UNIVERSIDADE DE BRASÍLIA

FELIPE MOTTA

AVALIAÇÃO DO DESENVOLVIMENTO INFANTIL DE FILHOS DE MÃES COM INFECÇÃO POR SARS-CoV-2 DURANTE A GESTAÇÃO

BRASÍLIA

2024

UNIVERSIDADE DE BRASÍLIA
FACULDADE DE MEDICINA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS

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Orientador: Prof. Dr. Alexandre Anderson de Sousa Munhoz Soares

BRASÍLIA
2024

Motta, Felipe
Ma Avaliação do desenvolvimento infantil de filhos de mães com infecção por SARS-CoV-2 durante a gestação / Felipe Motta; orientador Alexandre Anderson de Sousa Munhoz Soares. -- Brasília, 2024.
135 p.

Tese(Doutorado em Ciências Médicas) -- Universidade de Brasília, 2024.

1. Neurodesenvolvimento. 2. Pediatria. 3. SARS-CoV-2. 4. Bayley-III. 5. COVID-19. I. Anderson de Sousa Munhoz Soares, Alexandre , orient. II. Título.

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Dedico este trabalho à minha família

AGRADECIMENTOS

Agradeço ao meu orientador, Prof. Dr. Alexandre Anderson de Sousa Munhoz Soares, por ter compartilhado de sua experiência acadêmica, bem como de sua expertise na obtenção, análise e apresentação de dados científicos. Sua capacidade de nos motivar foi fundamental para superarmos os desafios encontrados ao longo do caminho.

Agradeço especialmente ao Dr. Geraldo por sua dedicação aos pacientes e a nossa pesquisa mesmo diante de todas as dificuldades. Agradeço ainda a Maria Eduarda pelo otimismo e confiança na equipe PROUDEST-BORN. O Agradeço ao Dr. David Araújo Júnior por toda contribuição na concepção da radiologia pediátrica do estudo.

Agradeço a toda a equipe multidisciplinar do nosso ambulatório, radiologista, enfermeiras, fonoaudiólogas, terapeutas ocupacionais e psicólogas, sua experiência com pacientes pediátricos e constante colaboração foram de grande importância para o sucesso deste projeto.

Agradeço profundamente a cada família que participou deste estudo. Mesmo em um período de grande turbulência mundial, vocês se dedicaram incansavelmente ao acompanhamento de seus filhos no ambulatório de pediatria.

“Às vezes, o próprio sonho pode mantê-lo seguro ao longo de toda a estrada.”

(Traduzido do original *Sometimes a dream itself can keep you safe all along the roads* por Keane)

RESUMO

Fundamentos: A infecção materna pelo SARS-CoV-2 não apenas expõe o feto e o neonato aos efeitos diretos do vírus, mas também às possíveis complicações associadas à resposta inflamatória materna. Ainda há lacunas significativas no entendimento dos impactos da exposição intrauterina ao SARS-CoV-2, especialmente no que diz respeito ao neurodesenvolvimento das crianças. Compreender esses aspectos é crucial para orientar políticas de saúde pública e intervenções clínicas que possam mitigar potenciais danos ao desenvolvimento infantil.

Metodologia: Crianças de mães que tiveram infecção por SARS-CoV-2 durante a gravidez (confirmada por RT-PCR de swabs nasofaríngeos) foram avaliadas em consultas ambulatoriais prospectivas e analíticas realizadas aos 6 e 12 meses após o parto. O desenvolvimento foi avaliado com as Escalas Bayley-III usando um limiar de corte definido como >85 no escore de índice. Diferenças entre os grupos foram avaliadas por análise de variância usando o teste post hoc de Bonferroni.

Resultados: Duzentos e sessenta e sete crianças foram avaliadas, a maioria nascida a termo e com peso ao nascimento adequado para a idade gestacional. Nesse cenário, atrasos no desenvolvimento foram observados em 26% das crianças em pelo menos um domínio. O domínio da linguagem foi particularmente afetado, com prejuízos observados em crianças expostas ao SARS-CoV-2 mais próximas do momento do parto, assim como aquelas que tiveram sintomas graves durante a gravidez devido à exposição ao vírus. Filhos de mães com menor grau de instrução - mães que não possuíam diploma universitário - também apresentaram pior desempenho na avaliação com escala de desenvolvimento neuropsicomotor. Esses achados foram estatisticamente significativos ($p < 0,05$).

Conclusões: Crianças nascidas de mulheres com infecção por SARS-CoV-2 durante o período da pandemia de COVID-19, avaliadas aos 6 e 12 meses, apresentaram atraso na linguagem. Filhos de mulheres com COVID-19 grave apresentaram escores mais baixos nas escalas de avaliação referentes ao domínio da linguagem. Crianças nascidas de mulheres sem ensino superior também apresentaram pontuação abaixo da média no domínio linguagem da escala Bayley-III.

Palavras-chaves: neurodesenvolvimento, SARS-CoV-2, pediatria, COVID-19, Bayley-III

ABSTRACT

Background: Maternal infection by SARS-CoV-2 not only exposes the fetus and newborn to the direct effects of the virus but also to the potential complications associated with the maternal inflammatory response. The long-term implications for the neuropsychomotor development of exposed children are yet to be fully understood. Understanding these aspects is crucial to guide public health policies and clinical interventions that can mitigate potential harm to child development.

Methodology: Children of mothers who had a SARS-CoV-2 infection during pregnancy (confirmed by RT-PCR of nasopharyngeal swabs) were evaluated in prospective and analytical outpatient consultations conducted at 6 and 12 months postpartum. Development was assessed using the Bayley-III Scales, with a cut-off threshold defined as >85 on the index score. Differences between groups were evaluated by analysis of variance using Bonferroni's post hoc test.

Results: Two hundred and sixty-seven children were evaluated, most of whom were born at term and with a birth weight appropriate for gestational age. In this cohort, developmental delays were observed in 26% of the children in at least one domain. The language domain was particularly affected, with impairments observed in children exposed to SARS-CoV-2 closer to the time of delivery, as well as in those whose mothers had severe symptoms during pregnancy due to virus exposure. Children of mothers with lower educational levels – those without a university degree – also showed poorer performance in the neuropsychomotor development assessment scale. These findings were statistically significant ($p < 0.05$).

Conclusions: Children born to women with SARS-CoV-2 infection during the COVID-19 pandemic, evaluated at 6 and 12 months, showed language delays. Children of women with severe COVID-19 had lower scores on the language domain assessment scales. Children born to women without higher education also scored below average in the language domain of the Bayley-III scale.

Keywords: neurodevelopment, SARS-CoV-2, pediatrics, COVID-19, Bayley-III

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1. INTRODUÇÃO

1.1 Revisão da literatura

A pandemia e a exposição ao SARS-CoV-2 durante a gestação

Desde o final de 2019, um novo coronavírus foi identificado como o agente desencadeador de um surto de pneumonia na cidade de Wuhan, localizada na província de Hubei, na China. Este vírus se disseminou rapidamente, inicialmente resultando em uma epidemia local e, posteriormente, se transformando em uma pandemia global. Em fevereiro de 2020, a Organização Mundial da Saúde (OMS) oficializou o nome da doença como COVID-19, que é a abreviação para doença causada pelo coronavírus 2019 [1]. O patógeno responsável pela COVID-19 é conhecido como coronavírus da síndrome respiratória aguda grave 2 (SARS-CoV-2), anteriormente denominado 2019-nCoV [2].

Informações provenientes de Wuhan revelam que cerca de 36,4% dos pacientes hospitalizados exibem anomalias neurológicas [3]. A origem precisa dessas condições neurológicas na COVID-19 ainda não está completamente esclarecida, mas parece ser influenciada por diversos fatores, como os efeitos diretos do vírus, respostas autoimunes, inflamação generalizada e outros mecanismos.

Os coronavírus (CoV) constituem uma família de vírus envelopados com genoma de RNA de fita simples, classificados em quatro gêneros principais: alfa, beta, gama e delta. Os CoV alfa, beta e delta têm a capacidade de infectar mamíferos e são reconhecidos por sua propensão a cruzar barreiras de espécies, resultando em doenças respiratórias graves em humanos. Exemplos dessas síndromes incluem a Síndrome Respiratória do Oriente Médio (MERS), causada pelo MERS-CoV, a Síndrome Respiratória Aguda Grave (SARS), causada pelo SARS-CoV, e a COVID-19, causada pelo SARS-CoV-2 [4].

O conhecimento sobre as consequências da infecção pelo SARS-CoV-2 e da síndrome clínica multisistêmica da COVID-19 está em constante evolução com a realização de novos estudos clínicos.

Além disso, complicações vasculares decorrentes da inflamação sistêmica e da disfunção do sistema de coagulação podem aumentar significativamente o risco de doenças cerebrovasculares, meningoencefalite e encefalopatia hipóxico-isquêmica, particularmente em adultos. Entretanto, o impacto dessas condições em crianças ainda não está completamente esclarecido na literatura científica [5].

A infecção materna pelo SARS-CoV-2 apresenta um cenário desafiador não apenas devido aos potenciais efeitos diretos do vírus sobre o feto, mas também pelas complicações associadas à resposta inflamatória tanto materna quanto fetal, incluindo o uso de terapias antivirais. As implicações de longo prazo para o desenvolvimento neurológico pós-natal e eventuais alterações estruturais ainda carecem de investigações abrangentes. Ainda há muitas lacunas a serem preenchidas quanto aos efeitos da infecção durante a gestação, no puerpério e no desenvolvimento de longo prazo de crianças nascidas de mães infectadas. [6]

A compreensão das sequelas de longo prazo no sistema nervoso de crianças afetadas pela COVID-19 está em fase de desenvolvimento contínuo. Este processo inclui o estudo de possíveis alterações morfológicas e funcionais que poderiam impactar negativamente o neurodesenvolvimento. Estudos clínicos longitudinais, empregando métodos objetivos para avaliação do neurodesenvolvimento e técnicas avançadas de imagem, são cruciais para esclarecer os desfechos clínicos possíveis.

A possibilidade de distúrbios no neurodesenvolvimento em crianças expostas intraútero ao SARS-CoV-2 suscita uma preocupação considerável. Dada a grande quantidade de indivíduos expostos, até mesmo um pequeno aumento no risco de desenvolvimento neuropsicomotor adverso poderia ter um impacto substancial em termos de saúde pública [7-13]. De acordo com estimativas, mais de 59 milhões de pessoas nos Estados Unidos e 300 milhões em todo o mundo foram diagnosticadas com COVID-19, incluindo mais de 155.500 mulheres grávidas nos Estados Unidos [13]. Com aproximadamente 140 milhões de nascimentos vivos ocorrendo anualmente em todo o mundo e uma prevalência de positividade para o SARS-CoV-2 em mulheres grávidas chegando a 15% em grandes centros urbanos [14], o número de crianças expostas à infecção materna por COVID-19 *in utero* poderia atingir até 20 milhões por ano, especialmente devido à baixa adesão inicial à vacinação contra a COVID-19 entre as gestantes [15,16].

Estudos de acompanhamento, incluindo um monitoramento de 12 meses de mais de 7.000 partos em um grande sistema hospitalar, onde mais de 200 gestações foram expostas à COVID-19, sugerem que a infecção pré-natal pelo SARS-CoV-2 está associada a um aumento no risco de diagnósticos de distúrbios do neurodesenvolvimento na prole [17]. Existem várias vias potenciais pelas quais a infecção materna pelo SARS-CoV-2 pode impactar o cérebro fetal em desenvolvimento [18]:

- Ativação imune materna: Durante as janelas críticas de neurodesenvolvimento na gestação, a ativação do sistema imunológico da mãe pode ter efeitos adversos no desenvolvimento neurológico do feto. [19]

- Infecção fetal direta: A transmissão transplacentária do vírus pode levar à infecção direta dos tecidos neurológicos fetais.[19]

- Comprometimento da função placentária: A disfunção placentária pode resultar em desfechos adversos na gravidez, como restrição de crescimento fetal, descolamento ou hematoma retroplacentário e parto prematuro, todos associados a um maior risco de lesões neurológicas. [18-20]

Essas possíveis vias destacam a complexidade das interações entre a infecção materna por SARS-CoV-2 e o desenvolvimento fetal, reforçando a necessidade de vigilância contínua e pesquisas aprofundadas para entender completamente os riscos e mecanismos envolvidos. O impacto potencial em saúde pública é significativo, dado o número expressivo de crianças potencialmente afetadas globalmente.

Em suma, as interações entre a infecção pelo SARS-CoV-2, as respostas inflamatórias e os potenciais impactos no sistema nervoso de fetos e crianças cujas as mães foram infectadas durante a gestação, requer uma abordagem científica rigorosa e contínua. A investigação detalhada desses aspectos é fundamental para orientar estratégias de manejo clínico e intervenções terapêuticas eficazes.

A Fisiopatologia do acometimento Neurológico pelo SARS-CoV-2

A complexidade das complicações neurológicas associadas à COVID-19 é

profundamente influenciada pela interação intricada entre o vírus SARS-CoV-2, respostas imunológicas do hospedeiro e possíveis fatores genéticos predisponentes. Essas complicações podem se apresentar de várias formas ao longo do tempo, desde sintomas agudos durante a fase inicial da doença até manifestações crônicas que persistem além da fase de infecção inicial. [21]

Inicialmente, é comum observar complicações neurológicas agudas em pacientes severamente afetados pela COVID-19, especialmente aqueles que desenvolvem comprometimento respiratório grave ou falência de múltiplos órgãos, exigindo hospitalização intensiva. Nestes casos críticos, a hipoxemia decorrente da doença pode desencadear uma gama de distúrbios neurológicos agudos, exacerbados por distúrbios metabólicos associados aos efeitos adversos de tratamentos medicamentosos administrados durante a terapia. Estudos post-mortem têm consistentemente identificado lesões no sistema nervoso central como infartos cerebrais e nódulos microgliais, refletindo uma resposta inflamatória acentuada no tecido cerebral [22-24].

Além disso, a disfunção imunológica desempenha um papel crucial no desenvolvimento de sintomas neurológicos associados à COVID-19. Pacientes gravemente enfermos frequentemente apresentam sinais de inflamação sistêmica severa, caracterizada pela liberação excessiva de citocinas pró-inflamatórias como TNF-alfa e IL-6. Estas citocinas podem atravessar a barreira hematoencefálica, afetando diretamente o sistema nervoso central e contribuindo para sintomas como confusão mental e alteração de consciência (16-19). A ativação da microglia e a ocorrência de neuronofagia são comuns em casos graves, sugerindo uma resposta imunológica localizada no cérebro [25-29].

Adicionalmente, estudos neuropatológicos estão investigando a possível invasão direta do SARS-CoV-2 no sistema nervoso central, com detecção do vírus em amostras cerebrais. No entanto, ainda há incertezas sobre a relação entre essa detecção viral e a gravidade das alterações neuropatológicas observadas, incluindo a controversa hipótese de endotelite cerebral induzida pelo vírus, destacando a necessidade urgente de mais pesquisas para elucidar esses mecanismos [30-34].

Por outro lado, a COVID-19 tem sido associada ao desenvolvimento de condições autoimunes, como a síndrome de Guillain-Barré e a encefalite autoimune, nas quais o sistema imunológico ataca erroneamente o tecido neural. A evolução

temporal dos sintomas neurológicos em relação aos sintomas iniciais da infecção sugere que essas condições podem surgir como complicações parainfecciosas, acrescentando complexidade ao quadro clínico [35,36].

Por fim, crescem as preocupações sobre o potencial de neurodegeneração associado à COVID-19. Estudos indicam que a infecção pode causar alterações estruturais e funcionais no cérebro, possivelmente predispondo os pacientes ao desenvolvimento de doenças neurodegenerativas a longo prazo. Biomarcadores de disfunção neuronal foram identificados em indivíduos com sintomas neurológicos persistentes após a recuperação da infecção, enfatizando a importância de monitoramento contínuo e de estudos adicionais para uma compreensão completa do impacto a longo prazo da COVID-19 no sistema nervoso [37,38].

Em síntese, a compreensão abrangente das complicações neurológicas associadas à COVID-19 é essencial para o desenvolvimento de estratégias de tratamento eficazes e para mitigar os efeitos adversos a longo prazo em pacientes afetados pela doença. A continuidade da pesquisa é fundamental para esclarecer os mecanismos patológicos subjacentes e orientar intervenções clínicas direcionadas, visando melhorar os resultados clínicos e funcionais dos pacientes.

A “COVID longa”

Pacientes que se recuperam de formas graves da COVID-19 ou que passaram por hospitalização devido à doença frequentemente relatam sintomas neurológicos que persistem por períodos prolongados. Além disso, mesmo pacientes que apresentaram quadros agudos mais leves da COVID-19, sem necessidade de hospitalização por pneumonia ou hipoxemia, também podem relatar a persistência de sintomas neurológicos e sistêmicos por períodos estendidos. [39-43]

Os sintomas neurológicos que mais comumente interferem nas atividades diárias incluem comprometimento cognitivo, parestesia (sensação de formigamento ou dormência), cefaleia (dor de cabeça), disgeusia (alteração ou perda do paladar), anosmia (perda do olfato), mialgia (dores musculares), tontura, visão turva e zumbido. Quando esses sintomas persistem por mais de quatro semanas após a infecção inicial, eles podem ser classificados como parte da síndrome conhecida como "COVID longa", também referida como "sequelas pós-agudas da infecção por SARS-CoV-2".[44]

Além disso, há relatos de que a infecção prévia por COVID-19 pode desencadear a manifestação de sintomas de doenças neurodegenerativas em indivíduos que eram previamente assintomáticos. Um estudo realizado na Suécia indicou que a demência, assim como muitas outras doenças crônicas, foi subdiagnosticada e subtratada durante a pandemia. [45]

No que diz respeito à neuroimagem, a ressonância magnética cerebral tende a ser normal em pacientes que apresentam sintomas neurológicos persistentes após a infecção por COVID-19. Contudo, estudos que utilizaram neuroimagem funcional, especificamente a tomografia por emissão de pósitrons de fluorodesoxiglicose (FDG-PET), identificaram diversas áreas com redução no metabolismo da glicose em alguns pacientes com sintomas prolongados. As regiões afetadas incluem áreas de interesse neurológico como o giro orbitofrontal, o hipocampo, a amígdala, o tálamo e o córtex insular. [46,47]

Esses achados indicam que a COVID-19 pode ter efeitos duradouros no sistema nervoso central, independentemente da gravidade inicial da infecção, e que a pesquisa contínua é essencial para compreender completamente as implicações neurológicas de longo prazo da doença. [48,49]

Achados de Neuroimagem em COVID-19

Um amplo espectro de anormalidades em neuroimagem tem sido meticulosamente descrito em pacientes que desenvolvem encefalopatia relacionada à COVID-19. Essas anomalias não apenas refletem o impacto agudo da infecção pelo vírus SARS-CoV-2 no sistema nervoso central, mas também podem indicar a presença subjacente de condições adicionais, como acidente vascular cerebral (AVC), encefalite, síndrome de leucoencefalopatia posterior reversível (SLPR) e outras entidades neurológicas [50-52]. A análise dos estudos revela que aproximadamente metade dos pacientes examinados apresenta algum tipo de alteração significativa na neuroimagem, sendo as mais comuns as alterações no sinal cortical na sequência FLAIR, AVC isquêmico agudo, realce leptomeningeal discreto e manifestações sugestivas de encefalite [53,54]. Em uma recente série de casos envolvendo 190 pacientes com COVID-19 grave e sintomas de encefalopatia, foram identificadas anormalidades radiológicas significantes após a exclusão de lesões crônicas não

relacionadas e AVC isquêmico [50]. Entre essas anormalidades, destacam-se lesões multifocais hiperintensas na substância branca ou no lobo temporal medial, frequentemente associadas a micro-hemorragias. Além disso, foram relatadas lesões hiperintensas no esplênio do corpo caloso, tanto em adultos com encefalopatia relacionada à COVID-19 quanto em crianças com síndrome inflamatória multissistêmica [55,56].

A associação entre COVID-19 e eventos neurovasculares é complexa, envolvendo fatores pró-inflamatórios e de hipercoagulabilidade induzidos pela infecção viral, conforme indicam níveis elevados de marcadores como D-dímero em pacientes com COVID-19 grave e AVC isquêmico [57-60]. Esse contexto inflamatório e trombótico pode predispor tanto à formação de coágulos como à transformação hemorrágica de lesões isquêmicas, aumentando o risco de hemorragia intracraniana, especialmente em pacientes tratados com anticoagulação plena ou submetidos a oxigenação por membrana extracorpórea (ECMO) [61, 65].

Além dos eventos cerebrovasculares, outras síndromes neurológicas e neuromusculares têm sido relatadas em associação com COVID-19, ampliando a compreensão das manifestações neurológicas dessa doença viral. Estas incluem síndrome de Guillain-Barré, meningoencefalite viral, encefalomielite disseminada aguda (ADEM), mielite transversa aguda, mioclonia generalizada, síndrome de leucoencefalopatia posterior reversível (SLPR), síndrome de vasoconstrição cerebral reversível (RCVS), convulsões, miosite, neuropatias focais e multifocais, além de neuropatia e miopatia da doença crítica [66,67]. Cada uma dessas condições reflete uma interação complexa entre o vírus SARS-CoV-2 e o sistema nervoso, com manifestações clínicas variáveis que exigem uma abordagem multidisciplinar para o diagnóstico e tratamento adequados. A investigação contínua dessas manifestações neurovirais é crucial para aprimorar estratégias de manejo clínico e otimizar os resultados para os pacientes afetados.

1.2. COVID-19 e sintomas neurológicos em crianças

Manifestações Neurológicas em Crianças expostas ao SARS-CoV-2

Os sintomas da COVID-19 em recém-nascidos e pacientes pediátricos são

predominantemente leves a moderados, assemelhando-se à gripe comum. No entanto, uma minoria desenvolve formas graves da doença respiratória, necessitando de suporte respiratório. O entendimento desses desdobramentos ainda está em desenvolvimento à medida que mais informações são coletadas e analisadas.

Um conjunto crescente de evidências sugere que a infecção pelo SARS-CoV-2 pode causar sequelas neurológicas agudas e crônicas em populações pediátricas. Um estudo recente no Reino Unido indica que 3,8% das crianças hospitalizadas com COVID-19 desenvolvem complicações neurológicas, as quais podem ter consequências a longo prazo. Estas complicações variam desde alterações comportamentais, alucinações e encefalopatia até condições mais graves como estado epiléptico, encefalite, síndromes de Guillain-Barré/desmielinização aguda, coreia e psicose [68].

Manifestações neurológicas têm sido documentadas em crianças hospitalizadas com COVID-19 aguda, representando um risco significativo à vida [69-71]. Em um estudo multicêntrico transversal envolvendo 15.137 crianças hospitalizadas com COVID-19, observou-se que 7% apresentaram uma ou mais complicações neurológicas. Estas complicações incluíam convulsões febris em 3,8%, convulsões não febris em 2,3% e encefalopatia em 2,2%. Complicações neurológicas menos comuns, mas igualmente graves, incluíram abscesso cerebral, meningite bacteriana e infarto cerebral [69]. Outros estudos relataram uma gama de manifestações neurológicas, como acidente vascular cerebral, infecção/desmielinização do sistema nervoso central, síndrome de Guillain-Barré e suas variantes, edema cerebral agudo fulminante, dores de cabeça, fraqueza, anosmia, ageusia e delírio [72-73].

Embora a maioria das crianças com infecção sintomática aguda não-grave pelo SARS-CoV-2 se recupere dentro de uma a duas semanas após o início dos sintomas, há casos em que a deterioração clínica ocorre subitamente após cerca de uma semana, exigindo reavaliação clínica urgente, idealmente em centros médicos especializados no cuidado pediátrico de COVID-19 [74]. Em um estudo de coorte prospectivo, onde um adulto responsável relatava sintomas via aplicativo móvel, a duração média da doença em 1.734 crianças que testaram positivo para SARS-CoV-2 foi de seis dias (intervalo interquartil [IIQ] de 3 a 11 dias), em comparação com três dias (IIQ de 2 a 7 dias) em uma coorte pareada de crianças que testaram negativo

para o vírus [196]. Aproximadamente 2% dos pacientes de cada grupo procuraram atendimento em pronto-socorro ou foram hospitalizados. Notou-se que a duração média da doença pelo SARS-CoV-2 foi menor em crianças de 5 a 11 anos do que em crianças de 12 a 17 anos (5 versus 7 dias).

Um problema persistente na interpretação desses dados é a escassez de estudos com controles pareados, que poderiam ajudar a entender se essas sequelas refletem efeitos diretos do SARS-CoV-2 ou são simplesmente consequências de qualquer doença grave que requer hospitalização. A análise de alguns casos, utilizando avaliações neurocognitivas, sugeriu que o padrão de déficits encontrados foi inespecífico, consistente com um contexto clínico multifatorial em hospitalizações complicadas. Contudo, os resultados sugerem a possibilidade de que o SARS-CoV-2, através de mecanismos inflamatórios, imunomediados ou de infecção direta do sistema nervoso central, possa ter um impacto prolongado ou permanente no cérebro desenvolvido e, portanto, também no cérebro em desenvolvimento.

Algumas complicações peculiares da COVID-19 em crianças incluem:

- *Síndrome Inflamatória Multissistêmica em Crianças (MISC):* Esta é uma condição rara, mas grave, associada à COVID-19, que pode apresentar características clínicas semelhantes às da doença de Kawasaki, síndrome de choque da doença de Kawasaki e síndrome do choque tóxico. Os sintomas incluem febre persistente, hipotensão, sintomas gastrointestinais, erupção cutânea, miocardite e achados laboratoriais de inflamação aumentada; os sintomas respiratórios podem estar ausentes.

- *Condição pós-COVID-19 ("COVID longa") em crianças:* O Centro de Controle e Prevenção de Doenças (CDC), a Academia Americana de Pediatria e a Organização Mundial da Saúde (OMS) utilizam o termo "condição pós-COVID-19" para descrever a ampla gama de sintomas e condições físicas e mentais persistentes, recorrentes ou novas que aparecem mais de 4 semanas após a infecção pelo SARS-CoV-2. Essa condição, também conhecida como "COVID longa" ou "sequelas pós-agudas da infecção por SARS-CoV-2", é caracterizada por sintomas como fadiga, alteração do olfato/anosmia e ansiedade, sendo mais frequente entre crianças com

condição pós-COVID-19 do que entre os controles [76-78].

A definição clínica consensual da OMS para esta condição, que pode ser aplicada a crianças de todas as idades, inclui [78]:

-Infecção pelo SARS-CoV-2 confirmada ou provável.

-Sintomas com duração maior ou igual a 2 meses, que surgiram inicialmente dentro de três meses após a COVID-19 aguda. Os sintomas podem ser de início recente ou persistentes e podem recorrer ao longo do tempo.

-Os sintomas afetam a função diária, como hábitos alimentares, atividade física, interação social e desempenho escolar.

-A coexistência de outros diagnósticos não exclui o diagnóstico de condição pós-COVID-19.

Observações iniciais de desfechos neurodesenvolvimentais adversos aos 3–6 meses, 1 ano e 1,5 anos em bebês e crianças expostos ao SARS-CoV-2 intraútero, e/ou nascidos durante a pandemia de COVID-19, têm sido descritas. Um estudo de coorte longitudinal com 57 bebês expostos ao SARS-CoV-2 no período pré-natal na China identificou déficits no domínio sócio-emocional em testes neurodesenvolvimentais aos 3 meses de idade [116]. Além disso, um relatório preliminar envolvendo 298 bebês nascidos de mulheres infectadas pelo SARS-CoV-2 durante a gravidez encontrou evidências de atraso no desenvolvimento em 10% dos bebês aos 12 meses de idade, embora nenhum dos estudos incluísse um grupo controle não infectado [105].

Outro estudo preliminar, que avaliou mais de 7.000 bebês nascidos durante a pandemia de COVID-19 de mães infectadas e não infectadas pelo SARS-CoV-2, identificou uma associação entre a exposição materna ao vírus e alterações no neurodesenvolvimento aos 12 meses [17]. Importante destacar que, embora a associação entre a exposição materna ao SARS-CoV-2 e o atraso do neurodesenvolvimento na prole tenha sido exacerbada pelo parto prematuro, a presença de distúrbios do neurodesenvolvimento não foi totalmente explicada pela prematuridade. Isso sugere a existência de um mecanismo de efeito mais específico, em que o SARS-CoV-2 contribui para complicações na gravidez, além da prematuridade.

Dada a diversidade das manifestações clínicas e o potencial para dano neurológico significativo em crianças, é essencial uma abordagem abrangente que previna sequelas

e promova o diagnóstico precoce, baseando-se em avaliações clínicas, laboratoriais e exames complementares de neuroimagem pediátrica.

