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¿CÓMO AFECTA LA ENFERMEDAD COVID-19 AL EMBARAZO Y A LA MUJER EMBARAZADA? ESTUDIO DE COHORTES PROSPECTIVO

TESIS DOCTORALTESIS

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Madrid, 2025

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Confirmo que Dña. María de las Nieves Rayo Navarro ha llevado a cabo, bajo mi supervisión, los estudios presentados en la Tesis: *¿Cómo afecta la enfermedad COVID-19 al embarazo y a la mujer embarazada? Estudio de cohortes prospectivo.*

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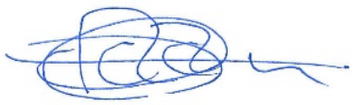

María del Mar Gil Mira
Madrid, 21 de marzo de 2025

Directora de tesis: Adriana Aquise Pino
Hospital Universitario de Torrejón
Madrid, España

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Adriana Aquise Pino
Madrid, 21 de marzo de 2025

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2019-nCoV	Nuevo coronavirus del 2019
Células NK	Células natural killer
CID	Coagulación intravascular diseminada
CLIA	Chemiluminescent immunoassay (quimioluminiscencia)
COVID-19	Enfermedad COVID-19
ECA2	Enzima convertidora de angiotensina 2
ELISA	Inmunoadsorción ligada a enzimas
HUF	Hospital Universitario de Fuenlabrada
HUT	Hospital Universitario de Torrejón
Ig	Inmunoglobulina
IL	Interleucina
IMC	Índice de masa corporal
INF	Interferón
LFA	Lateral Flow Inmunoassays (inmunocromatografía)
MERS-CoV	Coronavirus responsable del Síndrome Respiratorio de Oriente Medio
OMS	Organización Mundial de la Salud
PdA	Hospital Universitario Príncipe de Asturias
PoW	Prince of Wales Hospital
PRECORSE	Study for PREgnancy CORonavirus Serologic Evidence
Proteína E	Proteína de la envoltura

Proteína M	Proteína de membrana
Proteína N	Proteína de la nucleocápside
Proteína S	Glicoproteína S o Spikes
RBD	Receptor binding domain (Dominio de unión al receptor)
rRT-PCR	Técnica de reacción en cadena de la polimerasa con transcripción inversa en tiempo real
SARS-CoV1	Coronavirus responsable del síndrome respiratorio agudo grave tipo 1
SARS-CoV2	Coronavirus responsable del síndrome respiratorio agudo grave tipo 2
SC	Hospital Universitario San Cecilio
SDRA	Síndrome de dificultad respiratoria aguda
Sistema GALT	Tejido linfoide asociado al tracto gastrointestinal materno
TNF	Factor de necrosis tumoral
VdA	Hospital Universitario Virgen de la Arrixaca
VdH	Hospital Universitario Valle de Hebrón

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RESUMEN

Introducción

El coronavirus responsable del síndrome respiratorio agudo grave tipo 2 (SARS-CoV2) ha supuesto un desafío para la obstetricia debido a su potencial impacto para el embarazo y el resultado perinatal.

Objetivos

El objetivo fue evaluar la implementación de un protocolo preventivo frente a la infección en gestantes, analizar la seroprevalencia y seroconversión en los primeros meses de pandemia, y estudiar la posible asociación entre la infección y las complicaciones obstétricas. Finalmente, se investigó la posible transmisión vertical por la leche materna y la potencial inmunización pasiva del recién nacido.

Métodos

Diseño de estudio: estudio de cohortes prospectivo.

Población a estudio y procedimientos clínicos: tres cohortes prospectivas de diferentes centros hospitalarios.

- Primera cohorte: mujeres que acudieron a su visita de primer trimestre al Hospital Universitario de Torrejón (HUT) entre el 1 de enero y el 15 de mayo de 2020. Se recogió sangre materna en cada trimestre, posparto inmediato, al mes y seis meses posparto, calostro y leche madura a las 4-6 semanas, registrando los datos evolutivos y el desenlace perinatal. Se registraron los cambios de protocolos en HUT, el tipo de cita y contagios entre el personal sanitario.
- Segunda cohorte: mujeres que acudieron a su visita de primer trimestre del embarazo al Hospital Universitario de Fuenlabrada (HUF) entre el 1 de enero y el 15 de mayo de 2020. Se recogió sangre materna en el primer trimestre y los datos relativos al embarazo, parto y postparto.
- Tercera cohorte: mujeres positivas para SARS-CoV2 que dieron a luz en los hospitales Príncipe de Asturias (Madrid), San Cecilio (Granada), Valle de Hebrón (Barcelona) y Prince of Wales Hospital (Hong Kong, SAR), entre marzo del 2020 y marzo de 2021, recogiéndose calostro y leche a las 4-6 semanas posparto.

Análisis de muestras: se analizó la presencia del virus y de anticuerpos para estudiar la dinámica de la respuesta inmune en la gestante, tasa de contagiosidad y el impacto de los protocolos preventivos sobre ésta.

Resultado perinatal: se registró el momento de diagnóstico, la gravedad y las complicaciones del embarazo tanto en casos afectos como no.

Resultados

Los ajustes establecidos en las consultas de Obstetricia del HUT permitieron una continuidad asistencial, con un 23,5% de citas telefónicas y un 76,5% de presenciales, sin casos de infección sintomática entre los sanitarios de la unidad.

En el primer trimestre, se incluyeron 707 participantes (480 del HUT y 227 del HUF), detectándose anticuerpos frente a SARS-CoV2 en 58 de ellas (8,2% de seroprevalencia, similar a la nacional en ese momento). No hubo diferencias significativas en complicaciones obstétricas en función del estatus serológico.

En la cohorte de HUT, 31 mujeres (6,6%) presentaron anticuerpos frente a SARS-CoV2 en el primer trimestre frente a 66 (16,4%) que los presentaron en el tercer trimestre. La tasa de seroconversión en el tercer trimestre fue del 12,8%, siendo significativamente mayor que en el primer trimestre (p=0.003). No se encontraron diferencias significativas en la tasa de complicaciones obstétricas en función del trimestre en el que se produjo la infección.

No se detectó el virus en leche materna, pero sí anticuerpos frente al SARS-CoV2 en calostro y leche madura, independientemente del momento de la infección, siendo la inmunoglobulina (Ig) A el anticuerpo predominante.

Conclusiones

La adaptación rápida del protocolo clínico permitió mantener la atención obstétrica en el primer brote de la enfermedad COVID-19. La seroprevalencia en gestantes fue similar a la de la población general, aunque mayor en su tercer trimestre, sin diferencias en los resultados obstétricos según el estado serológico ni el momento de infección. No se detectó SARS-CoV2 en leche materna, siendo por tanto improbable la transmisión vertical por esta vía.

Los hallazgos de esta tesis pueden ayudar frente a futuras pandemias, facilitando un asesoramiento más específico a las mujeres embarazadas.

Palabras clave: coronavirus, SARS-CoV2, COVID-19, embarazo, anticuerpos, morbilidad, protocolo, seroprevalencia, pandemia, inmunoglobulinas, consultas, obstetricia.

ABSTRACT

Introduction

The coronavirus responsible for severe acute respiratory syndrome type 2 (SARS-CoV2) has been a challenge for obstetrics due to its potential impact on pregnancy and perinatal outcomes.

Objectives

The aim was to evaluate the implementation of a preventive protocol against infection in pregnant women, analyze seroprevalence and seroconversion in the first months of the pandemic, and examine the potential association between infection and obstetric complications. Finally, the possible vertical transmission through breast milk and the potential passive immunization of the newborn were investigated.

Methods

Study design: prospective cohort study.

Study Population and Clinical Procedures: Three prospective cohorts from different hospitals.

- First Cohort: Women who attended their first trimester control at the Hospital Universitario de Torrejón (HUT) between January 1 and May 15, 2020. Maternal blood was collected in each trimester, immediately postpartum, at one month and six months postpartum, as well as colostrum and mature milk at 4-6 weeks, recording the follow-up data and perinatal outcomes. Protocol changes at HUT, the type of visit, and infections among health care workers were also recorded.
- Second Cohort: Women who attended their first trimester control at the Hospital Universitario de Fuenlabrada (HUF) between January 1 and May 15, 2020. Maternal blood was collected in the first trimester. Data related to pregnancy, delivery, and postpartum was recorded.
- Third Cohort: Women who tested positive for SARS-CoV2 and gave birth at these hospitals: Príncipe de Asturias (Madrid), San Cecilio (Granada), Valle de Hebrón (Barcelona), and Prince of Wales (Hong Kong, SAR) hospitals between March 2020 and March 2021. Colostrum and milk were collected at 4-6 weeks postpartum.

Sample Analysis: The presence of the virus and antibodies was analyzed to evaluate the dynamics of the immune response in the pregnant woman, the transmission rate, and the impact of preventive protocols on it.

Perinatal Outcome: The time of diagnosis, severity of the infection and complications of the pregnancy were recorded, both in affected and non-affected cases.

Results

The changes implemented in the obstetrics outpatient service at the HUT ensured obstetric care, with 23.5% of consultations conducted by telephone and 76.5% conducted face-to-face, with no cases of symptomatic infection among the health care workers of the unit.

In the first trimester, 707 participants were included (480 from HUT and 227 from HUF), and antibodies against SARS-CoV2 were detected in 58 (8.2% seroprevalence, similar to the national rate at that time). No significant differences were found in obstetric complications based on serological status.

In the cohort from HUT, 31 (6.6%) women had antibodies against SARS-CoV2 in the first trimester, compared to 66 (16.4%) in the third trimester. The seroconversion rate in the third trimester was 12.8%, significantly higher than in the first trimester (p=0.003). No significant differences were found in the rate of obstetric complications based on the trimester in which the infection occurred.

The virus was not detected in breast milk, but antibodies against SARS-CoV2 were found in both colostrum and mature milk, regardless of the time of the infection. Immunoglobulin (Ig) A was the predominant antibody.

Conclusions:

The prompt adaptation of the clinical protocol ensured the continuity of obstetric care during the first outbreak of the COVID-19 infection. The seroprevalence in pregnant women was similar to the reported rate in general population, although higher in the third trimester, with no differences in obstetric outcomes based on serological status or the time of the infection. SARS-CoV2 was not found in breast milk, suggesting that vertical transmission through this route is unlikely.

The findings of this thesis may prove helpful in future pandemics, providing specific counseling for pregnant women.

Key words: coronavirus, SARS-CoV2, COVID-19, pregnancy, antibodies, morbidity, protocol, seroprevalence, pandemic, immunoglobulins, outpatient care, obstetrics.

INTRODUCCIÓN

INTRODUCCIÓN

El virus relacionado con el síndrome respiratorio agudo grave (SARS-CoV2) y la enfermedad que produce, enfermedad COVID-19, han supuesto un reto para los gobiernos de los países de todo el mundo y para la comunidad científica, cuyo principal objetivo ha sido proteger la salud pública y conseguir superar la emergencia sanitaria desencadenada por el virus. Debido a su rápida propagación por todo el mundo, su gran poder virulento y alto impacto en la salud pública, la pandemia de COVID-19 ha sido considerada como una de las peores pandemias producida por un virus respiratorio de la historia reciente de la humanidad. La COVID-19 supuso un verdadero desafío para los sistemas de salud, conduciendo a los centros sanitarios a una sobrecarga asistencial abrumadora, enfrentándose a la priorización de la atención médica, a la necesidad de aprendizaje y a la rápida adaptación de los protocolos de actuación, poniendo a prueba la resiliencia de todos los trabajadores del ámbito sanitario y la capacidad de reacción y colaboración internacional. Hoy en día, el SARS-CoV2 es aún considerado como una amenaza para la salud pública y motivo de preocupación en muchos países del mundo.

1. La pandemia COVID-19.

El día 31 de diciembre de 2019, la Comisión Municipal de Salud y Sanidad de Wuhan, en la provincia de Hubei, China, informó sobre la aparición de una serie de casos de neumonía de origen desconocido (1,2). Mediante la combinación de técnicas de secuenciación genómica nanopore y secuenciación masiva, y mediante la técnica de reacción en cadena de la polimerasa con transcripción inversa en tiempo real (rRT-PCR) de muestras de esputo broncoalveolar procedente de 3 pacientes enfermos en Wuhan, China, se logró aislar el agente etiológico coincidente en todas las muestras, siendo inicialmente denominado nuevo coronavirus del 2019 o 2019-nCoV (Figura 1)(3) y confirmado por las autoridades chinas el día 7 de enero de 2020 (2). Se trataba de un nuevo virus de alta capacidad infectiva formado por una sola cadena de ARN (monocatenario positivo), perteneciente a la familia de *Coronaviridae*, del género Betacoronavirus, subgénero Sarbecovirus, cuya secuencia genómica muestra un grado de coincidencia de 86,9% con la cepa de SARS en murciélagos (3–5) y, por lo tanto, sugiriendo un origen zoonótico de la infección. Renombrado como SARS-CoV2 (6), su secuencia genética fue compartida por las autoridades chinas el 12 de enero de 2020.

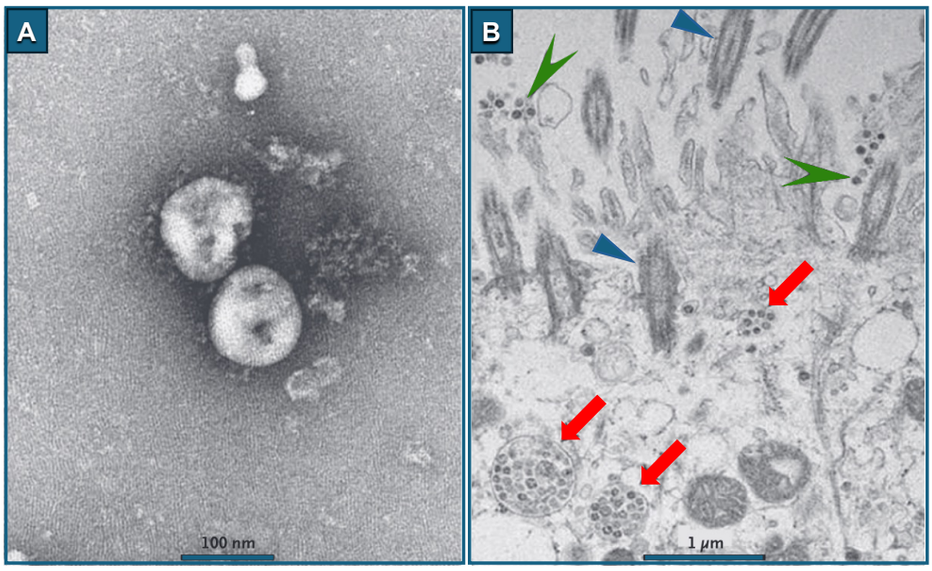

Figura 1. Visualización del 2019-nCoV mediante microscopio electrónico de transmisión. Panel A: imagen en negativo del 2019-nCoV. Panel B: partículas del 2019-nCoV en epitelio de vía aérea humana (las cabezas de flecha verdes señalan partículas extracelulares del virus; las flechas rojas señalan cuerpos de inclusión del virus en el citoplasma celular; los triángulos azules señalan los cilios). Imagen adaptada de Zhu y cols., 2020 (3).

