosticadas por ultrassom morfológico e que não assinarem o TCLE. Os casos de mal formação fetal diagnosticada ao nascimento também serão excluídos.

Avaliação dos Riscos e Benefícios:

Riscos: Os riscos são mínimos. As gestantes poderão se sentir constrangidas ao responderem as perguntas do questionário sobre perda involuntária de urina. Além disso, poderão ter desconforto mínimo no momento da coleta de sangue e urina. Visando reduzir os riscos, a coleta será realizada por uma equipe de saúde capacitada.

Benefícios: Os benefícios envolvem o acesso ao diagnóstico e orientações adequadas fornecidas por uma equipe especializada na área. Além disso, a pesquisa poderá gerar maiores conhecimentos que podem contribuir para melhor qualidade de vida em futuras gestantes com diabetes mellitus gestacional e incontinência urinária específica da gravidez.

Comentários e Considerações sobre a Pesquisa:

Projeto de Danielle Cristina Honorio Franca, sob orientação da Profª Emerita Marilza Vieira Cunha Rudge, ambas do Departamento de Ginecologia e Obstetrícia da Faculdade de Medicina de Botucatu(FMB) - UNESP., cujo objetivo é avaliar o papel da serotonina, melatonina e citocinas

inflamatórias como potenciais mediadores na interação DMG e IUEG. Trata-se de um projeto de pesquisa para obtenção de doutoramento. O projeto está escrito de forma clara e apresenta todos os dados de identificação dos autores, identificação de onde será realizado, descrição dos objetivos, detalhamento da metodologia, bem como as referências que fundamentam e justificam a importância do desenvolvimento do tema abordado. Trata-se de um projeto factível. O pesquisador não informa o custo da pesquisa será de R$8000,00, com financiamento próprio.

Considerações sobre os Termos de apresentação obrigatória:

- Folha de rosto: presente e adequadamente assinada
- Termo de anuência institucional: presente e adequada
- Projeto na integra
- Análise de viabilidade
- Cronograma adequado e apresenta coerência entre brochura e plataforma, com data prevista para coleta de dados a partir de julho de 2022.
- Biorepositório adequadamente elaborado
- TCLE adequado

Recomendações:

Não há

Conclusões ou Pendências e Lista de Inadequações:

Após análise em REUNIÃO ORDINÁRIA, o Colegiado deliberou APROVAÇÃO do PROJETO de Pesquisa apresentado.

Considerações Finais a critério do CEP:

Conforme deliberação do Colegiado, em REUNIÃO ORDINÁRIA do Comitê de Ética em Pesquisa FMB/UNESP, realizada em 02/05/2022, do PROJETO de Pesquisa apresentado encontra-se APROVADO.
O projeto de pesquisa deverá ter início somente após aprovação deste CEP.
Ao final da execução da pesquisa, o Pesquisador deverá enviar o Relatório Final de Atividades, na forma de Notificação, via Plataforma Brasil.

Atenciosamente,

Comitê de Ética em Pesquisa FMB/UNESP

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1916257.pdf	29/03/2022 15:17:10		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	29/03/2022 15:15:59	DANIELLE CRISTINA HONORIO FRANCA	Aceito
Declaração de Instituição e Infraestrutura	AnuenciaHcfmbSipe812022.pdf	29/03/2022 15:13:27	DANIELLE CRISTINA HONORIO FRANCA	Aceito
Outros	AnaliseDeViabilidadeDoProjetoDePesquisaSipe812022.pdf	29/03/2022 15:12:55	DANIELLE CRISTINA	Aceito
Declaração de Instituição e Infraestrutura	AceiteUnipex.pdf	29/03/2022 15:12:36	DANIELLE CRISTINA HONORIO FRANCA	Aceito
Declaração de Instituição e Infraestrutura	DeclaracaoArmazenamentoNaSalaDeCriogeniaUnipex.pdf	29/03/2022 15:12:23	DANIELLE CRISTINA HONORIO FRANCA	Aceito
Declaração de Instituição e Infraestrutura	TermoDeAnuenciaMaternidadeSipe622022.pdf	29/03/2022 15:12:01	DANIELLE CRISTINA HONORIO FRANCA	Aceito
Declaração de Instituição e Infraestrutura	TermoDeAnuenciaInstitucional.pdf	29/03/2022 15:11:40	DANIELLE CRISTINA HONORIO FRANCA	Aceito
Outros	QUESTIONARIOPERDADEURINA.pdf	29/03/2022 15:11:10	DANIELLE CRISTINA	Aceito
Folha de Rosto	FolhaDeRostoAssinada.pdf	29/03/2022 14:43:52	DANIELLE CRISTINA	Aceito
Projeto Detalhado / Brochura Investigador	PROJETODEPESQUISA.pdf	29/03/2022 14:39:55	DANIELLE CRISTINA HONORIO FRANCA	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

Endereço: Chácara Butignolli , s/n
Bairro: Rubião Junior **CEP:** 18.618-970
UF: SP **Município:** BOTUCATU
Telefone: (14)3880-1609 **E-mail:** cep@fmb.unesp.br

Continuação do Parecer: 5.395.627

BOTUCATU, 09 de Maio de 2022

Assinado por:
SILVANA ANDREA MOLINA LIMA
(Coordenador(a))

Endereço: Chácara Butignolli , s/n
Bairro: Rubião Junior **CEP:** 18.618-970
UF: SP **Município:** BOTUCATU
Telefone: (14)3880-1609 **E-mail:** cep@fmb.unesp.br

Anexo 3 - Termo de Ciência e Anuência do HCFMB

HOSPITAL DAS CLÍNICAS
FACULDADE DE MEDICINA DE BOTUCATU - UNESP

ANUÊNCIA DO HCFMB

DECLARO que tenho CIÊNCIA e AUTORIZO a realização do projeto de pesquisa intitulado
Serotonina, melatonina e citocinas inflamatórias como potenciais mediadores na interação Diabetes Mellitus Gestacional e Incontinência Urinária Específica da Gestação

a ser desenvolvido pelo (a) pesquisador (a)
Danielle Cristina Honório França

sob a orientação do (a)
Marilza Vieira Cunha Rudge

tendo como cenário de pesquisa o Hospital das Clínicas da Faculdade de Medicina de Botucatu (HCFMB).

Esta autorização está condicionada ao cumprimento do (a) pesquisador (a) aos requisitos da Resolução 466/12 CNS e suas complementares, comprometendo-se o (a) mesmo (a) a utilizar os dados pessoais dos participantes da pesquisa, exclusivamente, para os fins científicos, mantendo o sigilo e garantindo a não utilização das informações em prejuízo das pessoas e/ou das comunidades.