1.3 O Neurodesenvolvimento

O cérebro humano se compõe de estruturas extremamente complexas, e a formação dessa complexidade se deve ao resultado de uma sincronia ainda no período fetal entre fatores de proliferação, diferenciação, e migração celular que formarão as diferentes áreas anatômicas e funcionais do sistema nervoso [81]. A maturação desse sistema requer uma sequência de processos extremamente complexos, mais do que na formação de qualquer outra estrutura, fato esse que torna o cérebro único e bastante vulnerável a influências ambientais. [82]

Diante disso, qualquer alteração nessa complexa rede pode causar transtornos no desenvolvimento neuropsicomotor, os agentes que causam infecções congênitas podem causar surdez, cegueira, paralisia cerebral, dificuldade de aprendizado, transtornos do comportamento e epilepsia [83] . Se sabe ainda que a resposta imune materna exacerbada durante a gestação pode ser um fator determinante. Isso foi estudado durante a epidemia de influenza de 1957 na Finlândia, onde um número significativo de fetos expostas ao vírus no segundo trimestre de gestação desenvolveu esquizofrenia na vida adulta. [84]

Após essas observações, pesquisas foram iniciadas analisando a exposição fetal a agentes infecciosos como bactérias e vírus e as alterações no neurodesenvolvimento. Para tal, utilizaram-se modelos murinos nos quais observou-se que muito mais do que a presença do microrganismo em si, a resposta imune materna a um agente estressor foi fator desencadeante de citocinas pró inflamatórias no plasma materno, fator esse que resultou em déficits comportamentais em adolescentes e adultos que se assemelham aos sintomas de transtornos de desenvolvimento nessa prole. [84]

Diante disso, ficou evidente que a ativação da resposta imune materna, que normalmente acontece durante a metade ou final da gestação, é o momento mais crítico para o desenvolvimento cerebral, isso porque é nesse momento que a neurogênese pode ser afetada por fatores ambientais adversos. [85]

Essas evidências sugerem ainda que a ativação imune materna altera a proliferação e a diferenciação das células neuroepiteliais. Modificando, dessa forma, os locais de especialização celular no encéfalo. Foi demonstrado, por exemplo, que 2 a 8 horas após a exposição de fatores inflamatórios maternos, ocorre no feto uma diminuição da proliferação celular no córtex pré-frontal, com redução em um marcador de ativação mitótica. [86, 87,88]

É importante entender, ainda, que além dos fatores físicos, as alterações da saúde mental materna durante a gravidez também possuem impacto no desenvolvimento neurológico do bebê. Situações como a perda de um ente querido, desastres ambientais e estressores sociais (incluindo moradia inadequada, pobreza, desemprego, criminalidade, racismo e preconceito) são reconhecidos como causas potenciais de parto prematuro e desnutrição ao nascer. Esses fatores também podem reduzir a atividade das enzimas placentárias, aumentando a exposição do feto a hormônios do estresse, como o cortisol materno [89]. Sendo assim, doenças e estressores da saúde mental materna afetam o neurodesenvolvimento nos diversos domínios como cognitivo, processamento e conexões funcionais e estruturais do cérebro; o que envolve as amígdalas e o córtex pré-frontal, assim como o eixo hipotálamo-hipofisário; tais alterações levarão a um risco aumentado de transtornos do comportamento durante a vida da criança exposta. [90, 91]

Faz-se importante destacar que o inverso também é verdadeiro. Estudos indicam que gestantes submetidas a altos níveis de estresse quando comparadas entre grupos com e sem suporte adequado de seu parceiro(a) apresentam um possível aumento na exposição fetal ao cortisol no grupo sem suporte adequado. Ademais, há evidências de que a atividade física e um ambiente favorável reduzem as taxas de parto prematuro e melhoram os escores cognitivos e de linguagem em testes de desenvolvimento aplicados aos 12, 18 e 24 meses de vida nas famílias estudadas [92]

1.4 As Escalas Bayley de desenvolvimento em bebês e crianças – 3ª edição

Acredita-se que 15% das crianças possuem algum atraso no desenvolvimento neuropsicomotor. Tal diagnóstico ocorre em crianças que

sofreram algum evento em períodos cruciais do neurodesenvolvimento, como: fetal, neonatal, e nas primeiras semanas de vida. Identificar esse atraso é crucial, pois o curso da doença, seja ele cura ou diminuição da intensidade, pode ser modificado quanto antes ocorra uma intervenção. [93]

O primeiro ano de vida é essencial para o desenvolvimento global da criança, com aquisições significativas que influenciam seu prognóstico futuro. É imperativo diagnosticar precocemente tanto danos permanentes, como a encefalopatia crônica não-progressiva, quanto formas sutis de atraso no desenvolvimento, estabelecendo imediatamente um plano terapêutico. Evidências indicam que diagnósticos e intervenções precoces sobre atrasos no desenvolvimento reduzem significativamente o impacto desses problemas na vida futura das crianças. [94]

Entre os diversos testes para desenvolvimento, as Escalas Bayley de Desenvolvimento de Bebês e Crianças são utilizadas com frequência nas pesquisas com crianças de risco. É um exame que requer treinamento do profissional para a sua aplicação, e é necessário experiência no desenvolvimento infantil e em outras avaliações do desenvolvimento por parte do avaliador.

O teste Bayley, desenvolvido por Nancy Bayley da Universidade de Berkeley em 1969, foi inicialmente conhecido como a Escala Mental do Primeiro Ano de Vida da Califórnia. Em 1993, os dados normativos foram atualizados com a publicação da segunda versão. Em 2006, foi lançada a terceira edição, Bayley III, que apresenta qualidades psicométricas aprimoradas e escalas mais detalhadas e específicas. [95]

Contudo, ainda era necessário um método no qual coubesse a avaliação de forma global (abrangendo os aspectos motores, cognitivos, sociais e de linguagem), e que incorporasse as diferenças econômicas, étnicas e culturais no Brasil. Dessa forma, em 2016, as Escalas Bayley de Desenvolvimento de Bebês e Crianças em sua 3 ª edição foram validadas para o Brasil. Durante o processo de adaptação, foram considerados diversos fatores para assegurar a adequação cultural e linguística do instrumento. Elementos específicos da cultura americana, como jogos tradicionais e músicas, foram substituídos por equivalentes brasileiros. Essas modificações facilitaram a compreensão tanto por parte dos pacientes quanto dos cuidadores, garantindo que os itens do teste fossem relevantes e

culturalmente apropriados. [96]

Essas escalas são consideradas padrão-ouro e reconhecidas internacionalmente tanto para pesquisa quanto para prática clínica. Isso porque são capazes de avaliar de forma individual os diversos domínios, tendo uma base teórica robusta e ao mesmo tempo adequada qualidade e confiabilidade dos instrumentos. [97]

Dito isso, as escalas são aplicadas de forma individual e por um profissional da pediatria devidamente capacitado e com experiência em desenvolvimento infantil. A avaliação pode ter de 60 a 90 minutos de duração, a depender da idade do indivíduo avaliado. Para a realização do teste, um kit com material teórico ao avaliador e objetos, brinquedos e imagens para o avaliado são utilizados de forma padronizada assim como um questionário. (Figuras 1 e 2).

Figura 1 – Componentes do Kit Bayley-III
Stimuli Book - Livro de Estímulo; *Stepping Path* – Caminho de passos; *Large Ball* – Bola grande; *Blocks* – Blocos; *Pegboard and Pegs*- Painel com pinos e ganchos; *Blue Puzzle Board & Block Set* - Quebra-cabeças azul e blocos; *Rolling Case* – mala de rodinhas; *Administration Manual*- Manual do avaliador; *Teddy Bear* – urso de pelúcia; *Doll* – Boneca; *Piggy Bank* – cofrinho ; *Lacing Card & Shoelace* – Cartão de montar e amarração; Registro de cada participante
Fonte: *https://www.pearsonassessments.com/store/usassessments/en/Store/Professional-Assessments/Behavior/Bayley-Scales-of-Infant-and-Toddler-Development-%7C-Third-Edition/p/100000123.html.* Acessado em Janeiro 2024.

As escalas acessam 5 domínios principais do desenvolvimento infantil em crianças de 16 dias até 42 meses e 15 dias de vida. A saber os domínios são: cognitivo, linguagem (receptiva e expressiva), motor (grosso e fino), socioemocional e comportamento adaptativo. Os dois últimos são avaliados através de questionários preenchidos pelos cuidadores, sendo por isso considerados como complementares na avaliação e pouco utilizados em pesquisa ou para tomada de decisão na prática clínica.

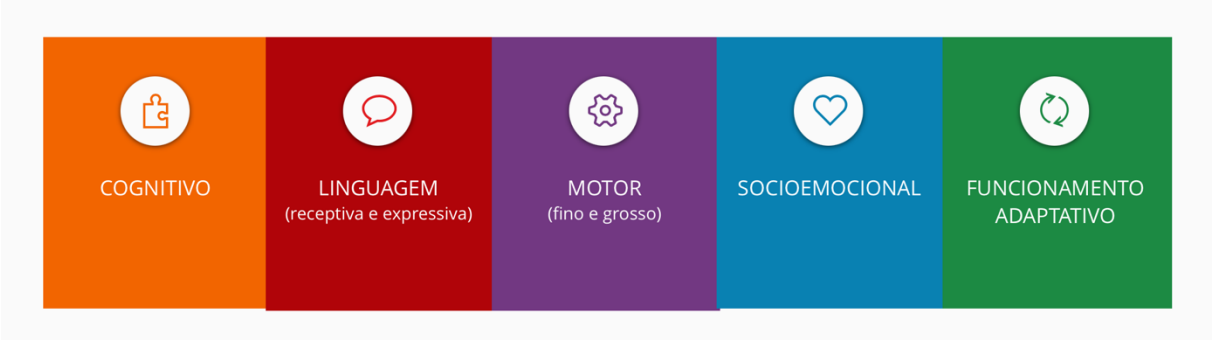


Figura 2 – Domínios de avaliação das escalas Bayley-III.
Fonte:*https://www.pearsonassessments.com/store/usassessments/en/Store/Professional-Assessments/Behavior/Bayley-Scales-of-Infant-and-Toddler-Development-%7C-Third-Edition/p/100000123.html.* Acessado em Janeiro 2024.

Vale ainda explicar os aspectos principais de avaliação de cada domínio. Na avaliação da linguagem, a comunicação é subdividida em expressiva - que diz respeito ao aspecto não verbal, ao vocabulário e ao desenvolvimento morfossintático; e comunicação receptiva - que avalia como a criança entende o vocabulário e os mais diferentes sons.

O domínio motor pode ser dividido em motor grosseiro - que são os atos de sentar, levantar, caminhar; e motor fino - que compreende atividades mais complexas como pegar pequenos objetos, manipular os objetos (empilhar blocos, virar páginas, ou inserir pinos em orifícios) e a coordenação das mãos com a visão.

O aspecto cognitivo da avaliação compreende avaliar a capacidade de a criança de pensar, aprender e lembrar informações.

Dessa forma uma pontuação numérica é obtida, e o valor é comparado com uma tabela idade específica; dessa forma uma pontuação final é obtida. De acordo com a literatura, o valor que melhor contempla sensibilidade e especificidade é >

85 para se considerar um exame adequado para idade. Valores inferiores a esse são considerados sugestivos de atraso do desenvolvimento, e devem ser mais bem avaliados pela equipe multiprofissional, sugerindo que uma intervenção direcionada deverá acontecer. [97]

2. Justificativa

A pandemia da COVID-19 trouxe à tona uma série de preocupações sobre os efeitos do vírus na saúde das gestantes e de seus filhos. Estudos iniciais já indicaram possíveis consequências para a saúde geral das crianças nascidas de mães infectadas, mas a compreensão detalhada dos impactos no desenvolvimento neuropsicomotor ainda é limitada. Este trabalho busca investigar como a exposição ao SARS-CoV-2 durante a gestação pode influenciar o desenvolvimento das crianças, oferecendo uma visão sobre os possíveis efeitos a longo prazo da pandemia.

Além de explorar a relação entre a infecção materna e o desenvolvimento infantil, a pesquisa examina como diferentes fatores, como o momento da infecção, a gravidade da doença materna e o nível educacional da mãe, podem influenciar esses efeitos. Essa abordagem visa fornecer informações úteis para aprimorar as estratégias de acompanhamento e suporte para crianças expostas ao vírus, contribuindo para uma melhor compreensão das necessidades dessas crianças e ajudando a orientar práticas de cuidado mais adequadas.

3. Objetivos

2.1. Objetivo primário

Determinar os efeitos da infecção por SARS-CoV-2 sobre o neurodesenvolvimento e suas respectivas manifestações em filhos de mulheres expostas à infecção pelo SARS-CoV-2 durante a gestação.

2.2. Objetivos secundários

- Caracterizar associações da exposição ao SARS-CoV-2 durante a pandemia no desenvolvimento neuropsicomotor de crianças filhas de mães infectadas em qualquer fase do período gestacional.
- Mensurar os efeitos da exposição ao SARS-CoV-2 sobre os resultados da escala padrão-ouro de desenvolvimento neuropsicomotor, categorizando os possíveis graus de atraso.
- Avaliar fatores associados a pior o prognóstico do desenvolvimento baseados em: momento da infecção durante a gestação, gravidade da doença materna e escolaridade materna

4. Metodologia

Este estudo faz parte de um projeto maior, chamado PROUDEST (Pregnancy Outcome and Child Development – Effects of SARS-CoV-2 Infection Trial). O projeto é dividido em dois segmentos: o obstétrico (PREGNANT) e o pediátrico (BORN). Seu objetivo é analisar os efeitos da infecção por SARS-CoV-2 sobre o crescimento e desenvolvimento a longo prazo de crianças cujas mães foram expostas ou não ao vírus durante a gravidez. O estudo proposto representa uma das fases do PROUDEST- BORN.

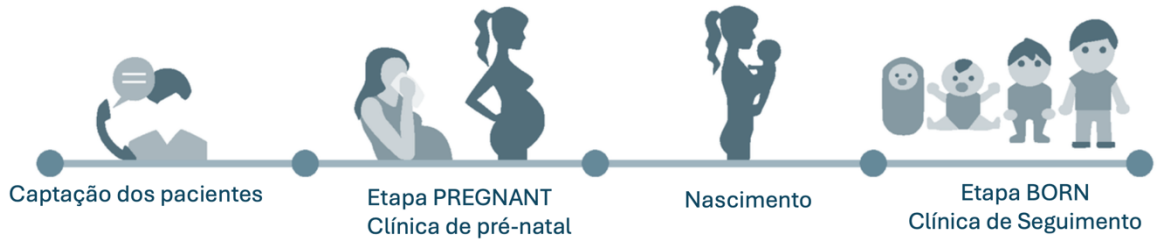


Figura 3 – Etapas do projeto PROUDEST.
Fonte: Acervo do pesquisador.

4.1 Pacientes

4.1.1 – Delineamento, local e período do estudo

Este é um estudo de coorte prospectivo, comparativo e analítico. Refere-se ao acompanhamento de lactentes filhos de mães expostas à infecção pelo SARS-CoV-2 durante a gestação. O recrutamento de casos teve início em maio de 2020. O período de realização do estudo foi de junho de 2020 a dezembro de 2022, na cidade de Brasília-DF, no Hospital Universitário de Brasília.O estudo PROUDEST foi conduzido pela equipe de perinatologia do Hospital Universitário de Brasília (HUB-UnB). A divulgação para captação de pacientes foi realizada por meio de jornais televisivos e mídias sociais. Gestantes que testaram positivo para a infecção por SARS-CoV-2 (por RT-PCR ou IgM positivo com IgG negativo na sorologia) foram convidadas a realizar o pré-natal em nossa estrutura, que inclui ambulatórios, centro de parto humanizado, centro obstétrico, maternidade e ambulatórios de pediatria. O pré-natal, o nascimento e as consultas de puericultura das crianças foram realizados conforme as diretrizes

das sociedades brasileiras de ginecologia, obstetrícia e pediatria.

Os locais envolvidos no estudo englobam dois hospitais na cidade de Brasília, Distrito Federal. Se a gestante estivesse infectada pelo vírus no momento do parto, o nascimento acontecia no Hospital Regional da Asa Norte – hospital que concentrou os casos positivos de SARS-CoV-2 na Secretaria de Saúde do Distrito Federal durante a pandemia. Se a gestante não estivesse infectada no momento do parto, o nascimento e o atendimento pré-natal eram realizados no Hospital Universitário de Brasília, com seguimento em ambulatório criado para o estudo PROUDEST.

A coleta de dados ocorreu de Junho de 2020 a dezembro de 2022 e foi realizada no mesmo dia das consultas pré-agendadas. Dessa forma, os dados foram coletados na admissão em nosso serviço e durante as consultas de puericultura.

4.1.2 Cálculo amostral

Para calcular o tamanho da amostra, foi necessário considerar que inicialmente não havia dados definitivos sobre a prevalência de infecção por SARS-CoV-2 em grávidas e crianças na população brasileira. De acordo com dados do Ministério da Saúde em 14 de maio de 2020, o Distrito Federal apresentava 758,5 casos por 100.000 habitantes e uma taxa de mortalidade de 10,1 por 100.000 habitantes, sem informações específicas para gestantes. Além disso, o número de nascimentos anuais no Distrito Federal em 2018, o dado mais recente disponível naquele momento, foi de 43.313 recém-nascidos.

Considerando uma população de 2.500.000 habitantes, um número anual de nascimentos de 43.313 recém-nascidos, e uma prevalência presumida de 10% de infecção por SARS-CoV-2 em gestantes (com um intervalo de confiança de 95% e um erro alfa de 5%), o cálculo resultou em uma amostra mínima necessária de 188 participantes. Além disso, foi estimada uma perda amostral de até 20% durante o acompanhamento pediátrico. [98]

4.1.3 Critérios de inclusão e exclusão

Critérios de inclusão

- Comprovação de infecção materna pelo SARS-CoV-2 durante a gestação (RT

PCR ou IgM positivo).

- Ter Idade Gestacional ao nascimento > ou igual a 34 semanas.

Critérios de exclusão

- Indícios ou confirmação de síndromes genéticas;
- Idade gestacional ao nascimento < 34 semanas e/ou cuidados de uti neonatal, uso de acesso central, drogas vasoativas, antibióticos, ventilação mecânica (invasiva ou não-invasiva);
- Suspeita ou confirmação de outras infecções congênitas, como toxoplasmose, sífilis, rubéola, herpes, Chagas e zika vírus;
- Impossibilidade de acompanhamento sequencial até os dois anos de vida.

4.1.5 Divisão dos grupos

Os pacientes foram agrupados em momentos distintos de análise quanto a três características:

- Gravidade da doença materna (critérios da OMS em maio de 2020) [99]:

- Gestantes não graves: sem uso de oxigenioterapia, ausência de internação em UTI e/ou uso de drogas vasoativas;
- Gestantes Graves/Críticas: necessitaram utilizar oxigenioterapia, internação em UTI e/ou uso de drogas vasoativas.

- Momento de infecção por SARS-CoV-2 durante a gestação:

- 1º trimestre: até 13 semanas e 6 dias de idade gestacional;
- 2º trimestre: entre 14 semanas e 27 semanas e 0 dias de idade gestacional;
- 3º trimestre: a partir de 27 semanas e 1 dia;
- Periparto: infecção no momento do parto.

- Escolaridade Materna

- Com Ensino superior (diploma universitário);

- Ausência de Ensino superior (sem diploma universitário).

4.2 Metodologia

4.2.1 Acompanhamento pediátrico

Um total de 295 crianças foram inscritas no projeto. Dentre essas, 18 crianças eram de mães sem registro de infecção por SARS-CoV-2 durante a gestação, e 2 crianças apresentaram sífilis congênita. Adicionalmente, outras 8 crianças foram excluídas do acompanhamento ambulatorial devido à perda de seguimento, sendo consideradas como perdas para o estudo. Sendo assim o resultado final foi de 267 pacientes.

Os responsáveis eram ainda acolhidos, termo de consentimento livre e esclarecido explicados e assinados pelos cuidadores, assim como o seguimento a longo prazo da criança. Uma vez admitidos no ambulatório, na primeira consulta foram obtidos dados completos sobre o pré-natal (número de consultas, sorologias, exames de imagem, patologias maternas prévias e durante a gestação, uso de medicações contínuas, necessidade de internação hospitalar, exames de imagem fetal intraútero e comprovação de infecção); As crianças recebiam um mesmo código para identificação dos dados nas diferentes variáveis analisadas.

O atendimento foi realizado de forma multiprofissional. Inicialmente, os auxiliares de enfermagem realizaram medidas antropométricas como checagem de administração de medicações e apoio à amamentação; Em seguida as crianças eram divididas para atendimento médico, coordenado por pediatras e realizado com apoio de residentes de pediatria; outro grupo realizava a avaliação do neurodesenvolvimento com a equipe de terapia ocupacional. Após essa etapa ser concluída, os grupos trocavam os atendimentos.

Para facilitar no entendimento das idades para realização dos testes e exames propostos, utilizamos a idade gestacional corrigida para prematuridade (descontamos o valor faltante do cálculo, 40 semanas subtraído da idade gestacional ao nascimento, e o valor encontrado é subtraído da idade cronológica do bebê).

Por exemplo para um bebê que nasceu de 34 semanas de idade gestacional:

- o cálculo seria 40 semanas – 34 semanas = 6 semanas,

- *Logo aos 8 meses de idade cronológica o lactente seria classificado como:*

8 meses – 6 semanas = 6 meses e 2 semanas (idade corrigida)

Na consulta médica, os dados de antropometria foram plotados nas curvas da Organização mundial de saúde e em caso de recém-nascidos prematuros nas curvas Intergrowth-21 [100]; além disso, o calendário vacinal era checado, condições de moradia e financeira, hábitos de sono, alimentação, medicações, alguma queixa aguda em relação a saúde da criança, tempo de tela, hábitos intestinais e rede de apoio a família também eram avaliadas.

As consultas e atendimentos foram realizados mensalmente durante os 6 primeiros meses de vida, e posteriormente a cada 2 meses até completar 12 meses e semestralmente entre 12 e 24 meses. Tínhamos ainda um canal de comunicação direta com os pais, de forma virtual, para eventuais adiantamentos de consultas.

Todos os diagnósticos e tratamentos realizados foram feitos conforme preconizado pela Sociedade Brasileira de Pediatria.

Para os diagnósticos de doenças mais encontradas neste trabalho, utilizamos os seguintes critérios:

4.2.1.1 Dermatite atópica: critérios estabelecidos pela Academia Americana de Dermatologia. Esses critérios incluem três categorias principais: essenciais, importantes e associadas. Os critérios essenciais envolvem a presença de prurido de leve a severo, dermatite eczematosa com morfologia típica e padrões específicos de idade, como envolvimento facial, cervical e dos extensores em lactentes e crianças até os quatro anos de idade, além de lesões flexurais atuais ou anteriores em qualquer faixa etária, e um curso crônico ou recorrente da doença. Critérios importantes incluem o início dos sintomas antes dos dois anos de idade, histórico pessoal ou familiar de condições como rinite alérgica, asma, alergias alimentares ou dermatite atópica, e presença de pele seca dentro do último ano. Já as características associadas englobam respostas vasculares atípicas, como palidez facial e dermografismo branco além de condições dermatológicas específicas como queratose pilar, pitiríase alba, palmas hiperlineares e ictiose. [101]

4.2.1.2 Rinite Alérgica: Para o diagnóstico de rinite alérgica, utilizamos principalmente critérios clínicos estabelecidos com base na presença de sintomas característicos, tais como acessos de espirros, rinorreia, obstrução nasal, prurido

nasal, gotejamento pós-nasal, tosse, irritabilidade e fadiga, conforme descrito na literatura [90,91]. A história clínica sugestiva, incluindo a presença de fatores de risco, é fundamental para fundamentar o diagnóstico inicial. Achados de exame físico que apoiam o diagnóstico incluem sinais como edema da mucosa nasal, palidez da mucosa e congestão nasal. [102]

4.2.1.3 Alergia a proteína do leite de vaca: Para esse diagnóstico, consideramos crianças que estavam em dieta de restrição (ou a mãe em caso de aleitamento materno exclusivo) e que tinham diagnóstico dado por gastropediatria via relatório médico.

4.2.1.4 Lactente sibilante (Sibilância recorrente do lactente: persistência de sibilos por 30 dias ou mais, ou a presença de três ou mais episódios de sibilância em período de seis meses. [103]

4.2.2 Avaliação do neurodesenvolvimento

O desenvolvimento neuropsicomotor foi avaliado por meio da aplicação das escalas Bayley Scales of Infant Development III - Bayley III. Neste trabalho, o teste foi dividido em três escalas independentes: cognitiva, linguagem, motora. [104]

O desempenho das crianças foi avaliado utilizando um instrumento dividido em três escalas independentes, com cada item recebendo um escore de 0 ou 1. Os avaliadores, cegos para os grupos aos quais os bebês foram distribuídos, aplicaram o instrumento. Quatro avaliadores treinados participaram do processo, e a confiabilidade entre eles foi testada em 10 crianças que não faziam parte do estudo, resultando em um excelente índice de correlação (ICC = 90).

As crianças foram avaliadas aos 6 e 12 meses de idade corrigida. O escore bruto foi obtido por meio da soma dos itens realizados pela criança. O escore bruto foi convertido em pontos padronizados, obtendo-se index score (IE) com uma média de 100 e desvio-padrão de 15. Com base no IE, os lactentes podem ser classificados como: desempenho dentro dos limites normais (85 a 114); desempenho atrasado (IS < 85)

4.2.3 Análise Estatística

Foram calculados valores de média, desvio padrão e/ou porcentagem de frequência para todas as variáveis. Testes de normalidade e homoscedasticidade foram realizados para verificar a distribuição da amostra. Para comparar os valores médios dos domínios Cognitivo, Linguístico e Motor entre dois grupos, utilizou-se o teste t de Student para amostras independentes. Quando a comparação envolveu mais de dois grupos, foi aplicada a análise de variância (ANOVA).

Nos casos em que o teste ANOVA apresentou significância, realizaram-se comparações múltiplas com correção de Bonferroni, relatando-se os valores de p. Considerou-se um valor de $p < 0,05$ como significativo. As análises foram realizadas utilizando o programa SAS 9.4.

4.2.4. Aspectos éticos

Todos os participantes do estudo em ambas as fases forneceram consentimento informado por escrito antes de sua inclusão, de acordo com a Declaração de Helsinque e a Resolução 466/2012 do Conselho Nacional de Saúde do Brasil, que regulamenta pesquisas envolvendo seres humanos. Esse processo assegurou a adesão aos princípios éticos fundamentais, garantindo a proteção dos direitos e do bem-estar dos participantes.

Este estudo foi devidamente registrado na Plataforma do Registro Brasileiro de Ensaios Clínicos (ReBEC, RBR-65qxs2) e recebeu aprovação da Comissão Nacional de Ética em Pesquisa do Brasil (CONEP, CAAE 32359620.0.0000.5558). A aprovação pela CONEP confirma que o estudo atende aos padrões éticos e regulamentares exigidos para a condução de pesquisas envolvendo seres humanos no Brasil.

5. Resultados

5.1 Perfil da Amostra avaliada

A Tabela 1 apresenta a caracterização dos participantes do estudo e suas famílias, totalizando 267 participantes. A idade gestacional média ao nascimento foi de 38,40 (±1,66) semanas, e 90,3% dos bebês nasceram a termo. O peso médio ao nascimento foi de 3137,80 (±532,84) gramas, e 80,1% dos bebês tinham um peso adequado para a idade gestacional. A maioria dos participantes era do sexo feminino (51,3%) e o comprimento médio ao nascimento foi de 51,46 (±3,65) cm. A idade média das mães ao nascimento foi de 30,52 (±6,34) anos, e 72,6% dos cuidadores não tinham nível de escolaridade superior. Um dado importante é que o número de prematuros da amostra foi de 9,7% (n = 26 pacientes) e não houve diferença estatisticamente significativa quando comparado ao grupo de lactentes de termo.

Tabela 1. População estudada

Características dos participantes	**Total = 267**
Termo, n (%)	241(90,3)
Idade Gestacional, (semanas), média ± DP	38,40 (±1.66)
Feminino, n (%)	137(51,3)
Peso de Nascimento, (gramas)	3.137,80 (±532,84)
Adequados para idade gestacional, n (%)	214 (80,1)
Comprimento ao nascimento, (cm), média ± DP	51,46 (±6,69)
Perímetro cefálico, (cm), média ± DP	35,50 (±3,62)
Idade maternal no momento do parto (anos), média ± DP	30,52 (±6,34)
APGAR (Primeiro Minuto)	
< 7, n (%)	25 (9,0)
≥ 7, n (%)	243 (91)
APGAR (Quinto minuto)	
< 7, n (%)	2 (0,7)
≥ 7, n (%)	265 (99,3)

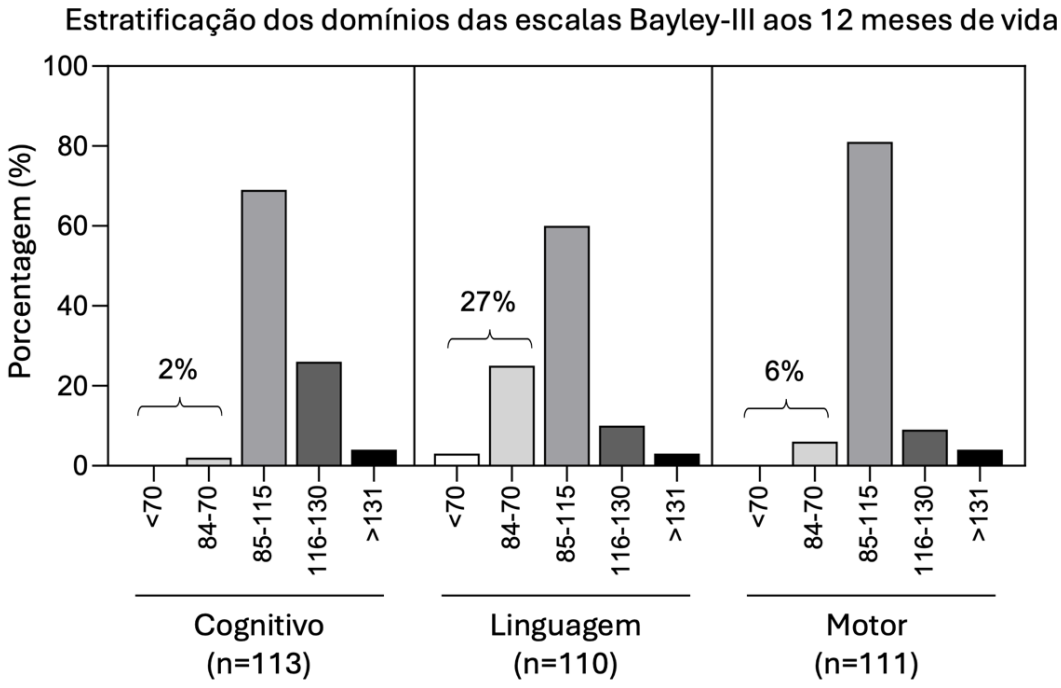
Momento da infecção maternal por SARS-CoV -2	
1º. Trimestre, n (%)	51 (19,1)
2º. Trimestre, n (%)	92 (34,5)
3º. Trimestre, n (%)	104(38,9)
Infecção no Nascimento, n (%)	20 (7,5)
Gravidade da doença materna pelo SARS-CoV -2	
Não grave, n (%)	226 (84,6)
Grave, n (%)	41 (15,4)
Nível educacional dos cuidadores	
Ausência de diploma universitário, n (%)	194 (72,6)
Com diploma universitário n (%)	55 (20,6)
Não quiseram informar n (%)	18 (6,8)

É importante descrever ainda que não foi encontrada diferença estaticamente significante entre os escores APGAR (indicando as condições de nascimento), doenças que as crianças desenvolveram durante o primeiro ano de vida e os escores Bayley-III.

5.2 Avaliação do Neurodesenvolvimento

Ao analisar os resultados do neurodesenvolvimento dos participantes aos 6 e 12 meses de idade, observamos que aos 6 meses de idade, 7% dos participantes apresentaram atrasos cognitivos, 26% tiveram atrasos de linguagem e 11% atrasos motores. Já aos 12 meses de idade, foram observados atrasos cognitivos em 2% dos participantes, atrasos de linguagem em 25% e atrasos motores em 6,3%. A porcentagem de crianças aos 12 meses com atraso de acordo com cada grupo de análise pode ser vista na Figura 5.