El Comité de Emergencias del Reglamento Sanitario Internacional declaró el brote del renombrado coronavirus SARS-CoV2 como Emergencia de Salud Pública de Importancia Internacional el 30 de enero de 2020 (2). El día 31 de enero de 2020 se comunicó el primer caso de la enfermedad por dicho agente en España, en la isla de La Gomera, en Gran Canaria: un paciente contagiado tras haber estado en contacto con un infectado por el virus en Alemania, momento a partir del cual el número de casos creció rápidamente en nuestro país (7). La Organización Mundial de la Salud (OMS) declaró el 11 de marzo de 2020 como pandemia a la infección producida por el SARS-CoV2, denominando a la enfermedad provocada por este virus como COVID-19, debido a su rápida e incontrolable propagación. En ese momento, la COVID-19 afectaba a más de 118.000 personas en más de 110 países por todo el mundo (Figura 2), con un 3,6% de mortalidad en ese momento (2).

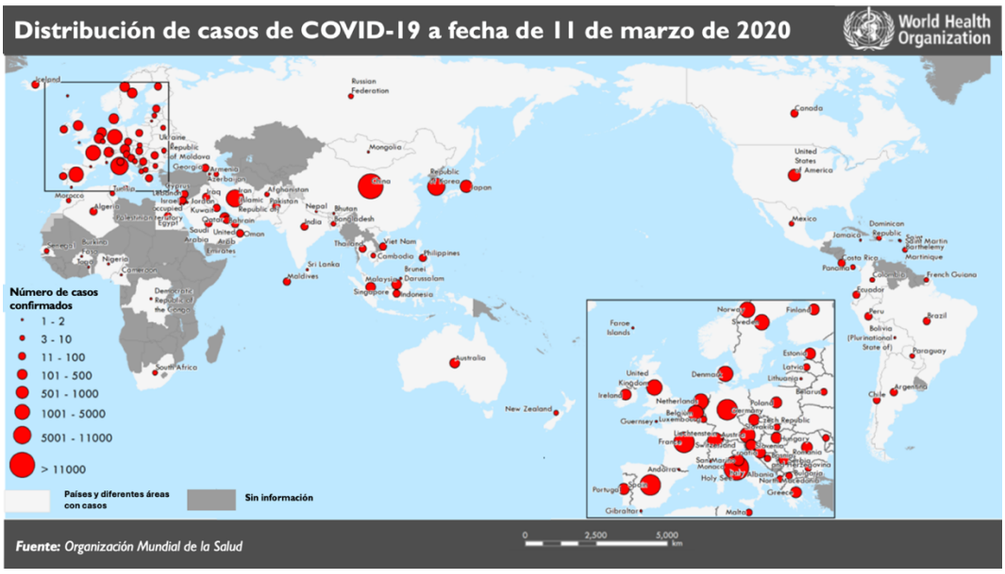

Figura 2. Territorios con casos confirmados de COVID-19 reportados a fecha de 11 de marzo de 2020. Imagen adaptada de la OMS (8).

2. Virología del SARS-CoV2.

Para poder hacer frente a la pandemia, fue fundamental profundizar en la estructura y origen del virus. El SARS-CoV2 es un virus que envuelve su contenido de RNA monocatenario en el interior de una característica corona de puntas o glicoproteínas denominadas S*pikes (S),* las cuales se encuentran distribuidas por toda la superficie del virus. Estas glicoproteínas son las responsables de la unión y fusión del virus con las membranas celulares del huésped. Dos tercios de su RNA codifica para 16 proteínas no estructuradas que van a interferir con el sistema inmune del huésped. El tercio restante del genoma del virus codifica las 4 proteínas estructurales esenciales: la anteriormente mencionada glicoproteína S o *Spike;* la proteína de membrana (M), responsable del transporte transmembrana de nutrientes, liberación de la partícula viral y eventual formación de su envoltura; las proteínas de nucleocápside (N) y las proteínas de envoltura (E) (9). La *Spike (S)* tiene 2 subunidades: la subunidad S1 incluye un dominio de unión al receptor (RBD) y un dominio amino terminal. El dominio RBD determina la unión a la célula huésped y el grado de afinidad de la unión (10). La subunidad S2 colabora en dicha fusión (Figura 3)(9).

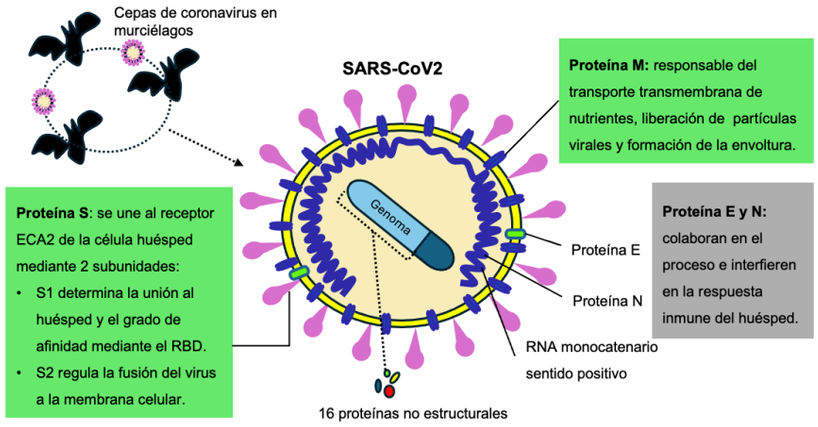

Figura 3. Estructura del SARS-CoV2. Imagen adaptada de Guo y cols., 2020 (9).

El SARS-CoV2 se transmite de persona a persona mediante contacto directo o por vía aérea, siendo la mucosa nasal y orofaríngea la principal puerta de entrada. Una vez en el interior del huésped, el primer paso para la infección es la unión del virus a las células epiteliales de las vías respiratorias, células epiteliales alveolares, células endoteliales y macrófagos alveolares, dado que expresan en la superficie celular un receptor específico, la enzima convertidora de angiotensina 2 (ECA2), que constituye el principal mecanismo de infección del SARS-CoV2. La infección se produce mediante la unión de la glicoproteína S del virus y el receptor ECA2, activándose la glicoproteína S y produciéndose la fusión y entrada del virus en la célula huésped (10). De esta manera, se libera el ARN genómico viral en el citoplasma, iniciando su replicación y formando nuevos viriones que migran hacia la membrana celular. Las partículas virales son liberadas masivamente por la célula y son capaces de infectar a nuevas células del huésped (9). La gran afinidad del SARS-CoV2 por la ECA2 y la presencia de este receptor en numerosos órganos del cuerpo humano, explica el gran potencial de producir un daño multiorgánico y la elevada contagiosidad del virus, incluso mayor que lo descrito con previos coronavirus (10,11).

3. Respuesta inmunológica frente al SARS-CoV2.

El sistema inmune es el responsable de reaccionar y actuar de manera coordinada frente a lo extraño y patógeno. Sin embargo, no funciona de la misma manera en todos los seres humanos y, por tanto, la gravedad de una enfermedad se ve influida por las comorbilidades de cada individuo, el envejecimiento del sistema inmune y la heterogeneidad de la respuesta inmune.

Tras la fusión del virus con el receptor ECA 2 de las células huésped, se produce la amplificación viral y su transmisión a células adyacentes, desencadenando una cascada inmunológica en la que se ven implicados el sistema inmunitario innato y el adaptativo (Figura 4)(10,12). La respuesta inflamatoria primaria innata implica a los macrófagos alveolares, monocitos, neutrófilos, el sistema complemento y las células Natural Killer (NK), que responden masivamente a la rápida replicación del SARS-CoV2. Los macrófagos M1 se encargan de la defensa mediante su capacidad fagocítica y liberación de citocinas proinflamatorias como interleucina 1 beta (IL-1β), IL-6, factor de necrosis tumoral alfa (TNF-α), interferón gamma (IFN-γ) y otras quimiocinas, activando el complemento y mastocitos, propiciando su degranulación y aumentando la permeabilidad vascular. Esto favorece la extravasación de monocitos que se convertirán en macrófagos, células dendríticas y neutrófilos para poder contener el virus y aumentar la inflamación. Los macrófagos M2, en cambio, son los encargados de resolver la inflamación mediante la activación de linfocitos T inmunosupresores (linfocitos Th o CD4+), inhibiendo la fagocitosis y favoreciendo la tolerancia inmunológica.

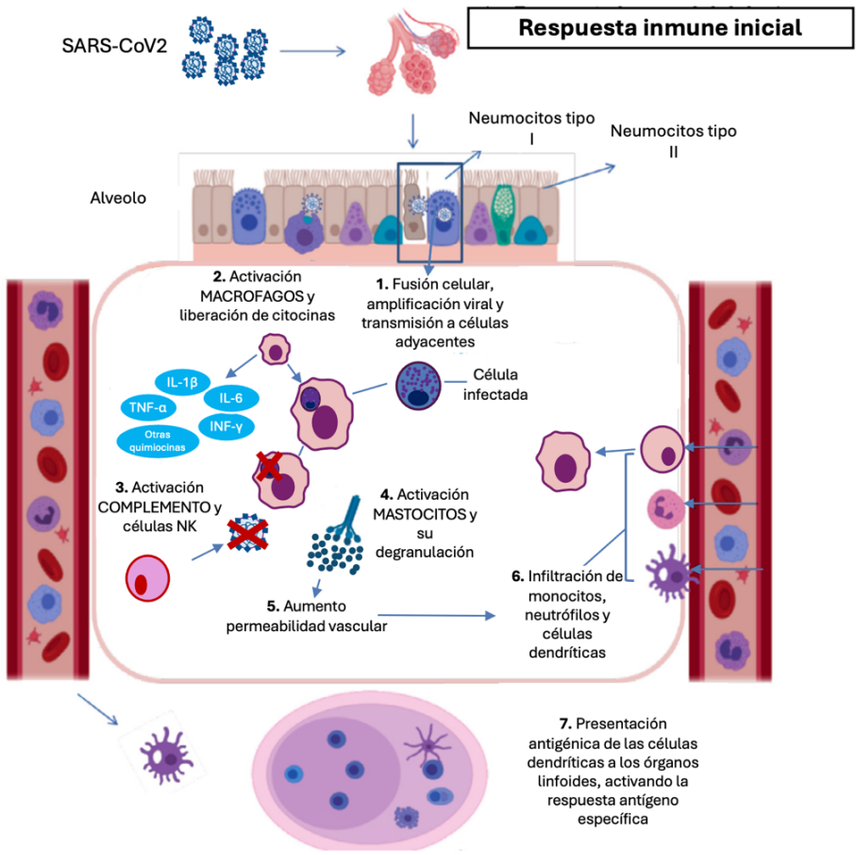

Figura 4. Respuesta inmunológica inicial frente a la invasión por SARS-CoV2. Imagen adaptada de Sanz y cols., 2021 (10).

Posteriormente, las células dendríticas maduran y migran hasta órganos linfoides para la presentación antigénica viral, entrando en escena la respuesta inflamatoria primaria adaptativa mediante activación y proliferación linfocitaria: los linfocitos Th (inmunosupresores) regulan la respuesta inmune mediante la liberación selectiva de citocinas, y su activación depende de la respuesta del sistema inmune innato. Los linfocitos Tc (citotóxicos o CD8+) se encargan de eliminar las células infectadas y los linfocitos B son los responsables de la respuesta humoral específica, mediante el reconocimiento específico de los complejos antigénicos virales (10,13). En muchos casos, el huésped tolera la fase inflamatoria primaria superando la infección mediante el cese de la cascada inflamatoria y de la replicación viral y gracias a la aparición de los anticuerpos específicos frente al virus (memoria antigénica) propia de la respuesta inmunológica secundaria (11,12). Esta respuesta coordinada y eficiente está presente en casos asintomáticos o con síntomas leves-moderados.

Sin embargo, en algunos casos la respuesta inmune es inadecuada: retraso en la activación linfocitaria aumentando así la replicación viral, apoptosis de las células huésped e hiperactivación anormal de la respuesta inflamatoria, dando lugar a un estado crítico o la muerte (Figura 5)(10).

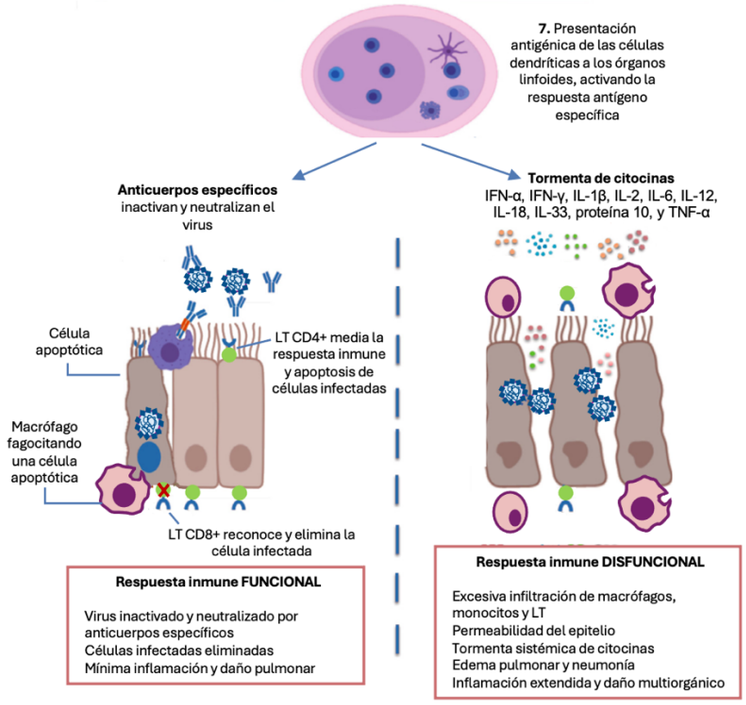

Figura 5. Modelo explicativo de los tipos de respuesta inmunológica frente a la invasión por SARS-CoV2. Imagen adaptada de Sanz y cols., 2021 (10).

Este fenómeno de hiperactivación perpetúa la cascada inflamatoria produciendo una tormenta inflamatoria (IFN-α, IFN-γ, IL-1β, IL-2, IL-6, IL-12, IL-18, IL-33, proteína 10, y TNF-α, entre otras citocinas), capaz de desencadenar una hiperactivación de la cascada de coagulación. Esto puede conducir al paciente a una coagulación intravascular diseminada (CID) en el contexto de un fallo multiorgánico, una situación catastrófica que conduciría con el tiempo a su muerte (9,11,12). Afortunadamente, en algunos casos el sistema inmune es capaz de recuperarse de la infección, y el estado de hiperrespuesta inmunológica e hiperactivación de la coagulación regresa de manera gradual a su estado basal. Esta fase postinfecciosa puede mantenerse en el tiempo durante varios meses, asociando o no síntomas residuales como la fibrosis pulmonar o complicaciones cardiovasculares, que pueden ocurrir hasta en un 15-20% de los casos (12).

Los linfocitos B son los responsables de la defensa humoral y requieren un tiempo de acción más largo (entre 5 y 14 días) para producir anticuerpos específicos, durante una primoinfección, mientras que el sistema innato contiene la infección. Las partículas del SARS-CoV2 activan dos poblaciones de linfocitos B: linfocitos B secretores de IgM actúan de manera masiva y poco específica, sin producir memoria antigénica. Por otro lado, otros linfocitos B cooperan con linfocitos T previamente activados (respuesta timo-dependiente) y sufrirán un proceso lento de diferenciación para producir IgA, IgG e IgE y para formar linfocitos B memoria. Tras las primeras 24h de la infección, se producen grandes cantidades de IgM, incrementándose con el paso de los días. A partir de los primeros 5-15 días, comienzan a producirse IgA e IgG. Todos los pacientes que sufren la infección producen anticuerpos neutralizantes frente al virus, pero no siempre en altas concentraciones o de manera eficiente (10).