DECLARO ainda, que o projeto de pesquisa não deverá acarretar nenhum ônus financeiro para o SUS ou para o participante da pesquisa. Caso contrário, tenho a liberdade de retirar minha anuência a qualquer momento da pesquisa sem penalização alguma.

Antes de iniciar a pesquisa, o (a) pesquisador (a) deverá apresentar a esta Instituição o Parecer Consubstanciado devidamente aprovado, emitido por Comitê de Ética em Pesquisa Envolvendo Seres Humanos, credenciado ao Sistema CEP/CONEP.

Botucatu, 22 de Março de 2022

Prof. Assoc. André Luis Balbi
Superintendente do HCFMB

Sipe: 81/2022
ID HCFMB: 1371823
- Acadêmico/ rotina hospitalar
- Acadêmico/ com fomento
- ✓ Acadêmico/ sem fomento
- Patrocinado/UPECLIN
- Aguardando fomento
- Outros

Superintendência do Hospital das Clínicas
Distrito de Rubião Júnior s/n – CEP 18618 970
Botucatu | São Paulo | Brasil
Tel. (14) 3811-6215 | 3811-6218 | 3811-6100 | Fax. 3882-5387
www.hcfmb.unesp.br | pesquisadgaa@hcfmb.unesp.br

Anexo 4 – Termo de Ciência e Anuência da Responsável pelo Serviço da Enfermaria de Obstetrícia

HC FMB

DGAA

Ciência e Anuência dos Gestores da Área

Título do projeto de pesquisa: "Serotonina, melatonina e citocinas inflamatórias como potenciais mediadores na interação Diabetes Mellitus Gestacional e Incontinência Urinária Específica da Gestação"

Pesquisador principal: MSc. Danielle Cristina Honório França

Orientadora: Profa. Dra. Marilza Vieira Cunha Rudge

Vigência do Projeto: 04/07/2022 a 04/07/2024

Área de realização do projeto: Maternidade-HCFMB

Responsável pela área: Profa. Dra. Cláudia Garcia Magalhães

Período das atividades: 04/07/2022 a 04/07/2023

Atividades que serão desenvolvidas na área:

(Exemplos: entrevistas, aplicação de questionário, acesso à formulário próprio do serviço, realização de exames de imagem, laboratoriais (especificar a periodicidade dos exames) etc.

Serão realizadas entrevistas e aplicação de questionário em gestantes que estarão esperando para realização do exame de GTT e Perfil Glicêmico. Além disso, será solicitado a coleta de sangue e urina para as gestantes que aceitarem participar da pesquisa. Será fornecido todo material de assepsia e coleta. Será realizado também, após coleta, o acesso aos resultados dos exames GTT, Perfil Glicêmico, Glicose.

Para iniciar as atividades de pesquisa, o pesquisador deverá apresentar o Parecer Consubstanciado devidamente aprovado, emitido por Comitê de Ética em Pesquisa Envolvendo Seres Humanos (CEP) à área onde será desenvolvida a pesquisa e ao Departamento de Gestão de AtividadesAcadêmicas (DGAA). **Emendas** de estudos aprovados que envolvam o Complexo HCFMB, devem ser submetidas à apreciação do DGAA para obtenção de novo Termo de Anuência Institucional.

Seguindo a Resolução nº 580 de 22 de março de 2018, que regulamenta o disposto no itemXIII.4 da CNS nº466 de 2012 e estabelece especificidades éticas de pesquisas em Instituições do Sistema Único de Saúde (SUS), determina-se que os procedimentos de pesquisa NÃO podem ser confundidos com as atividades de assistência à saúde e NÃO devem acarretar ônus financeiro ao SUS ou participantes da pesquisa.

Eu, abaixo assinado, declaro ser de meu conhecimento o teor do projeto acima, cujo pesquisador tem a minha anuência em desenvolver as atividades descritas no projeto proposto, pertinentes à minha área de gestão.

Botucatu, 23 de março de 2022

Assinatura e Carimbo da chefia imediata do serviço

Anexo 5 – Termo de Anuência Institucional

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

ESCRITÓRIO DE APOIO À PESQUISA

Documento de Anuência Institucional

Em tendo cumprido as análises solicitadas pelo Fluxo Institucional de Projetos de Pesquisa da Faculdade de Medicina de Botucatu, informamos que a proposta de pesquisa "Serotonina, melatonina e citocinas inflamatórias como potenciais mediadores na interação Diabetes Mellitus Gestacional e Incontinência Urinária Específica da Gestação", protocolo 81/2022, que tem como autor principal "Danielle Cristina Honório França" sob orientação de "Marilza Vieira Cunha Rudge" está liberada para ser submetida ao Órgão de Ética da Instituição.

Atenciosamente

Profa. Associada Maria Cristina Pereira Lima
Diretora da Faculdade de Medicina

Botucatu, 29 de marÃ§o de 2022

Documento gerado pelo SIPE.
Unidade Responsável: EAP - Escritório de Apoio à Pesquisa
Funcionário responsável: Fernanda Chiuso Minicucci

Faculdade de Medicina de Botucatu
Av. Prof. Mário Rubens Guimarães Montenegro, s/n - Bairro: UNESP - Campus de Botucatu
CEP 18618-687 - Botucatu - SP - Tel. 14 3880 1001

Anexo 6 – Termo de Anuência e Ciência da UNIPEX

unesp UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO" FMB unipex UNIDADE DE PESQUISA EXPERIMENTAL

ACEITE

A Unidade de Pesquisa Experimental (UNIPEX) tem ciência e autoriza o desenvolvimento do Projeto de Pesquisa: **"Serotonina, melatonina e citocinas inflamatórias como potenciais mediadores na interação Diabetes Mellitus Gestacional e Incontinência Urinária Específica da Gestação"**, registrado no SIPE sob n° 81/2022, a ser conduzido pela pesquisadora: **Danielle Cristina Honório França** sob orientação de **Marilza Vieira Cunha Rudge**, previsto para execução no período de 04/07/2022 a 03/07/2024, após aprovação do respectivo Órgão de Ética. A pesquisadora responsável pelo projeto deve seguir as normas de utilização da UNIPEX, incluindo as normas de boas práticas laboratoriais e boas práticas em pesquisa. A UNIPEX conta com infraestrutura de salas de freezers e tanques de nitrogênio líquido, possibilitando o armazenamento de amostras animais e/ou humanas em condições ideais. A UNIPEX não se responsabiliza pela aquisição de materiais permanentes, de consumo ou equipamentos específicos necessários ao desenvolvimento do projeto de pesquisa. Pesquisas que requerem análises especializadas executadas pela equipe de profissionais qualificados da UNIPEX, estarão sujeitas ao pagamento de taxas de prestação de serviços e custos de manutenção de animais de experimentação. A prestação de serviços segue o estabelecido na Instrução Normativa nº 001/2019, que dispõe sobre a Normatização de prestação de serviços nos laboratórios/biotérios da Unidade de Pesquisa Experimental.