Figura 5. Estratificação dos domínios cognitivo, de linguagem e motor das escalas Bayley-III aos 12 meses após o nascimento.



5.2.1 Gravidade da doença materna

Em termos de gravidade da doença das mães, 84,6% apresentaram COVID19 leve segundo critério da OMS, enquanto 15,4% apresentaram doença grave. Vale ressaltar, que não foram encontradas anomalias nos testes de triagens neonatais para

as crianças que foram submetidas à avaliação Bayley-III.

Foi realizada uma comparação entre a gravidade da doença materna e os domínios cognitivo, linguagem e motor da Bayley-III aos 6 e 12 meses (Tabela 2). Os resultados indicam que não houve diferença significativa no desempenho cognitivo aos 6 meses entre o grupo com doença materna grave e o grupo com doença materna leve.

Aos 12 meses, o grupo com doença materna grave teve escores cognitivos mais baixos em comparação com o grupo com doença materna não grave, embora essa diferença não tenha sido estatisticamente significativa.

Em relação ao desenvolvimento da linguagem, não houve diferença significativa entre o grupo com doença materna grave e o grupo com doença materna não grave aos 6 meses. No entanto, aos 12 meses, o grupo com doença materna grave teve escores de linguagem significativamente mais baixos em comparação com o grupo com doença materna não grave. Em termos de desenvolvimento motor, não houve diferença significativa no desempenho aos 6 ou 12 meses entre o grupo com doença materna grave e o grupo com doença materna não grave.

5.2.2 Momento da infecção

Na Tabela 1, é possível observar a distribuição dos grupos por trimestre em que a infecção ocorreu, no primeiro em 19,1%, segundo 34,5% e terceiro 38,9%, e 7,5% dos participantes estavam infectados no momento do parto. Quando analisados os escores de desenvolvimento quanto ao momento da infecção, diferenças estatisticamente significantes foram encontradas quando comparados os trimestres entre si, principalmente no grupo de infecção no momento do parto.

Diante disso, apresentamos na Tabela 3 a comparação entre o trimestre de infecção materna e os domínios cognitivo, linguagem e motor dos bebês aos 6 e 12 meses. Aos 6 meses, não foram observadas diferenças significativas em nenhum dos domínios entre os três trimestres ou ao nascimento.

Contudo, aos 12 meses, houve uma diferença significativa no desenvolvimento da linguagem entre os bebês de acordo com o período gestacional de exposição ao SARS-CoV-2 (p=0,0452): o grupo de mães infectadas durante o primeiro trimestre,

quando comparado com as gestantes infectadas no momento do parto, apresenta escores mais altos e com significância estatística. Em relação aos outros domínios, não foram observadas diferenças significativas aos 12 meses.

A correção de Bonferroni foi usada para múltiplas comparações, e o valor de p foi relatado para cada comparação de grupo. Os resultados deste estudo sugerem que o momento da infecção materna durante a gravidez pode ter um impacto diferencial no desenvolvimento infantil, particularmente no domínio da linguagem e em crianças expostas ao vírus no período compreendido entre o momento do parto e até 14 dias antecedentes.

5.2.3 Escolaridade dos cuidadores

No que diz respeito à escolaridade dos cuidadores, a Tabela 4 mostra a comparação entre os níveis de escolaridade dos cuidadores e as variáveis dos domínios cognitivo, linguístico e motor aos 6 e 12 meses. Foram encontradas diferenças significativas nos escores de linguagem aos 6 meses de bebês nascidos de mães com nível superior de escolaridade, apresentando escores significativamente mais altos (p=0,0139 e p=0,0877, respectivamente).

Em termos de escores motores e cognitivos, não foram encontradas diferenças significativas entre os dois grupos aos 6 ou 12 meses (p=0,5441 e p=0,6272, respectivamente). Esses achados sugerem que a educação materna pode ter um impacto maior no desenvolvimento da linguagem durante o primeiro ano de vida em crianças expostas ao vírus SARS-CoV-2 durante a vida fetal.

Tabela 2. Resultado das escalas Bayley-III para os domínios cognitivo, linguagem, e motor com 6 e 12 meses, de acordo a severidade da infecção materna por SARS-CoV-2 na gestação.

Domínios Bayley-III *	**Gravidade da doença maternal por SARS-CoV-2**		**p-valor#**
	Não grave	**Grave**	**T-Student**
Cognitivo			
6 meses	101.9±16.4	99.7±17.5	0.6248
12 meses	111.6±12.5	105.6±13.8	0.0703
Linguagem			
6 meses	93.3±14.8	91.3±14.8	0.6289
12 meses	98.3±16.3	**89.6±15.3#**	**0.0378**
Motor			
6 meses	105.2±16.6	100.8±17.4	0.3338
12 meses	101.9±13.2	102.5±16.3	0.8784

* Os dados são expressos como valores médios ± desvio padrão. # valor de p das comparações múltiplas realizadas pelo teste t de Student é indicado. A diferença significativa no domínio de linguagem aos 12 meses na comparação entre infecção materna severa versus não severa é destacada em formato negrito.

Tabela 3. Resultado das escalas Bayley-III para os domínios cognitivo, linguagem, e motor com 6 e 12 meses, de acordo com o momento da infecção materna pelo SARS-CoV-2 na gestação

Domínios Bayley-III *	Momento da Infecção materna por SARS-CoV-2				p-value#
	Primeiro Trimestre	Segundo Trimestre	Terceiro Trimestre	No Parto	ANOVA
Cognitivo					
6 meses	101.5±15.0	100.0±10.9	105.1±20.9	91.7±11.7	0.1473
12 meses	110.5±13.0	109.1±11.1	113.2±13.5	105.5±15.4	0.2926
Linguagem					
6 meses	95.0±14.7	89.9±13.4	95.0±15.1	90.9±18.6	0.4728
12 meses	101.4±16.3	95.2±15.0	99.2±17.0	**85.0±14.6#**	**0.0452**
Motor					
6 meses	107.0±19.7	103.7±15.2	105.4±16.2	97.8±16.9	0.5551
12 meses	101.2±12.2	99.5±9.8	104.8±16.7	102.1±16.3	0.3979

* Os dados são expressos como valores médios ± desvio padrão. # Valor de p das comparações múltiplas realizadas por ANOVA com correção de Bonferroni. A diferença significativa no domínio da linguagem aos 12 meses na comparação entre infecção materna no 1º trimestre versus infecção materna no momento do nascimento é destacada em formato negrito.

Tabela 4. Resultado das escalas Bayley-III para os domínios cognitivo, linguagem, e motor com 6 e 12 meses, de acordo com nível educacional dos cuidadores.

Domínios Bayley-III*	Nível Educacional dos cuidadores		p-valor#
	Sem diploma universitário	Com diploma universitário	T-Student
Cognitivo			
6 meses	102.7±18.8	101.7±12.6	0.7507
12 meses	111.4±13.9	108.8±12.2	0.3027
Linguagem			
6 meses	90.1±13.8	**97.2±14.7#**	**0.0139**
12 meses	94.1±15.3	99.5±17.7#	0.0877
Motor			
6 meses	104.4±17.5	106.4±16.4	0.5441
12 meses	102.5±14.4	101.2±13.7	0.6272

*Os dados são expressos como valores médios ± desvio padrão. # valor de p das comparações múltiplas realizadas pelo teste t de Student é indicado. A diferença significativa no domínio da linguagem aos 6 e 12 meses na comparação entre Cuidador sem diploma universitário versus com diploma universitário é destacada em formato negrito.

6. Discussão

Este estudo observou que mulheres expostas ao SARS-CoV-2 durante a gravidez tiveram filhos com atraso no desenvolvimento neuropsicomotor, especificamente na área da linguagem. Para isso, os indivíduos estudados (todos no contexto de uma pandemia e isolamento social) foram divididos de acordo com o momento da infecção, a gravidade da infecção nas gestantes e a educação dos cuidadores. Se observou que aos 6 meses de vida, houve um atraso no desenvolvimento da linguagem em crianças cujos cuidadores não possuíam ensino superior comparadas com aquelas que possuíam. E aos 12 meses, foi observado um atraso na linguagem em: crianças cujas mães foram infectadas com o vírus no momento do parto em relação a outros períodos gestacionais e naquelas com sintomas graves e críticas pela infecção do SARS-CoV-2 em comparação àquelas com sintomas leves.

Estes achados destacam as intrincadas interações entre a exposição ao SARS-CoV-2 durante o período fetal, o isolamento social na pandemia e os desdobramentos no desenvolvimento neuropsicomotor em crianças, oferecendo insights valiosos para a compreensão dos impactos a longo prazo da infecção materna por SARS-CoV-2. [5,6]

Nesse contexto, uma vez diagnosticada a infecção por SARS-CoV-2 em uma mulher grávida, é necessário um acompanhamento ordenado e seriado dessas crianças, com foco na avaliação do desenvolvimento para diagnosticar e intervir precocemente e assim reduzir o impacto do atraso desse domínio do neurodesenvolvimento, já que a linguagem atua como um motor para muitas outras habilidades a serem adquiridas na infância e na vida adulta.

Ainda não está claro se existe uma conexão definitiva entre a exposição pré-natal ao SARS-CoV-2 e os distúrbios do neurodesenvolvimento na prole. Em parte, isso se deve ao fato de que a maioria das crianças nascidas de mulheres infectadas ainda é muito jovem para um diagnóstico de muitas condições patológicas do neurodesenvolvimento. Apesar das limitações significativas dos conjuntos de dados epidemiológicos e clínicos na definição de causalidade ou mecanismo, esses dados

preliminares demonstram o potencial da exposição pré-natal ao SARS-CoV-2 para impactar os desfechos precoces de neurodesenvolvimento.

Num trabalho muito bem desenhado, os autores revelaram uma diferença no desenvolvimento motor grosso e fino e nas habilidades de interação social em bebês nascidos durante a pandemia em comparação com aqueles nascidos antes da pandemia. Esses resultados sugeriram que a infecção materna por SARS-CoV-2 durante a gravidez está associada a um atraso no desenvolvimento social e motor aos seis meses de idade. Atrasos nesses domínios do neurodesenvolvimento não foram observados no presente estudo. No entanto, eles apoiam a necessidade de monitorar crianças nascidas durante a pandemia de SARS-CoV-2 para avaliar sequelas a longo prazo. [105]

Em outro estudo recente, testes de desenvolvimento foram administrados de forma indireta, solicitando aos pais que preenchessem questionários e se comparou um grupo exposto ao vírus e um grupo controle com características semelhantes, mas pré pandemia. Apesar de observar diferenças nas pontuações de desenvolvimento entre bebês expostos e aqueles não expostos, esses estudos não encontraram diferenças estatisticamente significativas entre os grupos em relação ao trimestre de infecção durante a gravidez ou à gravidade da doença materna. No entanto, crianças nascidas durante a pandemia apresentaram uma redução considerável no desempenho verbal, motor e cognitivo geral em comparação com aquelas nascidas antes da pandemia, independentemente da exposição ao vírus. [106]

Porém um importante limitador dos estudos citados anteriormente seria a comparação com um grupo controle que não viveu o cenário de pandemia. Isso porque, o isolamento social, a ansiedade e depressão oriundas dos diversos lockdowns, lutos por pessoas queridas, angústias sobre situação financeira são gatilhos estressores para atrasos no desenvolvimento, como foi relatado no trabalho realizado durante a epidemia do Zika vírus. [107]

Já no que diz respeito ao momento da infecção materna, Firestein e colaboradores [109] não encontraram diferenças entre o trimestre da infecção materna e atrasos de desenvolvimento. Porém no trabalho de Edlow e colaboradores [17] onde analisou-se 222 crianças de mães infectadas pelo SARS-CoV-2 durante a gestação e as comparou com 7550 crianças não expostas intraútero ao vírus durante a pandemia, se concluiu que o diagnóstico de atraso do desenvolvimento neuropsicomotor foi mais

comum no grupo exposto ao vírus, com resultado estatisticamente significativo especialmente no grupo exposto no 3º trimestre da gestação. Nesse caso os domínios com desempenho alterado foram os da linguagem e motor. Semelhante ao encontrado no presente estudo que para os pacientes que as mães apresentaram a doença durante o período periparto, os escores para desenvolvimento neuropsicomotor foram considerados como alterados, especialmente no domínio da linguagem.

Quando os dados foram analisados utilizando a gravidade dos sintomas maternos, no nosso estudo, nas gestantes com doença grave as crianças tiveram menores escores para subdomínio motor e linguagem com 6 e 12 meses respectivamente. Assim como na coorte de Firestein e colaboradores [109] que utilizando telessaúde e um questionário de desenvolvimento ASQ-3, não observaram associação entre diminuição dos escores de desenvolvimento com sintomas leves ou assintomáticos pela infecção do SARS-CoV-2 na gestação. Diferente do trabalho de Martinez et al [115] no qual os sintomas de severidade da doença materna não tiveram impacto estatístico para a escala de desenvolvimento utilizada.

Além disso, uma pesquisa recente revelou um preocupante aumento nas disparidades sociais preexistentes durante o período da pandemia. Nessa pesquisa que entrevistou 500 cuidadores de origens desfavorecidas em termos de educação, revelou que seus filhos de 0 a 2 anos eram menos propensos a participar de atividades enriquecedoras. E essa privação de engajamento em atividades pode contribuir para atrasos no desenvolvimento, como observado também no presente estudo, particularmente entre crianças cujos cuidadores não possuíam diploma universitário. [111]. Esses achados destacam a necessidade de maior apoio e recursos disponíveis para famílias vulneráveis durante tempos de crise.

Um outro estudo que seguiu crianças de 1 a 3 anos e de 3 a 5 anos de idade observou que em famílias desfavorecidas socioeconomicamente expostas à pandemia, as crianças foram mais propensas a apresentar um desenvolvimento infantil abaixo do esperado até os 5 anos de idade. Isso reforça a importância crítica da estimulação infantil e dos fatores socioeconômicos no neurodesenvolvimento infantil. [112]

A literatura ainda não chegou a um consenso sobre se os lockdowns pandêmicos, a falta de interação entre pais e filhos, a depressão parental e o uso de máscaras podem por si só influenciar negativamente o desempenho nas escalas de

neurodesenvolvimento em crianças ou se de fato são as vias de exposição a doença e infecção materna prováveis causas de transtornos do desenvolvimento infantil. O mais provável é que múltiplos mecanismos combinados sejam responsáveis pelos atrasos do desenvolvimento observados nos diversos estudos.

Há evidências que um importante papel é desempenhado pelos mecanismos envolvidos na exposição ao SARS-COV-2 durante a vida fetal, tal aspecto fica mais evidente ao analisarmos os estudos de imagem nesse cenário. Para Norman et al (2021) [87] que avaliou através de RNM Crânio em 55 indivíduos expostos ao SARS-CoV-2 no período fetal, observou que os mesmas apresentavam alteração de espessura na área cerebral responsável pela linguagem.

Um outro estudo do nosso grupo [113] foi pioneiro ao utilizar a escala ouro de avaliação de neurodesenvolvimento e o uso de ultrassonografia com elastografia. Nesse trabalho os dados de elastografia em crianças expostas e não expostas ao SARS-CoV-2 durante a pandemia apontou diferenças estatisticamente significativa no que diz respeito a redução no coeficiente de elasticidade no grupo exposto ao vírus, especialmente da substância branca profunda. Esses pacientes que apresentaram alteração do coeficiente de elasticidade (sugere uma inflamação prévia) na substância branca profunda encefálica tiveram também alteração nas escalas Bayley-III indicando atraso no desenvolvimento no subdomínio linguagem com 12 meses.

Uma provável hipótese seria de que a exposição viral seria responsável por alterações na quantidade de mielina no tecido cerebral (que apresentam menor coeficiente de elasticidade) ou por um leve edema na mielina, no qual a inflamação aumentaria a quantidade de água no parênquima cerebral o que levaria a alterações na rigidez do parênquima cerebral.

Esses achados iniciais sugerem a necessidade de monitorar as crianças expostas no período fetal ao SARS-CoV-2 com objetivo de acessar sequelas a longo prazo e programar possíveis intervenções e terapias para esses pacientes. [109]

Diante disso, há consistência em com um razoável corpo de literatura demonstrando associação entre a a exposição pré-natal ao SARS-CoV-2 e diagnósticos de atraso do neurodesenvolvimento aos 12 meses. Isso porque os resultados clínicos (Escalas de desenvolvimento), imagenológicos e imunológicos, incluindo estudos humanos e animais, relacionam infecções virais maternas e

ativação imunológica materna com distúrbios do desenvolvimento na prole de forma mais tardia na vida, e alguns dos quais podem ser previstos já no primeiro ano de vida. [114,116]

Dado o objetivo de pesquisa delineado neste estudo, que abrange a trajetória de desenvolvimento em crianças expostas ao SARS-CoV-2 *in utero* usando as escalas Bayley-III ao longo de um período de um ano, bem como uma análise comparativa baseada em fatores como o momento da exposição fetal, a gravidade da infecção materna e o nível de educação materna, é imperativo continuar investigando os atrasos no desenvolvimento.

7. Limitações

Este estudo possui como principais limitações o tamanho amostral relativamente reduzido e o fato de não ter acompanhado um grupo controle. Entendemos que, devido ao contexto de pandemia no qual o estudo foi iniciado, durante o isolamento social e ainda sem vacinas, o medo da população de frequentar uma clínica inserida dentro de um complexo hospitalar (utilizando transporte público, logo com possível contato entre a díade mãe-bebê e portadores assintomáticos) tornou um grupo de controle inviável. Um grupo de controle pré-pandêmico não foi utilizado porque, dentro do cenário de desenvolvimento infantil, a presença/ausência de isolamento social pode comprometer os resultados do desenvolvimento. Assim, optamos por comparar os grupos entre si (usando o momento da infecção na gestante, a gravidade da doença materna e o nível de escolaridade dos cuidadores).

A maioria da nossa amostra foi composta por pacientes ambulatoriais, de modo que a gravidade da infecção materna foi predominantemente leve e as crianças foram acompanhadas em um único centro, reduzindo a propriedade de generalização de nossos achados.

8. **Conclusão**

- Filhos de gestantes expostas ao SARS-CoV-2 durante a gravidez no período de pandemia de COVID-19 apresentaram atrasos no desenvolvimento neuropsicomotor, especialmente na linguagem. Tal achado foi observado em crianças cujas mães estavam infectadas durante o momento do parto e nas que tiveram condições graves de infecção.

- As crianças cujos cuidadores não têm formação universitária possuem maior probabilidade de apresentar atrasos no desenvolvimento da linguagem, sugerindo o impacto das disparidades socioeconômicas no desenvolvimento infantil durante a pandemia.

- Faz-se importante o acompanhamento contínuo e a intervenção precoce em crianças expostas ao SARS-CoV-2 durante a gestação, para diagnosticar e diminuir os possíveis impactos no desenvolvimento a longo prazo, especialmente em habilidades de linguagem, que são fundamentais para o desenvolvimento infantil e posterior vida adulta.

- Essas descobertas abrem um espectro de possibilidades de pesquisa sobre os efeitos na saúde fetal e infantil. A descrição das consequências da infecção no acompanhamento de longo prazo fornece uma melhor compreensão da doença e sua influência no sistema nervoso central.

9. Produção científica (artigos publicados)

- Motta F.; Araujo DA, Fernandes GM, Castro MEC, Sasaki LMP, et al. Neuropsychomotor development of children exposed in utero to SARS-CoV-2 during COVID-19 pandemic period. The Journal of Pediatrics. Frontiers in Pediatrics, 2024. (Apêndice A - Qualis Capes A1 – FI 3,9). Consoante ao regimento do PPGCM-UnB. – submetido.

- Motta F.; Araujo DA, Fernandes GM, Castro MEC, Sasaki LMP, et al. Neuroimaging assessment of pediatric cerebral changes associated with SARS-CoV-2 infection during pregnancy. Frontiers in Pediatrics, v. 11, p. 01, 2023. (Apêndice A - Qualis Capes A2 – FI 3,24).

- *Fernandes GM, Motta F.;* Araujo DA, , Castro MEC, Sasaki LMP, et al. Pregnancy Outcomes and Child Development Effects of SARS-CoV-2 Infection (PROUDEST Trial): Protocol for a Multicenter, Prospective Cohort Study. JMIR Research Protocols [JMIR Res Protoc 2021 | vol. 10 | iss. 4 | e26477]. (Apêndice B – PROTOCOLO PROUDEST - Qualis Capes B2 – FI 1,25).

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- Apresentação oral sobre o tema em congresso internacional (4th joint European Neonatal Societies Congress – Milão, Itália).

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APÊNDICE A – ARTIGO ORIGINAL NEURODESENVOLVIMENTO – Fator impacto 3.9

The Journal of Pediatrics

Neuropsychomotor development of children exposed in utero to SARS-CoV-2 during COVID-19 pandemic period.

--Manuscript Draft--

Manuscript Number:	
Article Type:	Original Article
Keywords:	child development; COVID-19; pandemic; SARS-CoV-2; neuropsychological tests; Infant
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Keywords:

SARS-CoV-2 pandemic, neurodevelopment, Bayley-III, COVID-19

Fundings:

This research was funded by Fundo COVID-19 UnB em Ação: Ações emergenciais para combate à COVID-19 e mitigação das consequências da pandemia, Grant 7155, Project Eficácia de um protocolo de testagem RT-PCR para SARS-CoV-2 sobre a preservação da força de trabalho em saúde, durante a pandemia COVID-19 no Brasil: ensaio clínico randomizado, de grupos paralelos. OTN received a fellowship for productivity in research from CNPq.

Neuropsychomotor development of children exposed in utero to SARS-CoV-2 during COVID-19 pandemic period.

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1 Introduction

Numerous scientific studies have been conducted on the relationship between obstetric complications and SARS-CoV-2 infection[1]. However, the existing literature demands a comprehensive understanding on the neurological and psychiatric effects of the infection on children who were exposed to coronavirus during the fetal period since the gap in this research area is evident.[2,3]

Previous research on neurodevelopment in infants born with congenital infections, such as the Zika virus, suggests that even without brain damage or birth defects, children may experience impaired neurodevelopment due to the inflammatory cytokine response and placental immune activation, which can interfere with brain growth and plasticity.[4,5]

A longitudinal study in Spain demonstrated that newborns prenatally exposed to SARS-CoV-2 had lower neurodevelopmental scores, particularly in motor development and interactive behavior. [6]Furthermore, Suffreyet al.[7] found that children born during the pandemic had lower than average scores in developmental tests in 2022. As advocated elsewhere[2, 6], further research is warranted to elucidate the full extent of these effects.

The Bayley Scales in their third edition (Bayley-III) are the most used assessment tool for infant development.[8] These scales were translated to Portuguese and validated in the Brazilian context in 2006 and have since then become the gold standard for monitoring children's developmental progress.[9,10]

This study aims to (1) comprehensively examine the development of children exposed in utero to SARS-CoV-2 by describing their progress during one year of follow up, and (2) to compare the development of the newborns according to clinical aspects of their fetal exposure to SARS-CoV2 and to health and social conditions of the maternal

life mother. The findings of this study are expected to contribute significantly to our understanding of the effects of prenatal exposure to SARS-CoV-2 on infant neurodevelopment so to clinical practice and public health policies.

3 Material and Methods

3.1 Population

This is a prospective cohortstudy performed at the University Hospital of Brasília, in Brasília, Brazil. Children were eligible for enrolment from the first 15 days of life to 12 months of age if born from June 2020 to November 2020, and whose mothers had SARS-CoV-2 infection proven by nasopharyngeal RT-PCR during pregnancy. The primary endpoint was defined as the evaluation of the Bayley-III at 12 months of life.

Exclusion criteria for the studyencompassed need to admitted to the Neonatal Intensive Care Unit admission, congenital malformations and chromosomalabnormalities diagnosed prior to maternal SARS-CoV-2 infection, anddiagnosis of syphilis, cytomegalovirus, rubella, human immunodeficiency virus, Chagas disease, viral hepatitis (A, B,or C)or HTLV (1 or 2), either clinically or serologically detected, at any stage of pregnancy.

All participants underwent mandatory neonatal screenings in Brazil, including biological screening (TANDEM methodology), pulse oximetry test with subsequent echocardiogram if abnormalities were detected, auditory screening (evoked otoacoustic emissions), and ophthalmic fundus examination.

The evaluation took place with pregnant women infected by SARS-CoV-2 in Brazil between June 2020 and April 2022. The guardians of each participant signed an informed consent form and were informed of the test results. Children with developmental delays were oriented about treatment and were referred to a rehabilitation center.

3.2 Collection procedures, instruments, and analyzed variables

The trial's general description has beenpreviously described[3]. In this manuscript, we provide a summary of the overall methodology employed in the study. Information from the pregnant women was collected and analyzed duringinitial consultations, whereas data on infants and children were gathered during follow-up visits. Maternal infection severity was graded as non-severe, severe, and critical according to the criteria of the World Health Organization,and pregnant women were divided into 2 groups: non-severe, and severe (including critical)groupsfor analysis purposes. The trimester of infection was defined as follows: 1st trimester was up to 13 weeks and 6 days, 2nd trimester was from 14 weeks to 26 weeks and 6 days, and 3rd trimester was from 27 weeks on. The caregiver's level of education was determined according to the presence or absence of a university degree.The general description of this trial is provided in the companion article. Here, we present a summary of the overall methods.

The Bayley-III were applied to assess child neurodevelopment. The original version of the Bayley-III presents appropriate parameters of validity and reliability, in addition to good sensitivity and specificity indices to identify children with developmental delay.[10,11] For this study, the motor, cognitive, and language subscales were used, not using the complementary scales (socio-emotional, and adaptive behavior). The instrument was applied by four trained evaluators. The inter-rater reliability was measured in 10 children who were not part of the study, obtaining an excellent correlation index (ICC=90).

To evaluate the performance of the investigated children, a score of 0 or 1 was attributed to each item on the scale. The weighted average of the scores obtained by the

Bayley-III at 6 and 12 months was used. For statistical analysis, the raw score was converted into standardized points, obtaining the index score (IE) with a mean of 100 and a standard deviation of 15, with a cutoff threshold to indicate developmental delay set below 85. [12,13]

3.3 Statistical analysis

Mean, standard deviation, and/or frequency percentage values were calculated for all variables. Normality tests and homoscedasticity tests were performed to verify the distribution of the sample. Mean values of the Cognitive, Language, and Motor domains were compared between groups using the analysis of variance (ANOVA) test. In cases where the ANOVA test proved to be significant, multiple comparisons with Bonferroni correction were performed and the p-values reported. A p-value of< 0.05 was considered significant. The SAS 9.4 program was used.

4 Results

Table 1 presents the characterization of the study participants and their families, with a total of 267 participants. The mean gestational age at birth was 38.40 (±1.66) weeks, and 90.3% of the babies were born full-term. The mean birth weight was 3137.80 (±532.84) grams, and 80.1% of the babies had an appropriate weight for their gestational age. More of the participants were female (51.3%), and the mean birth height was 51.46 (±3.65 cm. The mothers' mean age at birth was 30.52 (±6.34) years, and 72.6% of the caregivers had a no- bachelor's degree level education. In terms of the severity of illness of the mothers, 84.6% had a mild illness, while 15.4% had a severe illness. The trimester in which infection took place was first (19.1%), second (34.5%), and third (38.9%), and only 7.5% of the participants were infected at the time of delivery.Importantly, no abnormalities were found in the neonatal screening tests for children who underwent Bayley-III assessment.

It is important to describe that no statistical significance was found between the APGAR scores (1st and 5th minute) and the Bayley scores, indicating that there was no significancewith AGPAR scores and the cognitive, language, and motor development assessed by the Bayley-III.

The study also used the Bayley-III to assess the participants' development at 6 and 12 months of age. The results indicated that at 6 months of age, 7% of the participants experienced cognitive delays, 26% had language delays, and 11% had motor delays. As for 12 months of age, cognitive delays were observed in 2% of the participants, language delays in 25%, and motor delays in 6.3%. The percentage of children with a delay according to each group of analysis can be seen in Figure 1.

Table 2 presents a comparison between the trimester of maternal infection and the cognitive, language, and motor domains of infants at 6 and 12 months. At 6 months, no significant differences were observed in any of the domains between the three trimesters or at birth. At 12 months, there was a significant difference in language development between babies acoording gestacional period of exposure to SARS-CoV2 ($p=0.0452$), with infants born to mothers infected during the first trimester having higher scores than those born to mothers infected at birth. However, no significant differences were observed in cognitive or motor domains at 12 months.

The Bonferroni correction was used for multiple comparisons, and the p-value was reported for each group comparison. The results of this study suggest that the timing of maternal infection during pregnancy may have a differential impact on infant development, particularly in the language domain.

A comparison was carried out between the severity of maternal disease and Bayley-IIIcognitive, language, and motor domains at 6 and 12 months (Table 3). The Student's T-test was used to analyze the data, and the results indicate that there was no significant difference in cognitive performance at 6 months between the group with severe maternal disease and the group with mild maternal disease. At 12 months, however, the group with severe maternal disease had lower cognitive scores compared to the group with non-severe maternal disease, although this difference was not statistically significant.

Regarding language development, there was no significant difference between the group with severe maternal disease and the group with non-severe maternal disease at 6 months. However, at 12 months, the group with the severe maternal disease had significantly lower language scores compared to the group with non-severe maternal

disease. In terms of motor development, there was no significant difference in performance at 6 or 12 months between the group with severe maternal disease and the group with non-severe maternal disease.

Table 4 shows the comparison between caregiver education levels and cognitive, language, and motor domain variables at 6 and 12 months. Although no differences were found in terms of motor and cognitive developments between grups.

On the other hand, significant differences were found in language scores at both 6 and 12 months of babies born to mothers with a university degree having significantly higher scores ($p=0.0139$ and $p=0.0877$, respectively). In terms of motor scores, no significant differences were found between the two groups at either 6 or 12 months ($p=0.5441$ and $p=0.6272$, respectively). These findings suggest that maternal education may have a greater impact on language development than on cognitive and motor development during the first year of life in children exposed to the SARS-CoV-2 virus during fetal life.

5 Discussion

This study observed that women exposed to SARS-CoV-2 during pregnancy had children with delayed neuropsychomotor development, specifically in the language domain. For this, the individuals studied (all within the context of a pandemic and social isolation) were divided according to the moment of infection, the severity of the infection in the pregnant women, and the education of the caregivers. At 6 months of life, a delay in language development was observed in children whose caregivers did not have atertiary degree. At 12 months, a language delay was observed in children whose pregnant mothers were infected with the virus at the time of delivery and those with severe and critical conditions.