4. Clínica de la infección por SARS-CoV2.

El gran poder virulento del SARS-CoV2 radica en la alta afinidad de la subunidad S1-RBD del virus por la enzima ECA2 de las células huésped y la masiva replicación viral. Esta enzima está presente en las células de un gran número de órganos del cuerpo humano, tales como el cerebro, corazón, endotelio respiratorio, alveolos, hígado, riñones y otros, lo que explica el amplio abanico de síntomas que pueden estar presentes en el contexto de la enfermedad COVID-19 (12). La clínica más frecuente incluye fiebre, tos, disnea, fatiga y mialgia (14,15). Sin embargo, pueden aparecer síntomas más severos tales como miocarditis, enfermedad tromboembólica,

insuficiencia renal o la coagulación intravascular diseminada. Además, pueden aparecer complicaciones derivadas de la afectación pulmonar, como la neumonía o el síndrome de dificultad respiratoria aguda (SDRA), producido por el daño alveolar difuso y que conlleva inevitablemente al ingreso en Unidades de Cuidados Intensivos, con alto riesgo de muerte de la persona afectada (9). Los síntomas más frecuentes en mujeres gestantes son la fiebre y la tos (16).

Por el contrario, existe también un elevado porcentaje de pacientes que padecen la infección de manera asintomática o con una clínica leve, viéndose infradiagnosticados debido a la falta de estudios de seroprevalencia (17). Esto a su vez ha generado controversia en los datos publicados sobre la prevalencia de la enfermedad. El desarrollo de estudios de seroprevalencia a lo largo de los años ha sido clave para mejorar la tasa de detección de la infección. Además, eso ha permitido profundizar en la morbilidad asociada a la infección en los casos asintomáticos y ha contribuido a garantizar la seguridad de los trabajadores sanitarios, preparando el camino para posibles futuras pandemias. Así mismo, los estudios serológicos han permitido la detección precoz de la infección por SARS-CoV2 en población más susceptible como puede ser la mujer gestante, que presenta mayor susceptibilidad a infecciones respiratorias por los cambios fisiopatológicos inherentes a la gestación.

5. Coronavirus y la mujer gestante. Vista del pasado a la actualidad.

Previo a la COVID-19, la pandemia del coronavirus responsable del síndrome respiratorio agudo grave (SARS-CoV1) en 2002-2003, la del Influenza H1N1 y la pandemia del Síndrome Respiratorio de Oriente Medio (MERS) en 2012, dieron lugar a emergencias sanitarias que afectaron de manera importante a la población gestante. A pesar de la escasez de datos publicados sobre mujeres gestantes con infección por SARS-CoV1 en la pandemia de 2002-2003, hubo una clara asociación entre el estado de embarazo de la mujer con una mayor severidad de la enfermedad, abortos e incluso, muerte materna (18). En 2012, tuvo lugar otra pandemia desencadenada por el coronavirus del síndrome respiratorio del medio este (MERS-CoV). Éste se propagó rápidamente por los países de la península arábica, llegando hasta Corea en 2015, según los escasos datos publicados (18). El MERS-CoV podía producir un abanico de síntomas desde la ausencia de éstos, a la presencia de tos, fiebre y astenia, hasta incluso clínica más severa como la neumonía y el síndrome respiratorio agudo grave (18). La tasa de mortalidad del SARS-CoV2 fue menor que la que se produjo por SARS-CoV1 y MERS, pero con una mayor contagiosidad debido a la gran afinidad por la ECA2

(11). Así como sucedió en la pandemia de SARS-CoV1, la gestación implicó un mayor riesgo de enfermedad severa, mortalidad materna y peores resultados perinatales, a pesar de la escasez de casos en embarazadas y los escasos conocimientos acerca de la fisiopatología de la infección en el embarazo (19–23). En ambas pandemias, se describieron mayores tasas de complicaciones obstétricas tales como el parto prematuro, restricción de crecimiento fetal, y muerte fetal y neonatal (12).

Por el aumento en la morbilidad obstétrica descrita en las pandemias previas del SARS-CoV1 y MERS, era de esperar que el nuevo coronavirus, el SARS-CoV2, tuviera un impacto similar en la evolución del embarazo. Esto puede ser debido a los cambios fisiológicos que se producen a nivel cardiovascular, respiratorio e inmunológico durante la gestación y que implican una mayor predisposición a la infección por diversos patógenos respiratorios con una mayor severidad. Durante la gestación, se producen algunos cambios en el sistema inmune y su respuesta a los patógenos: aumento de la población de linfocitos B, aumentando la respuesta humoral sobre la respuesta celular; disminución de las células natural killer (NK); disminución de la producción de INF-1; aumento de la progesterona en sangre, con sus propiedades inmunomoduladoras; alteraciones en la respuesta primaria innata (24). A los cambios inmunológicos se suman el aumento del riesgo trombótico y los cambios anatómicos a nivel pulmonar como la elevación del diafragma, produciendo una disminución de la capacidad pulmonar y de aclaramiento de las secreciones. Algunos autores han sugerido que todos estos cambios podrían propiciar una mayor predisposición a la infección por virus respiratorios y a un mayor riesgo de complicaciones de la propia infección (24–26). A pesar de los cambios en el cuerpo de la mujer gestante y de lo vivido con las anteriores pandemias, no hay unanimidad en los datos publicados sobre el impacto del actual SARS-CoV2 en la gestación.

Uno de los primeros metaanálisis publicados sobre el SARS-CoV2 en el embarazo objetivó una mayor tasa de parto prematuro en gestantes infectadas, así como una mayor tasa de preeclampsia, partos por cesárea y muerte fetal (27). Desde entonces, un creciente número de publicaciones han salido a la luz compartiendo sus datos sobre el impacto de la infección por COVID-19 en la gestación (12,14,15,17,27–32), generando una notable controversia debido a la discrepancia de sus resultados. Mientras algunos autores describieron en las gestantes infectadas por SARS-CoV2 un elevado porcentaje de complicaciones obstétricas, tales como el parto prematuro, la rotura prematura de membranas pretérmino y el bajo peso al nacer (15,27–29,32), otros autores discreparon al no encontrar diferencias significativas en la tasa de morbilidad

obstétrica entre las gestantes que padecieron la infección por el virus y las que no lo hicieron (14,17,30).

De igual manera, es todavía punto de debate y discrepancia el impacto del momento en el que se produce la infección por SARS-CoV2, sugiriendo un aumento en la tasa de mortalidad fetal, neonatal y parto prematuro en las infecciones que se producen durante el primer trimestre, y un aumento en las alteraciones de crecimiento fetal y bajo peso al nacimiento, si la infección ocurre en el tercer trimestre (27,33,34). Sin embargo, es importante recalcar la infraestimación del diagnóstico, ya que la gran mayoría de las mujeres incluidas en los estudios eran pacientes sintomáticas a las que se realizaba rRT-PCR en un momento determinado. Hasta la fecha, escasos estudios han analizado la influencia del virus en diferentes momentos de la gestación: Di Mascio y cols. (34), mediante el diagnóstico por rRT-PCR, observó una tasa significativamente mayor de parto prematuro, muerte fetal y muerte neonatal en las gestaciones infectadas con menor edad gestacional, considerando así la edad gestacional a la que se produce la infección como el mejor predictor de morbilidad perinatal, corroborándolo Piekos y cols. posteriormente con sus resultados (33).

Debido a un posible riesgo aumentado de infección y complicaciones durante la gestación, y a la elevada proporción de casos asintomáticos o con síntomas leves, era crucial el desarrollo de otras técnicas para mejorar la tasa diagnóstica de la infección y poder valorar el impacto del virus en el embarazo en función del momento de infección. En el momento de realización de esta tesis, únicamente Accurti y cols. ha utilizado la serología para comparar la morbilidad obstétrica en un mismo grupo de gestantes: un grupo con serología positiva frente a SARS-CoV2 en su primer trimestre frente a las que positivizaron en su tercer trimestre, sin objetivarse diferencias en la tasa de morbilidad obstétrica (35).

Las técnicas serológicas son una herramienta muy poderosa para avanzar en la investigación, realizar un seguimiento más estrecho del embarazo afecto por la infección por SARS-CoV2 y poder dar luz a la controversia y discrepancia en esta área.

6. Diagnóstico de la infección.

La respuesta inflamatoria e inmunológica producida por la infección, sugirió que la titulación de anticuerpos frente al SARS-CoV2 podría ser considerada como una herramienta de gran ayuda en el diagnóstico y seguimiento de la infección. A pesar de que el estándar de oro para el diagnóstico es la técnica de rRT-PCR en muestra nasofaríngea (36), el desarrollo de otros procedimientos era crucial para afinar la

detección de la infección. La titulación de anticuerpos permitía la detección de la infección en pacientes con síntomas leves y asintomáticos, permitiendo realizar un seguimiento de todos los pacientes afectos por la COVID-19 y no solo los sintomáticos. A su vez, la detección precoz de los casos de infección por SARS-CoV2 permitía la promoción de medidas de prevención, seguridad y protección global.

Las técnicas serológicas proporcionan una herramienta rápida y efectiva para la detección de infecciones de patógenos respiratorios, y se basan en la alta inmunogenicidad de las proteínas S y las proteínas N, liberando los diferentes anticuerpos o Ig: IgA, IgG e IgM (37). Cuando se produce la invasión por el SARS-CoV2, se produce en primer lugar la liberación de ARN viral al huésped. Esto ocurre coincidiendo con el inicio de los síntomas. Posteriormente, y de una manera muy precoz, aparecen las IgA e IgM, marcadores de primoinfección. La IgA cobra una especial importancia, puesto que se caracteriza por recubrir la mucosa respiratoria y digestiva, siendo la primera defensa inmunológica frente a virus. Es el anticuerpo predominante en las secreciones (saliva, secreciones respiratorias, digestivas, genitourinarias y leche materna) y es la que se ve elevada de manera más precoz en suero, desde el día 4-6 del inicio de los síntomas. La IgM es la inmunoglobulina de mayor tamaño y, por tanto, la encontramos exclusivamente a nivel intravascular. Va aumentando hasta su pico máximo desde el día 6-14 del inicio de los síntomas, disminuyendo progresivamente junto con la IgA hasta niveles indetectables sobre el día 21 (38). A partir del día 14, comienza la elevación de la IgG, llegando a niveles máximos sobre el día 21 desde el inicio de los síntomas hasta la 7ª semana (Figura 6). Al ser un anticuerpo de menor tamaño, se puede encontrar a nivel extravascular, siendo característico su paso transplacentario, proporcionando así inmunidad al feto. Así como la IgA e IgM son marcadores de primoinfección, la IgG es considerada la responsable de la inmunidad específica y a largo plazo, permaneciendo detectable hasta 12 semanas después del inicio de los síntomas (11,38).

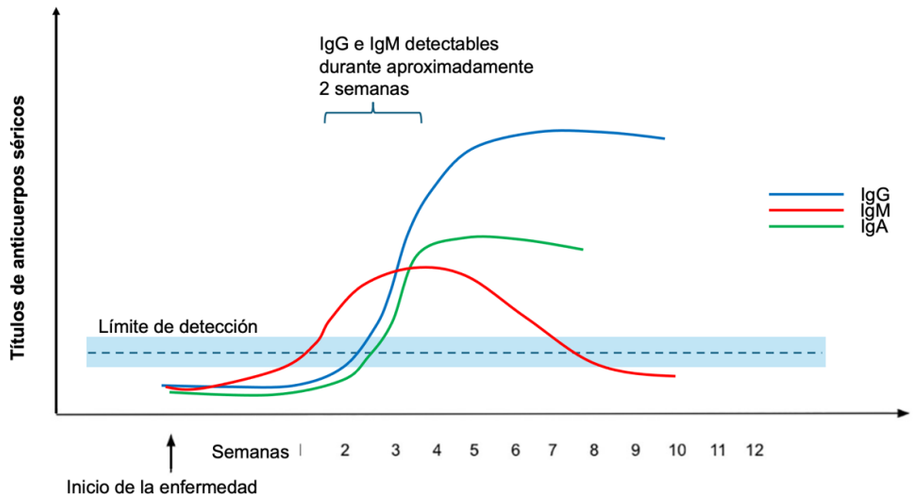

Figura 6. Dinámica serológica de las IgM, IgA e IgG desde el inicio de la infección. Imagen adaptada de Post y cols., 2020 (38).

La rápida identificación del virus y la difusión de la secuenciación genómica del mismo junto con la colaboración internacional fue fundamental para intentar controlar la pandemia, proporcionando mayor certeza sobre el número de casos confirmados con enfermedad COVID-19, y promoviendo el aislamiento y contención de la misma (9). Su diagnóstico se realiza mediante la PCR (Reacción de la Polimerasa en Cadena), una técnica basada en la amplificación de fragmentos de ADN, mediante ciclos de subidas y bajadas de temperatura, permitiendo así ampliar las secuencias de ADN disponibles para ser detectadas posteriormente mediante fluorescencia. Dado que el material genético del SARS-CoV2 es ARN, el primer paso es convertirlo a ADN (por transcripción inversa, RT, reverse transcription) para poder realizar el procedimiento de la PCR. Por ello, a la técnica de PCR utilizada en el SARS-CoV2 se le llama rRT-PCR (36,39). Es un procedimiento muy establecido y con alta especificidad para el diagnóstico de la infección debido a la elección concreta de zonas del genoma exclusivas del virus a detectar.

Sin embargo, la rRT-PCR tenía ciertas desventajas a la hora de la detección de la enfermedad, obteniendo unas tasas generales de sensibilidad al inicio de la pandemia de 45-60% (40), y una tasa de falsos negativos que variaba entre un 2% a un 58% (41), lo cual explica la preocupación y duda sobre si la rRT-PCR debería seguir siendo el Gold Standard para el diagnóstico de la infección por SARS-CoV2 (42). La técnica precisaba personal cualificado dado el riesgo de contaminación de la muestra, así como instrumentación especializada, no disponible en muchos centros sanitarios. Por este

motivo, la técnica tenía una baja reproducibilidad y fiabilidad, lo que dificultaba aún más su estandarización para su realización por personal no cualificado (39,43). Otra de las limitaciones importantes de este procedimiento era su coste económico. Esto, sumado a todo lo previo, explica que la técnica estuviese limitada a los centros sanitarios para pacientes con sintomatología moderada o grave, generando así una infraestimación de la tasa de detección real de la infección por SARS-CoV2 (44). Estas limitaciones explicaron la importancia del desarrollo de otros métodos diagnósticos complementarios para la infección por SARS-CoV2, con el objetivo de mejorar la tasa de detección y seguimiento de la infección por SARS-CoV2. Si bien es cierto, el avance en la investigación y el desarrollo de numerosos kits para realizar el procedimiento de la rRT-PCR han mejorado notablemente las tasas de sensibilidad de la prueba en los últimos años, alcanzando hasta un 100% de sensibilidad en algunos casos (40,45).