A coordenadora do projeto é responsável por registrar a pesquisa na Comissão Técnica Nacional de Biossegurança (CTNBio) e/ou no Sistema Nacional de Gestão de Patrimônio Genético (SISGEN), caso seja aplicável. A UNIPEX não se responsabiliza por tais registros.

Caso o projeto inclua a manipulação de amostras contaminadas com patógenos de alto risco, o projeto estará sujeito à aprovação da Comissão de Biossegurança da Unipex e toda proteção dos ambientes laboratoriais e laboratoristas, será de responsabilidade da coordenadora do projeto. O início das atividades de pesquisa está condicionado às recomendações da Pró-Reitoria de Pesquisa e Comitê COVID-19 da Unesp, as quais são seguidas na unidade de pesquisa.

O início das atividades de pesquisa está condicionado às recomendações da Pró-Reitoria de Pesquisa e Comitê COVID-19 da Unesp, as quais são seguidas na unidade de pesquisa.

Botucatu, 25 de março de 2022.

Professora Associada Patrícia Pintor dos Reis
Coordenadora
Unidade de Pesquisa Experimental (UNIPEX)
Faculdade de Medicina de Botucatu (FMB)
Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP)

Faculdade de Medicina de Botucatu
Unidade de Pesquisa Experimental – UNIPEX
Rua: Prof. Dr. Walter Maurício Correa, s/n
Distrito de Rubião Júnior - CEP 18618-681 Botucatu São Paulo Brasil
Tel /VOIP - 55 14 38801064 e 38801743 e-mail secretaria.unipex.fmb@unesp.br

Anexo 7 – Termo de Anuência e Ciência de Armazenamento em Sala de Criogenia da UNIPEX

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

DECLARAÇÃO

Declaro, para os devidos fins, que a Unidade de Pesquisa Experimental (UNIPEX) da Faculdade de Medicina de Botucatu, possui infraestrutura de sala de ultrafreezers e tanques de nitrogênio líquido, com capacidade de armazenamento de amostras biológicas. Nossa infraestrutura conta com monitoramento remoto, em tempo real, garantindo a segurança de amostras biológicas armazenadas em biorrepositórios individuais sob responsabilidade dos pesquisadores da unidade.

Declaro ainda que o projeto de pesquisa intitulado: **"Serotonina, melatonina e citocinas inflamatórias como potenciais mediadores na interação Diabetes Mellitus Gestacional e Incontinência Urinária Específica da Gestação"**, sob responsabilidade da pesquisadora **Danielle Cristina Honório França** utilizará a referida infraestrutura da UNIPEX para o armazenamento de amostras biológicas, após aprovação do Comitê de Ética em Pesquisa da Faculdade de Medicina de Botucatu, sendo que os pesquisadores são responsáveis pela coleta e pelo biorrepositório das amostras.

Professora Associada Patrícia Pintor dos Reis
Coordenadora
Unidade de Pesquisa Experimental (UNIPEX)
Faculdade de Medicina de Botucatu (FMB)
Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP)

Faculdade de Medicina de Botucatu
Unidade de Pesquisa Experimental – UNIPEX
Rua: Prof. Dr. Walter Maurício Correa, s/n
Distrito de Rubião Júnior - CEP 18618-681 Botucatu São Paulo Brasil
Tel /VOIP - 55 14 38801611 e 38801743 e-mail secretariaunipex@fmb.unesp.br

Anexo 8 – Questionário Incontinência Urinária

INCONTINÊNCIA URINÁRIA

"Muitas mulheres e gestantes se queixam que escapa urina sem querer e molham um pouco a calcinha ou chegam a molhar até mesmo a roupa".

Isso já aconteceu com você?

( ) Sim ( ) Não

Já aconteceu de você perder urina, sem querer, quando espirrou, tossiu ou fez esforço?

( ) Sim ( ) Não

Já aconteceu de você perder urina, sem querer, quando teve muita vontade de urinar e não conseguiu chegar ao banheiro?

( ) Sim ( ) Não

Se você respondeu SIM para uma ou mais perguntas acima responda as perguntas abaixo.

International Consultation on Incontinence Questionnaire – Short Form (ICIQ-SF)

Com que frequência você perde urina?

(1) Nunca
(2) Uma vez por semana ou menos
(3) Duas ou três vezes por semana
(4) Uma vez ao dia
(5) Diversas vezes ao dia
(6) O tempo todo

Gostaríamos de saber a quantidade de urina que você pensa que perde:

(1) Nenhuma
(2) Uma pequena quantidade
(3) Uma moderada quantidade
(4) Uma grande quantidade

Em geral, quanto que perder urina interfere em sua vida diária?

Entre 0 (não interfere) e 10 (interfere muito)

Anotar o valor 0-10: _____

Quando você perde urina? (Assinale todas as alternativas que se aplicam a você)

(1) Nunca
(2) Perco antes de chegar ao banheiro
(3) Perco quando tusso ou espirro
(4) Perco quando estou dormindo
(5) Perco quando estou fazendo atividades físicas
(6) Perco quando terminei de urinar e estou me vestindo
(7) Perco sem razão óbvia
(8) Perco o tempo todo

Incontinence Severity Index (ISI)

Com qual frequência você apresenta perda de urina?

(1) Menos de uma vez ao mês
(2) Algumas vezes ao mês
(3) Algumas vezes na semana
(4) Todos os dias e/ou noites

Qual quantidade de urina você perde cada vez?

( ) Gotas

( ) Pequeno jato

( ) Muita quantidade

Anexo 9 – Disciplinas cursadas

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Botucatu

ATESTADO

Atestamos, para os devidos fins, que DANIELLE CRISTINA HONÓRIO FRANÇA, RA nº TOC210412, RG nº 5932951, expedido pela SSP/GO, realizou as seguintes atividades programadas junto ao Programa de Pós-Graduação em Tocoginecologia, Curso de Doutorado, reconhecido pela Portaria MEC número 609/2019 de 14/03/2019.