Given these findings, once the SARS-CoV-2 infection has been diagnosed in a pregnant woman, an orderly and serial follow-up of these children is necessary, with a strong focus on developmental assessment to diagnose and intervene early and reduce the impact, since language acts as a driver of many other skills to be acquired in childhood and adult life.[13]

In two recent studies, developmental tests were administered indirectly by asking parents to complete surveys. Despite observing differences in developmental scores between infants exposed to SARS-CoV-2 and those not exposed, these studies did not find any statistically significant differences between the groups in relation to the trimester of infection during pregnancy or the severity of the maternal disease.[5,14]However, children born during the pandemic exhibited a considerable reduction in verbal, motor, and general cognitive performance compared to those born prior to the pandemic, regardless of their exposure to the virus.[14]

Furthermore, a recent survey has uncovered a concerning increase in pre-existing social disparities during the pandemic period. Specifically, the survey, which interviewed 500 parents from disadvantaged backgrounds in terms of education, revealed that their 0-to-2-year-old children were less likely to participate in enriching activities. This deprivation of engaging in activities may contribute to developmental delays, as was observed in the current study, particularly among children whose mothers did not hold a university degree.[15, 16] These findings highlight the need for greater support and resources to be made available to vulnerable families during times of crisis.[17,18]

Shuffrey et al. [5]revealed a difference in gross and fine motor development and personal/social scores in pandemic babies compared to those born before the pandemic. These results suggest that maternal SARS-CoV-2 infection during pregnancy is associated with delayed social and motor development at six months of age. These initial findings were not observed in the present study. However, they support the need to monitor children born during the SARS-CoV-2 pandemic to assess long-term sequelae (irrespective of intrauterine exposure or not to SARS-CoV-2).[18]

Our study has limitations. It has a small sample size from a single center. We understand that due to the social context in which the study was initiated, during lockdown, and as yet with no vaccines, the population's fear of attending an outpatient clinic (using public transportation, possible contact of the mother-baby dyad with asymptomatic carriers) made a control group unfeasible. A control group was not used and compared to pre-pandemic groups because, within the child development scenario, the presence/absence of social isolation can compromise child development results.

Thus, we chose to compare the groups among themselves (using the time of infection of the pregnant woman, the severity of maternal illness, and the caregiver's education).

Possible interventions for these children and the impact of readjusting neurodevelopment scores, especially language, should also be monitored.

Given the research objective outlined in this study, which encompasses a comprehensive examination of the developmental trajectory in children exposed to SARS-CoV-2 in utero using the Bayley-III over a one-year period, as well as a comparative analysis based on factors such as the timing of fetal exposure, maternal infection severity, and maternal education level, it is imperative to pursue further investigation into developmental delays and understand the right moment for intervention and if the intervention can change the course of the delay. Additionally, the evaluation of children’s development within a cohort of vaccinated pregnant women becomes paramount, as vaccinated individuals demonstrate distinctive responses to SARS-CoV-2 infection and associated conditions. This evaluation will enable an assessment of whether such children are susceptible to developmental delays.

Figure Captions

Figure 1. Stratification of Bayley-IIIcognitive, language, and motor domains at 6 and 12 months after birth. Data are presented as percentage of children with Bayley-III, categorized as follows: <70 IS (well below average; <−2 SD), 84–70 IS (below average; −1 to – 2 SD), 85–115 IS (average; −1 to 1 SD), 116–130 IS (above average; 1–2 SD) and >131 (well above average; >2 SD). IS: index score; SD: standard deviation.

Acknowledgements

This work was supported by the Conselho Nacional de Desenvolvimento Tecnológico (CNPq), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and the University of Brasília (Grant # 7155, *"Fundo COVID-19 UnB em Ação: Ações emergenciais para combate à COVID-19 e mitigação das consequências da pandemia", "Eficácia de um protocolo de testagem RT-PCR para SARS-CoV-2 sobre a preservação da força de trabalho em saúde durante a pandemia COVID-19 no Brasil: ensaio clínico randomizado",* and Grant # 7110, *"Amamentar em tempos de pandemia de COVID-19: um protocolo para avaliação do impacto do SARS-COV-2 no sucesso do aleitamento materno"*).The study was carried out by students and professor enrolled in Post-graduate Programs within the Programa de Pós-graduação em Ciências Médicas da Universidade de Brasília (UnB), supported by theCoordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Funding

This research was funded by Fundo COVID-19 UnB em Ação: Ações emergenciais para combate à COVID-19 e mitigação das consequências da pandemia, Grant 7155, Project Eficácia de um protocolo de testagem RT-PCR para SARS-CoV-2 sobre a preservação da força de trabalho em saúde, durante a pandemia COVID-19 no Brasil: ensaio clínico randomizado, de grupos paralelos. OTN received a fellowship for productivity in research from CNPq.

Role of the funding source

The funder did not play any role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Declarations of interest

The authors report no conflict of interest

Ethics approval

This study observed the ethical principles stated in the Helsinki Declaration involving research with human beings. The final protocol was approved by the Institutional Review Board of the Medical Faculty of the University of Brasília (CAAE 32359620.0.0000.5558) in May 2020, with additional approval granted by the Comissão Nacional de Ética em Pesquisa (CONEP) in October 2021. All participants provided written informed consent to participate in the study.

Informed consent

All participants provided written informed consent to participate in the study.

Data availability

All relevant data are within the manuscript and its Supporting Information files.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used Google translator by Google in order to English correction. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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Table 4. Bayley-III cognitive, language, and motor domains at 6 and 12 months, according to the caregiver education level

Bayley-II Domains*	Caregiver Education Level		p-value#
	No University degree	University degree	T-Student
Cognitive			
6 months	102.7±18.8	101.7±12.6	0.7507
12 months	111.4±13.9	108.8±12.2	0.3027
Language			
6 months	90.1±13.8	**97.2±14.7#**	**0.0139**
12 months	94.1±15.3	99.5±17.7#	0.0877
Motor			
6 months	104.4±17.5	106.4±16.4	0.5441
12 months	102.5±14.4	101.2±13.7	0.6272

* Data are expressed as mean values ± standard deviation. # p-value of multiple comparisons carried out by T-Student test. Significant difference of language domain at 6 and 12 months for comparison between Caregiver with No University *vs* University degree is underscored by bold format.

The table presents data on the cognitive, language, and motor development of infants assessed using the Bayley-III at 6 and 12 months after birth. The data is categorized based on the caregiver's education level, specifically whether they have a university degree or not. The data presented are expressed as mean values ± standard deviation, and the p-values indicate the statistical significance of the observed differences. A p-value < 0.05 is considered statistically significant. In this case, the significant differences in the language domain at both 6 and 12 months between caregivers with no university degree and those with a university degree are highlighted in bold format.

Table 3. Bayley-III cognitive, language, and motor domains at 6 and 12 months, according to the severity of mother SARS-CoV-2 infection

Bayley-II Domains*	Severity of Mother SARS-CoV-2 Infection		p-value#
	Non-Severe	Severe	T-Student
Cognitive			
6 months	101.9±16.4	99.7±17.5	0.6248
12 months	111.6±12.5	105.6±13.8	0.0703
Language			
6 months	93.3±14.8	91.3±14.8	0.6289
12 months	98.3±16.3	**89.6±15.3#**	**0.0378**
Motor			
6 months	105.2±16.6	100.8±17.4	0.3338
12 months	101.9±13.2	102.5±16.3	0.8784

* Data are expressed as mean values ± standard deviation. # p-value of multiple comparisons carried out by T-Student test. Significant difference of language domain at 12 months for comparison between Severe *vs* Non-Severe maternal infection is underscored by bold format.

The table presents data on the cognitive, language, and motor development of infants assessed using the Bayley-III at 6 and 12 months after birth. The data is categorized based on the severity of maternal SARS-CoV-2 infection, specifically non-severe and severe infections. The data presented are expressed as mean values ± standard deviation, and the p-values indicate the statistical significance of the observed differences. A p-value < 0.05 is considered statistically significant. In this case, the significant difference in the language domain at 12 months between severe and non-severe maternal infections is highlighted in bold format.

Table 2. Bayley-III cognitive, language, and motor domains at 6 and 12 months after birth, according to the time of maternal SARS-CoV-2 infection

Bayley-II Domains*	Time of mother SARS-CoV-2 Infection				p-value#
	1st trimester	2nd trimester	3rd trimester	At Delivery	ANOVA
Cognitive					
6 months	101.5±15.0	100.0±10.9	105.1±20.9	91.7±11.7	0.1473
12 months	110.5±13.0	109.1±11.1	113.2±13.5	105.5±15.4	0.2926
Language					
6 months	95.0±14.7	89.9±13.4	95.0±15.1	90.9±18.6	0.4728
12 months	101.4±16.3	95.2±15.0	99.2±17.0	**85.0±14.6#**	**0.0452**
Motor					
6 months	107.0±19.7	103.7±15.2	105.4±16.2	97.8±16.9	0.5551
12 months	101.2±12.2	99.5±9.8	104.8±16.7	102.1±16.3	0.3979

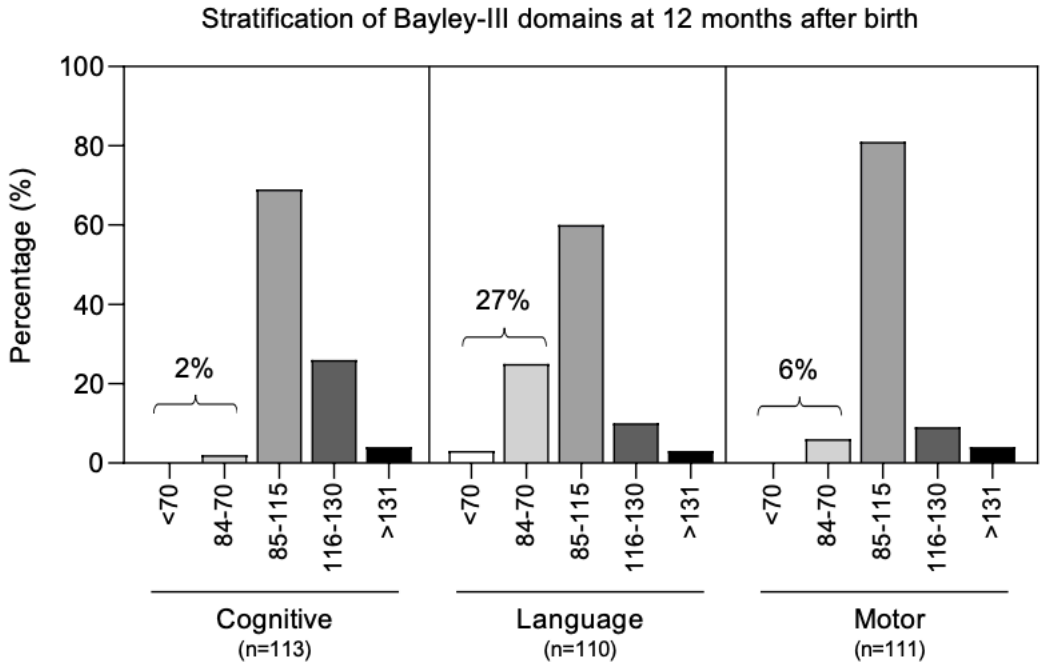
* Data are expressed as mean values ± standard deviation. # p-value of multiple comparisons carried out by ANOVA with Bonferroni correction. Significant difference of language domain at 12 months for comparison between maternal infection at 1st trimester *vs* maternal infection at birth is underscored by bold format.

The table presents data on the cognitive, language, and motor development of infants assessed using the Bayley-III at 6 and 12 months after birth. The data is categorized based on the timing of maternal SARS-CoV-2 infection, specifically during the 1st trimester, 2nd trimester, 3rd trimester, or at delivery. The data presented are expressed as mean values ± standard deviation, and the p-values indicate the statistical significance of the observed differences, with $p < 0.05$ considered statistically significant.

Table 1. Study population

Newborn Features	**Total = 267**
Full term, n (%)	241(90.3)
Gestational age, (weeks), mean ± SD	38.40 (±1.66)
Female, n (%)	137(51.3)
Birth weight, (grams)	3,137.80 (±532.84)
Appropriate weight for gestational age, n (%)	214 (80.1)
Birth height, (cm), mean ± SD	51.46 (±6.69)
Cephalic perimeter, (cm), mean ± SD	35.50 (±3.62)
Mother age at delivery, (years), mean ± SD	30.52 (±6.34)
APGAR Score (1st minute)	
< 7, n (%)	25 (9.0)
≥ 7, n (%)	243 (91)
APGAR Score (5th minute)	
< 7, n (%)	2 (0.7)
≥ 7, n (%)	265 (99.3)
Time of Mother SARS-CoV -2 Infection	
1st Trimester, n (%)	51 (19.1)
2nd Trimester, n (%)	92 (34.5)
3rd Trimester, n (%)	104(38.9)
At delivery, n (%)	20 (7.5)
Severity of Mother SARS-CoV -2 Infection	
Non-severe, n (%)	226 (84.6)
Severe, n (%)	41 (15.4)
Caregiver Education Level	
No University degree, n (%)	194 (72.6)
University degree, n (%)	55 (20.6)
Not reported, n (%)	18 (6.8%)

Figure 1. Stratification of Bayley-III cognitive, language, and motor domains at 6 and 12 months after birth.



APENDICE B - ARTIGO ORIGINAL COM DADOS DE NEUROIMAGEM

Qualis Capes A2 – FI 3,24

frontiers | Frontiers in Pediatrics

TYPE Original Research
PUBLISHED 24 May 2023
DOI 10.3389/fped.2023.1194114

OPEN ACCESS

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RECEIVED 26 March 2023
ACCEPTED 26 April 2023
PUBLISHED 24 May 2023

CITATION
Alves de Araujo Junior D, Motta F, Fernandes GM, Castro MECD, Sasaki LMP, Luna LP, Rodrigues TS, Kurizky PS, Soares AADSM, Nobrega OdT, Espindola LS, Zaconeta AM, Gomes CM, Martins-Filho OA, Albuquerque CPd and Mota LMHd (2023) Neuroimaging assessment of pediatric cerebral changes associated with SARS-CoV-2 infection during pregnancy.
Front. Pediatr. 11:1194114.
doi: 10.3389/fped.2023.1194114

Neuroimaging assessment of pediatric cerebral changes associated with SARS-CoV-2 infection during pregnancy

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Background: SARS-CoV-2 infection and perinatal neurologic outcomes are still not fully understood. However, there is recent evidence of white matter disease and impaired neurodevelopment in newborns following maternal SARS-CoV-2 infection. These appear to occur as a consequence of both direct viral effects and a systemic inflammatory response, with glial cell/myelin involvement and regional hypoxia/microvascular dysfunction. We sought to characterize the consequences of maternal and fetal inflammatory states in the central nervous system of newborns following maternal SARS-CoV-2 infection.

Methods: We conducted a longitudinal prospective cohort study from June 2020 to December 2021, with follow-up of newborns born to mothers exposed or not exposed to SARS-CoV-2 infection during pregnancy. Brain analysis included data from cranial ultrasound scans (CUS) with grayscale, Doppler studies (color and spectral), and ultrasound-based brain elastography (shear-wave mode) in specific regions of interest (ROIs): deep white matter, superficial white matter, corpus callosum, basal ganglia, and cortical gray matter. Brain elastography was used to estimate brain parenchymal stiffness, which is an indirect quantifier of cerebral myelin tissue content.

Results: A total of 219 single-pregnancy children were enrolled, including 201 born to mothers exposed to SARS-CoV-2 infection and 18 from unexposed controls. A neuroimaging evaluation was performed at 6 months of adjusted chronological age and revealed 18 grayscale and 21 Doppler abnormalities. Predominant findings were hyperechogenicity of deep brain white matter and basal ganglia (caudate nuclei/thalamus) and a reduction in the resistance and pulsatility indices of intracranial arterial flow. The anterior brain circulation (middle cerebral and pericallosal arteries) displayed a wider range of flow variation than the posterior circulation (basilar artery). Shear-wave US elastography analysis showed a reduction in stiffness values in the SARS-CoV-2

exposed group in all analyzed regions of interest, especially in the deep white matter elasticity coefficients (3.98 ± 0.62) compared to the control group (7.76 ± 0.77); p-value < 0.001.

Conclusion: This study further characterizes pediatric structural encephalic changes associated with SARS-CoV-2 infection during pregnancy. The maternal infection has been shown to be related to cerebral deep white matter predominant involvement, with regional hyperechogenicity and reduction of elasticity coefficients, suggesting zonal impairment of myelin content. Morphologic findings may be subtle, and functional studies such as Doppler and elastography may be valuable tools to more accurately identify infants at risk of neurologic damage.

KEYWORDS

COVID, SARS-CoV-2, pregnancy, neonatology, ultrasound, elastography, neuroimaging

1. Introduction

Maternal infection with SARS-CoV-2 during pregnancy may expose the fetus to both direct and indirect systemic effects triggered by the virus (1). The consequences of the maternal and fetal inflammatory response with the production of potentially cytotoxic cytokines, in addition to the effect of the use of antiviral medications, have not been adequately studied to date (1, 2).

There is evidence that vascular complications may result from the potential hyperactivation of inflammatory factors and coagulation system dysfunction, particularly D-dimer and platelet abnormalities, increasing the risk of cerebrovascular disease, myelination defects, and hypoxic-ischemic encephalopathy following exposure to SARS-CoV-2 (3, 4). The potential consequences of changes in intracranial blood flow dynamics and cerebral hypoxia, mediated by systemic inflammatory response syndrome (SIRS), are still poorly elucidated in the pediatric age group (5). This study aimed to investigate the effects of maternal SARS-CoV-2 infection on the brains of infants exposed to SARS-CoV-2 infection during pregnancy, focusing on brain morphological changes, intracranial blood flow dynamics, and parenchymal composition/stiffness analysis. Additionally, we sought to assess the clinical and neurodevelopmental outcomes of newborns following maternal SARS-CoV-2 infection.

2. Methods

2.1. Study design and population

A prospective, comparative, and analytical cohort study was conducted with the follow-up of newborns born to mothers exposed or not exposed to SARS-CoV-2 infection during pregnancy. The study population consisted of 219 children, of whom 201 were in the group of newborns born to women infected by SARS-CoV-2 at different stages of pregnancy. The control group consisted of 18 newborns born to women who remained serologically negative for SARS-CoV-2 until the end of the neonatal period. Study recruitment was from May 2020 to June 2022, during the COVID-19 pandemic, with planned clinical, neurological, and psychomotor follow-up until December 2022. Clinical follow-up was performed monthly until 6 months of age and then quarterly until 24 months of age. A global pediatric assessment and a neuro-psychomotor development diagnostic scale (Bayley III scale) were administered quarterly. Neuro-ultrasonography, color/spectral Doppler, and shear-wave elastography studies were performed at 6 months of adjusted chronological age, and follow-up evaluation was completed 4 weeks later for the abnormal cases. Detailed maternal clinical characteristics were also prospectively collected.

The case group included exposed newborns born to mothers infected by SARS-CoV-2 during pregnancy (RT-PCR or positive IgM). The control group included unexposed neonates with no maternal infection with the SARS-CoV-2 virus during pregnancy, no symptoms, and negative IgG and IgM serology at the end of pregnancy. Unexposed control subjects had negative IgG serology at 6 months of adjusted chronological age. Exclusion criteria for the study sample were evidence or confirmation of genetic syndromes; suspected or confirmed other congenital infections, such as toxoplasmosis, syphilis, rubella, herpes, Chagas, and Zika; discontinuation of clinical follow-up before the age of 2 years.

2.2. Neuroimaging data

The study groups were evaluated for morphometric, hemodynamic, and cerebral tissue elasticity parameters using high-frequency ultrasonography. A cranial ultrasound scan (CUS) was performed through the anterior fontanelle at 6 months of adjusted chronological age and repeated 4 weeks later at a follow-up exam in case of abnormal findings at the first CUS. Further examinations were planned thereafter, if indicated, according to an individualized schedule based on the persistence of abnormal findings and their clinical correlation. Only data from the first ultrasound scan of each infant were considered for the purpose of statistical analysis. All CUS were performed or supervised by the same operator (DA). A Philips Affiniti 70 ultrasound system, equipped with a 5–7.5 MHz convex probe

first- and fifth-minute APGAR scores in the control group are 8 (IQR: 7–8) and 9 (IQR: 9–9), respectively. With our cut-offs of 37 weeks for prematurity and 2,500 g for low birth weight, we have 16.6% (3) pre-term and 11.1% (2) low-birth weight individuals in the control group; the case group has similar frequencies with 14.8% (26) pre-term and 16.5% (29) low-birth weight individuals. Among the comorbidities found within the groups, the most frequent were anemia, bronchospasm, malnutrition, obesity, rhinitis, dermatitis, cow's milk protein allergy (CMPA), and gastroesophageal reflux disease (GERD). A supplemental table in Appendix C is provided for reference, demonstrating that groups display a similar profile of comorbidities.

3.2. Findings by imaging modality

3.2.1. Grayscale ultrasonography (structural US)

An association was found between maternal SARS-CoV-2 infection and white matter involvement in their children, with increased echogenicity in grayscale studies. Among the 201 examinations performed in the case group, 18 examinations showed abnormalities in B-mode analysis (8.9%), with deep white matter disease in the totality of these 18 abnormal cases (100%). To a lesser extent, we also saw mild alterations in the basal ganglia (caudate nuclei and thalamus), with abnormal caudothalamic echogenicity in 2 (11.1%) of 18 abnormal B-mode cases, concurrent with the deep white matter findings.

Supplementary Figure A1 summarizes the three main planes for cranial image acquisition and ultrasonographic analysis of deep white matter changes. It also shows CUS B-mode and Doppler velocimetry studies, analyzing three major intracranial arteries (the middle cerebral, pericallosal, and basilar arteries).

An equally significant finding of the morphometric US studies was the persistence of increased echogenicity in the affected areas at the routine second-look ultrasound study, performed 4 weeks after the initial study, in all the abnormal cases. On re-evaluation, it was possible to characterize the clear extension of the affected areas, with additional abnormalities in the basal ganglia—in total, the caudate nuclei and thalami. It is also noteworthy that there were no individuals in the control group (18 out of 219) with grayscale ultrasound alterations.

3.2.2. Hemodynamic abnormalities (Doppler velocimetry)

In the exposed group, 21 out of 201 (10.4%) subjects presented with abnormal hemodynamic patterns, showing a reduction in the resistance (RI) and pulsatility (PI) indices in the blood flow of the major intracranial arteries. We conducted separate analyses of three main intracranial arteries: the middle cerebral artery, the pericallosal artery, and the basilar artery, the former two representing hemodynamic parameters for the anterior intracranial circulation, and the basilar artery velocimetry as an estimate of posterior circulation flow data.

Supplementary Figures A2–A4 demonstrate the Doppler velocimetric scan with spectral curves for the analysis of the flow of three major intracranial arteries (middle cerebral, pericallosal, and basilar arteries).

A significant trend of reduction in both resistance and pulsatility indices of arterial intracranial flow in SARS-CoV-2-exposed children were observed for both anterior and posterior circulation arteries, which was positively correlated with the severity of maternal infection. Significant decreases in RI and PI were found in cases of critical SARS-CoV-2 gestational infection, with mean PI values of 1.09 for the MCA (middle cerebral artery), 0.98 for the PA (pericallosal artery), and 1.04 for the BA (basilar artery).

Table 1 shows the neuroimaging parameters according to the severity of maternal infection (COVID-19 categories according to WHO classification).

When both analyses, mode-B ultrasound, and Doppler scan findings, were integrated and cross-matched with the categories of maternal infection severity, a positive correlation of abnormal neuroimaging results that increased proportionally with the severity of maternal infection, and a peak of abnormal neuroimaging results in children whose mothers had critical SARS-CoV-2 infection during pregnancy could be identified. These data are summarized in Table 2.

A second trend in the hemodynamic data was identified in this analysis, related to the duration of SARS-CoV-2 infection during pregnancy. A significant reduction in both the resistance and pulsatility indices of intracranial arterial flow positively correlated with the last trimester of maternal SARS-CoV-2 infection, as shown in Table 3.

TABLE 1 Imaging parameters distributed according to the severity of maternal infection (COVID-19 categories according to WHO classification).

Variable[a]	COVID-19 severity scale—WHO			ANOVA	Multiple comparisons *p*-value[b]		
	Mild ($n = 165$)	Severe ($n = 23$)	Critical ($n = 8$)	*p*-value	Mild to severe	Mild to critical	Severe to critical
MCA RI	0.77 ± 0.09	0.75 ± 0.10	0.64 ± 0.14	0.0011	0.8394	0.0010	0.0260
MCA PI	1.64 ± 0.50	1.47 ± 0.38	1.09 ± 0.45	0.0039	0.3789	0.0064	0.1713
Pericallosal RI	0.70 ± 0.08	0.68 ± 0.10	0.61 ± 0.09	0.0062	0.7004	0.0070	0.1185
Pericallosal PI	1.29 ± 0.31	1.19 ± 0.25	0.98 ± 0.24	0.0113	0.4744	0.0181	0.2833
Basilar RI	0.72 ± 0.07	0.69 ± 0.09	0.64 ± 0.09	0.0032	0.1692	0.0093	0.3330
Basilar PI	1.34 ± 0.29	1.24 ± 0.28	1.04 ± 0.28	0.0121	0.4389	0.0208	0.3229

MCA, middle cerebral artery; RI, resistance index; PI, pulsatility index.
[a]values expressed as mean ± standard error.
[b]p-values for multiple comparisons adjusted with Bonferroni correction.

TABLE 2 Neuroimaging parameters are distributed according to the COVID-19 severity scale—WHO classification.

Variable[a]	COVID-19 severity scale—WHO[a]			Pearson correlation (CI 95%)	*p*-value*
	Mild (*n* = 166)	Severe (*n* = 23)	Critical (*n* = 8)		
Intracranial Doppler				0.23 (0.06; 0.40)	<0.001
Abnormal	11 (6.63)	5 (21.74)	5 (62.50)		
Normal	155 (93.37)	18 (78.26)	3 (37.50)		
Ultrasonography				0.24 (0.06; 0.42)	<0.001
Abnormal	9 (5.42)	5 (21.74)	4 (50.00)		
Normal	157 (94.58)	18 (78.26)	4 (50.00)		

[a]Values expressed in frequency (%).
*p-value calculated with the Cochran-Armitage trend test.

TABLE 3 Neuroimaging parameters (ultrasound B-mode and Doppler analysis) distributed according to the trimester of SARS-CoV-2 infection during pregnancy.

Variable[a]	Gestational trimester of SARS-CoV-2 infection[a]					
	1st (*n* = 27)	2nd (*n* = 58)	3rd (*n* = 95)	Peripartum (*n* = 21)	Pearson Correlation (CI 95%)	*p*-value*
Intracranial arteries Doppler					0.43 (0.32; 0.54)	<0.001
Abnormal	0 (0.00)	1 (1.72)	6 (6.32)	14 (66.67)		
Normal	27 (100.00)	57 (98.28)	89 (93.68)	7 (33.33)		
Ultrasound B-mode					0.41 (0.30; 0.53)	<0.001
Abnormal	0 (0.00)	1 (1.72)	4 (4.21)	13 (61.90)		
Normal	27 (100.00)	57 (98.28)	91 (95.79)	8 (38.10)		

[a]Values expressed in frequency (%).
*p-value calculated with the Cochran-Armitage trend test.

The hemodynamic evaluation data show a significant correlation between the resistance/pulsatility indices in the main intracranial arteries and the trimester of maternal infection, with the highest proportional frequency of abnormal results observed in cases of peripartum infection (defined as a period equal to or less than 14 days between infection with SARS-CoV-2 and the date of delivery). Among the pregnant women infected during this period, 66% had abnormal Doppler velocimetry, and nearly 62% had abnormal cranial ultrasound in grayscale.

3.2.3. Elastography abnormalities (shear-wave ultrasound-based)

The functional studies based on shear-wave elastography were performed in five regions of interest (ROIs) and "E" cut-off references were adopted according to previous recent literature (6–8), as there is no definitive normality parameter for elastography studies in the pediatric brain.

A significant relationship was found between maternal exposure to SARS-CoV-2 and elastography changes, mainly in the cerebral deep white matter and basal ganglia, in terms of stiffness alterations, with a decrease of the elastic modulus (*E*) in the SARS-CoV-2-exposed group when compared to controls. Table 4 shows these findings categorized by ROIs.

The SARS-CoV-2 group had significantly lower "*E*" coefficients in specific brain areas, including the deep/periventricular white matter and the splenium of the corpus callosum. The basal ganglia (caudate nuclei and thalamus), superficial white matter, and cortical gray matter also showed stiffness variations associated with SARS-CoV-2 exposure, although to a lesser extent.

TABLE 4 Neuroimaging parameters of children distributed between the group exposed to SARS-CoV-2 infection during gestation (cases) and the non-exposed (control) group, according to the specific regions of interest (ROIs) for elastography analysis: deep white matter, frontal white matter, caudate/thalamus, corpus callosum, and frontal cortex.

Variable[a]	Groups		*p*-value*
	Cases (*n* = 201)	Control (*n* = 18)	
Elastography—DWM	3.98 ± 0.62	7.76 ± 0.77	<0.001
Elastography—FWM	3.31 ± 0.59	4.69 ± 0.85	<0.001
Elastography—caudate/thalamus	5.45 ± 0.64	6.46 ± 0.96	<0.001
Elastography—corpus callosum	4.53 ± 0.39	7.93 ± 0.88	<0.001
Elastography—frontal cortex	5.62 ± 0.57	6.59 ± 0.66	<0.001

DWM, deep white matter; FWM, frontal white matter.
[a]Values expressed in kilopascal, as mean ± standard error.
*p-value calculated by Mann-Whitney test.

A significant dose-response relationship was found between exposure to SARS-CoV-2 during pregnancy and the presence of neuroimaging abnormalities, including grayscale, Doppler, and elastography modalities.

The neuroimaging parameter means were also compared between groups (SARS-CoV-2 exposure vs. non-exposure) using an analysis of covariance (ANCOVA) model. In this ANCOVA model, the neuroimaging parameter measures (hemodynamic indices and elastic modulus) were considered dependent variables, the group (SARS-CoV-2 exposure vs. non-exposure) was considered the independent variable, and the measures of GA (gestational age) and BW (birth weight) were considered covariates.