Las técnicas serológicas validadas ayudaron a detectar casos asintomáticos, permitiendo su aislamiento para disminuir el riesgo de contagio, desarrollar estudios epidemiológicos sobre la infección en diferentes poblaciones, localizar comunidades de riesgo y evaluar de manera prospectiva la eficacia de las vacunas frente al SARS-CoV2 (36,37,43,46). En este contexto, se desarrollaron rápidamente distintas técnicas serológicas como la inmunoadsorción ligada a enzimas (ELISA), la inmunocromatografía (Lateral Flow Inmunoassays o LFA) y técnicas de quimioluminiscensia (Chemiluminescent Immunoassay o CLIA), capaces de detectar diferentes inmunoglobulinas, incluyendo IgA, IgM e IgG (36,47), variando levemente la tasa de sensibilidad y especificidad en función del test utilizado y del momento en el que se realizó la técnica. ELISA y CLIA han demostrado similares tasas de sensibilidad y especificidad (en torno a un 84,3-97,8% y 96,6-99,7%, respectivamente). Una menor sensibilidad se ha objetivado con el test LFA NG-test (en torno a un 66%), a pesar de que mantiene una elevada especificidad (96,6%)(47). Sin embargo, el test combinado LFA IgG-IgM ha demostrado mejorar la tasa de sensibilidad diagnóstica (88,6%), con una especificidad algo menor, del 90,6% (48). En cuanto al momento de realización de las técnicas serológicas, la sensibilidad y especificidad mejoraron cuando la técnica se realizó más allá de los 14 días del inicio de la clínica, si la hubo (hasta un 100% sensibilidad utilizando cualquiera de las 3 técnicas; y una especificidad de 95,8% con ELISA, y de 98% con CLIA-Abbott o LFA NG-test)(36).

Gracias a la detección de anticuerpos frente al SARS-CoV2, la asistencia sanitaria ha podido adaptarse a la situación de la pandemia COVID-19 garantizando, por un lado, la seguridad de los trabajadores sanitarios, al detectar casos asintomáticos o con clínica

leve; y por otro, ofreciendo a las mujeres embarazadas la mejor asistencia posible, especializada y enfocada a las posibles complicaciones de la infección por SARS-CoV2. Gracias a las técnicas serológicas, se ha podido estudiar incluso la presencia de anticuerpos frente al SARS-CoV2 en la leche materna, investigando la transmisión de inmunidad desde la madre al recién nacido. Al inicio de la pandemia, una de las primeras medidas adoptadas en los paritorios de muchos lugares del mundo fue la separación de los recién nacidos de las madres diagnosticadas con COVID-19 debido al desconocimiento y confusión sobre el posible riesgo de transmisión al neonato. Poco a poco, esta medida quedó obsoleta por los numerosos beneficios que conlleva la lactancia materna, siendo uno de ellos, la inmunización del recién nacido a través de las inmunoglobulinas secretadas en la leche materna (49). Concretamente, la IgA tiene un papel fundamental en este proceso. Ésta es segregada por el tejido linfoide asociado al tracto gastrointestinal materno (sistema GALT), transportada a las glándulas mamarias e incorporada en la leche materna. Cuando el recién nacido mama, la IgA comienza a cubrir y sellar el tracto respiratorio y digestivo del bebé para evitar el paso de los agentes patógenos y su infección, constituyendo así la primera defensa del bebé frente a los virus (50,51). Así mismo, la controversia generada por la posible presencia del virus de manera activa en la leche materna también fue aplacada por los datos publicados por diferentes autores, quienes no detectaron RNA del SARS-CoV2 en la lecha materna (52,53). Gracias a la perseverancia de la comunidad científica en su labor investigadora, la lactancia materna ha pasado de ser suprimida al principio de la pandemia COVID-19, a ser promovida en estas situaciones. Esta idea se ha visto apoyada por los datos que se aportan en la presente tesis, al reflejar el proceso de inmunización de los recién nacidos de madres con la infección a través de la lactancia materna, siempre y cuando ésta se realice bajo las medidas de seguridad e higiene recomendadas por la OMS (54).

7. Adaptación de la asistencia sanitaria en Obstetricia.

La alta contagiosidad del nuevo coronavirus ha supuesto un reto sin precedente para la asistencia sanitaria, cuyo objetivo ha sido trabajar incesablemente por la salud de la población, intentando proporcionar la mejor atención médica posible, adaptando la actividad asistencial a la pandemia, fomentando la educación sanitaria y prevención, y favoreciendo la investigación. Además, es fundamental recalcar el riesgo infeccioso al que los profesionales sanitarios se vieron expuestos. Un estudio nacional de seroprevalencia en España realizado en mayo de 2020 describió una seroprevalencia frente al SARS-CoV2 del 4,6%. En este estudio, la seroprevalencia descrita en Madrid

se encontraba por encima de la seroprevalencia a nivel nacional, siendo del 11,5% (55), siendo aún más elevada la seroprevalencia descrita en el municipio de Torrejón de Ardoz (20,18% en la población general y ligeramente superior en mujeres: 20,27%)(56). Así mismo, la seroprevalencia de la infección por SARS-CoV2 descrita entre los profesionales sanitarios varió entre un 16,6% - 17,2% - 21% en diferentes centros hospitalarios españoles (57–59).

A diferencia de otros servicios que paralizaron su actividad casi por completo durante el pico de la pandemia con el fin de minimizar el riesgo de contagio, la gestación exigía una continuidad asistencial que no podía interrumpirse en ninguna circunstancia. Debido a la importancia del seguimiento obstétrico y dada la mayor prevalencia de la infección en Torrejón de Ardoz (56) en comparación con el resto de la comunidad de Madrid y el resto del país, el primer objetivo del Servicio de Obstetricia del Hospital Universitario de Torrejón ha sido el desarrollo e implementación precoz de un protocolo clínico que garantizase el adecuado seguimiento de la gestación durante la crisis sanitaria. El objetivo principal de este protocolo ha sido llevar a cabo la reestructuración de las consultas de obstetricia y establecer las medidas necesarias para garantizar la seguridad de las gestantes, minimizando el riesgo de contagio de los profesionales implicados en la asistencia. Este protocolo se ha desarrollado en base a la literatura disponible en cada momento, actualizándose constantemente a medida que surgía nueva evidencia científica, y permitiendo así continuar el seguimiento y cuidado esencial del embarazo, parto y puerperio. Por este motivo, uno de los factores determinantes para poder adecuar las medidas de seguridad necesarias para minimizar el riesgo de contagio tanto de las gestantes como de los profesionales, fue conocer la incidencia y prevalencia de la enfermedad en nuestra población.

8. Controversias e incógnitas.

Con el fin de dar luz a la controversia generada alrededor del impacto del SARS-CoV2 en el embarazo, en la presente tesis se intentará dar respuesta a las dudas que han ido apareciendo: ¿Cuál fue la prevalencia real a lo largo del embarazo, durante la primera y segunda ola de la pandemia COVID-19? ¿Implica la infección por SARS-CoV2 en el embarazo un mayor riesgo obstétrico y perinatal? ¿Cómo influye el trimestre en el que se produce la infección, en el curso del embarazo? ¿Existe una transmisión de la inmunidad a través de la leche materna?

HIPÓTESIS Y OBJETIVOS

HIPÓTESIS Y OBJETIVOS

Hipótesis de partida

El SARS-CoV2 es un virus de nueva aparición con alta tasa de contagio. ¿Tiene la infección por SARS-CoV2 un impacto sobre el curso de la gestación, parto y postparto?

Objetivos

Objetivos generales:

1. Evaluar la implementación clínica de un protocolo preventivo frente a la infección por SARS-CoV2.
2. Estudiar la seroprevalencia de la infección en gestantes durante los primeros meses de la pandemia COVID-19 y compararla con aquella reportada en población no gestante.
3. Evaluar la dinámica de seroconversión en la población gestante.
4. Analizar si la tasa de complicaciones del embarazo aumenta en gestaciones diagnosticadas de COVID-19 durante el embarazo (serología positiva) y si influye el momento en que se produce la infección.
5. Examinar la posible transmisión vertical del virus mediante leche materna o, por el contrario, la posible inmunización pasiva del recién nacido.

Objetivos específicos:

- Describir la estructuración de citas en la consulta de obstetricia.
- Determinar la tasa de infección del personal sanitario en las consultas de obstetricia.
- Determinar la tasa de seropositividad en los diferentes momentos epidemiológicos y según las medidas de confinamiento aplicadas.
- Comparar la tasa de preeclampsia, diabetes mellitus gestacional, parto prematuro y alteraciones del crecimiento fetal en embarazos con diagnóstico de SARS-CoV2 y sin él.
- Comparar la tasa preeclampsia, diabetes mellitus gestacional, parto prematuro y alteraciones del crecimiento fetal en embarazos con diagnóstico de SARS-CoV2 en el primer y en el tercer trimestre.
- Determinar la presencia de SARS-CoV2, IgG, IgM e IgA en calostro y leche madura a las 4-6 semanas tras el parto.

MÉTODOS

MÉTODOS

La presente Tesis es un compendio de cuatro publicaciones, basadas en los resultados de una línea de investigación para el estudio de la infección por SARS-CoV2 en la gestación.

1. Diseño de estudio

Estudio de cohortes prospectivo.

2. Población a estudio

Para la realización de este estudio se recogieron datos de tres cohortes prospectivas en gestantes de varios centros hospitalarios de la Comunidad de Madrid, Andalucía, Murcia, Cataluña, y un centro en Hong Kong, SAR.

- Primera cohorte: Se invitó a participar a todas las mujeres que acudieron a su visita de primer trimestre del embarazo al Hospital Universitario de Torrejón (HUT) entre el 1 de enero y el 15 de mayo de 2020.
- Segunda cohorte: Se invitó a participar a todas las mujeres que acudieron a su visita de primer trimestre del embarazo al Hospital Universitario de Fuenlabrada (HUF) entre el 1 de enero y el 15 de mayo de 2020.
- Tercera cohorte: Se invitó a participar a todas las mujeres que parieron en los hospitales Príncipe de Asturias (PdA, Madrid), San Cecilio (SC, Granada), Virgen de la Arrixaca (VdA, Murcia), Valle de Hebrón (VdH, Barcelona) y Prince of Wales Hospital (PoW, Hong Kong, SAR), entre marzo de 2020 y marzo de 2021.

3. Procedimientos clínicos

En la primera cohorte (HUT), se registraron los datos clínicos para cada participante, incluyendo la edad materna, el índice de masa corporal (IMC) al inicio del embarazo, los datos relativos al embarazo, parto y postparto, la edad gestacional al momento de la infección por SARS-CoV2 si la hubo, así como la gravedad de la enfermedad. Desde el diagnóstico de la infección se realizó un seguimiento mensual en consultas de obstetricia para el diagnóstico precoz de complicaciones. En todas las visitas se realizó toma de constantes maternas y ecografía para valoración del crecimiento y bienestar fetal. Se registraron todos los datos evolutivos, posibles complicaciones (enfermedades hipertensivas del embarazo, diabetes gestacional, restricción de crecimiento fetal, parto prematuro, rotura prematura de membranas y muerte fetal) así como el desenlace perinatal. En todas las participantes se recogió

sangre materna a las 11-13 y a las 35-37 semanas del embarazo, en el posparto inmediato (hasta 4 días tras el parto) y a las 4-6 semanas posparto. Tras el parto y en la visita del posparto se recogió además calostro y leche madura respectivamente. Durante el periodo del estudio se registraron de manera sistemática todos los cambios en los protocolos clínicos del hospital, así como el tipo de consultas y los contagios entre el personal sanitario.

En la segunda cohorte (HUF), se registraron los mismos datos clínicos para cada participante que en la primera cohorte, a excepción de las visitas de control, que no se realizaron de manera protocolizada. En todas las participantes se recogió sangre materna a las 11-13 semanas.

En la tercera cohorte (PdA, SC, VdA, VdH y PoW), se recogieron las variables demográficas en el momento de la infección, así como los detalles de la infección por SARS-CoV2, incluyendo el momento de la infección y su gravedad. En todas las participantes se recogió sangre materna y calostro en los primeros 4 días tras el parto y sangre y leche maternas a las 4-6 semanas posparto.

Todos datos se pseudonimizaron y se ingresaron en una base de datos común y segura.

4. Manejo y análisis de muestras

Las muestras de sangre materna se recogieron en tubos de 8 mL con gel separador y activador de coágulo, se centrifugaron durante cinco minutos a 3500 g y, posteriormente, se recogió el suero. Las muestras de leche materna (de 0,1 a 1,0 mL) se recogieron mediante extracción manual, siguiendo estrictas precauciones de contacto para evitar la contaminación (uso de mascarilla facial e higiene de manos). Tanto las muestras de suero como las de leche materna se dividieron en alícuotas de 0,5 mL (cuando fue posible) en tubos Eppendorf separados, etiquetados con un identificador único de paciente, y se almacenaron a -80ºC hasta su análisis posterior.

Las muestras almacenadas en Barcelona se analizaron localmente al final del período de reclutamiento. Las muestras de los demás centros se enviaron sin ningún procesamiento adicional, en hielo seco y en envíos nocturnos al laboratorio SynLab Diagnósticos Globales en Madrid: cada mes desde los centros españoles y en un único envío desde Hong Kong, después de realizarse la prueba de rRT-PCR localmente al final del período de reclutamiento.

Se analizó la presencia del virus SARS-CoV2 tanto por diagnóstico directo (análisis de la presencia del virus SARS-CoV2) como por diagnóstico indirecto (detección de anticuerpos contra el virus).

Las muestras de suero se descongelaron y se analizaron para detectar anticuerpos específicos frente al SARS-CoV2. En el estudio de campo de la presente tesis doctoral se utilizaron, por un lado, la técnica ELISA con el test Euroimmunn (Euroimmunn Medizinische Labordiagnostika AG, Lubeck, Germany) para la determinación de IgA e IgG frente a SARS-CoV2 (60) y, por otro lado, la técnica CLIA con el test Abbott (Abbott Ireland Diagnostics Division Finisklin, Ireland) para la determinación de IgM and IgG (36). Según ficha técnica de ambas casas comerciales, el uso combinado de ambos tests confiere una sensibilidad diagnóstica del 66,7% antes del décimo día del inicio de la clínica, y del 100% realizado posteriormente. La especificidad descrita con el uso combinado de los test asciende a un 97,5% (36,60).

Las muestras de leche materna se descongelaron en el laboratorio y se centrifugaron a 800 g durante 15 minutos. Se eliminó la capa de grasa y el sobrenadante se transfirió a un nuevo tubo. La centrifugación se repitió dos veces para asegurar la eliminación completa de células y grasa (61). Posteriormente, la leche desnatada y acelular se analizó en busca de IgM, IgA e IgG específicas frente al RBD de la proteína S1 de la espícula del SARS-CoV2 (61).

Todo el equipamiento y los reactivos utilizados en los análisis contaron con el marcado CE (Conformité Européenne).

5. Diagnóstico de COVID-19 y clasificación

El diagnóstico de la enfermedad por COVID-19 se realizó mediante rRT-PCR en muestras de saliva de la orofaringe profunda o hisopado nasofaríngeo, análisis serológico o mediante pruebas de detección rápida de antígenos. Se consideró positividad para la infección por SARS-CoV2 según análisis serológico en los siguientes escenarios: IgG positiva, IgA e IgG positivas, IgM e IgG positivas, o IgA e IgM positivas. Cuando se detectó IgA o IgM positiva de forma aislada, la clasificación del caso como exposición, infección reciente o falso positivo se realizó de acuerdo con la historia clínica de la paciente.

La gravedad de la COVID-19 se clasificó como asintomática, leve (cuando no fue necesario el ingreso hospitalario) y grave (cuando se diagnosticó una neumonía y se requirió hospitalización)(62).

6. Análisis estadístico

Los datos descriptivos se expresaron como mediana e intervalo intercuartílico, así como en proporciones (frecuencias absolutas y relativas). Se utilizó la prueba de Mann-Whitney y la prueba exacta de Fisher para comparar los grupos según los desenlaces, en el caso de las variables continuas y categóricas, respectivamente.