Disciplinas	Ano	Período letivo	Créditos	Carga Horária	Freq	Conceito
A captação de Recursos Externos para Pesquisa	2022	1º Semestre	2	30	100	A
Seminários de Projetos de Teses	2022	1º Semestre	3	45	100	A
Special Topics: Mechanisms of Metabolic Disorders (MMD)	2022	1º Semestre	4	60	100	A
Neoplasias malignas específicas da mulher: Da aplicabilidade clínica à publicação	2022	2º Semestre	2	30	100	B
Tópicos Especiais: Marcadores da miopatia diabética gestacional (MiDG) – Achados inéditos	2023	2º Semestre	3	45	85	A

1 crédito correspondente a 12 horas de atividades programadas até 13/9/99 (Res. UNESP 46, DE 18/6/91)
1 crédito correspondente a 15 horas de atividades programadas a partir de 14/9/99 (Res. UNESP 45, DE 14/9/99)
CONCEITOS:
A - Excelente; B - Bom; C - Regular D/R - Reprovado I - Incompleto T - Transferência
OBS: AE - Disciplina cursada como aluno especial
TP - Disciplina cursada no programa
T - Disciplina cursadas fora do Programa
M - Disciplina cursada como portador de título de Mestre

PROFICIÊNCIA EM LÍNGUAS: INGLÊS Data da Aprovação: 10/09/2021

DATA DO EXAME GERAL DE QUALIFICAÇÃO:

ATIVIDADE COMPLEMENTAR:

Conjunto de atividades complementares realizadas de acordo com o regulamento do Programa

INÍCIO: 30/08/2022 TÉRMINO: 17/10/2024 CARGA HORÁRIA: 270.0 horas

Documento emitido às 20:54 do dia 23/05/2025
Código de autenticidade: 30E6-138E-0F16-63B3-F948-E112-A718-CB96
Documento válido até às 20:54 do dia 22/07/2025

ATENÇÃO: Este é um documento oficial da Pós-graduação da UNESP, com autenticação eletrônica através da página https://app.unesp.br/sispg/autenticidade-documento, que dispensa carimbo e assinatura.

Faculdade de Medicina - Câmpus de Botucatu -
Av.: Prof. Mário Rubens Guimarães Montenegro, s/nº, 18618687

Anexo 10 – Artigos publicados da pesquisa

BBA - Molecular Basis of Disease 1869 (2023) 166737

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Review

Integration of nutrigenomics, melatonin, serotonin and inflammatory cytokines in the pathophysiology of pregnancy-specific urinary incontinence in women with gestational diabetes mellitus

Danielle Cristina Honorio França [a,*], Eduardo Luzía França [b], Luis Sobrevia [a,c,d,e,f,g], Angélica Mércia Pascon Barbosa [a,h], Adenilda Cristina Honorio-França [b], Marilza Vieira Cunha Rudge [a,*], Diamater Study Group [1]

[a] Department of Gynecology and Obstetrics, Botucatu Medical School (FMB), São Paulo State University (UNESP), Botucatu 18618-687, Brazil
[b] Institute of Biological and Health Science, Federal University of Mato Grosso (UFMT), Barra do Garças 78605-091, Brazil
[c] Cellular and Molecular Physiology Laboratory (CMPL), Department of Obstetrics, Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago 8330024, Chile
[d] Department of Physiology, Faculty of Pharmacy, Universidad de Sevilla, E-41012 Seville, Spain
[e] Faculty of Medicine and Biomedical Sciences, University of Queensland, Herston, QLD 4029, Australia
[f] Department of Pathology and Medical Biology, University of Groningen, 9713GZ Groningen, the Netherlands
[g] Tecnologico de Monterrey, Eutra, The Institute for Obesity Research (IOR), School of Medicine and Health Sciences, Monterrey 64710, Mexico
[h] Department of Physiotherapy and Occupational Therapy, School of Philosophy and Sciences, São Paulo State University (UNESP), Marília 17525-900, Brazil

ARTICLE INFO

Keywords:
Hormones
Immunomodulation
Hyperglycemia
Involuntary loss of urine
Epigenetic

ABSTRACT

Gestational diabetes mellitus is an important public health problem and has been associated with the development of pregnancy-specific urinary incontinence. The interaction is related to hyperglycemia, and inflammatory and hormonal patterns, which favor functional alterations in different organs and systems. Several genes associated with human diseases have been identified and partially characterized. Most of these genes are known to cause monogenic diseases. However, about 3 % of diseases do not fit the monogenic theory due to the complex interactions between multiple genes and environmental factors, as in chronic metabolic diseases such as diabetes. The nutritional, immunological, and hormonal patterns associated with changes in maternal metabolism may influence and contribute to greater susceptibility to urinary tract disorders. However, early systematic reviews have not yielded consistent findings for these associations. This literature review summarizes important new findings from integrating nutrigenomics, hormones, and cytokines in women with Gestational diabetes mellitus and pregnancy-specific urinary incontinence. Changes in maternal metabolism due to hyperglycemia can generate an inflammatory environment with increased inflammatory cytokines. This environment modulated by inflammation can alter tryptophan uptake through food and thus influence the production of serotonin and melatonin. As these hormones seem to have protective effects against smooth muscle dysfunction and to restore the impaired contractility of the detrusor muscle, it is assumed that these changes may favor the onset of urinary incontinence specific to pregnancy.

1. Nutrigenomic and intrinsic factors

Nutritional research has long sought knowledge about nutrients to meet the body's essential needs. However, in recent years a new strand has emerged to seek alternatives to improve the health of individuals through food. Thus, modern nutritional research aims at health promotion, performance improvement, and disease prevention. Thus came the concept of the development of nutritionally enriched or functional foods, which are part of the diet and contains, in addition to essential nutrients, bioactive components capable of reducing the risk of diseases.

* Corresponding authors.
E-mail addresses: daniellechfranca@gmail.com (D.C.H. França), eduardo.franca@ufmt.br (E.L. França), lsobrevia@uc.cl (L. Sobrevia), adenilda.franca@ufmt.br (A.C. Honorio-França), marilzarudge@gmail.com (M.V.C. Rudge), diamaterstudy@gmail.com (Diamater Study Group).
[1] Collaborators of the Diamater Study Group are provided in the Acknowledgments.