TABLE 5 Neuroimaging parameters of infants distributed between the group exposed to SARS-CoV-2 infection during gestation (cases) and the unexposed group (control), controlled by GA (gestational age) and BW (birth weight), according to the specific regions of interest (ROIs) for elastography analysis: deep white matter, frontal white matter, caudate/thalamus, corpus callosum, and frontal cortex; *p*-value calculated by ANCOVA model.

Variable	Groups—mean value[a] ± standard error		Comparison between groups	
	Cases (*n* = 201)	Control (*n* = 18)	Difference [CI 95%]	*p*-value*
MCA—RI	0.76 ± 0.01	0.79 ± 0.01	−0.03 [−0.07; −0.00]	0.0451
MCA—PI	1.59 ± 0.03	1.65 ± 0.08	−0.04 [−0.08; −0.00]	0.0434
Pericallosal artery—RI	0.70 ± 0.00	0.73 ± 0.01	−0.03 [−0.06; −0.00]	0.0277
Pericallosal artery—PI	1.26 ± 0.02	1.40 ± 0.05	−0.14 [−0.25; −0.03]	0.0123
Basilar artery—RI	0.71 ± 0.00	0.73 ± 0.02	−0.02 [−0.04; 0.01]	0.2324
Basilar artery—PI	1.31 ± 0.02	1.32 ± 0.07	−0.01 [−0.14; 0.14]	0.9585
Elastography—DWM	3.98 ± 0.04	7.77 ± 0.11	−3.80 [−4.03; −3.57]	<0.001
Elastography—FWM	3.31 ± 0.04	4.69 ± 0.11	−1.37 [−1.60; −1.14]	<0.001
Elastography—caudate nucleus/thalamus	5.46 ± 0.05	6.45 ± 0.12	−0.99 [−1.24; −0.74]	<0.001
Elastography—corpus callosum	4.56 ± 0.03	7.92 ± 0.08	−3.38 [−3.56; −3.20]	<0.001
Elastography—frontal cortex	5.61 ± 0.04	6.60 ± 0.10	−0.99 [−1.20; −0.78]	<0.001

Mean values adjusted by ANCOVA model. MCA, middle cerebral artery; RI, resistance index; PI, pulsatility index; DWM, deep white matter; FWM, frontal white matter.
[a]Results are expressed in kilopascals, as mean ± standard error.
*p-values for comparison between groups were calculated using ANCOVA model, with GA and BW as covariates.

As shown in Table 5, the neuroimaging parameters of the patients present significant differences between the two groups, even after controlling for GA and BW. According to the data, the mean value of deep white matter elasticity in the group exposed to SARS-CoV-2 is 3.98 ± 0.04, while in the group without SARS-CoV-2, it is 7.77 ± 0.11. The difference between the two groups is −3.80 with a 95% confidence interval of [−4.03, −3.57] and a *p*-value of less than 0.001. This means that there is a statistically significant difference between the two groups for this parameter, indicating that patients exposed to SARS-CoV-2 during pregnancy have lower values for deep white matter elasticity compared to those not exposed to SARS-CoV-2. In contrast, the parameters for a single vessel (basilar artery) interestingly did not show a significant difference between the two groups, when adjusted for GA and BW. Considering the basilar artery RI, the difference between the two groups is −0.02 with a 95% confidence interval of [−0.04, 0.01] and a *p*-value of 0.2324.

4. Discussion

4.1. General evidence

A systemic inflammatory response to the SARS-CoV-2 virus and consequent endothelial damage has been implicated in COVID-19 pathogenesis, with replicated evidence in many studies in both biochemical and clinical settings (9–11). Although there is extensive epidemiologic evidence of systemic COVID-19 effects (12, 13), the neurologic consequences of SARS-CoV-2 exposure in the pediatric group are still uncertain, and current evidence is mostly based on case reports (14, 15). It is not clear whether and to what extent the blood-brain barrier functions as a protective factor in blocking inflammatory cytokines (16–19).

Our study provides evidence that SARS-CoV-2 infection during pregnancy may be associated with both structural and functional brain damage in infants. The most recurrent findings were characterized in the cerebral deep white matter, although all other ROIs demonstrated some degree of change. These changes were manifested by increased regional echogenicity on B-mode studies, a reduction in the corresponding resistance/pulsatility of intracranial arterial flow, and a decrease in the cerebral elastic modulus. The reduced stiffness in the cerebral tissue, especially in the deep white matter, may represent a decreased amount of tissular myelin in the central nervous system, a crucial element for adequate neurodevelopment in children. Few neuroimaging studies have been conducted in this area with pediatric subjects, so our results provide unprecedented evidence based on structural and functional abnormalities.

4.2. Ultrasonographic findings (gray scale)

Structural neuroimaging scans in our study have repeatedly demonstrated white matter involvement in abnormal cases in SARS-CoV-2-exposed subjects. To date, there are published case series (20, 21) reporting a similar pattern of involvement in COVID-19, but no longitudinally designed studies with SARS-CoV-2-exposed and unexposed control groups correlating neuroimaging findings and clinical follow-up.

Because there is exceptional collateral circulation in the brain vasculature in the neonatal period and early childhood, the pattern of parenchymal involvement in these subjects tends to be less severe in the cortical gray matter (unlike in adults). In response to vascular and/or hypoxic encephalic injury, the deep white matter is one of the first areas of the brain affected during this early period of life (22–24).

This evidence was replicated in our results, as both deep white matter and basal ganglia areas presented as regions of higher echogenicity in abnormal B-mode scans when compared to

controls (the unexposed group). In our sample, 18 individuals whose mothers were infected by SARS-CoV-2 during pregnancy manifested some degree of white matter disease, of which 16 (88.8%) had exclusive white matter involvement and two (11.2%) subjects had concomitant involvement of deep white matter and cerebral basal ganglia (thalami and caudate nuclei). Another significant finding of the morphometric US studies was the persistence of increased echogenicity in the affected areas at the routine follow-up ultrasound study, performed 4 weeks after the initial scan, in all the abnormal cases. At re-evaluation, it was possible to characterize an increase in the extent of the affected areas in three individuals (16.6%) who evolved from initial exclusive deep white matter lesions to additional abnormalities in the basal ganglia, in total, the caudate nuclei and thalami.

Although the correlation of basal ganglia changes with the clinical COVID-19 syndrome is still unclear, it is thought to play a role in the long-lasting damage that some infants have shown, manifesting as late-onset post-COVID-19 symptoms, with delayed neurological development and failure to achieve neuropsychomotor milestones at specific ages (25, 26).

4.3. Hemodynamics findings (Doppler evaluation)

Our data regarding intracranial blood flow analysis in both groups suggest a relevant trend of decrease in RI (resistance) and PI (pulsatility) indices in the SARS-CoV-2-exposed group when maternal infections occur in the last 14 days of gestation and critical cases. This fact is thought to be a consequence of systemic adaptation to the persistent inflammatory condition that may be present even after the first 14 days of acute viral symptoms (27, 28). Cases of early maternal infection with SARS-CoV-2 during pregnancy, especially in the first and second trimesters, would allow sufficient time for arterial flow autoregulation to settle and the systemic inflammatory response to subside.

Such hemodynamic adaptation findings have been widely reported in the literature for other conditions predisposing to brain injury, such as hypoxic-ischemic injury, metabolic damage, and systemic inflammatory conditions (SIRS—systemic inflammatory response syndrome) (29, 30), generally indicating situations in which the brain has increased metabolic demands and a significant increase in intracranial blood flow is required. In fetal life, an analogous situation is classically demonstrated in cases of fetal intrauterine growth-restriction (IUGR), when the fetal arterial flow is redirected to the intracranial circulation to the detriment of visceral and peripheral flow (31–33).

Unlike other viral infections with the well-known transplacental transmission, such as human cytomegalovirus (CMV), rubella virus, parvovirus B19, and Zika virus (ZIKV), the worst pregnancy outcomes in SARS-CoV-2 infection were observed in late-stage pregnancies. This finding is consistent with the current literature, as current evidence does not demonstrate that SARS-CoV-2 represents efficient transplacental virus transmission or direct fetal neuronal damage (34, 35).

4.4. Elastography findings (shear-wave elastography assessment)

To our knowledge, no previous study has assessed elastography parameters of the brain parenchyma in infants exposed to SARS-CoV-2 during pregnancy. The few publications in the pediatric literature include small case series of healthy individuals aimed at suggesting standard elastography values for normal brain parenchyma in neonates (36, 37). Other similar studies have been conducted in mice with anatomopathological correlations (38, 39). Experiments in mice achieved a significant level of agreement with human brain values, presumably related to the very similar elasticity coefficients/energy densities (p) of mouse and human brains.

When the elastography data of our study groups were analyzed, significant differences were found between SARS-CoV-2-exposed newborns and the unexposed group in terms of the elastic modulus of the brain parenchyma. All regions of interest (ROIs) showed a reduction in the elasticity coefficient/Young's modulus (E) in the SARS-CoV-2-exposed group.

The elastography pattern differences between both groups were more pronounced in the DWM deep white matter zone (ROI number 1) when compared to other regions of analysis such as subcortical white matter and the frontal cortex. A plausible hypothesis is related to differences in the tissular composition of these regions, with a predominance of myelin in the deep white matter (40). Considering also the age of the subjects (6 months of adjusted chronological age), our ROI at the DWM was expected to be myelinated at this stage, different from the subcortex or frontal cortical zones (41–43). These elements suggest that brain findings related to SARS-CoV-2 exposure during pregnancy may be due, to some extent, to changes in the amount of myelin in the cerebral tissue, knowing that those with less myelin present a decrease in their elasticity coefficients, corresponding to a reduction in stiffness. Another possible mechanism could be mild intra-myelinic edema, in which the inflammation causes an increased water content in the cerebral tissue, thus leading to a decrease in tissue stiffness.

Our findings are consistent with recent studies investigating the impact of SARS-CoV-2 infection during pregnancy on pediatric neurodevelopment. Regarding neuroimaging, a study published in October 2021 aimed to assess the association between maternal SARS-CoV-2 infection during pregnancy and offspring brain development using MRI scans (44). The study followed 55 infants born to mothers with SARS-CoV-2 infection during pregnancy. The researchers found that infants born to mothers with SARS-CoV-2 infection during pregnancy had reduced cortical thickness in the left superior temporal gyrus, which is an important brain region for language and social communication. Abnormal cortical thickness in this region has been associated with neurodevelopmental conditions such as autism spectrum disorders. The study suggests that maternal SARS-CoV-2 infection during pregnancy may affect offspring brain development, particularly in brain regions important for language and social communication.

Protocols with a more clinical focus included a study published in January 2022 (45) that followed 205 children born to mothers with SARS-CoV-2 infection during pregnancy and found that children born to mothers with SARS-CoV-2 infection during pregnancy had an increased risk of developmental delay at 12 months of age compared to children born to mothers without SARS-CoV-2 infection. Another study published in August 2021 (46) found that children born to mothers with severe or critical COVID-19 during pregnancy had a higher risk of cognitive, motor, and language developmental delays at 6 months of age compared to children born to mothers without COVID-19. The study followed 150 infants born to mothers with COVID-19 and 150 infants born to mothers without COVID-19.

It is worth noting that these studies have limitations, and more research is needed to fully understand the potential effects of SARS-CoV-2 infection during pregnancy on pediatric neurodevelopment. However, the findings suggest that healthcare providers should closely monitor children born to mothers with SARS-CoV-2 infection during pregnancy for any signs of developmental delays or neurodevelopmental disorders.

4.5. Limitations

Causal associations between SARS-CoV-2 infection and adverse perinatal outcomes have been suggested in clinical studies but have not been definitely established, as there are many potential confounding factors involved. Among these, we should emphasize that mothers infected by SARS-CoV-2 during pregnancy are often prone to gestational complications, including adverse birth conditions, preterm labor, and maternal and neonatal hypoxia—factors that may themselves lead to CNS damage. Controlling all of these factors can be challenging. Our study attempted to control for some of these possible biases with covariance analysis techniques. However, many characteristics related to clinical maternal status, such as gestational hypertension, diabetes, previous lung disease, and obesity, persisted in our sample for both groups and may affect neurodevelopmental outcomes in infants. It is also relevant to consider that most of our sample was composed of outpatients, so the severity of maternal infection was predominantly mild to moderate, and there was a quantitative disproportion between case and control groups, given the context of multiple lockdowns and subsequent SARS-CoV-2 vaccination—the latter being one of the exclusion criteria for the control group. We acknowledge the substantial difference in the sample size between the cases and controls, and the possibility of introducing bias as a result. Because infant outcomes of maternal SARS-CoV-2 exposure during pregnancy are poorly defined to date, an accurate prospective sample size estimation for cases and controls was not feasible. However, a *post hoc* analysis was performed to estimate the number of controls needed to maintain a probability of error (alpha) of 0.05 with a power of 0.8 using the relative frequency of abnormal imaging findings in the cases. We used a likelihood ratio test to estimate the sample size needed for controls and found that $N = 9$. Thus, we believe that the control group in our study is sufficient for our research questions. Furthermore, the prospective recruitment of our controls involved randomly selecting individuals from a large representative population in our universal public health system.

5. Conclusion

SARS-CoV-2 infection during pregnancy is associated with encephalic changes in a relevant proportion of cases, predominantly affecting the cerebral deep white matter (DWM). The characteristic SARS-CoV-2-related pediatric leukopathy is manifested in neuroimaging with increased echogenicity and decreased elasticity coefficients in the DWM, i.e., reduced stiffness. These findings open up a spectrum of research possibilities regarding their effects on fetal, neonatal, and childhood health. The description of the consequences of infection in long-term follow-up may provide a better understanding of the disease and its impact on the central nervous system.

Future research using correlated axial methods, such as magnetic resonance imaging and tractography, may contribute to predicting brain areas more vulnerable to SARS-CoV-2-related encephalopathy and delineating regions with a propensity for decreased myelination. By understanding the neuroimaging correlates of SARS-CoV-2 infection in the perinatal period, this study could provide a more complete picture of the presentation pattern in the brain of SARS-CoV-2-exposed individuals during early childhood. The characterization of pediatric brain areas with a higher risk of neurological damage following maternal SARS-CoV-2 infection will allow the evaluation of clinical correlates and the early prevention of neurodevelopmental sequelae.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Research Ethics Committee of the University of Brasilia School of Medicine (Certificate Number 32359620.0.0000.5558). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

Study design: DA, FM, GF, MC, LS, CA, and LM. Advisory committee: LL, TR, PK, LE, AZ, AS, and ON. Funding

acquisition: LE, AZ, CG, OM-F, and LM. Sample collection, clinical appointments, and data collection: DA, FM, GF, MC, and LS. Data analysis: DA, FM, LL, TR, CA, and LM. Writing and revision of the manuscript: DA, FM, LL, TR, CA, and LM. All authors have participated sufficiently in the work to take responsibility for its content. All authors contributed to the article and approved the submitted version.

Funding

The study was supported by the Conselho Nacional de Desenvolvimento Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the University of Brasília (UnB) (Grant # 7155, "Fundo COVID-19 UnB em Ação: Ações emergenciais para combate à COVID-19 e mitigação das consequências da pandemia"). This study was funded by Cnpq/CAPES.

Acknowledgments

The research project involves doctoral students and professors affiliated with the Medical Sciences Post-Graduation Program of the University of Brasília (UnB), supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The authors express their gratitude University Hospital of Brasília (HUB) for the use of its facilities. The authors also thank the University of Melbourne and the Austin Health Library for technical support with literature searches and statistical analysis. ON and OM-F received a research fellowship from the Conselho de Desenvolvimento Científico e Tecnológico (CNPq; Brazil).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2023.1194114/full#supplementary-material.

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APÊNDICE C – PROTOCOLO PROUDEST - Qualis Capes A4 – FI 1,25

Protocol

Pregnancy Outcomes and Child Development Effects of SARS-CoV-2 Infection (PROUDEST Trial): Protocol for a Multicenter, Prospective Cohort Study

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Abstract

Background: A growing body of evidence suggests that SARS-COV-2 infection during pregnancy may affect maternal-fetal outcomes and possibly result in implications for the long-term development of SARS-CoV-2–exposed children.

Objective: The PROUDEST (Pregnancy Outcomes and Child Development Effects of SARS-CoV-2 Infection Study) is a multicenter, prospective cohort study designed to elucidate the repercussions of COVID-19 for the global health of mothers and their children.

Methods: The PROUDEST trial comprises 2 prospective, sequential substudies. The *PREGNANT* substudy will clinically assess the effects of SARS-CoV-2 infection on pregnancy, childbirth, and puerperium from a mechanistic standpoint to elucidate the pregnancy-related inflammatory and immunological phenomena underlying COVID-19. Pregnant women aged 18-40 years who have been exposed (proven with laboratory tests) to SARS-CoV-2 (group A; n=300) will be compared to control subjects with no laboratory evidence of in-pregnancy exposure to the virus (group B; n=300). Subjects exposed to other infections during pregnancy will be excluded. The *BORN* substudy is a long-term follow-up study that will assess the offspring of women who enrolled in the prior substudy. It will describe the effects of SARS-CoV-2 exposure during pregnancy on children's growth, neurodevelopment, and metabolism from birth up to 5 years of age. It includes two comparison groups; group A (exposed; n=300) comprises children born from SARS-CoV-2–exposed pregnancies, and group B (controls; n=300) comprises children born from nonexposed mothers.

Results: Recruitment began in July 2020, and as of January 2021, 260 pregnant women who were infected with SARS-CoV-2 during pregnancy and 160 newborns have been included in the study. Data analysis is scheduled to start after all data are collected.

Conclusions: Upon completion of the study, we expect to have comprehensive data that will provide a better understanding of the effects of SARS-CoV-2 infection and related inflammatory and immunological processes on pregnancy, puerperium, and infancy. Our findings will inform clinical decisions regarding the care of SARS-CoV-2–exposed mothers and children and support the development of evidence-based public health policies.

Trial Registration: Brazilian Register of Clinical Trials RBR65QXS2; https://ensaiosclinicos.gov.br/rg/RBR-65qxs2

International Registered Report Identifier (IRRID): DERR1-10.2196/26477

(JMIR Res Protoc 2021;10(4):e26477) doi: 10.2196/26477

KEYWORDS

SARS-CoV-2; COVID-19; pregnancy; neonate; children; outcome; development; prospective; cohort; women; fetus; baby; implication

Introduction

Background

The natural history of COVID-19, a disease caused by SARS-CoV-2, is still being written. Individuals infected with SARS-CoV-2 may present with a broad spectrum of clinical manifestations, from no symptoms to dramatically progressive disease symptoms that eventually result in death [1,2]. Some individuals develop intense inflammatory and procoagulant responses that can result in severe pulmonary damage, which is the main cause of COVID-19 morbidity and mortality.

The pathophysiological phenomena that take place in other human tissues that are potentially targeted by SARS-CoV-2 need further investigation. A central nervous system viral tropism has been postulated based on reports of neurological events such as stroke, acute hemorrhagic encephalopathy, seizures, and the loss of smell and taste [3,4].

To date, little is known about the effects of COVID-19 on women during pregnancy and puerperium [5,6] and its consequences for women's offspring (from the neonatal period through the first years of life) [7-9]. Thus, there is still a lack of a robust evidence base for the proper management of these mothers and children.

Data from previous epidemics of viral-induced respiratory distress syndrome have shown that pregnant and puerperal women have a high risk of developing life-threatening clinical outcomes [10]. These generally worse outcomes have been attributed to physiological changes in the immune and cardiopulmonary systems that occur during pregnancy [11]. Examples of epidemics include the H1N1 influenza, SARS-CoV (severe acute respiratory syndrome coronavirus), and MERS-CoV (Middle East respiratory syndrome coronavirus) epidemics, during which pregnancy mortality rates reached 27% [10,12,13].

Despite the structural similarities between coronaviruses, the initial reports for pregnant women with COVID-19 showed lower rates of intensive care unit admission, orotracheal intubation, and death during the SARS-CoV-2 outbreak than those during the SARS-CoV and MERS-CoV outbreaks [9,13]. However, more recent papers have shown higher morbidity and mortality rates among pregnant women than those among nonpregnant women. Several aspects of embryo implantation [14], placental development [15], and delivery dynamics [16] seem to be impaired by the inflammatory response driven by immune cell subtypes at the maternal-fetal interface [17]. Such phenomena may precipitate preeclampsia, spontaneous abortion, intrauterine growth restriction, and premature birth [18-21].

Most of the available data on pregnant women exposed to SARS-CoV-2 were obtained during the second half of pregnancy. Thus, SARS-CoV-2 infection during all stages of pregnancy, including the early stages of gestation, has not been fully investigated. Nevertheless, as the disease spreads worldwide, more women are being exposed to the virus during early gestation and midgestation, and new data have been accruing [22].

Studies evaluating the vertical transmission of SARS-CoV-2 are still inconclusive [6,23-25]. Investigations of placentas from women infected with SARS-CoV-2 have suggested that there is a low likelihood of viral transplacental transmission. However, the potentially hazardous effects of inflammatory and prothrombotic environments on placental function and, consequently, fetal growth could not be ruled out [26-28].

To date, the few reports on postnatally infected neonates have shown that they exhibit either no symptoms or mild clinical forms of COVID-19 with favorable outcomes. However, the younger the infant is, the higher the risk of critical outcomes [22,29].

Maternal SARS-CoV-2 infection would potentially expose a fetus not only to direct viral effects but also to the placental inflammatory response and the maternal cytokine storm [5,7,22]. Such processes and their consequences have not been extensively studied. The understanding of these phenomena should contribute to the proper management of children born to mothers infected with SARS-CoV-2.

A case series on the clinical aspects of newborns of COVID-19–exposed mothers reported a low risk of adverse outcomes for late pregnancy exposure and stated that there is a paramount need for close follow-ups [30]. Other reports have presented data showing no adverse effects on neonates born to mothers who tested positive for COVID-19. Furthermore, Liu et al [31] described 19 completely asymptomatic neonates from Wuhan, China.

A few studies however have reported that SARS-CoV-2 test–negative neonates born to mothers who tested positive and developed critical illnesses might present ominous clinical profiles. This is suggestive of the potential impact of inflammatory processes on fetal physiology. Romagano et al [32] reported a prevalence rate of 6.9% for symptomatic pregnant women infected with SARS-CoV-2 among 1053 deliveries at a large hospital network in New Jersey, United States. They reported that 8 pregnant women were critically ill and 7 neonates tested negative (via reverse transcriptase–polymerase chain reaction [RT-PCR]); 1 neonate was not yet delivered at the time of testing. All neonates were preterm and appropriately sized for their gestational age except for one (small for their gestational age). They were all separated from their mothers after delivery, and all of them developed respiratory distress and required neonatal intensive care unit admission. Anemia and hyperbilirubinemia of prematurity, temperature instability, and feeding problems were reported in some of the neonates.

Several other studies have reported symptoms among SARS-CoV-2 test–negative neonates born to mothers with COVID-19, such as rashes [33], facial ulceration [33], the need for noninvasive oxygen support [33], transient lymphocytopenia [34], impaired liver function [34], disseminated intravascular coagulation, and even multiple organ failure leading to death [35]. There are many critical questions regarding the standards of care for SARS-CoV-2–exposed pregnant women and their offspring that have yet to be answered, and guidelines are still being developed around the world [8,36,37]. Therefore, the overall purpose of this study is to describe the effects of in-pregnancy SARS-CoV-2 infection and related inflammatory and immunological phenomena on the health of SARS-CoV-2–exposed women and their offspring.

Objective

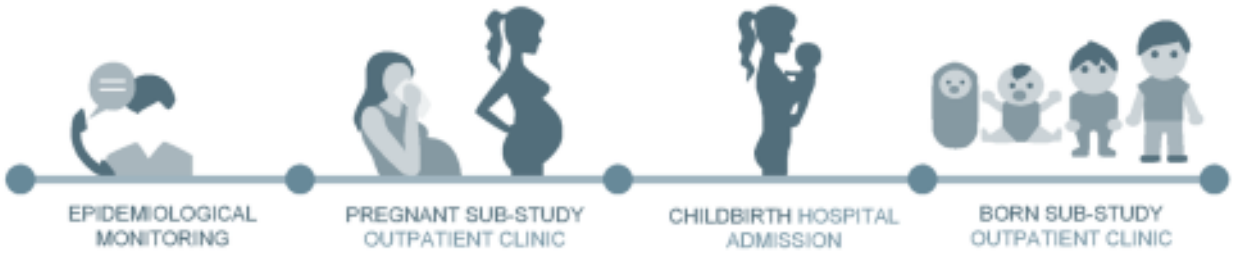
Our specific aims are (1) to study the effects of COVID-19 on maternal and obstetric morbidity and mortality, including those of indicators such as preeclampsia, abortion, fetal malformation, fetal growth, and premature birth; (2) to investigate the presence of SARS-CoV-2 and anti–SARS-CoV-2 antibodies in the cerebrospinal fluid (CSF) of women with symptomatic COVID-19 undergoing spinal anesthesia for a cesarean section; (3) to determine the serum proinflammatory and regulatory cytokine profiles of pregnant women with symptomatic COVID-19; (4) to determine the CSF proinflammatory and regulatory cytokine profiles of pregnant women with symptomatic COVID-19 undergoing spinal anesthesia for a cesarean section; (5) to study the histopathological markers of inflammatory and thrombotic phenomena in the placenta; (6) to study the correlations between the aforementioned serum and histologic biomarkers of COVID-19 and the outcomes of pregnancy, delivery, puerperium, and childbirth as well as the correlations between biomarkers and short- and long-term health outcomes during infancy; (7) to study the association between the use of maternal pharmacological therapy for treating COVID-19 and offsprings' health outcomes; (8) to evaluate the effects of COVID-19 exposure during different stages of pregnancy on fetal, neonatal, and infantile morbidity and mortality; and (9) to evaluate the effects of in-pregnancy COVID-19 exposure on children's somatic and neurological development and energy metabolism from birth up to 5 years of age.

Methods

Study Design

The PROUDEST (Pregnancy Outcomes and Child Development Effects of SARS-CoV-2 Infection Study) is a multicenter, longitudinal, prospective observational study that will be conducted in two sequential stages—the *PREGNANT* and *BORN* branches (or substudies). Each stage will have two parallel groups (exposed and nonexposed) for comparisons. The PROUDEST is designed to address the multifaceted questions surrounding the impact of COVID-19 exposure during pregnancy on the global health of mother-child dyads (Figure 1).

Figure 1. Pregnancy Outcomes and Child Development Effects of SARS-CoV-2 Infection Study follow-up flowchart.



The PREGNANT substudy will follow—until day 21 postpartum—pregnant women who are exposed to SARS-CoV-2 at any phase of gestation and compare them to a control group consisting of nonexposed pregnant women. The BORN substudy will follow the children of the women included in the preceding (PREGNANT) branch. These children will be allocated into two comparison groups (exposed and nonexposed) according to their mothers' in-pregnancy exposure status and will be followed up by a multidisciplinary team of health professionals from birth up to the age of 5 years. This team will conduct regular consultations every month up until the children reach 6 months of age, every 3 months up until the children reach 2 years of age, and every 6 months up until the children reach 5 years of age. Mothers and children may attend nonscheduled visits, as needed, for clinical reasons as well as specific appointments for conducting the procedures and tests described in this protocol.

The PROUDEST will be conducted from July 2020 to December 2026 in Brasília, Brazil. The recruitment of pregnant and newborn dyads will be carried out by using data from the Epidemiological Surveillance Center of the Federal District. These mother-fetus dyads will be followed up to childbirth (and puerperium in the case of mothers) until December 2021, which is when the last included dyads are expected to undergo childbirth in two different hospitals—the University Hospital of Brasília and Asa Norte Regional Hospital (the reference public medical center for COVID-19 in the Federal District in Brazil). Both hospitals are located in central urban areas and are included in the Brazilian public health system (Sistema Único de Saúde), which primarily serves the low-income population. Thus, the results of the PREGNANT substudy will be published as soon as the analyses are completed. The children will be followed from childbirth up to December 2026, which is when the last admitted neonate will turn 5 years old. As the BORN substudy is lengthy, partial results may be disclosed during the course of the study, but the final data set will be made available in the second half of 2026.

Pregnant women included in the study must be aged >18 years. COVID-19 exposure will be defined as a first-time RT-PCR test, serology test, or rapid test that returns positive results during pregnancy and is confirmed by a second test. Nonexposure to COVID-19 will be defined as asymptomatic pregnant women with negative serology tests (immunoglobulin G [IgG] and immunoglobulin M [IgM] tests), which will be conducted at 14-21 days postpartum.

Pregnant women with preexisting chronic diseases (except diabetes and hypertension); those taking continuous medications; those who consume tobacco, alcohol, or other drugs; and those with other suspected or confirmed congenital infections will be excluded.

Neonates whose mothers qualified for inclusion in the PREGNANT substudy (had these women been screened) may also be admitted to the BORN substudy, even if their mothers did not participate in the preceding branch.

Children initially assigned to the control (nonexposed) group who later become infected with SARS-CoV-2 (as confirmed via laboratory tests) during follow-up will be excluded from all analyses (from the time of SARS-CoV-2 infection diagnosis onward). However, they will continue to receive assistance under the same standards until the end of the study.

Sample Size Calculation

No precise data are available on the prevalence of SARS-CoV-2 infection among pregnant women in Brazil, but international reports have estimated that up to 15.3% of all pregnancies have been exposed to the virus [38]. Recent data have indicated a birth rate of 44,195 newborns per year in the Federal District [39]. Thus, after considering an "infinite" population (>20,000 pregnant women), assuming a 15% prevalence of SARS-CoV-2–exposed pregnancies, and accounting for a confidence level of 95% and a margin of error of 5%, the minimum sample size for a random sample of SARS-CoV-2–exposed women would be 195. This calculation expectedly yielded a similar number for a random sample of SARS-CoV-2–exposed children. If we set the expected dropout rate for the BORN substudy to 20%, the required number of SARS-CoV-2–exposed mothers (those giving birth to the BORN participants) would increase to 234.

Our sampling approach however is not truly random; it is based on convenience, as eligible subjects will present to the recruitment centers. The aforementioned calculations only serve as a reference for avoiding the overestimation of the inclusion of participants. Given the limited amount of available knowledge regarding the effects of SARS-CoV-2 infection on pregnancy and child development, which resulted in the eminently exploratory character of our study, we adopted an "as much as feasible, but no more than reasonable" approach for defining the sample size.