Se empleó el coeficiente Kappa de Cohen para evaluar la concordancia entre calostro y suero. El estadístico Kappa se calculó sin ponderación; se consideró un nivel de concordancia muy bueno cuando fue > 0,80; bueno, entre 0,80 y 0,60; moderado, entre 0,60 y 0,40; pobre, entre 0,40 y 0,20; y muy pobre cuando fue < 0,20 (63). Se realizó un análisis de regresión logística univariable para evaluar si la presencia de inmunoglobulinas en el calostro se asociaba con la presencia de inmunoglobulinas en el suero materno, la gravedad de los síntomas maternos o el tiempo transcurrido desde la infección. Se calcularon los odds ratio (OR) y el intervalo de confianza (IC) del 95% (64). Por último, se utilizó la prueba de McNemar para evaluar la evolución de cada tipo de inmunoglobulina en todas las muestras emparejadas de calostro y leche madura; esta prueba reporta valores p basados en la distribución chi cuadrado con 1 grado de libertad.

El nivel de significación se estableció en 0,05. Para todos los análisis de datos se utilizó el software estadístico R, versión 4.1.2 (Viena, Austria)(65).

7. Consideraciones éticas

El estudio fue aprobado por los Comités de Ética de la Investigación con Medicamentos de los Hospitales Universitarios Torrevieja y Elche-Vinalopó (NCT 2020.028), del Hospital Universitario Valle de Hebrón (PR(AMI)181/2020), y del Prince Wales Hospital (sin número proporcionado), y posteriormente validado por los respectivos Comités de Ética de Investigación Locales de los centros participantes. Se proporcionó información verbal y escrita sobre el estudio a las mujeres, por parte de un miembro del equipo médico, durante el embarazo, el parto o inmediatamente después del nacimiento. Se obtuvo el consentimiento informado por escrito de cada participante.

ARTÍCULOS PUBLICADOS

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Estudio 1.- **Application of a new protocol for providing obstetric care in an outpatient service during the COVID-19 pandemic in a public hospital in Madrid, Spain**

Rayo MN, Fernández-Buhigas I, Ferrer E, Arrébola M, Gil MM and Santacruz B. Front Med (Lausanne). 2022 Aug 3;9:902640.

Estudio 2.- **PRECORSE study: Seroprevalence of severe acute respiratory syndrome coronavirus 2 in the first trimester of pregnancy during the first wave of the COVID-19 pandemic and subsequent pregnancy complications-A cohort study**

Aquise A, **Rayo N**, Fernández-Buhigas I, Alfonso A, Pagola N, Rodriguez M, de Miguel L, Santacruz I, Valor S, Poon LC, Gil MM, Santacruz B. Int J Gynaecol Obstet. 2023 Oct;163(1):326-328.

Estudio 3.- **Maternal COVID-19 Serological Changes-Comparison between Seroconversion Rate in First and Third Trimesters of Pregnancy and Subsequent Obstetric Complications: A Cohort Study**

Rayo MN, Aquise A, Fernandez-Buhigas I, Gonzalez-Gea L, Garcia-Gonzalez C, Sanchez-Tudela M, Rodriguez-Fernandez M, Tuñon-Le Poultel D, Santacruz B, Gil MM. Viruses. 2023 Dec 5;15(12):2386.

Estudio 4.- **Anti-SARS-CoV-2-specific antibodies in human breast milk following SARS-CoV-2 infection during pregnancy: a prospective cohort study**

Fernández-Buhigas I, **Rayo N**, Silos JC, Serrano B, Ocón-Hernández O, Leung BW, Delgado JL, Fernández DS, Valle S, De Miguel L, Silgado A, Tanoira RP, Rolle V, Santacruz B, Gil MM, Poon LC. Int Breastfeed J. 2024 Jan 18;19(1):5.

Application of a new protocol for providing obstetric care in an outpatient service during the COVID-19 pandemic in a public hospital in Madrid, Spain **Rayo MN**, Fernández-Buhigas I, Ferrer E, Arrébola M, Gil MM and Santacruz B. Front Med (Lausanne). 2022 Aug 3;9:902640. doi: 10.3389/fmed.2022.902640
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Application of a new protocol for providing obstetric care in an outpatient service during the COVID-19 pandemic in a public hospital in Madrid, Spain

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Objective: To evaluate the clinical implementation of a preventive COVID-19 protocol regarding re-organization of appointments and documented infections among health workers in an obstetric outpatient service.

Methods: Descriptive analysis of the antenatal care at our obstetric outpatient service and infection rates among health care providers from March 19th to May 22nd, 2020. Appointments were divided into telephone calls or face-to-face examinations. A pre-consultation triage was implemented to identify suspected SARS-CoV2 infected women to reschedule them 14 days later or, if the consultation was non-delayable, to use complete Personal Protective Equipment (PPE). Firstly, the number of face-to-face appointments, telephone appointments, and COVID-19 diagnoses in pregnant women were analyzed. Secondly, the number of obstetricians and nurses diagnosed with SARS-CoV2 infection and their serologic status during universal screening in May 2020 were recorded.

Results: One thousand eight hundred forty-two obstetric appointments were scheduled during this period, including 432 (23.5%) telephone appointments (96.53% according to clinical protocol, 1.62% symptomatic patients advised to stay at home, and 1.85% COVID-19 confirmed cases), and 1,410 (76.5%) face-to-face appointments (9.7% did not attend due to fear of getting the infection, 3.1% were lost-to-follow-up, 0.5% were rescheduled due to COVID-19 symptoms and 86.7% who did attend). Of the 1,223 women attending their hospital appointment, 3.6% screened positive at the triage (72.7% rescheduled and 27.3% seen with PPE). 43 rRT-PCR-SARS-CoV2 tests were performed, and two tested positive. No COVID-19 symptoms were reported among health workers at the outpatient obstetric service, and only one nurse presented immunoglobulin (Ig)G anti-SARS-CoV2.

Conclusion: A prompt implementation of a preventive protocol in a hospital obstetric outpatient service, including triage, hygienic and preventive

measurements, and rescheduling pregnancy appointments, reduces the percentage of health workers affected by SARS-CoV2.

KEYWORDS

pregnancy, protocol, COVID-19 pandemic, obstetric care, SARS-CoV2, outpatient care

Introduction

On December 31st, 2019, the Authorities of the People's Republic of China reported several pneumonia cases of unknown etiology to the WHO in Wuhan, a city located in the Chinese province of Hubei (1). A week later, they confirmed that it was a new coronavirus named SARS-CoV2, and the disease it causes was named COVID-19 (1). The disease was transmitted similarly to influenza, SARS, or MERS virus, a person-to-person transmission through respiratory drops produced by coughing, sneezing, or speech in close contact. It was also reported that transmission could occur by touching a contaminated surface or any contaminated mucosa (2, 3). Although the contagion mainly occurred through symptomatic people, presymptomatic or very slightly symptomatic patients were also reported infectious (4, 5).

The first case in Spanish territory appeared on January 31st, but it was not until February 24th when the infection with transmission among the population was verified. The first case of local transmission was detected at Hospital Universitario de Torrejón in Madrid, a patient who had been admitted for pneumonia of unknown origin. From that moment, the city of Torrejón de Ardoz, where the hospital is located, became one of the hotspots for spreading infection in the Madrid area. For this reason and to assess the number of people that had been infected during the first peak of the pandemic, the city hall conducted a universal antibody screening undertaken by 75% of the city population (from 1 year old onwards) from May 29th to June 5th, 2020 (6). It was reported that 20.2% of the people presented anti-SARS-CoV2 immunoglobulin (Ig)G (20.3% in women), and 5.1% showed anti-SARS-CoV2 IgM. In contrast, in the subgroup of women of fertile age (15–44 years old), these percentages were 17.1 and 4.5%, respectively.

Since the onset of the disease, one of the major concerns has been the severe risk faced by health care providers, and many different strategies have been implemented to avoid their massive infection. Obstetric outpatient service were high-risk places for exposure since long periods of close contact with the patients are usually required to perform antenatal scans and examinations. Therefore, obstetricians and midwives constituted a particular risk group.

This study aimed to describe the clinical implementation of a preventive protocol in terms of first, re-organization of appointments and hospital visits, and second, the infection rate among health care workers in a hospital obstetric outpatient service during the first peak of the COVID-19 pandemic.

Materials and methods

Study design and population

This is a descriptive analysis of the antenatal care provided in the obstetric outpatient service at Hospital Universitario de Torrejón, from March 19th to May 22nd, 2020, during the peak of the first wave of the pandemic in Madrid, Spain. The number of face-to-face appointments, telephone appointments, and COVID-19 diagnoses in pregnant women during that period were analyzed.

Additionally, a universal screening by serological analysis of anti-SARS-CoV2 IgM and IgG was performed on all hospital health workers at the end of May 2020. The number of obstetricians and nurses attending pregnant women at the outpatient service who were diagnosed by rRT-PCR-SARS-CoV2 or presented IgG or IgM anti-SARS-CoV2 at the end of May was recorded.

Intervention

When the first patient was diagnosed with COVID-19 infection at the hospital on February 24th, 2020, obstetricians assisting pregnant women began to develop a preventive protocol to ensure safety among health care workers and patients. The first protocol version was implemented on March 7th, and the only change applied was related to contact measures. All personnel attending pregnant women in consultations were requested to wear a surgical mask during the entire examination and to take special care in hand hygiene before and after examining each pregnant woman. In addition, it was recommended that a pregnant woman with respiratory symptoms should wear a surgical mask. Since March 14th, the professionals' face mask was changed to an FFP2 (filtering face pieces type 2) if available, and a double pre-consultation triage was implemented. In addition, the government made strict confinement of the population mandatory, so an entire new prenatal care protocol was established to minimize hospital visits and ensure

TABLE 1 Triage questionnaire.

- Have you had or have a fever or feverish sensation in the last week?
- Have you had or have a persistent cough in the last week?
- Have you had or have muscle pain in the last week?
- Have you had or have general discomfort in the last week?
- Does food taste nothing or have you lost the smell?
- Are you or have you been positive for COVID-19? If the answer is yes:
  - Have been passed >5 weeks since the diagnosis?
- Do you live or are you in close contact with a patient with COVID-19?

pregnancy care on March 19th. The description of the protocol was as follows:

Accompaniment

No companion was allowed in the examination room. The access to the hospital was only limited to the strict necessarily.

Triage from the admission department

All pregnant women who required an appointment at the unit received a telephone text message 2–4 days before the visit confirming whether the consultation was face-to-face or by telephone. They were also advised to contact the hospital if they had any symptoms related to the COVID-19 disease. Furthermore, all pregnant women scheduled for a face-to-face appointment were contacted 24 h before their visit to conduct a 7-questions interview concerning COVID-19 (Table 1). If any question was answered positively, the pregnant woman was asked to stay home and wait for the health professional to call her the next day.

Triage upon arrival at consultations

Before entering the outpatient service, a receptionist was performing the same questionnaire (Table 1) again. If all the items in the survey were negative, she was allowed to enter the examination room. The patient was provided with a surgical mask, hand washing facilities, and latex gloves, and she was instructed to keep the security distance (2 empty chairs) in the waiting area.

If any item was positive, she was considered a possible SARS-CoV2 case, and the obstetrician was informed. The procedure was then as follows:

- If the symptoms were severe (fever higher than 37.5 degrees, dyspnea, severe cough, general malaise), the obstetrician referred the patient to the emergency department for clinical assessment without further evaluation, and subsequent management was assessed later.
- If the symptoms were mild, the patient was either rescheduled or assessed with complete PPE if that was a non-delayable appointment (Table 2).

TABLE 2 Rescheduling algorithm for patients screening or testing positive for COVID-19.

Appointment missed	Action
First-trimester appointment	Rescheduled for US scan 5 weeks later. - Normal ultrasound: cell-free DNA test for aneuploidy screening. - Abnormal* ultrasound: invasive testing.
20–22 weeks anomaly scan	- Fetal anatomy examination by US performed in the first trimester: rescheduled 14 days later. - No fetal anatomy examination by US in the first trimester: anomaly scan rescheduled before 22^{+3} weeks (with PPE).
35–37 growth scan or any other clinically guided scan	Rescheduled 3 weeks later.
Fetal monitoring at 40–41 weeks	- rRT-PCR-SARS-CoV-2 test. - Telephone appointment: ○ General well-being, fetal movements, and presence of eventualities inquiry. ○ Delivery plan. - Labor induction will be scheduled with all the security measures established if positive rRT-PCR-SARS-COV2.
Invasive test	- Suspected cases: delay the procedure 3 weeks. - Confirmed cases: delay 4 weeks after acute illness. - The procedure cannot be delayed: ○ Isolated room ○ Health workers with PPE ○ The minimum needed personnel The risk-benefit was individually assessed.

*Abnormal ultrasound refers to major fetal defects such as holoprosencephaly, omphalocele, megacystis, etc.
US, ultrasound; PPE, personal protection equipment.

Follow-up of non-complicated pregnancies

Appointments at the hospital were limited to the strictly necessary. All the appointments were divided into face-to-face and telephone consultations. Those appointments where complementary tests were not required (ultrasound, physical examination, etc.) were carried out by telephone (Table 3). Complementary tests were always performed simultaneously (ultrasound scans, blood tests), and telephone calls were arranged for results if needed.

TABLE 3 Pregnancy monitoring scheme*.

Face-to-face	Telephone
- Symptoms derived ultrasounds. - First-trimester scan and aneuploidy screening (including blood sampling). - 20–22 weeks anomaly scan. - Second-trimester blood sampling (+/- anti-D immunoglobulin administration). - Routine growth scan at 35–37 weeks, Streptococcus B-Agalactiae screening, and blood sampling. - Fetal cardiotocography monitoring at postdates.	- Results from blood analyses or other prenatal tests (including aneuploidy screening). - 30–32 weeks midwife appointment. - 38–39 weeks midwife appointment.

*High-risk cases were managed individually.

TABLE 4 Preparation and cleaning of obstetrics room.

Deep cleaning

Ultrasound rooms should be cleaned thoroughly (double cleaning) each morning before the arrival of the patients and each evening at the end of the session using CDC-approved cleaners. This cleaning includes:

- Computer, keyboard and mouse, printer, door handles, stretcher, chairs, armchairs, ultrasound machines, light switches.
- Tensiometer and weight scale.
- Fetal monitors.

Ultrasound transducers

- The use of ultrasound transducers was limited to only multi-frequency transabdominal ones (one per machine).
  - If other transducers were needed (i.e., transvaginal probe): double cleaning should be carried out before storing again.
- The transducers in use will be covered with a protector (cover, glove) and will be cleaned with CDC-approved cleaners between patients.

Intermediate cleaning (before the next patient was called into the room)

- Hand wash with soap and warm water or with an antimicrobial cleaner for at least 20 s.
- Ultrasound transducers and cables disinfection
- Patient's bed and chair disinfection
- Change disposable gloves (latex-free, polyurethane)
- Two pairs of gloves when handling dirty clothes or sheets. Hand wash for at least 20 seconds afterward.

Ultrasound scans in suspected or confirmed COVID-19 cases

Whenever possible, the obstetric visit was delayed until 14 days after the end of the respiratory symptoms or, at least, the resolution of the active disease (Table 2). If the examination could not be delayed, it was carried out at the end of the session, limiting the number of health workers and the examination time and wearing full PPE (FFP2 or FFP3 masks if available, protected by surgical mask or screen, waterproof gown, double glove, and glasses). The subsequent disinfection of the scanning room was complete, including the ultrasound machine (Table 4).