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International Journal of
Molecular Sciences

MDPI

Article

Serotonin and Interleukin 10 Can Influence the Blood and Urine Viscosity in Gestational Diabetes Mellitus and Pregnancy-Specific Urinary Incontinence

Danielle Cristina Honório França [1], Adenilda Cristina Honorio-França [2,*], Kênia Maria Rezende Silva [2], Fernanda Cristina Bérgamo Alves [1], Gabriela Bueno [1], Sarah Maria Barneze Costa [1], Aron Carlos de Melo Cotrim [2], Angélica Mércia Pascon Barbosa [1,3], Eduardo Luzía França [2], Marilza Vieira Cunha Rudge [1,*] and The Diamater Study Group [1,†]

[1] Department of Gynecology and Obstetrics, Botucatu Medical School, São Paulo State University, Botucatu 05508-070, SP, Brazil; daniellechfranca@gmail.com (D.C.H.F.); fernandacbalves15@gmail.com (F.C.B.A.); gabriela.bueno24@unesp.br (G.B.); sarah.barneze@unesp.br (S.M.B.C.); angelicapascon@gmail.com (A.M.P.B.); diamaterstudy@gmail.com (The Diamater Study Group)

[2] Biological and Health Sciences Institute, Federal University of Mato Grosso, Barra do Garças 78605-091, MT, Brazil; keniarezende1@gmail.com (K.M.R.S.); aroncarlosbg@gmail.com (A.C.d.M.C.); eduardo.franca@ufmt.br (E.L.F.)

[3] Department of Physiotherapy and Occupational Therapy, School of Philosophy and Sciences, São Paulo State University, Marília 17525-900, SP, Brazil

* Correspondence: adenilda.franca@ufmt.br (A.C.H.-F.); m.rudge@unesp.br (M.V.C.R.)

† Membership of The Diamater Study Group is provided in the Acknowledgments.

Abstract: Serotonin and interleukin 10 (IL-10) may play a role in gestational diabetes mellitus. Hyperglycemic environment, the detrusor musculature of the bladder and pelvic floor muscles may become damaged, leading to urination problems and urine viscosity in pregnant women with gestational diabetes mellitus and pregnancy-specific urinary incontinence. Urine and blood samples were collected from pregnant women between 24 and 28 weeks of gestation. The serotonin concentration and cytokine IL-10 levels were evaluated in plasma and urine. In the total blood and urine, the viscosity was evaluated in the presence and absence of exogenous serotonin and IL-10. The plasma serotonin levels decreased, while the urine serotonin levels increased in the normoglycemic incontinent (NG-I), hyperglycemic continent (GDM-C), and hyperglycemic incontinent (GDM-I) groups. The IL-10 in the plasma decreased in the GDM-I group and was higher in the urine in the NG-I and GDM-I groups. The blood viscosity was higher, independently of urinary incontinence, in the GDM groups. The serotonin increased the blood viscosity from women with GDM-C and urine in the NG-I, GDM-C, and GDM-I groups. Blood and urine in the presence of IL-10 showed a similar viscosity in all groups studied. Also, no difference was observed in the viscosity in either the blood or urine when in the presence of serotonin and IL-10. These findings suggest that serotonin and IL-10 have the potential to reduce blood viscosity in pregnant women with gestational diabetes and specific urinary incontinence, maintaining values similar to those in normoglycemic women's blood.

Keywords: hormone; immunomodulation; cytokines; rheology; involuntary loss of urine

check for updates

Citation: França, D.C.H.; Honorio-França, A.C.; Silva, K.M.R.; Alves, F.C.B.; Bueno, G.; Costa, S.M.B.; Cotrim, A.C.d.M.; Barbosa, A.M.P.; França, E.L.; Rudge, M.V.C.; et al. Serotonin and Interleukin 10 Can Influence the Blood and Urine Viscosity in Gestational Diabetes Mellitus and Pregnancy-Specific Urinary Incontinence. *Int. J. Mol. Sci.* **2023**, *24*, 17125. https://doi.org/10.3390/ijms242417125

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1. Introduction

Gestational diabetes mellitus (GDM) is an important metabolic disease with increased insulin counter-regulatory hormones generating glucose intolerance, an inflammatory environment, abnormalities in oxidative stress, and altering the profile of released cytokines and other hormones [1,2].

In maternal hyperglycemia, the effects on cellular metabolism can affect systems and tissues, like the pelvic floor muscles (PFMs) and the urinary system, with pre-disposition to muscle weakening, atrophy, and pregnancy-specific urinary incontinence (PSUI) [3].

metabolites

MDPI

Article

Melatonin and Inflammatory Cytokines as Modulators of the Interaction Between Gestational Diabetes Mellitus and Pregnancy-Specific Urinary Incontinence

Danielle Cristina Honório França [1], Eduardo Luzia França [2], Adenilda Cristina Honório-França [2,*], Kênia Maria Rezende Silva [2], Adriele Ataídes de Queiroz [2], Tassiane Cristina Morais [3], Emanuelle Carolina Honorio França [2], Carolina Neiva Frota de Carvalho [1], Danny Laura Gomes Fagundes-Triches [2], Angélica Mércia Pascon Barbosa [1,4], Iracema de Mattos Paranhos Calderon [1], Luis Sobrevia [1,5,6,7] and Marilza Vieira Cunha Rudge [1,*]

[1] Department of Gynecology and Obstetrics, Botucatu Medical School (FMB), São Paulo State University (UNESP), Botucatu 18618-687, Brazil; danielle.franca@unesp.br (D.C.H.F.); carolina.n.carvalho@unesp.br (C.N.F.d.C.)
[2] Institute of Biological and Health Science, Federal University of Mato Grosso (UFMT), Barra do Garças 78605-091, Brazil; eduardo.franca@ufmt.br (E.L.F.)
[3] School of Sciences, Santa Casa de Misericórdia de Vitória (EMESCAM), Vitória 29045-402, Brazil
[4] Department of Physiotherapy and Occupational Therapy, School of Philosophy and Sciences, São Paulo State University (UNESP), Marilia 17525-900, Brazil
[5] Cellular and Molecular Physiology Laboratory (CMPL), Department of Obstetrics, Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago 8330024, Chile
[6] Department of Physiology, Faculty of Pharmacy, Universidad de Sevilla, E-41012 Seville, Spain
[7] Faculty of Medicine and Biomedical Sciences, University of Queensland, Herston, QLD 4029, Australia
* Correspondence: adenilda.franca@ufmt.br (A.C.H.-F.); m.rudge@unesp.br or marilzarudge@gmail.com (M.V.C.R.)