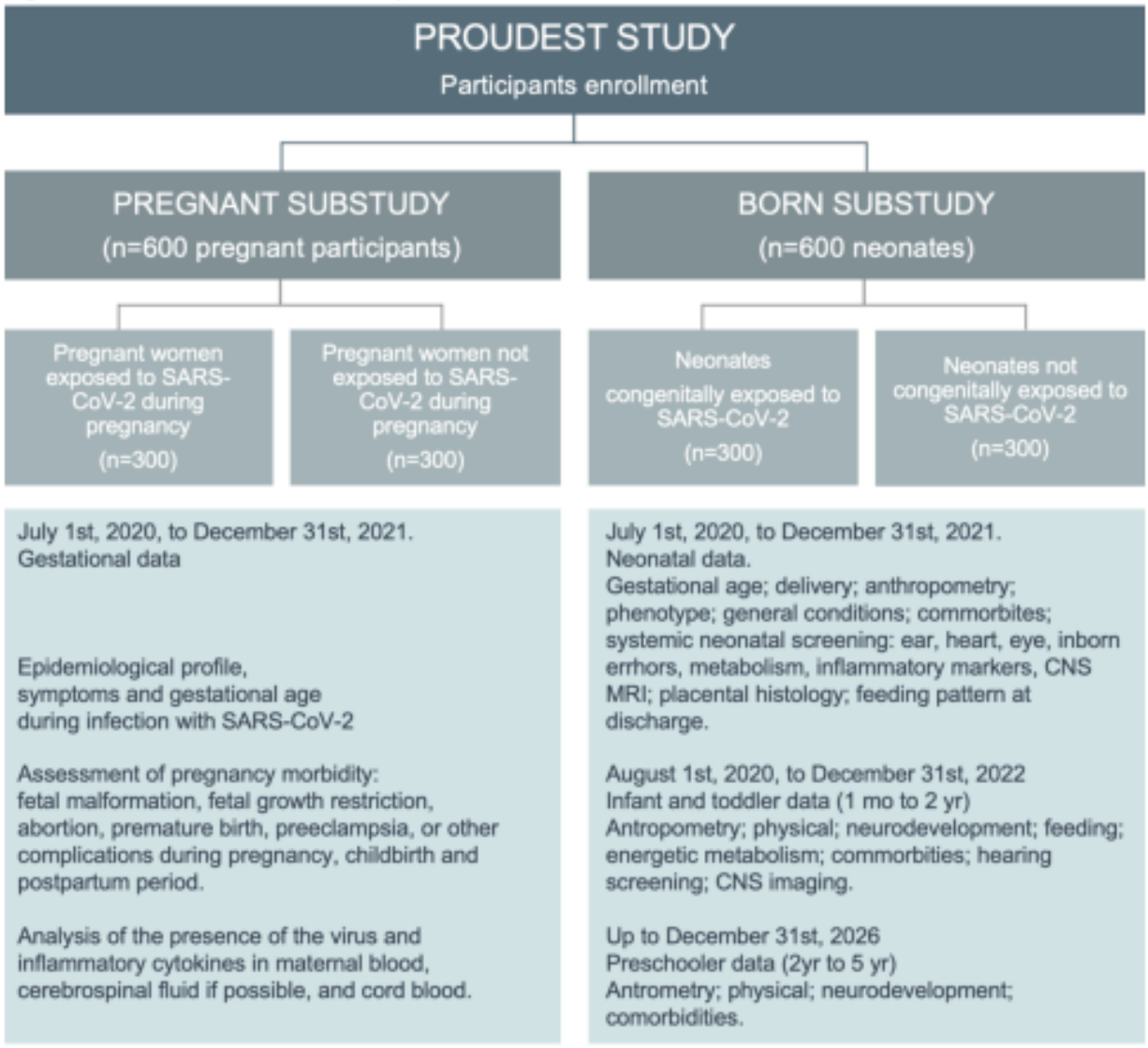
We set an a priori number of 300 SARS-CoV-2–exposed women for the PREGNANT phase. This will result in the inclusion of the expected 300 SARS-CoV-2–exposed children in the BORN phase. The subject allocation rate between the exposed and control groups was 1:1. This indicated the need for an additional 300 mothers and 300 children to constitute the nonexposed control groups. Hence, the overall sample size of the PROUDEST was set to 1200 participants (600 mother-child dyads consisting of 300 SARS-CoV-2–exposed mother-child dyads and 300 control dyads).

To promote participant retention and completed follow-ups at pregnancy and pediatric outpatient clinics, we will actively search for patients via phone and email.

Procedures

A host of clinical, psychological, neurodevelopmental, biochemical, histological, and imaging assessments will be conducted in accordance with the PROUDEST protocol (Figure 2).

Figure 2. PROUDEST study design. CNS: central nervous system; MRI: magnetic resonance imaging; PROUDEST: Pregnancy Outcomes and Child Development Effects of SARS-CoV-2 Infection Study.



Prenatal data from both the pregnant women and their fetuses will be gathered during the follow-up at the Pregnancy Outpatient Clinic of the University Hospital of Brasilia. These data will consist of the medical and sociodemographic data of the mothers; gestational age; symptoms, interventions, and outcomes related to COVID-19 (for SARS-CoV-2–exposed participants); congenital infection screening results; hypertensive disorders and other pregnancy-specific morbidities; general health assessments; general physical examinations; routine clinical biochemistry tests; and ultrasound scans. These scans will be performed between gestational weeks 11-13 and gestational day 6, from gestational week 22 to gestational week 24, and on a monthly basis in the third trimester of pregnancy to assess fetal growth and morphology, placental morphology, amniotic fluid volume, and dopplerfluxometry results. Maternal blood will be collected at the first prenatal consultation, regardless of gestational age. Antenatal consultations will occur monthly up to week 34, every 2 weeks between weeks 34 and 36, and then weekly up to delivery. During pregnancy, psychological risk assessments and mental health screens will be performed with the Beck Depression Inventory during the first prenatal consultation. [40]. Individual psychological care will be provided to pregnant women who have a Beck Depression Inventory score of >12. Mothers will also be physically and psychologically evaluated between days 7 and 21 postpartum during the PREGNANT substudy and after the BORN substudy.

At childbirth, assessments will be conducted to identify the occurrence of dysfunctional labor and the premature rupture of membranes, the type of birth, and delivery outcomes. We will also conduct physical examinations and classifications of the newborns and anthropometry. The early initiation of breastfeeding, the need for neonatal intensive care, and the type of interventions will also be identified. Maternal blood, CSF from women undergoing spinal anesthesia for a cesarean section, and umbilical cord blood samples will be collected.

CSF will be collected immediately before the infusion of the medicine for spinal anesthesia, which will be injected via a sterile syringe at an average dosage of 0.5 ml. CSF will be

collected in a 4-mL cryotube. Blood samples from mothers and the umbilical cord will be collected in a heparinized tube and centrifuged immediately, and the plasma will be stored in 4-mL sealed cryotubes. CSF and blood samples will be subsequently stored at −80 °C for later analysis. The assessment of blood cell counts, inflammation markers (C-reactive protein and procalcitonin), biochemistry (alanine aminotransferase, aspartate aminotransferase, ferritin, alkaline phosphatase, and lactic dehydrogenase) and SARS-CoV-2 tests (RT-PCR and IgM and IgG antibody tests) will be carried out.

Circulating cytokine levels will be evaluated with the Luminex Bio-Plex Pro Human Cytokine 27 platform (Bio-Rad Laboratories). The cytokine profile assessment will analyze chemokines (CXC motif chemokine ligand [CXCL] 8, CC motif chemokine ligand [CCL] 11, CCL3, CCL4, CCL2, CCL5, and CXCL10), proinflammatory cytokines (interleukin [IL]-1β, IL-6, tumor necrosis factor, IL-12p70, interferon γ, IL-17A, and IL-15), regulatory cytokines (IL-1Ra, IL-4, IL-5, IL-9, IL-10, and IL-13), and cell growth factors (IL-2, IL-7, basic fibroblast growth factor, platelet-derived growth factor, vascular endothelial growth factor, granulocyte colony-stimulating factor, and granulocyte-macrophage colony-stimulating factor). All procedures will be performed according to the manufacturer's recommendations.

Analyses will be performed at the clinical biochemistry laboratories of the hospitals where delivery occurred (blood cell count, C-reactive protein, procalcitonin, and routine biochemistry assessments), the Central Laboratory of the Federal District Department of Health (Laboratório Central de Saúde Pública; SARS-CoV-2 tests), and the University of Brasilia laboratory (cytokine profile assessments).

The placenta will be subjected to fresh histopathological analyses for assessing possible morphological and histological changes that may be associated with SARS-CoV2 infection. Histopathological analysis will be conducted according to the Amsterdam protocol [41].

Peripheral blood and CSF samples from the newborns will only be collected if there is clinical need; this will not be done per the routine research protocol. If such specimens are made available, they will also be subjected to the aforementioned analyses.

All newborns will undergo neonatal screening tests in accordance with the recommendations of the Ministry of Health of Brazil. Five drops of blood will be collected on filter paper for the national neonatal screening program. This blood sample will be collected after 48 hours of life and will be used to screen for the following diseases: phenylketonuria, congenital hypothyroidism, biotinidase deficiency, cystic fibrosis, and congenital adrenal hyperplasia. After 24 hours of life and before discharge, a pulse oximetry test will be performed. Oxygen saturation in the right upper limb and one of the lower limbs will be measured. The pulse oximetry test will be considered normal if oxygen saturation is ≥95% and the difference between the limbs is not ≥3%. Hearing screening will be performed between 36 and 48 hours of life by analyzing otoacoustic emissions.

After hospital discharge, all neonates will be followed up at the Pediatric Outpatient Clinic of the University Hospital of Brasilia. Child growth and neurodevelopment will be assessed at all visits. The first visit will be scheduled to occur on day 15 postpartum. Afterward, visits will be conducted monthly during the first 6 months of life. Thereafter, visits will be scheduled every 3 months until the children reach 12 months of age and every 6 months until the children reach 5 years of age. Nonscheduled visits may occur due to urgent clinical needs. The outpatient clinic staff (a multidisciplinary team) will be composed of pediatricians, psychologists, occupational therapists, speech therapists, physiotherapists, and nurses. The psychological effects of SARS-CoV-2 infection on mothers will be assessed with the Edinburgh Postnatal Depression Scale [42]. This assessment will occur more than once until their children reach 6 months of age. Breastfeeding and weaning patterns, dietary habits, nutritional status, and vaccinal status will be assessed throughout the study.

The assessment of children's neurodevelopment will be carried out until they reach the 60th month of life. This assessment will analyze cognitive, motor, socioemotional, and language-related aspects and adaptive behavior. The evaluation will be conducted by using the Bayley III Child Development Scale (ie, the version validated for Brazilian infants) [43]. From the age of 2.5 years onward, aspects related to intellectual performance will also be assessed by using the Wechsler Preschool and Primary Intelligence Scale, third edition at 6-month intervals [44].

Central nervous system imaging assessments will be carried out via transcranial ultrasound doppler scans, which will be performed between the 15th and 90th day of the child's life. A brain magnetic resonance imaging scan will be performed if altered cephalic perimeter measures, neurological development delays, or abnormal ultrasound doppler scan findings are identified.

Blood will be collected from SARS-CoV-2–exposed children aged 12 and 24 months to assess their metabolic profiles, which will be used to identify the long-term effects of SARS-CoV-2 infection on systemic metabolism that are potentially driven by past viral exposure and associated inflammatory responses. The examination will consist of assessments for energy metabolism markers (serum lipids, glucose, and insulin), thyroid function markers (thyroid-stimulating hormone and free thyroxine), bone metabolism markers (parathyroid hormone, calcium, phosphate, alkaline phosphatase, and 25-hydroxyvitamin D), adrenal tonus indicators (adrenocorticotropic hormone and basal cortisol), and renal function markers (blood urea nitrogen, creatinine, and urine analysis results).

All newborns will undergo extended hearing screening. Evoked otoacoustic emission and brainstem auditory-evoked potential tests will be performed during the child's first year of life.

Statistical Analysis

All data will be stored in REDCap (Research Electronic Data Capture; Vanderbilt University), which is a tool for building and managing web-based surveys and databases. All variables will be summarized via standard descriptive techniques according to their type and distribution. For the bivariate

analysis, differences in categorical variables between the exposed and unexposed groups will be verified with the Chi-square test or Fisher exact test, whereas differences in continuous variables between the groups will be assessed with the Student *t* test or the Mann-Whitney U test.

For dichotomous outcomes, binomial regression models, which will be adjusted based on the unbalanced and relevant background features of the comparison groups, will be used to estimate the relative risks between the exposed and nonexposed groups. Partial correlation and general linear models will be used to assess the associations between continuous outcome variables and covariates; adjustments for imbalances will be made as appropriate. A P value of $<.05$ will be considered significant. The control group will be composed of nonexposed mother-child dyads that meet the inclusion criteria. However, those with positive serology tests (IgG and IgM tests conducted at 14-21 days postpartum) will not be included in the control group. The groups will not be matched or paired based on age or other variables. However, any differences between the groups will be adjusted later via statistical means.

Ethics Approval and Consent to Participate

The PROUDEST was approved by the Research Ethics Committee of the University of Brasilia School of Medicine (Certificado de Apresentação de Apreciação Ética 32359620.0.0000.5558) [45]. It was also registered in the Brazilian Register of Clinical Trials [46]. All pregnant women participating in the PROUDEST are required to sign an informed consent form to join the PREGNANT branch. Likewise, the participation of the children in the BORN branch will require signed, informed consent from their mothers. The 6-month reports on the study's status and its partial results will be made available to the institutional Research Ethics Committee and may be publicly consulted upon request.

Availability of Data and Materials

At the time of the publication of this protocol, study enrollment and data collection have already started, but we have not completed the participant recruitment and data analysis phases. Therefore, data sharing is not yet feasible, as no data sets have been generated or analyzed at this stage of the study. As partial data become available, they will be displayed in the Brazilian Register of Clinical Trials [46].

Results

The PROUDEST is in the data collection phase. Study recruitment started in July 2020. As of January 2021, a total of 260 pregnant women who were infected with SARS-CoV-2 during pregnancy and 180 newborns from hospitals in the Federal District in Brazil have been included in the study. Data analysis is scheduled to start after all data are collected.

Discussion

Study Implications

The PROUDEST offers comprehensive insight (from both the obstetric and pediatric perspectives) into the effects of SARS-CoV-2 infection on the global health of pregnant women and their offspring. Specifically, the study will fill the deep gap in knowledge about the consequences of SARS-CoV-2 infection during early gestation (ie, the period when the critical stages of embryogenesis take place), as women in all stages of pregnancy will be followed. The virus-induced inflammatory and immunological phenomena that occur in SARS-CoV-2–exposed mothers during this early period of life may have a particular impact on placental and fetal physiology or may even be associated with epigenetic signals. Therefore, these phenomena could conceivably affect the long-term outcomes of a child's growth, development, and metabolism.

A better understanding of these potential long-term consequences requires lengthy, prospective observational studies, such as the PROUDEST. This study will not only address the clinical outcomes associated with in-pregnancy exposure to COVID-19 but also evaluate a host of soluble tissue biomarkers (as described in this protocol) with the aim of comprehensively understanding the mechanisms underlying related clinical phenomena.

Our data will add to the overall clinical and basic knowledge base for COVID-19, and our ultimate goal is to provide grounds for better managing SARS-CoV-2–exposed pregnant women and their children through direct means or by setting the stage for additional studies. In fact, the PROUDEST opens up a broad spectrum of possibilities for further, multidisciplinary research on the effects of SARS-CoV-2 infection on maternal, fetal, and pediatric health. Furthermore, our results might prove to be relevant from a social perspective, as they may provide data that support the tailored development and implementation of health policies that are specifically oriented to this particular demographic group.

The study does have several limitations. Its observational nature limits inferences for causal associations to some extent. However, the cohort study design is the closest observational equivalent to a clinical trial in terms of analytical power, and the objective of the PROUDEST does not ethically allow for interventional experiments because such experiments would imply that pregnant woman will be randomized based on SARS-CoV-2 infection. Moreover, the purpose of the study is to characterize the clinical and pathophysiological phenomena associated with SARS-CoV-2 infection during pregnancy and infancy, not to test the efficacy of any intervention. Thus, we believe that a cohort study is the best possible study design for addressing our objectives. The lack of random allocation for comparison groups will be partially compensated by statistically adjusting for the observed imbalances. Vaccination for SARS-CoV-2 will not be an exclusion criterion because due to the vaccine's limited accessibility and availability, there are no feasible methods for estimating the proportion of pregnant women that will receive the vaccine. However, the effects of SARS-CoV-2 vaccination can be adjusted and analyzed in small control groups that have either received or not received the vaccine.

The protracted follow-up in the BORN substudy is expected to result in the dropout of several participants. We set a sample size for the study after taking into consideration a 20% loss to follow-up rate. This might ensure that a sizable number of

children are available for the final assessment when they reach age of 5 years. However, we cannot avoid survival bias in the long-term data. Nevertheless, given that the continuity of multidisciplinary assistance will be guaranteed for all children in the BORN branch throughout the study period regardless of their withdrawal from the analysis or (temporary) losses to follow-up, we expect that children experiencing health problems associated with in-pregnancy COVID-19 exposure will be less likely to drop out than those who are in perfect health. Therefore, we do expect to have a sufficient number of children in the long run for identifying developmental abnormalities (should they exist), even after some amount of dropout.

Several routine pediatric consultations will be emphasized in the study, such as checking the kind of alimentation that a child is receiving (human milk or formula milk) and promoting the practice of breastfeeding.

Conclusions

The PROUDEST is a long-term, prospective cohort study designed to provide a comprehensive analysis of the effects of in-pregnancy exposure to COVID-19 on women and their offspring from a clinical and pathophysiological standpoint. Our results might contribute to the improvement of the management of SARS-CoV-2–exposed mother-child dyads—through direct means or by setting the stage for future related studies—by providing knowledge on the clinical-pathophysiological phenomena associated with COVID-19 exposure among this particular population.

Acknowledgments

We are immensely grateful for the contributions of all participants among the mother-child dyads, medical students, residents, and health professional staff from the participating hospitals; their support is essential for conducting our study. The resources used in outpatients' follow-ups, ambulatorial consultations, and complementary exams (diagnostic imaging and laboratorial studies) are provided by the University Hospital of Brasilia, Asa Norte Hospital of Brasilia, and the Central Laboratory of Public Health of the Federal District. All public institutes are funded by the Brazilian health care system (Sistema Único de Saúde).

Authors' Contributions

GF, LS, and FM are cofirst authors. GMF, LS, FM, ACZ, and LMHM drafted and finalized the manuscript. CPA, GM, LS, ACZ, and MECC provided input. AASS, COA, DAAJ, JALJ, RMT, LCGC, and CPA critically reviewed the manuscript. All authors made insightful contributions. All authors have read and approved the manuscript.

Conflicts of Interest

None declared.

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Abbreviations

CCL: CC motif chemokine ligand
CNS: central nervous system
CSF: cerebrospinal fluid
CXCL: CXC motif chemokine ligand
IgG: immunoglobulin G
IgM: immunoglobulin M
IL: interleukin
MERS-CoV: Middle East respiratory syndrome coronavirus
PROUDEST: Pregnancy Outcomes and Child Development Effects of SARS-CoV-2 Infection Study
REDCap: Research Electronic Data Capture
RT-PCR: reverse transcriptase–polymerase chain reaction
SARS-CoV: severe acute respiratory syndrome coronavirus

Edited by G Eysenbach; submitted 13.12.20; peer-reviewed by JM Kerver, C Kaliaperumal; comments to author 27.01.21; revised version received 02.02.21; accepted 25.02.21; published 20.04.21

Please cite as:
Fernandes GM, Motta F, Sasaki LMP, Silva ÂPD, Miranda AM, Carvalho AOD, Gomides APM, Soares AADSM, Santos Jr ADCMD, Alves CDO, Gomes CM, Siracusa CCD, Araújo Jr DAD, Mendonça-Silva DL, Jesus JALD, Costa KN, Castro MECD, Kurizky PS, França PS, Tristão R, Pereira YR, Castro LCGD, Zaconeta AM, Albuquerque CPD, Mota LMHD
Pregnancy Outcomes and Child Development Effects of SARS-CoV-2 Infection (PROUDEST Trial): Protocol for a Multicenter, Prospective Cohort Study
JMIR Res Protoc 2021;10(4):e26477
URL: https://www.researchprotocols.org/2021/4/e26477
doi: 10.2196/26477
PMID: 33793409

APÊNDICE D - QUALIS CAPES A1 – FI 7.30

frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 12 April 2023
DOI 10.3389/fimmu.2023.1176898

OPEN ACCESS

EDITED BY
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SPECIALTY SECTION
This article was submitted to Cytokines and Soluble Mediators in Immunity, a section of the journal Frontiers in Immunology

RECEIVED 01 March 2023
ACCEPTED 30 March 2023
PUBLISHED 12 April 2023

CITATION
Fernandes GM, Sasaki LMP, Jardim-Santos GP, Schulte HL, Motta F, da Silva ÂP, de Carvalho AO, Pereira YR, Alves CdO, de Araújo Júnior DA, Mendonça-Silva DL, Costa KN, de Castro MEC, Lauand L, Nery RdR, Tristão R, Kurizky PS, Nóbrega OdT, Espindola LS, de Castro LCG, Alpoim PN, Godoi LC, Dusse LMSA, Coelho-dos-Reis JGA, Amaral LRd, Gomes MdS, Bertarini PLL, Brito-de-Sousa JP, Costa-Rocha IAd, Campi-Azevedo AC, Peruhype-Magalhães V, Teixeira-Carvalho A, Zaconeta AM, Soares AAdSM, Valim V, Gomes CM, de Albuquerque CP, Martins-Filho OA and da Mota LMH (2023) Panoramic snapshot of serum soluble mediator interplay in pregnant women with convalescent COVID-19: an exploratory study. *Front. Immunol.* 14:1176898. doi: 10.3389/fimmu.2023.1176898

Panoramic snapshot of serum soluble mediator interplay in pregnant women with convalescent COVID-19: an exploratory study

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Introduction: SARS-CoV-2 infection during pregnancy can induce changes in the maternal immune response, with effects on pregnancy outcome and offspring. This is a cross-sectional observational study designed to characterize the immunological status of pregnant women with convalescent COVID-19 at distinct pregnancy trimesters. The study focused on providing a clear snapshot of the interplay among serum soluble mediators.

Methods: A sample of 141 pregnant women from all prenatal periods (1st, 2nd and 3rd trimesters) comprised patients with convalescent SARS-CoV-2 infection at 3-20 weeks after symptoms onset (COVID, n=89) and a control group of pre-pandemic non-infected pregnant women (HC, n=52). Chemokine, pro-inflammatory/regulatory cytokine and growth factor levels were quantified by a high-throughput microbeads array.

Results: In the HC group, most serum soluble mediators progressively decreased towards the 2nd and 3rd trimesters of pregnancy, while higher chemokine, cytokine and growth factor levels were observed in the COVID patient group. Serum soluble mediator signatures and heatmap analysis pointed out that the major increase observed in the COVID group related to pro-inflammatory cytokines (IL-6, TNF-α, IL-12, IFN-γ and IL-17). A larger set of biomarkers displayed an increased COVID/HC ratio towards the 2nd (3x increase) and the 3rd (3x to 15x increase) trimesters. Integrative network analysis demonstrated that HC pregnancy evolves with decreasing connectivity between pairs of serum soluble mediators towards the 3rd trimester. Although the COVID group exhibited a similar profile, the number of connections was remarkably lower throughout the pregnancy. Meanwhile, IL-1Ra, IL-10 and GM-CSF presented a preserved number of correlations (≥5 strong correlations in HC and COVID), IL-17, FGF-basic and VEGF lost connectivity throughout the pregnancy. IL-6 and CXCL8 were included in a set of acquired attributes, named COVID-selective (≥5 strong correlations in COVID and <5 in HC) observed at the 3rd pregnancy trimester.

Discussion and conclusion: From an overall perspective, a pronounced increase in serum levels of soluble mediators with decreased network interplay between them demonstrated an imbalanced immune response in convalescent COVID-19 infection during pregnancy that may contribute to the management of, or indeed recovery from, late complications in the post-symptomatic phase of the SARS-CoV-2 infection in pregnant women.

KEYWORDS

chemokines, cytokines, growth factors, COVID-19, pregnancy

1 Introduction

In March 2020, the World Health Organization (WHO) characterized the outbreak of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) disease (COVID-19) as a pandemic, having since confirmed more than 655 million COVID-19 cases, 6.6 million of which resulted in death (1). SARS-CoV-2 is transmitted through airborne droplets, respiratory secretions, and direct contact. The clinical symptoms relating to the COVID-19 disease were primarily respiratory, and later reported as multisystemic effects. COVID-19 illness symptoms can be asymptomatic, mild, moderate, severe, or critical (2–4). Fever, cough, dyspnea, and myalgia were the most common mild symptoms. The pathogenesis of COVID-19 has been strongly associated with an unbalanced immune response; however, the pathophysiology of the disease remains under investigation (5–7).

Multiple studies concluded that pregnant women are a high-risk population for the COVID-19 disease. Infectious diseases in pregnancy are regularly considered a critical condition. Physiological changes during pregnancy have significant effects on the immune system, cardiopulmonary system and coagulation, and these changes may result in an altered response to COVID-19

infection (3, 8–11). Cytokine levels during pregnancy could be responsible for metabolic imprinting as cytokines are transferable from maternal to fetal circulation and are capable of modulating placental nutrient transfer. Maternal inflammation may induce metabolic reprogramming at several levels, from the periconceptional period onwards. Such processes and their consequences on the maternal and perinatal periods have not been extensively studied to date. Moreover, the maternal immune activation triggered by COVID-19 can have impacts for the mother, pregnancy outcome and offspring (12, 13). The understanding such phenomena should contribute to the proper management of children born to SARS-CoV-2-infected mothers (14).

The aim of the present study was to conduct a prospective observational study designed to characterize the immunological status of pregnant women with convalescent COVID-19, focusing on an overall snapshot of the interplay between serum soluble mediators.

2 Materials and methods

2.1 Study population

This cross-sectional observational study was conducted between July 2020 and December 2021 during the COVID-19 pandemic in the Federal District of Brazil during circulation of the SARS-CoV-2 B.1.1.28 and B.1.1.33 strains. A total of 141 participants were enrolled as non-probability convenience sampling, including pregnant women with convalescent SARS-CoV-2 infection (COVID, n=89) at 3-20 weeks after symptoms onset during the prenatal period (1st, 2nd and 3rd trimesters), together with a healthy control group composed of age-matched pre-pandemic non-infected pregnant women (HC, n=52).

The COVID-19 pregnant women were recruited at two public hospitals - the University Hospital of Brasília and the Asa Norte Regional Hospital, both public reference centers for COVID-19 in the Federal District of Brazil and participants of a large research project named PROUDEST (15). The COVID group comprised pregnant women aged 18-44 years, with a median age of 31 years. This group was further categorized into subgroups according to the pregnancy trimester, referred to as: 1st (n=7), 2nd (n=34) and 3rd (n=48). COVID-19 diagnosis was confirmed by a documented positive RT-PCR test using a nasopharyngeal swab or rapid test (Biomanguinhos, FIOCRUZ, Brazil) for IgM or IgG, during pregnancy. Most of the COVID-19 group (97%, 86 out of 89) presented the non-severe form of the disease. The most common symptoms were: Anosmia (68%), runny nose and/or nasal congestion (68%), headache (67%), ageusia (63%), myalgia (57%), cough (43%), fever (43%), dyspnea (31%), sore throat (31%), asthenia (22%), diarrhea (17%), nausea and vomiting (11%), joint pain (5%), dizziness (4%) and skin diseases (2%). SARS-CoV-2 infection during pregnancy was associated with important adverse maternal and neonatal outcomes, including gestational diabetes mellitus (37%), Apgar score at first minute ≤ 7 (22%), systemic arterial hypertension (18%), fetal restriction growth (11%), preterm labor (11%), acute fetal distress (8%), Apgar score at fifth minute ≤ 7 (5%) and preeclampsia (3%).

The HC group comprised a selected non-probability convenience sampling from a biorepository maintained at Grupo Integrado de Pesquisas em Biomarcadores, Instituto René Rachou, Fundação Oswaldo Cruz (FIOCRUZ-Minas), Belo Horizonte, Brazil. The HC group comprised pregnant women, aged 18- 42 years, with a median age of 28 years. The healthy control group was composed by primiparous with no previous history or current status of obesity, systemic arterial hypertension, diabetes mellitus and without records of pre-eclampsia. The HC group was further categorized into subgroups according to pregnancy trimester, referred to as: 1st (n=21), 2nd (n=10) and 3rd (n=21).

The Table 1 summarize the major demographic and clinical features of the study population.

TABLE 1 Demographic and clinical features of the study population.

Characteristics	GROUPS	
	Healthy Controls – HC (n=52)	COVID-19 – COVID (n=89)
Age, median (min-max)	28 (18-42)	31 (18-44)
Obstetric History		
Previous Pregnancies, median (min-max)	0% (0)	2 (0-6)
Abortions, median (min-max)	0% (0)	0 (0-2)
Complications* % (n)	0% (0)	24% (21)
Current Study		
Obesity % (n)	0% (0)	11% (10)
SAH % (n)	0% (0)	18% (16)
Diabetes % (n)	0% (0)	37% (33)
Pre-eclampsia % (n)	0% (0)	3% (3)

SAH, systemic arterial hypertension. *Obesity, SAH, diabetes, pre-eclampsia.

All study participants provided written informed consent prior to inclusion in accordance with the Helsinki Declaration and Resolution 466/2012 from the Brazilian National Health Council for research involving human subjects. This study was recorded on the Brazilian Registry of Clinical Trials Platform (ReBEC, RBR-65qxs2) and approved by the National Commission for Ethics in Research in Brazil (CONEP, CAAE 32359620.0.0000.5558). The anonymization strategy to protect the identity of participants was achieved by replacing the direct identifiers by standardized alphanumeric codes (*PRAxxxPNy and PRBxxxPNy*), where "*PR*" refer to the PROUDEST project name (15), "*A*" and "*B*" refers to the hospital unit, the "*xxx*" represent the sequential number of patient inclusion, "*PN*" refer to prenatal period and "*y*" the trimester of sample collection.

2.2 Biological samples

Whole blood sample (10 mL) were collected from each participant in vacuum tubes without anticoagulant by venipuncture at the first prenatal appointment or upon enrolment in the study. Serum samples were obtained by centrifugation (1400 x g, 10 min, 4°C) of original samples within 6 h after blood collection. The serum specimens were aliquoted and stored at -80°C until quantification of serum soluble mediators.