Results

From March 19th to May 22nd, 2020, 1842 obstetric appointments were scheduled (Figure 1). One thousand four hundred ten (76.5%) were face-to-face appointments and 23.4% were telephone appointments (432 patients). Of the telephone appointments, 417 (96.5%) were telephone calls according to clinical protocol, 7 (1.6%) corresponded to patients that had been advised to stay home as they had a positive item at the first triage, and 8 (1.9%) corresponded to patients who communicated that they were COVID-19 confirmed cases at the first triage. Of the face-to-face scheduled appointments, 137 (9.7%) patients did not attend because they feared getting infected; they were contacted by phone, and a follow-up was arranged. Six (0.5%) women contacted the hospital to report COVID-19 symptoms, and they were rescheduled according to protocol. 44 (3.1%) did not attend and did not answer phone calls, so they were lost to follow up. 1223 (86.7%) attended face-to-face appointments and had a second triage. 44 (3.6%) screened positive, of whom 32 (72.7%) were rescheduled, and 12 (27.3%) were seen on the day.

In 43 (97.7%) of the screened positive pregnant women, an rRT-PCR-SARS-CoV2 test was performed, and 2 of them tested positive. Additionally, 67 screened negative patients at first and second triages, had a planned cesarean section or induction of labor within the study period, and were tested by rRT-PCR-SARS-CoV2 on the day of the appointment according to protocol; none of them tested positive.

In this period, no symptoms of COVID-19 were reported among health care professionals in the outpatient obstetric service, where 12 doctors, 7 nurses/auxiliary nurses, and 3 receptionists worked daily. Anti-SARS-CoV2 IgG and IgM antibodies were only detected in one nurse following universal health care workers' screening, performed at the end of the study period. However, the onset of her symptoms occurred before she started to work in our unit.

Discussion

Main findings

The main findings of this study were that, first, a preventive protocol can be quickly implemented following an emergent disease outbreak, but this protocol must be

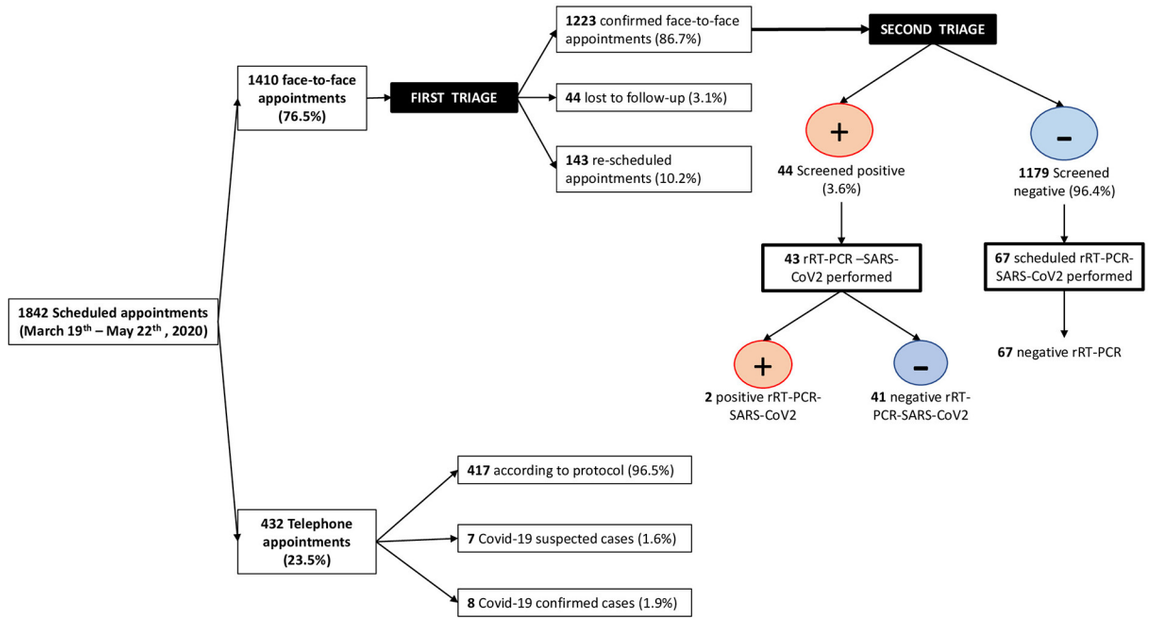

FIGURE 1
Patients diagram flow.

evolving to incorporate new scientific knowledge; second, the implementation of this new protocol in our obstetric outpatient service transformed about 23% of our face-to-face appointments into telephone calls; and third, as a result of the implemented preventive measures, only one health care worker was infected during the first peak of the pandemic.

Research in context

In Madrid region, official sources pointed out that 11,660 health care professionals, including 3,464 were doctors and 5,789 were nurses or auxiliary nurses, had tested positive by rRT-PCR-SARS-CoV2 (9.772 at hospitals and 1.678 at primary care), and 19 of them had died due to COVID-19 disease by June 15th, 2020 (7).

Two seroprevalence studies carried out at two major referral hospitals in Madrid reported that about 21% and 17% of their health care workers presented anti-SARS-CoV2 antibodies by May and June 2020, respectively (8, 9). The Spanish Ministry of Health sponsored a national seroprevalence study reporting an overall prevalence of antibodies in Spain of 4.6% and in Madrid region in particular of 11.5% in May 2020 (10), confirming that, similarly to other studies, the infection was more prevalent between health care professionals than in the general population (9, 11, 12). However, this was not translated into a higher proportion of infected professionals in our department; our only positive result corresponded to a nurse that was initially working in the emergency department, who presented with symptoms and, after recovery, was transferred to our unit.

Torrejón de Ardoz was a hotspot during the first pandemic's peak and one of the first places where people suffered from COVID-19 disease in Spain. The seroprevalence study carried out in the city showed that about 20% of the population presented anti-SARS-CoV-2 IgG, and about 23% presented any kind of antibody (IgM and IgG) by June 5th, 2020 (6). This study pointed out how severely affected this city was in comparison to the rest of Madrid region. If about 17% of the women of fertile age in the city were exposed to the virus during the study period, we could assume that about 300 (17% of the 1,842 appointments) of the scheduled women at our obstetric service were infected including about 200 (17% of 1,223) who had face-to-face appointments. However, we only identified 44 women as high risk of having COVID-19 in our triage system, and only two had a positive result from the rRT-PCR-SARS-CoV2 for the SARS-CoV2 test. A likely explanation for this lower rate is that first, asymptomatic patients were not tested, and second, most women with symptoms stayed at home. These results highlight the beneficial effect of the early implementation of our preventive protocol. Unfortunately, many other studies have reported delayed actions and much higher rates of infected professionals (4).

Strengths and limitations

One of the biggest concerns for implementing such a protocol was the decreased quality of care and the subsequent worsening in the perinatal outcomes. However, since that was not the aim of our study, we are not reporting on this. Another limitation relates to the lack of universal testing in pregnant women, which would have been crucial for assessing the usefulness of our protocol in terms of patient safety. However, antibody testing is not a definitive test to determine disease since false positives and negatives may occur.

However, our study presents some significant strengths. First, it is a complete cohort where all scheduled appointments were carefully reviewed, allowing accurate reporting on the protocol consequences in our obstetric service. Second, although not a diagnostic test, all health care professionals at our institution received universal screening for anti-SARS-CoV2 antibodies, which provides invaluable information regarding contagious rate.

Clinical implications

Since the early implementation of preventive measures was crucial to prevent the spread of the disease, our study has demonstrated the feasibility of rapidly rearranging all appointments within a unit by joining efforts from several professionals (doctors, nurses, and other health workers). Therefore, if a new infection outbreak happened in the future, we would be able to protect health workers and our pregnant population.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients/participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conceptualization, methodology, and writing—original draft: MR, IF-B, and MG. Data curation and formal analysis: MR and IF-B. Investigation and writing—review and editing: MR, IF-B, EF, MA, BS, and MG. Project administration: MG and BS. Supervision: IF-B, MG, and BS. Validation: MR, IF-B, MG, and BS. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

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PRECORSE study: Seroprevalence of severe acute respiratory syndrome coronavirus 2 in the first trimester of pregnancy during the first wave of the COVID-19 pandemic and subsequent pregnancy complications-A cohort study

Aquise A, **Rayo N**, Fernández-Buhigas I, Alfonso A, Pagola N, Rodriguez M, de Miguel L, Santacruz I, Valor S, Poon LC, Gil MM, Santacruz B. Int J Gynaecol Obstet. 2023 Oct;163(1):326-328.

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BRIEF COMMUNICATION

Obstetrics

PRECORSE study: Seroprevalence of severe acute respiratory syndrome coronavirus 2 in the first trimester of pregnancy during the first wave of the COVID-19 pandemic and subsequent pregnancy complications—A cohort study

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Keywords: antibodies, coronavirus, COVID-19, immunoglobulin, pandemic, pregnancy, SARS-CoV-2, seroprevalence

The current investigation was a multicenter, observational, ambispective study developed at two maternity units in Madrid, Spain. IgA, IgM, and IgG antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were analyzed from serum samples of archived first-trimester antenatal blood samples of 707 consecutive pregnant women between January 1 and May 15, 2020. Pregnant women who tested positive were included in a comprehensive follow-up program with obstetric visits every 4 weeks until delivery.

Unlike molecular tests (real-time reverse polymerase chain reaction), serological techniques are not only useful to confirm suspected cases of SARS-CoV-2 infection but will also detect mild or asymptomatic infections. Antibodies against SARS-CoV-2 were detected in 58 of 707 samples (seroprevalence of 8.20%): 49 (6.93%) positive for IgA, 16 (2.26%) for IgM, and 39 (5.52%) for IgG. Results were interpreted as "exposure to viral infection", "recent infection", and "past infection" according to immunoglobin status. The first blood samples in which antibodies against SARS-CoV-2 were present corresponded to the period of January 1 to 15, 2020, suggesting that the spread of the virus started in December 2019, with eight seropositive cases before the pandemic was declared (Figure 1).

Prevalence of anti–SARS-CoV-2 antibodies among first-trimester pregnant women during the peak of the COVID-19 pandemic was similar to that described in the general population in Spain. A nationwide population-based seroepidemiological study including 51 958 samples obtained between April 27 and May 11, 2020, reported a seroprevalence of 4.6%. However, the Madrid region showed a much higher rate of 11.5%.[1] Only two studies included first-trimester pregnant women: one including 769 nonconsecutive stored samples from women attending routine pregnancy blood testing, reporting a seroprevalence of 11.2%,[2] and one including 874 consecutive pregnant women attending the hospital for their first- and third-trimester blood testing, reporting a seroprevalence of 15% and 14%, respectively.[3] Reasons for the lower prevalence found in our study are: inclusion of patients from January and February 2020 when the prevalence was lower; the fact that these two studies were conducted at referral hospitals, showing a higher prevalence due to

Adriana Aquise and Nieves Rayo contributed equally to the work.

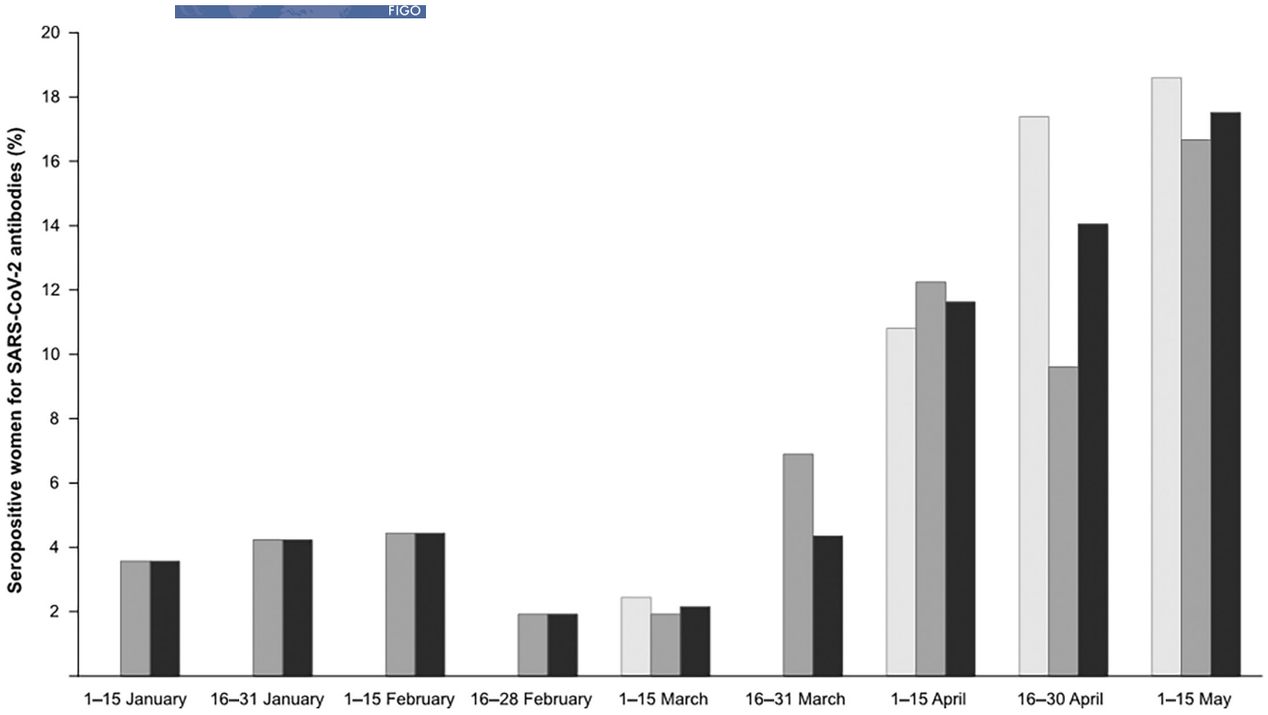

FIGURE 1 Rate of seropositive women for anti–severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies during the study period. Black bars: total prevalence of seropositive women during the study period; dark gray bars: seropositive women at Hospital Universitario de Torrejón; light gray bars: seropositive women at Hospital Universitario de Fuenlabrada.

centralization of COVID-19 cases; and that these studies mainly included third-trimester samples, when the immune status of pregnant women makes them more susceptible to infections.

Finally, rates of pregnancy complications were not significantly increased in seropositive patients. The main limitation of our study is that the small sample size may lack power to reliably evaluate the rates of pregnancy complications related to SARS-CoV-2 infection.

Increasing knowledge of SARS-CoV-2 seroconversion in pregnancy allows comprehensive follow-up for the early detection of possible complications and provides valuable information for future pandemics.

AUTHOR CONTRIBUTIONS

Adriana Aquise and Nieves Rayo participated in the design, planning, conduct, data collection, data analysis, and manuscript writing. Irene Fernánde-Buhigas, Ana Alfonso, and Natalia Pagola collaborated in the design and planning of the study, data collection, and manuscript reviewing. Miguel Rodriguez collaborated in the data analysis, performing the statistical analysis. Laura de Miguel, Irene Santacruz, and Santiago Valor collaborated in the design and planning of the study and performed the sample analysis and collected the data from Synlab Laboratory. Liona C. Poon and María M. Gil participated in the design, planning, writing, and revision of the manuscript. Belen Santacruz supervised and participated in the design, planning, conduct of the study, and revision of the article.