check for updates

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Abstract

Background: The pathogenesis of developing gestational diabetes mellitus (GDM) integrated with pregnancy-specific urinary incontinence (PSUI) may be related to immunological and hormonal factors. Inflammatory cytokines influence the function and regulation of the urinary tract, and changes in melatonin concentration are a predisposing factor for smooth muscle dysfunction and cystometric changes. Objective: This study examines the influence of melatonin, MT1 and MT2 receptors, and inflammatory cytokines in the blood and urine of pregnant women with GDM and PSUI. Methods: Two hundred sixty-nine pregnant women were approached during the diagnostic investigation of GDM and answered a specifically structured questionnaire about the involuntary loss of urine. According to these criteria, mothers were divided into four groups: continent normoglycemic (NG-C), incontinent normoglycemic (NG-I), continent GDM (GDM-C), and incontinent GDM (GDM-UI). Blood and urine samples were collected to determine the levels of melatonin, melatonin sulfate, melatonin receptors (MT1 and MT2), and inflammatory cytokines. Results: Blood level of melatonin and IL-10 was lower, but MT1, MT2, IL-1β, IL-8, and TNF-α were higher in GDM-UI compared with the NG-C group. The melatonin sulfate level was lower in the urine of the GDM-UI group compared with the NG-C group. Conclusions: Maternal hyperglycemia associated with urinary incontinence generates an inflammatory environment characterized by reduced melatonin and IL-10 and increased IL-1β, IL-8, and TNF-α in the blood of mothers with GDM with UI. This environmental condition may be involved in the pathogenesis of these pathologies.

Anexo 11 – Cursos complementares

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FMB 1963 BOTUCATU

CERTIFICADO

Certificamos que DANIELLE CRISTINA HONORIO FRANÇA participou do CURSO DE BIOSSEGURANÇA EM BIOTÉRIOS (2023) - UNIPEX, realizado no período de 10 de janeiro a 19 de dezembro de 2023 em Botucatu, São Paulo, na qualidade de ouvinte, com carga horária de 05 horas.

Botucatu, 19 de dezembro de 2023.

Prof. Titular Carlos Magno Castelo Branco Fortaleza
Diretor da Faculdade de Medicina de Botucatu
FMB - Unesp

Profª Associada Patricia Pintor dos Reis
Coordenadora do Evento
FMB - Unesp

Cep: 18.618-970 - Campus da Unesp/Rubião Júnior - Botucatu/SP - Fone: (14)3880-0000

UNIVERSIDADE ESTADUAL PAULISTA
JÚLIO DE MESQUITA FILHO - UNESP
CÂMPUS DE BOTUCATU
FACULDADE DE MEDICINA
Diretoria Técnica Acadêmica

Registrado sob Nº 1004
Fls.30 Livro 51
BOTUCATU 15 de Março de 2024.

PROGRAMAÇÃO

Aula Teórica - forma remota através da plataforma Moodle

Conceito de biossegurança em biotérios

Tipos de biotérios

Risco em experimentação animal

Equipamentos de proteção individual (EPIs) e coletivos (EPCs)

Preparo e uso de fármacos

Preparo do local

Aula Prática - Presencial - agendada com o professor (trazer jaleco)

July, 2022

To Whom It May Concern

This letter certifies that **DANIELLE CRISTINA HONORIO FRANÇA** (Brazil) has completed (in full) the activities of the postgraduate international course Mechanisms of Metabolic Disorders (MMD) 2022 (April 4 until July 8, 2022).

The MMD course was organized by the Cellular and Molecular Physiology Laboratory (CMPL) from the Faculty of Medicine, Pontificia Universidad Católica de Chile, and the University Medical Centre Groningen (UMCG), University of Groningen, The Netherlands.

The MMD course consisted of 49 lectures delivered by international experts, 21 seminars from MMD students, four conferences from journal Editors-in-Chief, six Rector and Vice-Rectors and three Ambassadors messages.

In-full attendance consisted of synchronic activities (online) in 28.5 chronological hours, equivalent to 57 academic hours (45 min per educational hour).

Having completed the requirement of in-full attendance and having delivered a seminar at the symposia part of the course programme, **DANIELLE CRISTINA HONORIO FRANÇA** is granted five (5) academic credits by the MMD course.

Sponsors: The International Union of Physiological Sciences (IUPS), Latin American Association of Physiological Sciences (ALACF), The Physiological Society (TPS), Sociedad Española de Ciencias Fisiológicas (SECF), Sociedad Chilena de Ciencias Fisiológicas (SCHCF), Bentham Science Publishers (BSP), SERNATUR (Chile), FONDECYT 1190316 (Chile), The Institute for Obesity Research, Tecnologico de Monterrey (Mexico).

Luis Sobrevia
MMD course coordinator
CMPL, Chile

Harry van Goor
MMD course coordinator
UMCG, The Netherlands

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CERTIFICADO

Certificamos que DANIELLE CRISTINA HONORIO FRANÇA participou do CURSO DE BIOSSEGURANÇA EM LABORATÓRIOS (2022) - UNIPEX, realizado no período de 21 de Fevereiro a 25 de Novembro de 2022 em Botucatu, São Paulo, na qualidade de ouvinte, com carga horária de 08 horas.

Botucatu, 25 de Novembro de 2022.

Prof. Titular Carlos Magno Castelo Branco Fortaleza
Diretor da Faculdade de Medicina de Botucatu
FMB - Unesp

Drª Marcia Tenório Delneri
Cordenadora do Evento
FMB - Unesp

Cep: 18.618-970 - Campus da Unesp/Rubião Júnior - Botucatu/SP - Fone: (14)3880-0000

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Diretoria Técnica Acadêmica

Registrado sob Nº 5839
Fls.189 Livro 40
BOTUCATU 15 de Agosto de 2023.

PROGRAMAÇÃO

Aulas teóricas - Plataforma Moodle
Apresentando a UNIPEX
Constituição da UNIPEX
Espaços e equipamentos multiusuários
Programa de Gestão da Qualidade
Normas de utilização dos espaços da UNIPEX

Módulo 1
Importância da biossegurança
Principais normas e regulamentações (legislações)
Definição de riscos laboratoriais
Classificação de riscos biológicos
Classificação de laboratórios

Módulo 2
Tríade de contenção de riscos
Equipamentos de proteção individual e coletivos

Módulo 3
Instalações laboratoriais
Boas práticas laboratoriais
Sinalizações

Aula prática - presencial
Técnica de lavagem das mãos
Demonstração de EPIs e EPCs
Sinalizações de risco

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FACULDADE DE MEDICINA

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CERTIFICADO

Certificamos que DANIELLE CRISTINA HONORIO FRANÇA participou da "Oficina Teórico-Prática de Inserção de DIU de cobre", realizada no dia 01 de Outubro de 2024 em Botucatu, São Paulo, na qualidade de ouvinte, com carga horária de 04 horas.