2.3 Quantification of serum soluble mediators

Serum soluble mediators were quantified by a high-throughput Luminex microbead multiplex assay (Bio-Plex Pro™ Human Cytokine 27-plex Assay, Bio-Rad Laboratories, Hercules, CA, USA). The manufacturer's instructions were followed to determine the concentrations of chemokines (CXCL8; CCL11; CCL3; CCL4; CCL2; CCL5; CXCL10), pro-inflammatory cytokines (IL-1β; IL-6; TNF-α; IL-12; IFN-γ; IL-15; IL-17), regulatory cytokines (IL-1Ra; IL-4; IL-5; IL-9; IL-10; IL-13) and growth factors (FGF-basic; VEGF; PDGF; G-CSF; GM-CSF; IL-2; IL-7). The assays were conducted in parallel batches by a trained technician at the flow cytometry facility at FIOCRUZ-Minas. The concentrations of serum soluble mediators (pg/mL) were obtained according to a 5-parameter logistic curve fit regression of standard curves.

2.4 Statistical analysis

Descriptive statistics were carried out using the Prism 8.0.2 software (GraphPad Software, San Diego, USA). Data normality was assessed using the Shapiro-Wilk test. Considering the nonparametric distribution of all data sets, multiple comparisons amongst HC and COVID subgroups were carried out using the Kruskal-Wallis followed by Dunn's post-test. Additionally, comparative analysis between HC and COVID at matching trimesters was performed using the Mann-Whitney test. In all cases, statistical significance was considered at $p<0.05$.

The serum soluble mediator signatures were calculated as the proportion (%) of pregnant women with serum levels above the reference values (cut-off) defined as the median Z-score of each soluble mediator detected for all HC along the 1st, 2nd and 3rd trimesters (CXCL8=-0.3; CCL11=-0.3; CCL3=-0.3; CCL4=-0.3; CCL2=-0.4; CCL5=-0.2; CXCL10=-0.2; IL-1β=-0.3; IL-6=-0.3; TNF-α=-0.3; IL-12=-0.3; IFN-γ=-0.4; IL-15=-0.5; IL-17=-0.4; IL-1Ra=-0.4; IL-4=-0.3; IL-5=-0.2; IL-9=-0.2; IL-10=-0.2; IL-13=-0.4; FGF-basic=-0.5; PDGF=-0.4; VEGF=-0.4; G-CSF=-0.2; GM-CSF=-0.4; IL-2=-0.4; IL-7=-0.3). Additionally, trimester-matching signatures were assembled, considering the reference values (cut-off) defined as the median Z-score of each soluble mediator detected for HC trimester subgroups at 1st (CXCL8 = 0.2; CCL11 = 0.1; CCL3 = 0; CCL4=-0.6; CCL2 = 0.4; CCL5=-0.2; CXCL10=-0.7; IL-1β=-0.2; IL-6 = 1.6; TNF-α=-0.1; IL-12=-0.2; IFN-γ=0.1; IL-15=-0.5; IL-17 = 0.1; IL-1Ra=0.1; IL-4=-0.5; IL-5=-0.1; IL-9 = 0.5; IL-10=-0.2; IL-13 = 0.2; FGF-basic=0.3; PDGF=0.2; VEGF=0.1; G-CSF=-0.2; GM-CSF=0.1; IL-2=-0.4; IL-7=-0.2), 2nd (CXCL8=-0.3; CCL11=-0.2; CCL3=-0.3; CCL4=-0.2; CCL2=-0.5; CCL5=-0.1; CXCL10=-0.6; IL-1β=-0.3; IL-6=-0.4; TNF-α=-0.3; IL-12=-0.3; IFN-γ=-0.4; IL-15 = 0.2; IL-17=-0.2; IL-1Ra=-0.5; IL-4 = 0.2; IL-5=-0.2; IL-9=-0.6; IL-10=-0.2; IL-13=-0.4; FGF-basic=-0.6; PDGF=-0.6; VEGF=-0.2; G-CSF=-0.1; GM-CSF=-0.6; IL-2=-0.1; IL-7=-0.2) and 3rd trimesters (CXCL8=-0.4; CCL11=-0.4; CCL3=-0.3; CCL4 = 0.3; CCL2=-0.5; CCL5 = 0.1; CXCL10 = 0.6; IL-1β=-0.4; IL-6=-0.3; TNF-α=-0.3; IL-12=-0.3; IFN-γ=-0.4; IL-15=-0.5; IL-17=-0.4; IL-1Ra=-0.6; IL-4=-0.3; IL-5=-0.2; IL-9=-0.8; IL-10=-0.3; IL-13=-0.4; FGF-basic=-0.6; PDGF=-0.7; VEGF=-0.4; G-CSF=-0.2; GM-CSF=-0.7; IL-2=-0.5; IL-7=-0.3). The serum soluble mediators displaying a proportion above 50% in pregnant women were included in the set of biomarkers with increased levels.

Heatmap constructs were assembled using conditional formatting in Microsoft Excel to illustrate the overall profile of serum soluble mediator signatures of the COVID and HC subgroups along the pregnancy trimesters. The ratio between the proportion of pregnant women with serum levels above the reference values in the COVID group in relation to HC (% COVID/%HC) was also assessed by comparative analysis.

Serum-soluble mediator networks were built based on correlation analysis (Pearson and Spearman rank tests) between pairs of serum-soluble mediators. Only significant strong correlations ($p<0.05$ and "r" scores $\geq |0.67|$) were employed to construct the comprehensive networks. The open-source Cytoscape software (available at https://cytoscape.org) was used to create cluster network layouts comprising the 4 categories of serum soluble mediators - chemokines, pro-inflammatory cytokines, regulatory cytokines, and growth factors. Descriptive analysis of

serum soluble mediator networks was performed by considering the ascendant number of strong correlations to identify the set of biomarkers with five or more strong correlations (≥ 5). Venn Diagram analysis (available at (http://bioinformatics.psb.ugent.be/webtools/Venn/) was performed to assess the preserved (common), lost or acquired (selective) serum soluble mediators with ≥ 5 strong correlations in COVID subgroups compared to trimester-matching HC.

The MATLAB software was employed for Principal Component Analysis (PCA). The PCA data was assembled to verify the ability of serum soluble mediators to cluster convalescent COVID-19 pregnant women from HC, as well as subgroups of COVID-19 as compared to trimester-matching HC. The PCA analysis enabled data dimensionality reduction.

3 Results

3.1 Levels of serum soluble mediators in convalescent COVID-19 at distinct pregnancy trimesters

The levels of chemokines, pro-inflammatory cytokines, regulatory cytokines and growth factors were measured in serum samples from pregnant women with convalescent COVID-19 at 3-20 weeks after symptoms onset (COVID) and compared with those detected in trimester-matching pre-pandemic non-infected pregnant women as a healthy control (HC). The results are presented in Figures 1 and 2.

In general, healthy pregnant women presented a progressive decrease in most serum soluble mediators towards the 2nd and 3rd pregnancy trimester, including: chemokines (CXCL8, CCL11, CCL3 and CCL2); pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, IL-12, IFN-γ, and IL-17); regulatory cytokines (IL-1Ra, IL-4, IL-5, IL-9, IL-10 and IL-13), and growth factors (FGF-basic, VEGF, PDGF, GM-CSF and IL-7). Conversely, progressive increases in CCL4, CCL5, CXCL10 and G-CSF were observed in the HC group. No difference was observed in the HC group for IL-15 and IL-2 (Figures 1, 2).

Overall, higher levels of the most soluble mediators were observed in convalescent COVID-19 pregnant women compared to the healthy controls, especially at the 2nd and 3rd trimesters, including higher levels of CXCL8; CCL11; CCL2; CCL3; IL-1β; IL-6; TNF-α; IL-12; IFN-γ; IL-17; IL-1Ra; IL-5; IL-9; IL-10; IL-13; FGF-basic; VEGF, and GM-CSF. Conversely, lower levels of CCL4, CCL5, CXCL10, G-CSF and IL-7 were observed towards the 2nd and 3rd trimesters in the COVID group compared to the HC group (Figures 1, 2).

Additional analysis amongst the COVID subgroups along the pregnancy trimesters demonstrated an inverted profile of CCL3, IL-1Ra and FGF-basic towards higher levels in the 3rd trimester (Figures 1, 2).

Supplementary Figure 1 summarizes the major changes observed in serum soluble mediators along the trimesters of healthy and convalescent COVID-19 pregnancy.

3.2 Serum soluble mediator signatures in convalescent COVID-19 at distinct pregnancy trimesters

Serum soluble mediator signatures were assembled as the percentage of pregnant women with serum levels above the reference values defined as the median Z-score of each soluble mediator detected in all healthy controls along the pregnancy. The results are presented in Figure 3.

Data analysis demonstrated that the proportion of healthy pregnant women with high levels of serum soluble mediators progressively decreased towards the 2nd and 3rd pregnancy trimesters. These data further corroborated that a healthy pregnancy course has a progressive decrease in most serum soluble mediators towards the 2nd and 3rd trimesters, except for CCL4, CCL5 and CXCL10 (Figure 3A).

On the other hand, the proportion of pregnant women with convalescent COVID-19 presenting high serum soluble mediator levels progressively increased from the 1st to the 3rd pregnancy trimester (Figure 3A). Heatmap constructs further illustrated that the major increase in serum soluble mediators observed in pregnant women with convalescent COVID-19 occurred in pro-inflammatory cytokines, namely IL-6, TNF-α, IL-12, IFN-γ and IL-17 (Figure 3B).

The profile of serum soluble mediators was further characterized as the ratio (%COVID/%HC), assessed by dividing the percentage of pregnant women with soluble mediator levels above the reference values observed in the COVID group by the percentage of trimester-matching HC patients. Using this strategy, the results confirmed that a larger set of biomarkers presented a high ratio (%COVID/%HC) towards the 2nd and 3rd trimester. In the 2nd pregnancy trimester, increased ratios were observed for IL-6, IFN-γ, IL-5 and GM-CSF (3x increase) in the COVID-19 group. A larger set of serum soluble mediators with increased ratios was identified for COVID-19 groups at the 3rd pregnancy trimester, including CXCL8, CCL11, IL-5 and PDGF (3x increase), CCL3, IL-1β, IFN-γ, IL-17 and IL-13 (4x increase), CCL2, TNF-α (7x) along with IL-1Ra, IL-9, GM-CSF and FGF-basic (5x, 9x, 9x, and 15x increase, respectively) (Figure 3C).

The signatures of serum soluble mediators were also assessed considering the reference values of trimester-matching healthy controls. The results are presented in the Supplementary Figure 2. Data reinforce that larger sets of serum soluble mediators with increased ratios were identified for the 2nd and 3rd pregnancy trimesters as compared with trimester-matching controls (Supplementary Figure 2).

3.3 Serum soluble mediator networks in convalescent COVID-19 at distinct pregnancy trimesers

Aimed at assessing a panoramic snapshot of serum soluble mediator interplay in pregnant women with convalescent COVID-19 and healthy controls, integrative networks were constructed

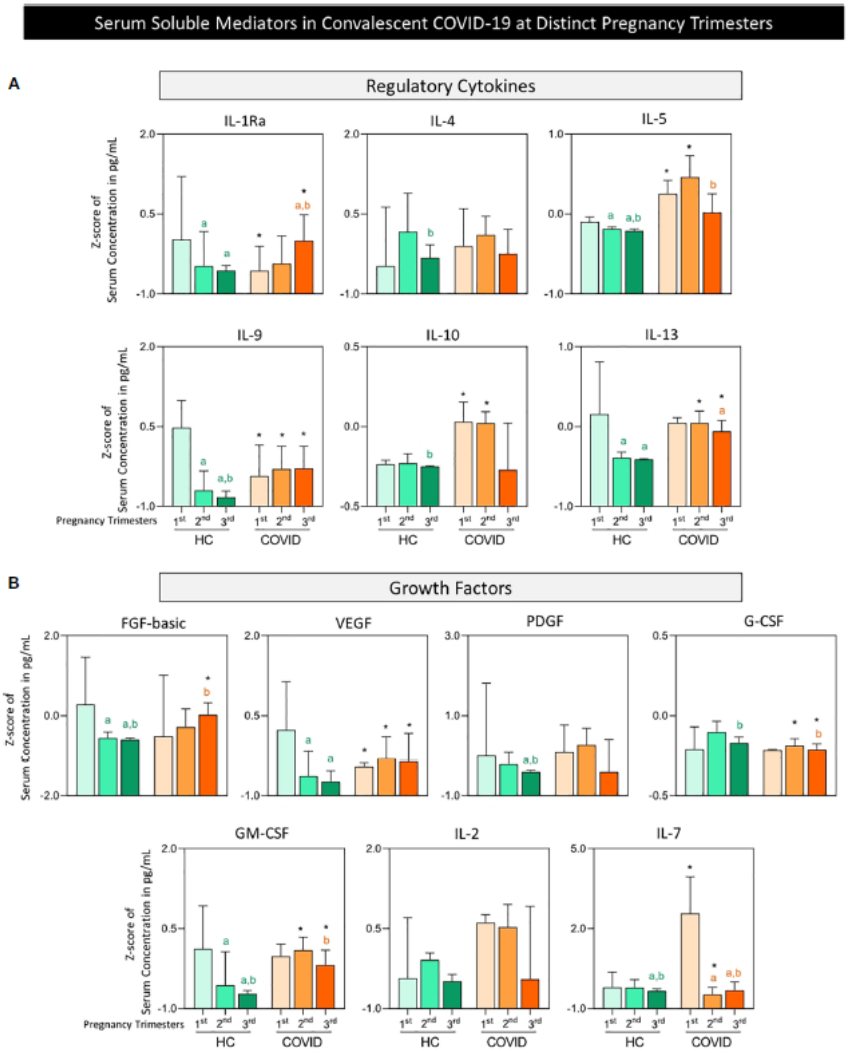

FIGURE 2
Serum regulatory cytokines and growth factors in convalescent COVID-19 patients at distinct pregnancy trimesters. The levels of: **(A)** regulatory cytokines (IL-1Ra, IL- 4, IL-5, IL-9, IL-10, IL-13), and **(B)** growth factors (FGF-basic, PDGF, VEGF, G-CSF, GM- CSF, IL-2, IL-7) were measured in serum samples from pregnant women with convalescent COVID-19 at 3-20 weeks after symptoms onset (COVID, n=89), with pre-pandemic non-infected pregnant women as a Healthy Control (HC, n=52). The HC and COVID-19 groups were further categorized into subgroups according to pregnancy trimester, referred to as: HC 1st (, n=21), HC 2nd (, n=10), HC 3rd (, n=21), and COVID 1st (, n=7), COVID 2nd (, n=34), COVID 3rd (, n=48). The measurements were taken by high-throughput multiplex bead array as described in Material and Methods. The results are presented in bar charts of median values and interquartile ranges for Z-score of serum concentration (pg/mL). Multiple comparative analysis was performed by Kruskal-Wallis followed by Dunn's post-test, and comparisons between COVID-19 and HC at matching pregnancy trimesters assessed by the Mann-Whitney test. In all cases, significance was considered at $p<0.05$. Intragroup differences were underscored by the letters "a" and "b" for comparisons with the 1st and 2nd trimesters, respectively. Inter-group differences at matching pregnancy trimesters were denoted by an asterisk (*).

correlations, respectively), with growth factor predominance at the 3rd trimester (42 strong correlations). Conversely, the COVID group displayed a predominance of regulatory cytokines in the 1st trimester (47 strong correlations) with growth factor predominance in the 2nd and 3rd trimesters (24 and 21, respectively) (Figure 4).

In general, convalescent COVID-19 infection during pregnancy leads to a loss of network connectivity, with fewer strong correlations and changes in the predominance of connectivity amongst the categories of serum soluble mediators (Figure 4).

3.4 Descriptive analysis of serum soluble mediator networks in convalescent COVID-19 patients at distinct pregnancy trimesters

In order to provide a more comprehensive overview of the network connectivity between serum soluble mediators in pregnant women with convalescent COVID-19 and healthy controls along the pregnancy trimesters, a descriptive Venn diagram analysis was

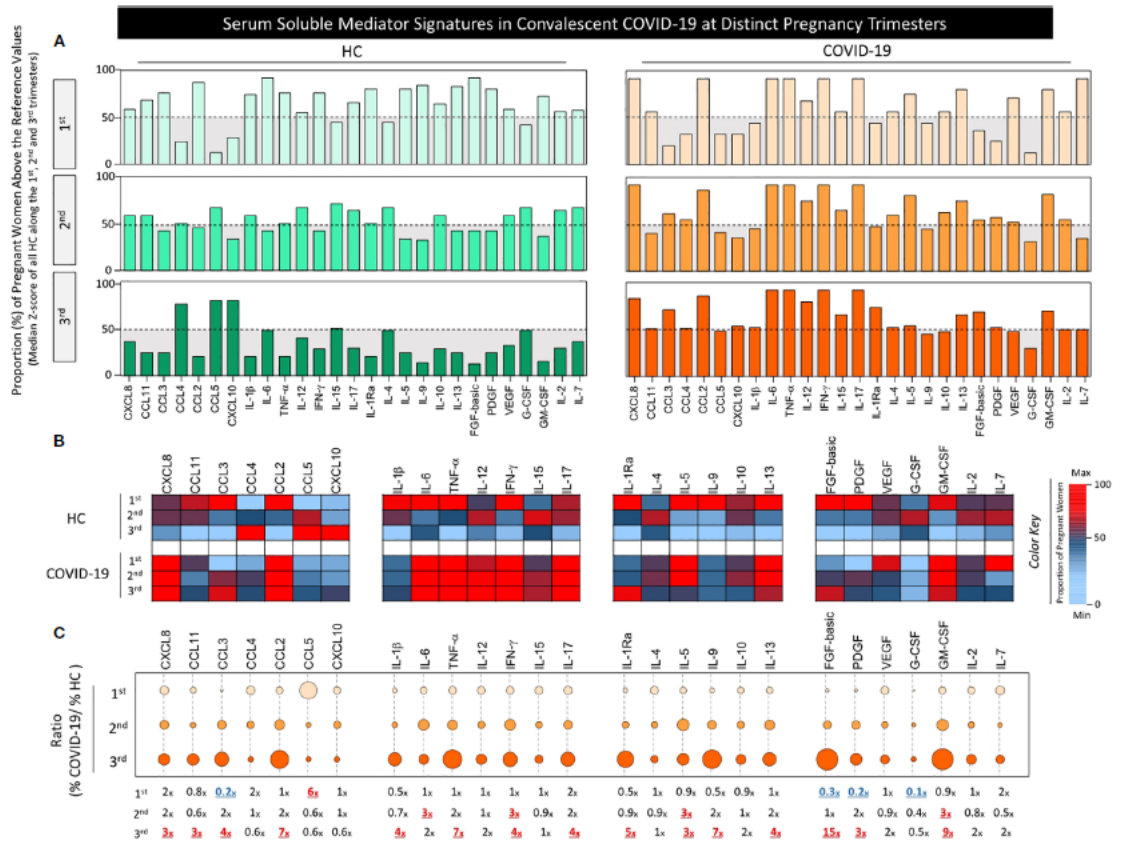

FIGURE 3
Serum soluble mediator signatures in convalescent COVID-19 patients at distinct pregnancy trimesters. Signatures of: serum chemokines (CXCL8, CCL11, CCL3, CCL4, CCL2, CCL5, and CXCL10), pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, IL-12, IFN-γ, IL-15, and IL-17), regulatory cytokines (IL-1Ra, IL-4, IL-5, IL-9, IL-10, and IL-13), and growth factors (FGF-basic, PDGF, VEGF, G-CSF, GM-CSF, IL-2, and IL-7) were assembled for pregnant women with convalescent COVID-19 at 3-20 weeks after symptoms onset (COVID, n=89) and for pre-pandemic non-infected pregnant women as a Healthy Control (HC, n=52). The HC and COVID-19 groups were further categorized into subgroups according to pregnancy trimester, referred to as: HC 1st (n=21), HC 2nd (n=10), HC 3rd (n=21) and COVID 1st (n=7), COVID 2nd (n=34), COVID 3rd (n=48). The measurements were taken by high-throughput multiplex bead array as described in Material and Methods. **(A)** The results are presented in bar charts showing the proportion (%) of pregnant women with serum levels above the reference values (cut-off) defined as the median Z-score of each soluble mediator detected for all HC along the 1st, 2nd and 3rd trimesters, as described in Material and Methods. The serum soluble mediators displaying a proportion of pregnant women above 50% (grey zone, dashed line) were included in the set of biomarkers with increased levels. **(B)** Heatmap constructs were further assembled to illustrate the overall profile of serum soluble mediator signatures of COVID and HC subgroups along the pregnancy trimesters. A color key was used to underscore the serum soluble mediators with decreased (proportion <50%, towards light blue), unaltered (proportion =50%, black) or increased levels (proportion>50%, towards red). **(C)** The ratio between the proportion of pregnant women with serum levels above the reference values in the COVID group in relation to HC (%COVID/%HC) was further calculated and presented in orbital graphs. The ratios of each serum soluble mediator along the 1st, 2nd and 3rd trimesters are provided in the figure, underscored as decreased (≤0.3x, blue), unaltered (0.4-2x, black) or increased (≥3x, red).

performed to identify the set of biomarkers with preserved (common), lost or acquired (selective) attributes with five or more (≥ 5) strong correlations in COVID subgroups as compared to the trimester-matching HC group. The results are presented in the Figure 5.

Heatmap constructs were assembled to organize the serum soluble mediators with an ascending order of strong correlations and identify the set of biomarkers with five or more (≥ 5) strong correlations at each pregnancy trimester in the COVID and HC groups (Figure 5A).

Data analysis demonstrated that the number of preserved attributes referred to as common in HC and COVID (≥5 strong correlations in HC and COVID) with five or more correlations progressively decreased from the 1st (n=12) to the 2nd (n=6) and 3rd trimesters (n=4). In detail: 1st: CCL11, CCL3, CCL2, IL-1β, IL-12, IL-15, IL-1Ra, IL-5, IL-9, IL-10, GM-CSF, and IL-2; 2nd: IL-6, TNF-α, IL-1Ra, IL-5, IL-10, GM-CSF and 3rd:IFN-γ, IL-1Ra, G-CSF, and GM-CSF.

The number of lost attributes referred to as HC-selective (≥5 strong correlations in HC and <5 strong correlations in COVID)

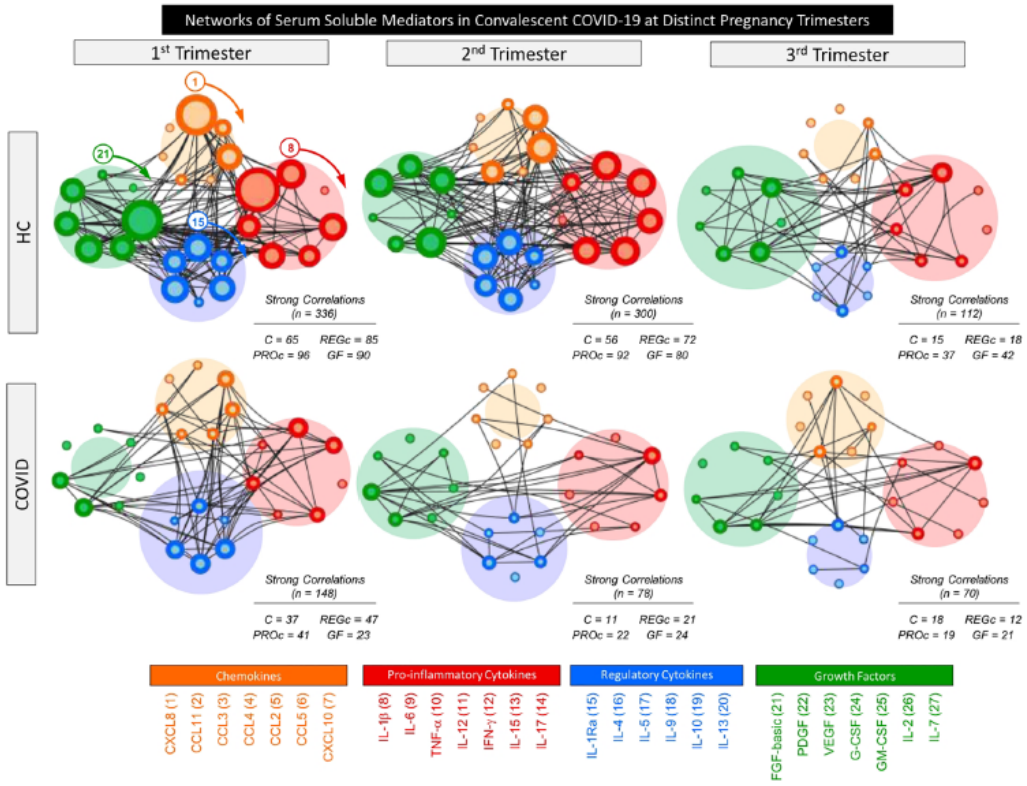

FIGURE 4
Networks of serum soluble mediators in convalescent COVID-19 patients at distinct pregnancy trimesters. Comprehensive networks were assembled for serum chemokines, pro-inflammatory cytokines, regulatory cytokines, and growth factors observed in pregnant women with convalescent COVID-19 at 3-20 weeks after symptoms onset (COVID, n=89), with pre-pandemic non-infected pregnant women as a Healthy Control (HC, n=52). The HC and COVID-19 groups were further categorized into subgroups according to pregnancy trimester, referred to as: 1st (HC=21 and COVID=7), 2nd (HC=18 and COVID=34) and 3rd (HC=21 and COVID=48). The measurements were taken by high-throughput multiplex bead array as described in Material and Methods. Data analyses were carried out by Pearson and Spearman rank tests with only significant strong correlations ($p<0.05$ and "r" scores $\geq |0.67|$) employed to construct the comprehensive networks. Cluster layout networks were assembled, comprising 4 categories of serum soluble mediators as follows: - Chemokines – C (orange nodes – 1=CXCL8; 2=CCL11; 3=CCL3; 4=CCL4; 5=CCL2, 6=CCL5 and 7=CXCL10); Pro-inflammatory – PROc (red nodes – 8= IL-1β; 9=IL-6; 10= TNF-α; 11=IL-12; 12= IFN-γ; 13=IL-15 and 14=IL-17); Regulatory cytokines – REGc (blue nodes – 15=IL-1Ra; 16=IL-4; 17=IL-5; 18=IL-9; 19=IL-10 and 20=IL-13), and Growth Factors – GF (green nodes – 21=FGF-basic; 22=PDGF; 23=VEGF; 24=GCSF; 25=GM-CSF; 26=IL-2 and 27=IL-7). Node border thickness is proportional to the number of strong correlations. Connecting edges (black lines) are used to link pairs of serum soluble mediators presenting significant correlations. The number of strong correlations (C, PROc, REGc and GF) observed for each network is provided in the figure and used for comparative analysis between COVID and HC, as well as amongst subgroups. The circular background area is proportional to the number of strong correlations of each cluster within the respective network.

was higher in the 2nd trimester (n=15) compared to 1st (n=10) and 3rd (n=9). In detail: 1st: CXCL8, TNF-α, IFN-γ, IL-17, IL-4, IL-13, FGF-basic, VEGF, G-CSF, and IL-7; 2nd: CXCL8, CCL11, CCL3, CCL2, IL-1β, IL-12, IFN-γ, IL-17, IL-4, IL-9, IL-13, FGF-basic, VEGF, G-CSF, and IL-7; 3rd: CCL11, CCL3, IL-1β, IL-12, IL-15, IL-17, IL-9, FGF-basic, and VEGF. A set of acquired attributes, named COVID-selective (<5 strong correlations in HC and ≥5 strong correlations in COVID) were identified in each trimester: 1st (n=3): CCL4, CCL5, and IL-6, 2nd: (n=1) IL-2, and 3rd (n=3) CXCL8, CCL2 and IL-6 (Figure 5B).

From an overall perspective, a pronounced decrease in network connectivity between serum soluble mediators was observed in convalescent COVID-19 infection during pregnancy as demonstrated by the fewer number of molecules establishing strong correlations driven by an imbalance between preserved, lost and acquired attributes in the COVID group. While IL-1Ra, IL-10 and GM-CSF presented a preserved number of correlations (≥5 strong correlations in HC and COVID), IL-17, FGF-basic and VEGF lost connectivity throughout pregnancy. IL-6 (at 1st and 3rd trimesters) and CXCL8 (at 3rd trimester) were included in a set of acquired

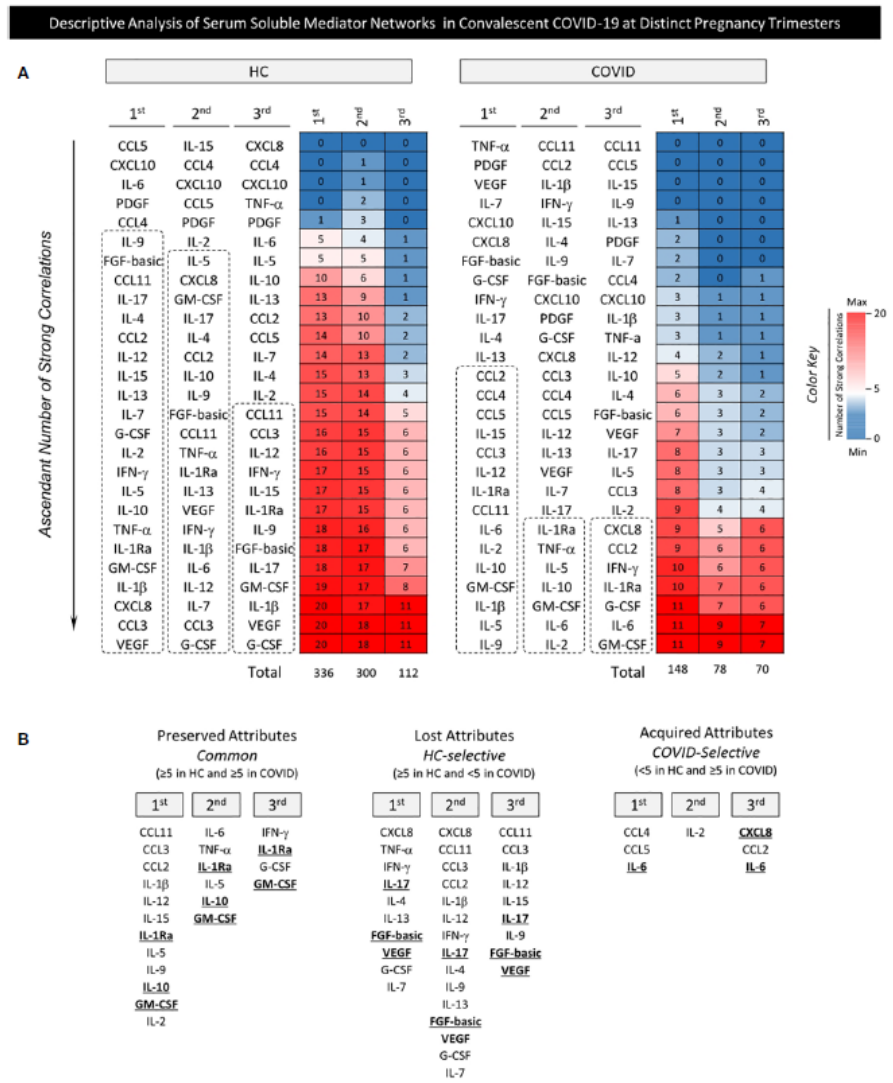

FIGURE 5
Descriptive analysis of serum soluble mediator networks in convalescent COVID-19 patients at distinct pregnancy trimesters. The overall profile of serum soluble mediator networks was assessed in pregnant women with convalescent COVID-19 at 3-20 weeks after symptoms onset (COVID, n=89) with pre-pandemic non-infected pregnant women as a Healthy Control (HC, n=52). The HC and COVID-19 groups were further categorized into subgroups according to pregnancy trimester, referred to as: 1st (HC=21 and COVID=7), 2nd (HC=10 and COVID=34), and 3rd (HC=21 and COVID=48). **(A)** The ascendant number of strong correlations was arranged to identify the set of biomarkers with five or more (≥ 5) strong correlations with other molecules at each pregnancy trimester in HC and COVID subgroups. Heatmap constructs were assembled to illustrate the overall profile of serum soluble mediator networks of COVID and HC subgroups along the pregnancy trimesters. A color key was used to underscore the serum soluble mediators with ≥ 5 strong correlations (towards red). **(B)** A summary of preserved (≥5 in HC and ≥5 in COVID), lost (≥5 in HC and <5 in COVID) or acquired (<5 in HC and ≥5 in COVID) attributes were identified by Venn diagram analysis. Attributes identified along the trimesters are highlighted by bold underline format.

attributes, named COVID-selective (≥5 strong correlations in COVID and <5 in HC) (Figure 5B, bold underline attributes).