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FUNDING INFORMATION

No funding was necessary to complete the study.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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Maternal COVID-19 Serological Changes-Comparison between Seroconversion Rate in First and Third Trimesters of Pregnancy and Subsequent Obstetric Complications: A Cohort Study

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Article

Maternal COVID-19 Serological Changes—Comparison between Seroconversion Rate in First and Third Trimesters of Pregnancy and Subsequent Obstetric Complications: A Cohort Study

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Abstract: Pregnant women are especially vulnerable to respiratory diseases. We aimed to study seroconversion rates during pregnancy in a cohort of consecutive pregnancies tested in the first and third trimesters and to compare the maternal and obstetric complications in the women who seroconverted in the first trimester and those who did so in the third. This was an observational cohort study carried out at the Hospital Universitario de Torrejón, in Madrid, Spain, during the first peak of the COVID-19 pandemic. All consecutive singleton pregnancies with a viable fetus attending their 11–13-week scan between 1 January and 15 May 2020 were included and seropositive women for SARS-CoV2 were monthly follow up until delivery. Antibodies against SARS-CoV-2 (IgA and IgG) were analyzed on stored serum samples obtained from first- and third-trimester routine antenatal bloods in 470 pregnant women. Antibodies against SARS-CoV-2 were detected in 31 (6.6%) women in the first trimester and in 66 (14.0%) in the third trimester, including 48 (10.2%) that were negative in the first trimester (seroconversion during pregnancy). Although the rate of infection was significantly higher in the third versus the first trimester (p = 0.003), no significant differences in maternal or obstetric complications were observed in women testing positive in the first versus the third trimester.

Keywords: SARS-CoV-2; pregnancy; morbidity

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1. Introduction

In early December 2019, a cluster of individuals suffering from pneumonia of unknown cause were identified in the city of Wuhan, Hubei Province, China. On 31 December 2019, the World Health Organization (WHO) was notified of these cases. Subsequently, the disease named COVID-19, a severe acute respiratory syndrome caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) [1], has spread rapidly throughout most countries in the world. On 11 March 2020, the WHO declared a global pandemic emergency due to COVID-19. Since the outset of the pandemic, the gold standard for the diagnosis of active SARS-CoV2 infection remains real-time reverse-transcription polymerase chain reaction (rRT-PCR), a technique that detects viral RNA in the nasopharynx. However, false negatives have been reported for this technique, mainly due to problems related to sample

collection and/or detection methods [2]. As a result, these limitations of the technique have led to some concerns as to whether rRT-PCR should be the gold standard test for the diagnosis of SARS-CoV2 infection. Conversely, serological tests bypass these technical challenges and, unlike rRT-PCR, are often faster, more cost-effective, user-friendly, and capable of identifying asymptomatic cases. As a result, they emerge as a valuable tool in gauging the pandemic's scope [2]. On the basis of these advantages, serological surveys have been suggested as a complementary approach to RT-PCR to improve its sensitivity and provide rapid identification, study, and isolation of infected people and their contacts to prevent the spread of the coronavirus.

Research on previous pandemics caused by seasonal influenza, Middle East respiratory syndrome (MERS), and SARS-CoV1 have provided valuable information to face this novel mutation of Coronavirus, labeled as SARS-CoV2. Data from the past two decades reveal that over 10,000 individuals have affected by SARS-CoV1 and MERS-CoV infections, with respective mortality rates of 10.5% and 34.4%. More importantly, these pandemics have demonstrated that pregnant women had a higher vulnerability to respiratory infections due to physiological changes in their immune and cardiopulmonary systems and that a different obstetric impact was observed according to the trimester in which the infection was acquired [3–5]. Unfortunately, a challenging problem arises in the attempt to determine the impact of this novel mutation of Coronavirus, labeled as SARS-CoV2. Despite the increasing number of published studies, the reported data are still insufficient to draw definite and unbiased conclusions regarding the impact of SARS-CoV2 infection on obstetric morbidity or the clinical relevance of the time at which the infection occurs. Hence, the testing of specific SARS-CoV2 antibodies have emerged as a potential solution to address this challenge more effectively. Conducting sequential serological tests during the first and third trimesters of pregnancy could serve as a valuable clinical approach. This method could reliably pinpoint the timing of infection and accurately assess the impact of COVID-19 on pregnancy based on the trimester in which the woman was infected. Moreover, understanding the SARS-CoV2 immune status of women among the pregnancy presents e a unique opportunity to determine a more precise incidence of SARS-CoV2 infection. This knowledge facilitates a comprehensive follow-up, enabling the prompt detection of any possible complications. It also ensures high-quality assessment and healthcare for these pregnancies.

In this study, we aimed to assess the immune status of a complete and consecutive cohort of pregnant women throughout the pregnancy (from the first to the third trimester) covering the first (between March and June 2020) and second (between June and December 2020) waves of the COVID-19 pandemic [6] in one of the hotspots of Madrid (Spain), as Torrejón de Ardoz was one of the first places where population suffered from the infection. In addition, we aimed to analyze the rates of obstetric complications in the group of women who got infected by this new Coronavirus in the first trimester compared to those who seroconverted in the second or third trimesters of pregnancy.

2. Materials and Methods

2.1. Study Design and Population

This was a longitudinal, observational, and ambispective study carried out between 1 January and 25 December 2020, at the Hospital Universitario de Torrejón (HUT), Madrid, Spain, as part of the PRECORSE study (Study for PREgnancy CORonavirus Serologic Evidence), as has been previously described [7].

In our center, a surplus of antenatal blood samples from all pregnant women is routinely frozen and stored at −80 °C degrees at the Biobank Network of the Region of Murcia (Spain), BIOBANC-MUR (reg. number: B.0000859) for clinical and for research purposes. After the COVID-19 pandemic outbreak, all available stored serum samples collected during the first-trimester routine analysis between 1 January and 15 May 2020 were identified. The samples corresponding to women who gave their written informed consent to participate in this study and fulfilled the inclusion criteria (women over 18 years

old, having singleton pregnancies with a nonmalformed life fetus, and having their pregnancy care in our Obstetric Unit), were retrieved from the freezers and transferred on dry ice to Synlab laboratory in Madrid, Spain, for determination of anti-SARS-CoV2 immunoglobulin A (IgA) and immunoglobulin G (IgG). These women were followed-up throughout their pregnancy according to the local protocol and, those testing positive in the first trimester were contacted and had monthly follow-ups in a specific clinic for maternal and fetal wellbeing and fetal biometry assessments. The surplus from their third-trimester routine blood was also tested for anti-SARS-CoV2 IgA and IgG antibodies. For every woman participating in our study, demographic characteristics, including age, ethnicity, body mass index, parity, smoking habits, medical disorders, and even blood type data were prospectively and thoroughly recorded at all hospital appointments throughout the pregnancy, until the last pregnant woman gave birth on 25 December 2020. All of the pregnant women included were classified according to their serological status in the first and third trimesters of pregnancy: those who were IgA or IgG anti-SARS-CoV2 positive in the first trimester of pregnancy ("positive serology 1T"); and those who were IgA or IgG anti-SARS-CoV2 positive in their third trimester, with a prior negative serology in the first trimester ("positive serology 3T"). Information about pregnancy outcomes (gestational hypertension, preeclampsia, gestational diabetes, fetal growth disorders, fetal anomalies, and other obstetrics complications, such as intrahepatic cholestasis, Rh isoimmunization, preterm birth, and shortened cervix) was meticulously collected from the hospital medical records and also by telephone interview if needed.

It is important to highlight that none of the participants had been vaccinated against SARS-CoV2, since the vaccine hadn't yet been developed and this infection marked their first known encounter with SARS-CoV-2.

The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement was used for reporting the results obtained in this study.

2.2. Laboratory Analysis and Interpretation

Determination of anti-SARS-CoV2 IgA and IgG was performed with an enzyme-linked immunosorbent assay (ELISA), providing semi-quantitative (extinction of the control patient sample/extinction of calibrator) serology results against the S1 domain of the spike protein of SARS-CoV2 in serum samples (Anti-SARS-CoV2 ELISA IgG and Anti-SARS-CoV2 ELISA IgA, Euroimmunn Medizinische Labordiagnostika AG, Lubeck, Germany). IgA and IgG were considered positive, indeterminate, and negative when the results were >1.1, 0.8 to 1.1, and <0.8, respectively, as recommended by the manufacturer (Supplementary Materials Table S1).

For anti-SARS-CoV2 IgG, sensitivity and specificity reported by manufacturers is 83.3% and 95.0% respectively in confirmed COVID-19 cases and, 70.8% and 96.6% respectively in suspected COVID-19 cases [8]. Overall sensibility and specificity reported for anti-SARS-CoV2 IgA are 86.7% and 82.7%, respectively [9].

2.3. Statistical Analysis and Data Management

The data are expressed as the median (interquartile range) for continuous variables and in proportions (absolute and relative frequencies) for categorical variables. The Mann–Whitney test and Fisher's exact test were used for comparing outcome groups for continuous and categorical data, respectively. The level of significance was set at 0.05. The statistical software package R was used for the data analyses [10], as well as table1 package [11].

2.4. Ethical Considerations

Approval from the local Research Ethics Committee Committee (Comité Ético de Investigación con Medicamentos de los Hospitales Universitarios Torrevieja y Elche-Vinalopó, No. Reg: 2020.028) was obtained prior to the start of the study. Signed informed consent was obtained from all pregnant women participating.

3. Results

3.1. Results from the First Trimester of Pregnancy

The surplus of routine first-trimester blood samples from 503 pregnant women was identified between 1 January and 15 May 2020, in the Hospital Universitario Torrejón in Madrid, Spain. Four hundred and eighty of the women were eligible, agreed, gave their consent to participate in the study, and had their blood samples from rutinary gestational analysis tested for anti-SARS-CoV2-specific antibodies. A total of 10 of the 480 pregnant women were excluded because of an insufficient amount of sample for analysis (n = 4) or lost to follow-up very early in their pregnancy (n = 6).

Finally, blood samples from 470 women were obtained (Table 1), including 31 (6.6%) samples that tested positive for SARS-CoV2 antibodies, either IgA, IgG, or both.

Table 1. Maternal baseline characteristics.

Variable	Positive Serology 1T (n = 31)	Positive Serology 3T (n = 48)	p-Value
Gestational age at delivery (weeks)	39.6 [38.9; 40.0] Missing: 2 (6.5%)	40.0 [38.6; 40.5] Missing: 0 (0%)	0.285
Maternal age (years)	35.0 [29.0; 38.0]	33.5 [29.8; 36.3]	0.398
Body mass index (kg/m^2)	22.9 [21.5; 26.4]	25.3 [22.8; 28.6]	0.110
Nulliparous	17 (54.8%)	20 (41.7%)	0.356
Race			
Black	1 (3.2%)	2 (4.2%)	1.000
Non-Hispanic White	22 (71.0%)	37 (77.1%)	0.601
Hispanic/Latin	7 (22.6%)	6 (12.5%)	0.352
Asian	0	1 (2.1%)	1.000
North African	1 (3.2%)	1 (2.1%)	1.000
Other	0	1 (2.1%)	1.000
Blood type			
A Positive	12 (38.7%)	15 (31.3%)	0.628
A Negative	1 (3.2%)	1 (2.1%)	1.000
O Positive	15 (48.4%)	21 (43.8%)	0.818
O Negative	1 (3.2%)	3 (6.3%)	1.000
B Positive	2 (6.5%)	6 (12.5%)	0.470
B Negative	0	0	1.000
AB Positive	0	1 (2.1%)	1.000
AB Negative	0	1 (2.1%)	1.000
Active smoking	0	5 (10.4%)	0.151
Chronic medical pathology			
None	24 (77.4%)	33 (68.8%)	0.451
Hypertensive disorders	1 (3.2%)	0	0.392
Diabetes mellitus	0	0	1000
Autoimmune or Immunological disorders	2 (6.5%)	0	0.151
Respiratory disease	1 (3.2%)	2 (4.2%)	1.000
Others	7 (22.6%)	15 (31.3%)	0.451

1T: first trimester; 3T: third trimester.

3.2. Results from the Third Trimester of Pregnancy

Of the 470 women with results from the first trimester testing, 7 had an early miscarriage (including 1 positive case in the first trimester), 4 had a late miscarriage, 3 terminated the pregnancy, and 54 were lost to follow-up (including 3 positive cases in the first trimester). Therefore, 402 samples were available for anti-SARS-CoV2-specific antibody testing in the third trimester, including 27 cases that were positive in the first trimester. A total of 66 (16.4%) of the 402 third-trimester samples tested positive, including 18 that had a positive result in the first trimester and 336 (83.6%) tested negative, including 9 that had a positive result in the first trimester. Therefore, SARS-CoV2 seroconversion during pregnancy occurred in 48 cases with complete follow-up (48, 12.8%, of the 375 negative pregnancies in the first trimester) (Figures 1 and 2), which is statistically significantly higher than the seroconversion rate in the first trimester ($p = 0.003$).

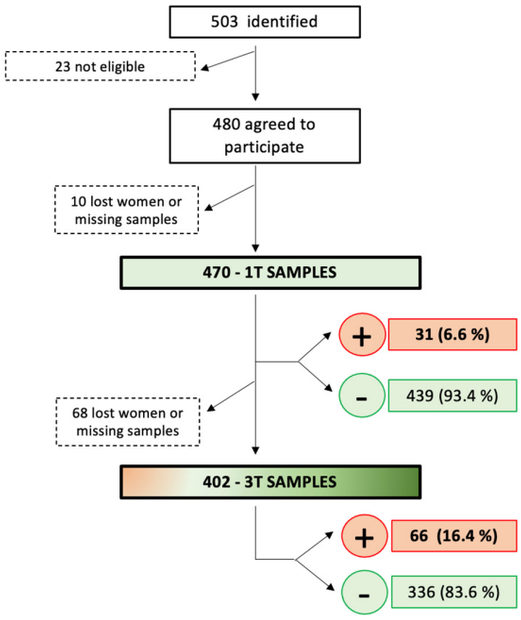

Figure 1. Patients' flowchart, study process, and COVID-19 seroprevalence rate in first and third trimesters of pregnancy. 1T: first trimester; 3T: third trimester.

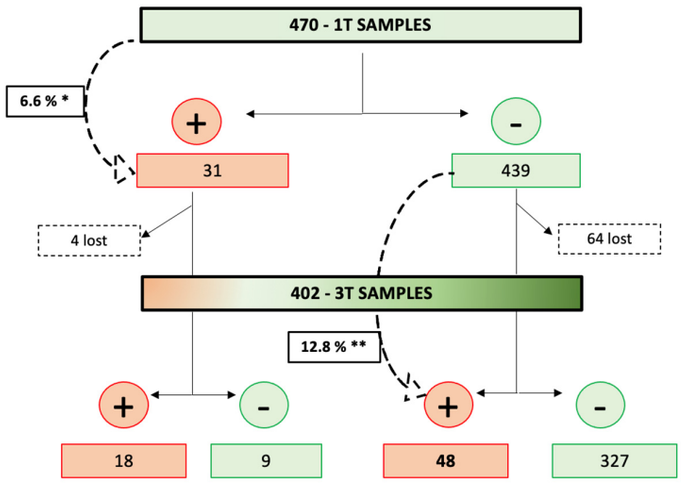

Figure 2. COVID-19 seroconversion rate throughout the pregnancies. 1T: first trimester; 3T: third trimester. * First-trimester seroconversion rate. ** Third-trimester seroconversion rate.