Botucatu, 18 de Dezembro de 2024.

Prof. Titular Carlos Magno Castelo Branco Fortaleza
Diretor da Faculdade de Medicina de Botucatu

Profa. Dra. Flávia Neves Bueloni Dias
Coordenadora do Evento

UNIVERSIDADE ESTADUAL PAULISTA
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CÂMPUS DE BOTUCATU
FACULDADE DE MEDICINA
Diretoria Técnica Acadêmica

Registrado sob Nº 7463
Fls.226 Livro 51
BOTUCATU 18 de Dezembro de 2024.

PROGRAMAÇÃO

Centro de Saúde Escola Vila dos Lavradores
01/10/2024
13h30 às 17h30
13:30 - Boas vindas
14h - Aula teórica: DIU de cobre: informações para indicação, inserção e seguimento seguros
15h - Treinamento em modelo inanimado
15h30 - Inserções nas pacientes selecionadas

CERTIFICADO
DE CONCLUSÃO

CONCEDEMOS ESTE CERTIFICADO A

DANIELLE CRISTINA HONÓRIO FRANÇA

Participante da turma: **A** , por concluir com com êxito o curso

Inserção, Revisão e Retirada do DIU

Promovido pela empresa CAROCI BRASIL, CNPJ: 33.863.029/0001-56.
Carga horária: 20 horas práticas, sendo realizadas nos dias 16
e 17 de outubro de 2024.

ABED
ASSOCIAÇÃO BRASILEIRA
DE EDUCAÇÃO A DISTÂNCIA

PROFª. DRA. ADRIANA CAROCI BECKER
COREN-SP78.412-ENF OBST
ADRIANA CAROCI BECKER

CEMICAMP

CERTIFICADO

Certificamos que Danielle Cristina Honório França **(CRM** SP 247984**)** participou do treinamento de inserção de Implanon NXT da Organon Brasil, no dia 29 de agosto do corrente ano, no Ambulatório de Planejamento Familiar do CAISM/UNICAMP.

Campinas, 29 de agosto de 2024

Prof. Dr. Luis Bahamondes
Professor Titular de Ginecologia

Anexo 12 – Apresentação oral em eventos



IV CONGRESSO CAPIXABA DE ENFERMAGEM

CERTIFICADO

Certificamos que

DANIELLE CRISTINA HONORIO FRANÇA

apresentou a conferência intitulada "Diabetes Mellitus gestacional e incontinência urinária: o potencial da melatonina para intervenções biotecnológicas" no Simpósio "Saúde materno-infantil: os pilares do desenvolvimento humano " durante o **IV Congresso Capixaba de Enfermagem**, promovido pela Escola Superior de Ciências da Santa Casa de Misericórdia de Vitória, EMESCAM entre os dias 30 de agosto e 02 de setembro de 2022, na modalidade virtual.

Vitória - ES, 02 de setembro de 2022.

Realização: emescam FAPES NAKP

Dr. Claudio Medina da Fonseca
Diretor da EMESCAM

Drª. Italla Maria Pinheiro Bezerra
Presidente do Evento
Coordenação do Curso de Enfermagem

Código do Certificado: 120283AE0ADB36210005751 · Verifique autenticidade em: www.e-certificado.com

Anexo 13 – Participação em eventos



CERTIFICADO

ESTE CERTIFICADO COMPROVA QUE

Danielle Cristina Honorio França

CONCLUIU COM ÊXITO A PARTICIPAÇÃO DA "SEMANA DA PÓS-GRADUAÇÃO DA UNESP: VENHA NOS CONHECER", FINANCIADA PELO EDITAL 31/2024 PROPG/UNESP, ENTRE 03/09/2024 E 04/09/2024, COM CARGA HORÁRIA DE 16 HORAS. DURANTE A ORGANIZAÇÃO, DEMONSTROU DEDICAÇÃO E EMPENHO EXEMPLARES.

Coordenadora Responsável

Coordenadora do Programa de Pós-Graduação em Tocoginecologia

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PG-TOCOGINECOLOGIA

Pró-Reitoria de Extensão Universitária e Cultura

PROPE PRÓ-REITORIA DE PESQUISA

PROPG

Verifique o código de autenticidade **1098718.8195162.6.8.049329924621519** em https://www.even3.com.br//documentos

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

CERTIFICADO

Certificamos que **Danielle Cristina Honório França** participou do evento **"A pesquisa na Unesp: Passado, Presente e Futuro"** realizado, no formato virtual, nos dias 18 e 25 de maio e 01 de junho de 2022, pela Pró-Reitoria de Pesquisa da Unesp, contabilizando carga horária total de 12 horas.

São Paulo, 01 de junho de 2022.

Prof. Edson Cocchieri Botelho
Pró-Reitor de Pesquisa


Simpósio
Saúde da Mulher
dasa educa
dasa oncologia
dasa genômica
Certificamos
DANIELLE CRISTINA HONORIO FRANÇA
por sua participação no Simpósio Saúde da Mulher, com carga horária total de 7 Horas
realizado do dia 02 de Agosto ao dia 04 de Agosto de 2022.
Dr. Leonardo Vedolin
Chief Medical Officer na Dasa
Dra. Fernanda Garozzo Velloni
Gerente Médica de Educação na Dasa

Verifique o código de autenticidade 1418563.8195162.6.8.523971328927073 em https://www.even3.com.br/documentos

V Seminário de Ética na pesquisa com seres humanos e com animais

Certificamos que **Danielle Cristina Honório França**, participou do evento **V Seminário de Ética na pesquisa com seres humanos e com animais**, organizado pelos Comitê de Ética em Pesquisa e Comissão de Ética no Uso de Animais do Instituto de Biociências, Letras e Ciências Exatas - Câmpus da UNESP de São José do Rio Preto. Realizado em 23 de março de 2022, na cidade de São José do Rio Preto/SP, contabilizando carga horária total de 6 horas.