3.5 Multivariate analysis of serum soluble mediators in convalescent COVID-19 patients at distinct pregnancy trimesters

Multivariate analysis of chemokines, pro-inflammatory cytokines, regulatory cytokines and growth factors was performed using PCA to verify the ability of serum mediators to cluster convalescent COVID-19 pregnant women apart from trimester-matching pre-pandemic non-infected pregnant women as a healthy control (HC). The results are presented in Figure 6. The PCA coordinates (2nd and 3rd principal components) demonstrated that although convalescent COVID pregnant women could be clustered apart from the HC when considering all trimesters together, the segregation profile was more evident when the COVID and HC

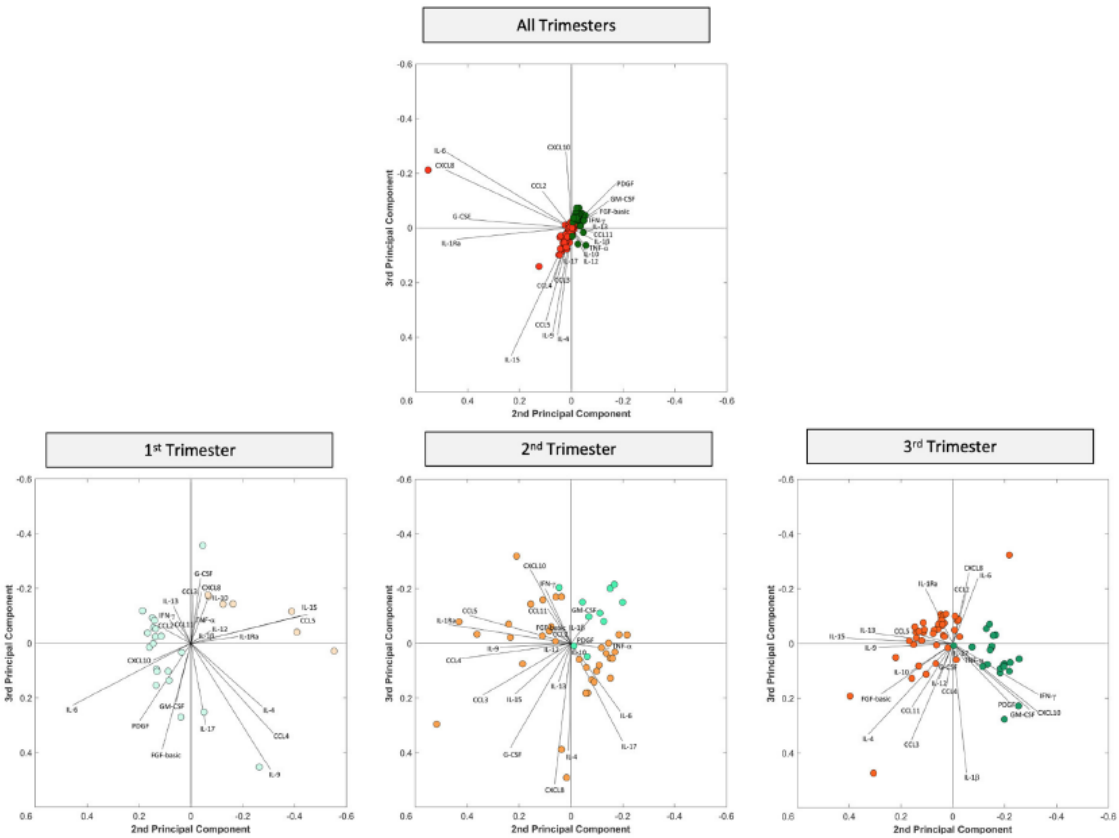

FIGURE 6
Multivariate analysis of serum soluble mediator networks in convalescent COVID-19 patients at distinct pregnancy trimesters. Multivariate analysis of serum chemokines, pro-inflammatory cytokines, regulatory cytokines and growth factors were performed to verify the ability of soluble mediators to cluster convalescent COVID-19 pregnant women at 3-20 weeks after symptoms onset (COVID, n=89), with pre-pandemic non-infected pregnant women as a Healthy Control (HC, n=52) as well as subgroups of COVID-19 and HC categorized according to pregnancy trimester, referred to as: HC 1st (○, n=21), HC 2nd (○, n=10), HC 3rd (○, n=21) and COVID 1st (○, n=7), COVID 2nd (○, n=34), COVID 3rd (○, n=48). The measurements were taken by high-throughput multiplex bead array as described in Material and Methods. Principal Component Analysis (PCA) was carried out by MATLAB software as described in Material and Methods. PCA coordinates (2nd and 3rd principal components) were used to compare and visualize the grouping of convalescent COVID-19 *vs* HC and subgroups according to pregnancy trimesters.

subgroups were compared at matching gestational trimesters (Figure 6). Vector analysis conducted in the 1st trimester indicated that CXCL8, CCL3, CCL5, IL-1β, TNF-α, IL-12, IL-15, IL-1Ra, IL-10, and G-CSF were associated with convalescent COVID-19 in pregnant women. Data from the 2nd trimester showed that most soluble mediators were vectors associated with differential distribution of convalescent COVID-19 in pregnant women, except for GM-CSF. Additionally, the PCA coordinates obtained from the 3rd trimester demonstrated that several soluble mediators were vectors related to convalescent COVID-19 in pregnant women, except for CXCL10, IL-1β, TNF-α, IFN-γ, PDGF and GM-CSF (Figure 6).

4 Discussion

Pregnancy triggers a unique immunological status, aiming to protect the fetus from maternal rejection and guarantee fetal development until birth. Several studies have reported that the immune system plays a balancing role during pregnancy with constant changes according to maternal and fetal demands (16, 17). Physiological changes in immune status during pregnancy are often characterized by alterations in cell-mediated immunity and humoral responses, from the 1st to 3rd pregnancy trimesters. Previous studies have demonstrated that successful implantation is associated with a transient increase in systemic proinflammatory

maternal pro-inflammatory rejection and guarantee fetal development until birth.

Our data demonstrated that IL-1Ra decreased in convalescent COVID-19 pregnant women in the 1st trimester but increased in the 3rd trimester. Previous studies reported that IL-1Ra levels increased during the inflammatory response to control acute inflammation and prevent immunopathological events (38). The IL-1 receptor antagonist (IL-1Ra) is an anti-inflammatory cytokine that blocks IL-1α and IL-1β functions and modulates their biological effects (39). It has been previously demonstrated in experimental models that high IL-1Ra levels at the beginning of pregnancy may lead to miscarriage due to impaired embryonic adhesion (40), and data from human studies showed that higher levels of circulating IL-1Ra have been reported in adverse pregnancy outcomes, including preeclampsia (41). Regarding the changes in IL-1Ra levels observed in convalescent COVID-19 along the pregnancy trimesters, our findings of preserved correlation profile between IL-1Ra and other soluble mediators throughout the pregnancy may suggest that an intricate microenvironment of soluble mediators is relevant to guarantee fetal development until birth.

Our data also demonstrated that GM-CSF presented preserved correlation with other soluble mediators throughout pregnancy. It was previously reported that after embryo implantation, GM-CSF participates in a network of cytokines and growth factors that regulate morphological and functional development of the placenta (42).

Conversely, despite increases in IL-17, FGF-basic and VEGF, loss of connectivity was observed throughout pregnancy. IL-17 up-regulates the expression of a variety of biological molecules with angiogenic properties including VEGF (43–47). VEGF plays a central role in vasculogenesis and angiogenesis, which augments vascular endothelial cell proliferation, migration, and survival. Moreover, data from previous studies have shown that IL-17 can induce placental oxidative stress and vascular dysfunction, resulting in hypertension and increased risk of preeclampsia (48). The loss of network connection of IL-17 and VEGF with other soluble mediators throughout the pregnancy may lead to intrinsic vascular dysfunction that results in impaired neonatal development. Post-natal follow-up studies may contribute to identifying impaired new-born growth and development related to altered angiogenesis.

Our data also demonstrated that IL-6 and CXCL8 were included in the set of attributes acquiring strong correlation in the 3rd pregnancy trimester, named COVID-selective correlations. Implications of IL-6 and CXCL8 in pregnancy-associated pathological conditions, such as pregnancy loss, preeclampsia, gestational diabetes mellitus, and infection/inflammation have been reported (35). These two soluble mediators are abundantly produced at the feto-maternal interface throughout pregnancy and have been shown to participate in several pregnancy-related events. Unbalanced expression/secretion of IL-6 and CXCL8 at the feto-maternal interface has been indicated in unexplained pregnancy loss (35). A study of the dynamic connections of the soluble mediator network in pre-eclampsia identified positive correlation between IL-6 and CXCL8, suggesting that these molecules are implicated in the pathophysiology of this pregnancy-associated disease (35, 49). Moreover, a meta-analysis and systematic review suggested a role of CXCL8 in shaping the immune microenvironment in gestational diabetes mellitus (50).

The present study has some limitations. The low number of pregnant women enrolled in each pregnancy trimester re-enforce the need to further validate our findings. This work was performed during circulation of the B.1.1.28 and B.1.1.33 SARS-CoV-2 strains and therefore, the impact of other variants on the immunological profiles remains to be addressed. Despite the pioneering approach of this exploratory investigation, the observational design with multiple comparisons without corrections for co-morbidities or other confounding variables also constitutes a study limitation that may interfere in the levels of systemic soluble mediators. Moreover, regardless the relevance of nutritional aspects ant the dietary inflammatory indices interfering in the immune response during pregnancy (51), we did not have the opportunity to address this issue in the present investigation.

In conclusion, the main finding of this study, a pronounced increase in serum levels of soluble mediators with decreased network interplay between them, portrayed an imbalanced immune response in convalescent COVID-19 infection during pregnancy that may contribute to the prevention or management of clinical course pregnancy complications.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by National Commission for Ethics in Research in Brazil (CONEP, CAAE 32359620.0.0000.5558). The patients/participants provided their written informed consent to participate in this study.

Author contributions

Study design: GF, LS, FM, CA, KC, MC, RT, ON, AS, CPA, AZ and LM. Advisory committee: CG, DM-S, PK, ON, LC, COA, and AZ. Funding acquisition: LE, OM-F and LM. Sample collection, experimental procedures, and data acquisition: ÂS, AC, YP, DA, LL, RN, PA, LG, LD, and JCdR. Data analysis: GF, LS, GJ-S, HS, LA, MG, PB, JBdS, IC-R, AC-A, VP-M, AC, and OM-F. Writing and reviewing the manuscript: GF, LS, GJ-S, HS, OM-F and LM. All authors contributed to the article and approved the submitted version.

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APÊNDICE E – FICHA CLÍNICA

FICHA PRIMEIRA CONSULTA - IGc /IC:

Consulta:

Nome:	
Cuidadores	

IDENTIFICAÇÃO			
IG: / Apresentação		PN: / PC / Compr.	
DN:		Termo em:	
Maternidade:		Dias de internação:	
Alta	Dia:	Peso:	Comprimento
Convênio:			

DADOS DA FAMÍLIA	
Religião:	
Idade:	Idade:
Profissão:	Profissão:
AF:	AF:
Casa:	Casa:
Animais:	Animais:
Cigarro:	Cigarro:

Rede de Apoio:	Rede de Apoio:

DIAGNÓSTICOS ANTERIORES	
1.	2.
3.	4.
5.	6.
7.	8.
9.	10.

TRIAGENS NEONATAIS		
T. pezinho		
Orelhinha		
Coração		
Olho		
Ortolani/Barlow	Fator de risco:	Resultado
USG Crânio		
DMO		
Anemia		

ESPECIALIDADES/ TERAPIAS			
Especialidade	Data	Diagnóstico	Retorno

ALIMENTAÇÃO ROTINA		
Café		
Lanche		
Almoço		
Lanche		
Jantar		
Ceia		

Eliminações
Sono
Bicos Artificiais

Vacinação

DESENVOLVIMENTO	
Motor Grosseiro	
Motor Fino	
Social	
Linguagem	

EXAME FÍSICO			
Peso:	Comprimento	PC	IMC

DIAGNÓSTICOS ATUAIS	
Clínico	
Vacinal	
Nutricional	
Crescimento	
Desenvolvimento	

CONDUTAS

ANEXO A – Parecer do CEP

PARECER CONSUBSTANCIADO DA CONEP

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Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeitos do SARS CoV-2 sobre gestação, parto, puerpério, período neontatal e desenvolvimento infantil: estudo de coortes, prospectivo multicêntrico

Área Temática:

Versão: 1

CAAE: 32359620.0.0000.5558

Instituição Proponente: EMPRESA BRASILEIRA DE SERVICOS HOSPITALARES - EBSERH

Patrocinador Principal: Financiamento Próprio

UNB - FACULDADE DE MEDICINA DA UNIVERSIDADE DE BRASÍLIA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeitos do SARS CoV-2 sobre gestação, parto, puerpério, período neontatal e desenvolvimento infantil: estudo de coortes, prospectivo multicêntrico

Área Temática:

Versão: 1

CAAE: 32359620.0.0000.5558

Instituição Proponente: EMPRESA BRASILEIRA DE SERVICOS HOSPITALARES - EBSERH

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.055.854

Apresentação do Projeto:

Trata-se de estudo observacional, longitudinal,prospectivo, que acompanhará mulheres com idade igual ou superior a 18 anos, de qualquer idade gestacional, no período de julho a dezembro de 2020 e os bebês nascidos dessas gestações, no período de junho de 2020 a junho de 2025, com o intuito de avaliar os efeitos da COVID-19 na gestação, puerpério, período neonatal e desenvolvimento infantil de crianças nascidas de mães sob infecção por COVID-19

Objetivo da Pesquisa:

Os autores relatam que pretendem determinar os efeitos da infeção por SARS-CoV-2 na gestação, parto e puerpério e comparar os achados clínicos, imageológicos, laboratoriais e de desenvolvimento a longo prazo entre filhos de mães expostas e não expostas à infecção pelo SARS-CoV-2 durante a gestação e ainda :

i. Avaliar o efeito da COVID-19 nos indicadores de morbidade e mortalidade materna; ii. Avaliar o efeito da COVID-19 nos indicadores de

morbidade obstétrica: aborto, malformação fetal, distúrbios do crescimento fetal, parto prematuro e patologias obstétricas, como pré-eclâmpsia.iii.

Investigar a presença do SARS-CoV-2 e/ou de anticorpos anti-SARS-CoV-2 no sangue materno, sangue do cordão umbilical e, naquelas mulheres

submetidas a raque anestesia para cesárea, no líquor. iv. Investigar a presença de marcadores

inflamatórios no sangue materno, sangue do cordão
umbilical e, naquelas mulheres submetidas a raque anestesia para cesárea, no líquor. v. Investigar a presença de sinais histológicos e/ou
marcadores moleculares no tecido placentário de mulheres infectadas pelo SARS-CoV-2vi. Avaliar o efeito da COVID-19 nos indicadores de
morbidade e mortalidade neonatal precoce; vii. Avaliar o efeito da COVID-19 nos indicadores de avaliação de sucesso do aleitamento materno;viii.
Avaliar o efeito da COVID-19 na saúde global de crianças filhas de mães infectadas em qualquer fase do período gestacional e neonatal precoce; ix.
Avaliar o efeito da COVID-19 no desenvolvimento somático, metabolismo energético e neurológico de crianças filhas de mães infectadas em
qualquer fase do período gestacional e neonatal precoce

Avaliação dos Riscos e Benefícios:

s autores referem que trata-se de estudo considerado de risco mínimo.

Existe risco de constrangimento por ter sido portadora de COVID-19. Para minimizar esse efeito, as participantes serão atendidas em um ambulatório especificamente criado para esse fim, além de que todos os dados serão mantidos em sigilo.

A coleta de sangue será realizada em uma veia do antebraço, com agulha e seringa estéreis. Há risco de equimose ou sangramento local inerente à coleta. O procedimento será realizado por profissional treinado, para minimizar qualquer complicação. O paciente será orientado sobre como proceder no caso de complicação relacionada ao procedimento.

Benefícios:

Os autores consideram que entre os benefícios, destacam-se primeiro os de caráter coletivo, pois através dos resultados obtidos será possível um melhor entendimento dos efeitos da doença durante a gestação e das suas consequências a longo prazo nos filhos de mulheres acometidas.

Um importante benefício para as mulheres é que será oferecido um acompanhamento pré-natal integral, com consultas, exames laboratoriais e de
imagem realizados no Hospital Universitário de Brasília (HUB), assim como a assistência ao parto nesse hospital.

Igualmente, aos filhos das participantes será garantido o acompanhamento do crescimento e desenvolvimento no ambulatório de pediatria.

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Comentários e Considerações sobre a Pesquisa:

Por meio de um estudo observacional pretende-se avaliar se: a exposição materna à infecção pelo vírus SARS-CoV-2 durante a gestação e puerpério está associada a riscos diretos à própria gestação, à gestante e ao feto em desenvolvimento? A gestação em si poderia exercer efeito protetor sobre o binômio mãe-feto em relação a formas graves da COVID-19? A exposição intraútero à infecção pelo vírus SARS-CoV2 está associada a alteração no desenvolvimento infantil?

Considerações sobre os Termos de apresentação obrigatória:

O projeto encontra-se elaborado de acordo com as normas e resoluçoes do sistema CEP/Conep com TCLE adequado, riscos e benefícios, critérios de inclusão e exclusão e cronograma.

Recomendações:

Recomenda-se : 1-atualizar o cronograma 2- considerando o auto custo do orçamento informar se haverá fonte de fomento 3- os exames complementares específicos para o projeto serão custeados pelo SUS ou haverá recursos financeiros para os mesmos? 4-Consta como beneficios :Um importante benefício para as mulheres é que será oferecido um acompanhamento pré-natal integral, com consultas, exames laboratoriais e de

imagem realizados no Hospital Universitário de Brasília (HUB), assim como a assistência ao parto nesse hospital.Igualmente, aos filhos das participantes será garantido o acompanhamento do crescimento e desenvolvimento no ambulatório de pediatria.5- Esse termo deverá ser retirado como benefício para os pacientes pois trata-se de procedimentos de rotina e direito dos pacientes.

Conclusões ou Pendências e Lista de Inadequações:

O colegiado considerou que com intuito de não atrasar o desenvolvimento do projeto o mesmo ficará como aprovado.Entretanto, as recomendações deverão ser atendidas e serão verificadas por ocasiao do relatório parcial.

Considerações Finais a critério do CEP:

Projeto apreciado na Reunião Ordinária do CEP-FM-UnB- 05/2020. Após apresentação do parecer do (a) Relator (a), aberta a discussão para os membros do Colegiado. O projeto foi Aprovado com as recomendações elencadas acima.

De acordo com a Resolução 466/2012-CONEP/CNS, itens X.1. - 3.b. e XI. -2.d, este Comitê chama a atenção da obrigatoriedade de envio do relatório parcial semestral e final do projeto de pesquisa para o CEP -FM, através de Notificações submetidas pela Plataforma Brasil, contados a partir da data de aprovação do protocolo de pesquisa e recomenda fortemente o atendimento das

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observações elencadas.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1556469.pdf	25/05/2020 14:40:33		Aceito
Cronograma	Cronograma_Proudest.docx	25/05/2020 14:28:52	GERALDO MAGELA FERNANDES	Aceito
Orçamento	Orcamento.docx	25/05/2020 14:26:43	GERALDO MAGELA FERNANDES	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_detalhado.docx	25/05/2020 14:24:27	GERALDO MAGELA FERNANDES	Aceito
Declaração de Pesquisadores	Carta_pesquisadores.pdf	25/05/2020 14:20:01	GERALDO MAGELA FERNANDES	Aceito
Declaração de Pesquisadores	resumo_estruturado.docx	25/05/2020 14:16:59	GERALDO MAGELA FERNANDES	Aceito
Declaração de Pesquisadores	12_Termo_de_responsabilidade.docx	25/05/2020 14:15:06	GERALDO MAGELA FERNANDES	Aceito
Declaração de Pesquisadores	Zaconeta.pdf	25/05/2020 14:07:51	GERALDO MAGELA FERNANDES	Aceito
Declaração de Pesquisadores		25/05/2020 14:07:41	GERALDO MAGELA FERNANDES	Aceito
Declaração de Pesquisadores		25/05/2020 14:07:27	GERALDO MAGELA FERNANDES	Aceito
Declaração de Pesquisadores	Karina.pdf	25/05/2020 14:06:58	GERALDO MAGELA FERNANDES	Aceito
Declaração de Pesquisadores	Cleandro.pdf	25/05/2020 14:06:45	GERALDO MAGELA FERNANDES	Aceito
Declaração de Pesquisadores	larissa.pdf	25/05/2020 14:06:31	GERALDO MAGELA FERNANDES	Aceito
Declaração de Pesquisadores		25/05/2020 14:06:04	GERALDO MAGELA FERNANDES	Aceito
Declaração de Pesquisadores	jose_alfredo.pdf	25/05/2020 14:05:43	GERALDO MAGELA FERNANDES	Aceito
Declaração de Pesquisadores	Lizandra.pdf	25/05/2020 14:05:20	GERALDO MAGELA FERNANDES	Aceito
Declaração de Pesquisadores	Andreza.pdf	25/05/2020 14:05:08	GERALDO MAGELA FERNANDES	Aceito
Declaração de Pesquisadores	gecilmara.pdf	25/05/2020 14:04:25	GERALDO MAGELA FERNANDES	Aceito
Declaração de		25/05/2020	GERALDO MAGELA	Aceito

ANEXO B - TCLE

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Convidamos o (a) Sr. (a) para participar como voluntário (a) da pesquisa "PREGNANCY OUTCOME AND CHILD DEVELOPMENT EFFECTS OF SARS-COV-2 INFECTION TRIAL", que significa "Estudo do impacto da infecção pelo novo coronavírus na gestação e desenvolvimento de crianças" que está sob a responsabilidade da pesquisadora Dra. LÍCIA MARIA HENRIQUE DA MOTA, Universidade de Brasília. Também participam deste estudo outros pesquisadores, na qualidade de Coordenadores: Dra. LIZANDRA MOURA PARAVIDINE SASAKI, Hospital Universitário de Brasília, e Dr. Geraldo Magela Fernandes, Universidade de Brasília.

Todas as suas dúvidas podem ser esclarecidas com os responsáveis por esta pesquisa. Apenas quando todos os esclarecimentos forem dados e você concorde com a realização do estudo, pedimos que rubrique as folhas e assine ao final deste documento, que está em duas vias. Uma via lhe será entregue e a outra ficará com o pesquisador responsável.

Você tem total liberdade para decidir participar ou recusar-se. Enfatizamos que a sua recusa não lhe trará qualquer prejuízo e que caso decida participar, também terá o direito de abandonar a pesquisa a qualquer momento, sem que ocorra nenhuma penalização.

INFORMAÇÕES SOBRE A PESQUISA:

- **Descrição da pesquisa**: Você foi convidada a participar desta pesquisa para estudarmos o impacto da pandemia pelo novo corona vírus 2019 - SARS-Cov-2- durante a gestação de seu filho (a) e o como pode influenciar no crescimento e desenvolvimento da criança. O objetivo deste estudo é acompanhar a evolução de pacientes gestantes que foram infectados pelo novo corona vírus 2019 (SARS-Cov-2) e verificar se há algum impacto na saúde do feto, na saúde materna e, após nascimento, acompanhar se há algum impacto na saúde da criança até os 5 anos de idade. A infecção pelo SARS-CoV-2 ainda é muito recente e se sabe muito pouco de sua influência na saúde das gestantes e nos

filhos de mães que foram contaminadas durante a gestação. Para participar será necessário, após a leitura e assinatura deste documento, que você faça o seu pré-natal no Hospital Universitário de Brasília (HUB) ou nos serviços supervisionados pela pesquisa e posteriormente leve o seu filho para o seguimento do crescimento e desenvolvimento no Ambulatório do HUB ou nos serviços supervisionados. Os procedimentos do estudo – coletas de informações relativas à sua saúde, exames laboratoriais, exames radiológicos, coleta de sangue – não são diferentes daqueles que você faria no pré-natal e na avaliação do Crescimento e Desenvolvimento do seu filho.

- **Período de participação no estudo:** após a primeira avaliação, você deverá retornar para as consultas de pré-natal conforme a rotina habitual, ou seja, mensalmente até a 34ª semana de gestação, quinzenalmente até a 36º semana e semanalmente até o parto. As consultas do seguimento da criança ocorrerão da mesma como orienta o Ministério da Saúde: 1º, 2º, 4º, 6º, 9º e 12º mês no primeiro ano de vida e após 1 ano, de 6 em 6 meses, até completar 5 anos de idade.
- **Riscos para o voluntário da pesquisa:** algumas gestantes podem ter vergonha ou medo de preconceito por terem adquirido a COVID-19. Para evitar isso, criamos um ambulatório de pré-natal especialmente para as participantes deste estudo, além de garantirmos que todos os dados serão tratados em segredo. A coleta de sangue para exames laboratoriais será realizada em uma veia do seu braço, com agulha e seringa esterilizadas, mas pode haver o risco de equimose (mancha roxa) ou sangramento local. Para reduzir esse risco, a coleta será realizada por uma pessoa com experiência. Além do sangue, a placenta e o sangue do cordão umbilical também serão encaminhados para estudos no laboratório. Caso você ou seu bebê precisem fazer uma anestesia ou punção na coluna por outro motivo, o líquido também será estudado. No caso da criança ela será submetida a uma ressonância magnética de crânio ainda na maternidade, o exame é indolor, será realizado com a criança dormindo, não será realizado se for necessária sedação. Não serão solicitados exames de sangue da criança que forem diferentes da rotina clínica habitual, exceto para pesquisa da presença do SARS-CoV-2 ou anticorpos relacionados no sangue. A criança também terá risco de hematoma (mancha roxa) e sangramento no local da coleta que é habitualmente no dorso da mão. Para diminuir estes

eventos a coleta desta amostra de sangue será feita junto com coletas de sangue que já seriam necessárias, como o teste do pezinho. Caso você ou a criança venha a apresentar as alterações relacionadas à coleta de sangue, você deverá fazer compressas de água quente em casa e se não melhorar, deve procurar o responsável por esta pesquisa no hospital ou através do telefone listado acima (pode fazer ligações a cobrar).

- Benefícios para o voluntário da pesquisa: o primeiro benefício deste estudo não é apenas para você, mas para toda a sociedade, pois permitirá que os médicos e gestores de saúde entendam melhor a doença e possam tratar melhor outras gestantes infectadas, assim como seus filhos. Outro benefício é que as participantes deste estudo que assim o desejarem terão todas as consultas de pré-natal, exames laboratoriais e ecografias realizadas no Hospital Universitário de Brasília, onde também poderão ter o parto. Igualmente, caso você participe do estudo, o seu filho poderá fazer todas as consultas de acompanhamento pediátrico no HUB.
- Sobre armazenamento e utilização de material biológico: o sangue que será coletado no seu braço, o sangue do cordão umbilical, o sangue da criança e a placenta servirão para que seja confirmado que você foi infectado pelo novo coronavírus, mas também pode ser utilizado para outros estudos, caso apareçam novas informações e que necessitem ser avaliadas. Ao assinar este documento você concorda que o seu sangue seja guardado e estudado em um outro momento. No entanto, mesmo assinando agora, você poderá voltar atrás a qualquer momento, retirando a autorização.
- Todas as informações desta pesquisa serão confidenciais (secretas) e serão repassadas apenas em reuniões ou publicações científicas, sem identificar as pessoas. Os dados coletados nesta pesquisa ficarão armazenados em pastas e no computador pessoal, sob a responsabilidade do pesquisador, no endereço acima informado, pelo período mínimo de 5 anos.
- A Sra. poderá solicitar, se assim quiser, o relatório final da pesquisa. Também, cópias de todos os resultados dos exames complementares realizados nesta pesquisa poderão ser solicitadas ao pesquisador.
- Nada lhe será pago e nem será cobrado para participar desta pesquisa, pois a aceitação é voluntária. Se houver necessidade, as despesas para a sua participação

serão assumidas pelos pesquisadores (ressarcimento de transporte). Em caso de dúvidas relacionadas aos aspectos éticos deste estudo, você poderá consultar o Comitê de Ética em Pesquisa do HUB-UnB no endereço: Setor de Grandes Áreas Norte 605 - Asa Norte, Brasília - DF, 70840-901.

Afirmo que recebi uma via deste termo de consentimento para ler antes de assiná-lo, que os detalhes do projeto foram explicados e que manterei uma cópia deste consentimento em meu poder.

Assinatura do participante ou representante

Assinatura do Pesquisador

Local Data