São José do Rio Preto/SP, 23 de março de 2022

Prof. Dr. Lauro Maia Amorim
Coordenador do Comitê de Ética em Pesquisa - IBILCE/UNESP

Profa. Dra. Patrícia Simone Leite Vilamaior
Presidente da Comissão de Ética no Uso de Animais - IBILCE/UNESP

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- **08h30 – Cerimônia de Abertura**
- **09h00 – Palestra - Tema: "Critérios para análise dos termos de consentimento e assentimento e a questão da garantia de indenização ao participante de pesquisa em caso de danos decorrentes da pesquisa". Prof. Dr. Paulo Henrique Condeixa de França.**
- **10h30 – Palestra - Tema: "Concea, CEUAs e o bem-estar animal". Dra. Ekaterina Akimovna Botovchenco Rivera.**
- **14h00 às 15h30 – Oficinas**
- **"Prática com os animais de laboratório" - Gracila Heitor de Oliveira.**
- **"Questões legais envolvendo o TCLE e o TALE" - Dr. Eudes Quintino de Oliveira Júnior.**
- **15h40 – 16h30 – ENCERRAMENTO e Palestra com tema "Ética em pesquisa na área de Humanidades" - Profa. Dra. Cláudia Maria Lima.**

DePaul University

CERTIFICATE OF PARTICIPATION

The 2022 Global Conversations Series, hosted by DePaul University in collaboration with faculty from international partner schools, offered students from across the globe the opportunity to connect and reflect together on interdisciplinary global issues.

For participation as a Faculty Facilitator in the 2022 Global Conversation Series (April 25-May 5, 2022), the Office of Global Engagement recognizes:

Danielle Cristina Honório França

UNIVERSIDADE ESTADUAL PAULISTA
CAMPUS DE BOTUCATU
FACULDADE DE MEDICINA

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CERTIFICADO

Certificamos que DANIELLE CRISTINA HONORIO FRANÇA participou do evento "Atenção Primária à Saúde e Meio Ambiente: Integrando Cuidados e Sustentabilidade ao Bem-Estar Comunitário", realizado nos dias 16 e 14 de Outubro de 2024 em Botucatu, São Paulo, na qualidade de ouvinte, com carga horária de 04 horas.

Botucatu, 25 de Novembro de 2024.

Prof. Titular Carlos Magno Castelo Branco Fortaleza
Diretor da Faculdade de Medicina de Botucatu
FMB - Unesp

Prof. Dr. Tiago Rocha Pinto
Coordenador do Evento
FMB-Unesp

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CÂMPUS DE BOTUCATU
FACULDADE DE MEDICINA
Diretoria Técnica Acadêmica

Registrado sob Nº 6687
Fls.208 Livro 51
BOTUCATU 11 de Dezembro de 2024.

PROGRAMAÇÃO

EVENTO ONLINE

16/10 a 17/10 das 19h às 21h
- Será transmitido através do canal do Youtube da Associação Paulista de Saúde Pública;
- Será enviado um e-mail com o link do canal com os dados da inscrição.

PRIMEIRA NOITE - MESA REDONDA
- Abertura: APSP, Liga e Pós-graduação da UNESP
- As questões climáticas e o impacto na saúde no Brasil
- Atenção Primária e intersecção com o meio ambiente: reflexões e atuação
- A atuação da APS no desastre do Rio Grande do Sul
- Discussão
- Encerramento.

SEGUNDA NOITE:
- Abertura
- Determinantes sociais e Racismo ambiental
- Descarte correto de resíduos infectantes/contaminados: Práticas sustentáveis que podem melhorar a saúde/prevenir o adoecimento - Experiência no pós-Covid
- Importância da educação ambiental na promoção da saúde e prevenção de doenças
- Discussão (abertura da mesa para discussão)
- Encerramento

Anexo 14 - Estágio Docência PAADES

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CERTIFICADO

Certificamos que o(a) discente Danielle Cristina Honório França, CPF nº 05163819119, participou do Programa de Atividades e Aperfeiçoamento em Docência no Ensino Superior - PAADES, na disciplina METODOLOGIA CIENTÍFICA de responsabilidade do Departamento de Economia, Administração e Educação do Câmpus de Jaboticabal, no periodo de 07/08/2023 à 09/12/2023 com a carga horária de 24h em atividades de apoio à docência e 6h em atividades de docência.

Maria Valnice Boldri
Pró-reitora de Pós Graduação
PROPG/UNESP

Celia Maria Giacheti
Pró-reitora de Graduação
PROGRAD/UNESP

O PAADES é regulamentado pela Resolução Unesp nº14, de 18 de abril de 2022 e pela Resolução Unesp nº 18, de 29 de junho de 2022.

Rua Quirino de Andrade, 215 - Centro - São Paulo/SP - CEP 01049-010 Pabx: +55 11 5627-0233

Pró-Reitoria de Pós Graduação

Certificado emitido em: 11/12/2023

Para ver cópia do certificado e conferir sua autenticidade acesse:

https://sistemas.unesp.br/paades/autenticidade-certificado e insira o código verificador 792C-748F-3566-E515-8A49

Anexo 15 – Declaração de outras defesas assistidas no programa

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CAMPUS DE BOTUCATU
FACULDADE DE MEDICINA
Pós-Graduação em Ginecologia, Obstetrícia e Mastologia
BOTUCATU, SP - Rubião Júnior - CEP 18618-970 - ☎ (14) 3880-1123- E-mail: pggo@fmb.unesp.br

DECLARAÇÃO

Declaro para os devidos fins que Emille Cristina Moreira Franca, aluno(a) do Programa de Pós-Graduação em Tocoginecologia, assistiu à Defesa de Tese de Doutorado de Valdete Cyprucida Ribeiro da Silva, com o tema Quimioterapia domiciliar e hospitalar com metotrexato/ácido fólico por 8 dias para neoplasia trofoblástica gestacional de baixo risco: uma comparação dos resultados em dois centros de referência terciários, no dia 15/12/2023.

Botucatu, 15 de dezembro de 2023.

Prof(a) Dr(a)
Orientador(a) da Tese

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FACULDADE DE MEDICINA
Pós-Graduação em Ginecologia, Obstetrícia e Mastologia
BOTUCATU, SP - Rubião Júnior - CEP 18618-970 - ☎ (14) 3880-1123- E-mail: pggo@fmb.unesp.br

DECLARAÇÃO

Declaro para os devidos fins que Daniele Cristina Honorio França, aluno(a) do Programa de Pós-Graduação em Tocoginecologia, assistiu à Defesa de Tese de Doutorado de Bruna Bologna Cotinelli, com o tema Avaliação do impacto do exercício aquático sobre a miopatia de ratas prenhes com diabetes mellitus., no dia 23/08/2024.

Botucatu, 23 de agosto de 2024.

Prof(a) Dr(a) Mailza V. C Rudge
Orientador(a) da Tese