3.3. Persistence of Antibodies among the Pregnancy

Of the 31 women with a positive serological test in the first trimester, 27 had their third-trimester blood samples tested for COVID-19 (1 had an early miscarriage and 3 were lost to follow-up). In addition, 18 (66.7%) of the 27 cases with complete follow-up still had a positive anti-SARS-CoV2 serology in the third trimester, while 9 of them (33.3%) had both negative IgG and IgA anti-SARS-CoV2 (Supplementary Materials Table S2).

3.4. Maternal Morbidity

Among the positive cases, there were no differences in baselines characteristics between pregnant women who had a positive serology in the first trimester (n = 31) and those who seroconverted in the third trimester of their pregnancy (n = 48) (Table 1).

The present study showed no statistically significant differences when comparing the maternal or obstetric morbidity (gestational hypertension, preeclampsia, gestational diabetes, fetal growth disorders, fetal anomalies, and other obstetrics complications, such as intrahepatic cholestasis, Rh isoimmunization, preterm birth, and shortened cervix) according to the trimester in which SARS-CoV2 seroconversion occurred (Table 2).

Table 2. Obstetric complications according to serology group.

	Positive Serology 1T (n 30 *)	**Positive Serology 3T (n 48)**	***p*-Value**
None	23 (76.7%)	33 (68.8%)	0.606
Gestational hypertension	1 (3.4%)	0	0.385
Preeclampsia	0	0	1.000
Gestational diabetes	3 (10.0%)	3 (6.3%)	0.670
Preterm birth	1 (3.3%)	2 (4.2%)	1.000
Other (cholestasis, Rh isoimmunization, shortened cervix, plaquetopenia. . .)	0	1 (2.1%)	1.000
Small for gestational age	0	4 (8.3%)	0.156
Fetal growth restriction	1 (3.3%)	3 (6.3%)	1.000
Fetal anomalies	1 (3.3%)	2 (4.2%)	1.000

* n = 30, as one woman was excluded from the positive serology 1T group (initially n = 31) due to early miscarriage in her first trimester of pregnancy. 1T: first trimester; 3T: third trimester; Rh: Rhesus.

4. Discussion

4.1. Main Findings of the Study

The main finding of this study is that, during the first COVID-19 pandemic peak, the seroconversion rate in the third trimester (12.8%) was double that in the first trimester (6.6%). However, obstetric or maternal complications did not differ between both groups. In addition, we demonstrated that about two-thirds (18/27) of the women with a positive serology in the first trimester, remained positive in the third trimester. This result highlights that naturally acquired immunity against SARS-CoV2 may last for several months.

4.2. Comparison with Previous Studies

We previously demonstrated that, in Madrid region, rate of SARS-CoV2 infection among pregnant women was similar to that reported in the general population [7]. A nationwide, population-based sero-epidemiological study carried out between 27 April and 11 May 2020, including 51,958 samples obtained from all Spanish regions, reported an overall SARS-CoV2 seroprevalence of 4.6%. However, there was a geographical variability, and Madrid showed a much higher seroprevalence rate of 11.5% [12] which is concordant with our findings.

Despite the growing number of published articles, only a few studies have evaluated the seroprevalence of SARS-CoV2 infection at different stages of pregnancy during the 2020 outbreak of COVID-19 in Spain. The reported prevalence of positive serological tests in pregnant women in our country varied from 15% in the first trimester [13], to 20% in the third trimester and delivery [13–16]. There is a smaller-scale study, carried out at three hospitals in New York, involving 149 women who were assessed for anti-SARS-CoV2 IgG antibodies during the first and second trimesters, as well as at the moment of delivery [17]. The outcomes from this study were similar to our own findings as the authors reported a seroprevalence rate of 12.1% during the first trimester and 16.1% during the second trimester. Notably, 71.4% of the women who tested positive during the first trimester remained positive at the time of delivery, which is similar to the 66.7% observed in our cohort. The notable increase in seroconversion rates during the third trimester compared to the first trimester might be explained by maternal immunological changes occurring in the latter stages of pregnancy. These adaptations potentially heighten susceptibility to certain infections [5,18,19]. However, in our study, this shift could be linked to the timing of the first trimester, which coincided with the pandemic's onset. Stringent preventive measures, especially for vulnerable groups like pregnant women, were rigorously implemented. As these measures eased during the 2020 summer, marking the end of extreme social isolation, a subsequent surge in COVID-19 cases occurred during the second wave, affecting the general population, including pregnant women.

Regarding obstetric and maternal morbidity, we expected a similar effect in pregnancy as that reported in the literature during previous pandemics (MERS, SARS-CoV-1 and influenza), with a higher rate of miscarriage when the infection took place in the first trimester of pregnancy and more cases of fetal growth restriction (FGR) when the infection occurred in late pregnancy [3–5]. However, the existing literature on obstetric morbidity among SARS-CoV-2-infected pregnant individuals presents conflicting findings demonstrating that our current understanding of COVID-19 infection across pregnancy trimesters remains limited. On the one hand and consistent with our results, certain authors have not identified statistically significant differences in obstetric complications. Cosma et al. did not observe elevated rates of early pregnancy loss in women infected with SARS-CoV-2 during their first trimester [20]. Similarly, Villalaín et al. and Juan et al. did not report an increased risk of adverse pregnancy outcomes such as FGR, preterm birth, or preeclampsia [21,22]. Conversely, numerous other studies have demonstrated higher rates of obstetric complications associated with SARS-CoV-2 infection, including preterm birth, premature rupture of membranes, low birth weight, and stillbirth [23–26].

Additionally, the current knowledge of COVID-19 infection in different trimesters of pregnancy is still limited. While efforts to compare obstetric morbidity based on the infection trimester have increased, there is ongoing debate. Several studies suggest a higher incidence of adverse fetal outcomes, including stillbirth, perinatal and neonatal death, and preterm birth in women infected during their first trimester. In contrast, infections in the third trimester seem associated with lower fetal growth percentile and higher rates of small for gestational age (SGA) fetuses [26,27].

However, there are still insufficient data assessing immunological status throughout pregnancy and, normally, only acute infection using rRT-PCR SARS-CoV2 has been assessed at a single time point. This could be leading to a selection bias, as the majority of the SARS-CoV2-infected population is actually asymptomatic and, therefore, no rRT-PCR will have been performed [5]. Di Mascio et al. [26] analyzed 388 pregnancies that had a positive rRT-PCR SARS-CoV2 test during pregnancy, describing how perinatal outcomes (stillbirth, perinatal and neonatal death, and preterm birth) were significantly worse with decreasing gestational age at the time of infection. In a retrospective study evaluating 882 positive pregnant women with rRT-PCR SARS-CoV2, including 85 women diagnosed in the first trimester, it was reported that gestational age at the time of infection was the best predictor for gestational age at delivery [27]. To the best of our knowledge, there is only one study that has been conducted to assess SARS-CoV2 serology during

both the first and third trimesters while examining the potential association between the presence of antibodies and pregnancy outcomes [28]. In this study, which involved 528 singleton pregnant women, the authors carried out serological assessments during the initial 11–13-week screening visit and again upon the admission for delivery. Data from pregnancy outcomes (gestational age at delivery, preterm birth before 34 weeks, hypertensive disorders, gestational diabetes, and abnormal fetal growth) were exhaustively collected to investigate the association between obstetric morbidity and SASRS-CoV2 infection. They did not discover any significant association between serological status and major obstetric complications. It must be pointed out that our study conducted a serological analysis in the third trimester of pregnancy, at 35–36 weeks, which likely provides a more comprehensive evaluation of newly emerging complications.

The high virulence of this novel SARS-CoV2, coupled with the still ongoing debate about its potential adverse effects on pregnancy, underscores the importance of continued scientific research. Researchers should remain committed to exploring existing data in order to be better prepared for potential future viral threats and to provide the pregnant population with a specific and high-quality assessment and healthcare. Serological SARS-CoV2 screening stands as a valuable tool that can provide high quality evidence regarding the natural progression of the disease, its severity and prognosis based on the timing of infection, enhancing the clinical management of those infected women. Nevertheless, we want to highlight that various other variables could contribute to the susceptibility to SARS-CoV-2 infection. Factors such as the employment status of pregnant individuals—whether on maternity or pregnancy leave—and the nature of their careers (onsite vs. remote work), along with the size of their household or the number of children in a family, are crucial elements that could significantly impact susceptibility to SARS-CoV-2 infection. Recognizing these social aspects as influential factors underscores the necessity for proactive investigation and their inclusion in research endeavors. By integrating these social dimensions, future studies can better equip us to confront and prevent infections in potential future pandemics.

4.3. Strengths and Limitations

The main strength of our study is the longitudinal follow-up of a consecutive sample of pregnant women who were in their first trimester of pregnancy during the COVID-19 first outbreak in Madrid, Spain, one of the most severely affected countries in Europe at that time. This allowed us to analyze two blood samples, corresponding to the first and third trimesters, coinciding with the first and second waves of the COVID-19 pandemic. Assessing anti-SARS-CoV-2 immunoglobulins in these distinct pregnancy phases provided a unique opportunity to enhance our understanding of maternal immune response adaptations, explore the impact of social security measures on COVID-19 incidence, and investigate the relevance of infection timing on obstetric outcomes.

However, there are some limitations in our work that warrant acknowledgment. We consider that the main limitation of our study relates to the small sample size of our cohort, which might be responsible for the lack of statistically significant differences in the results. Consequently, this has prevented us to perform any subgroup analysis. In addition, we did not record individual measures to prevent SARS-CoV2 infection, therefore we have assumed that women were compliant with governmental restrictions implemented during this period of the COVID-19 pandemic. Another possible limitation found in our study is the lack of information concerning social characteristics such as working routines and family members, including children living in the same residence, which could have been useful to better analyze both groups, as it might have an impact on the predisposition to suffer from SARS-CoV2 infection.

5. Conclusions

The main conclusion drawn in the present study is that the COVID-19 seroconversion rate was higher in third than in the first trimester of pregnancy, covering the first and

second waves of the COVID-19 pandemic, with the majority of the women infected during their first trimester remaining positive throughout gestation. The prompt implementation of SARS-CoV2 serological testing as part of the protocol in obstetric outpatient services in every trimester rutinary analysis would be able to detect asymptomatic cases and reflect an accurate COVID-19 seroconversion rate. While our study did not find any statically significant differences in maternal or obstetric complications based on the trimester of infection, larger studies, including social variables are still needed. Such studies are necessary to enhance preparedness for potential future viral threats and to mitigate the risk of disease contraction.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/v15122386/s1, Table S1: Serological and clinical characteristics of the 48 women with positive COVID third-trimester serology with prior negative testing in first trimester of pregnancy; Table S2: Pregnant women with persistent COVID-19 serology in the third trimester of pregnancy.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (Comité Ético de Investigación con Medicamentos de los Hospitales Universitarios Torrevieja y Elche-Vinalopó, Nº Reg: 2020.028, 29 June 2020), which was obtained prior to the start of the study. Signed informed consent was obtained from all participants. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement was used for reporting the results.

Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author. The data are not publicly available due to data protection regulations.

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Anti-SARS-CoV-2-specific antibodies in human breast milk following SARS-CoV-2 infection during pregnancy: a prospective cohort study

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Anti-SARS-CoV-2-specific antibodies in human breast milk following SARS-CoV-2 infection during pregnancy: a prospective cohort study

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Abstract

Background While the presence of SARS-CoV-2 in human breast milk is contentious, anti-SARS-CoV-2 antibodies have been consistently detected in human breast milk. However, it is uncertain when and how long the antibodies are present.

Methods This was a prospective cohort study including all consecutive pregnant women with confirmed SARS-CoV-2 infection during pregnancy, recruited at six maternity units in Spain and Hong Kong from March 2020 to March 2021. Colostrum (day of birth until day 4 postpartum) and mature milk (day 7 postpartum until 6 weeks postpartum) were prospectively collected, and paired maternal blood samples were also collected. Colostrum samples were tested with rRT-PCR-SARS-CoV-2, and skimmed acellular milk and maternal sera were tested against SARS-CoV-2 specific immunoglobulin M, A, and G reactive to receptor binding domain of SARS-CoV-2 spike protein 1 to determine the presence of immunoglobulins. Then, we examined how each immunoglobulin type in the colostrum was related to the time of infection by logistic regression analysis, the concordance between these immunoglobulins in the colostrum, maternal serum, and mature milk by Cohen's kappa statistic, and the relationship between immunoglobulin levels in mature milk and colostrum with McNemar.

Results One hundred eighty-seven pregnant women with confirmed SARS-CoV-2 infection during pregnancy or childbirth were recruited and donated the milk and blood samples. No SARS-CoV-2 was found in the human breast milk. Immunoglobulin A, G, and M were present in 129/162 (79·6%), 5/163 (3·1%), and 15/76 (19·7%) colostrum samples and in 17/62 (27·42%), 2/62 (3·23%) and 2/62 (3·23%) mature milk samples, respectively. Immunoglobulin A was the predominant immunoglobulin found in breast milk, and its levels were significantly higher in the colostrum than in the mature milk (p-value < 0.001). We did not find that the presence of immunoglobulins in the colostrum was associated with their presence in maternal, the severity of the disease, or the time when the infection had occurred.

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Conclusions Since anti-SARS-CoV-2 antibodies are found in the colostrum irrespective of the time of infection during pregnancy, but the virus itself is not detected in human breast milk, our study found no indications to withhold breastfeeding, taking contact precautions when there is active disease.

Keywords Human Breast Milk, Colostrum, SARS-CoV-2, COVID-19, Anti-SARS-CoV-2 specific antibodies

Background

On 11 March 2020, the COVID-19 pandemic was declared by the World Health Organization (WHO) [1]. Since then, extensive efforts have focused on evaluating the effects of the new coronavirus on pregnancy. At the very beginning of the pandemic, newborns were separated from their mothers with confirmed SARS-CoV-2 infection to protect them against the virus. Breastfeeding was avoided because it was unknown if the virus could be transmitted via human breast milk. To date, some studies have reported the presence of SARS-CoV-2 in human breast milk [2–7] while others have not [8–14], but the sample size of these studies is small.

Currently, most healthcare systems and international organizations such as the Centers for Disease Control and Prevention (CDC) recommend breastfeeding for all mothers with active or past infection of SARS-CoV-2, as there appear to be more benefits of breastfeeding than the potential risk of transmission through human breast milk. One of the most important reasons to recommend breastfeeding is the possible passive immunization in newborns against SARS-CoV-2 [15]. In particular, IgA is important because it coats and seals the neonate's respiratory and intestinal tracts to prevent microorganisms from entering the body and bloodstream, constituting the first defense against the virus [16, 17]. Several studies have reported the presence of anti-SARS-CoV-2 antibodies [18–25] in human breast milk. Pace et al. have demonstrated that the specific IgG, IgM, and IgA anti-SARS-CoV-2 antibodies in human breast milk can effectively neutralize SARS-CoV-2 infectivity [11]. However, it is uncertain when the antibodies become present and how long they last in human breast milk.

The aims of this study were first, to determine the presence of anti-SARS-CoV-2 virus and antibodies in colostrum and mature human breast milk in women who had SARS-CoV-2 infection during pregnancy or at the time of childbirth; second, to investigate the association between the anti-SARS-CoV-2 antibodies in human milk with the levels of anti-SARS-CoV-2 antibodies in maternal blood, the severity of SARS-CoV-2 infection and the time interval from active illness; and third, to evaluate how each immunoglobulin type evolved from the colostrum to the mature milk.

Methods

Study population

This was a prospective cohort study aiming to include all consecutive pregnant women with laboratory-confirmed SARS-CoV-2 infection by deep throat saliva (DTS) or nasopharyngeal swab (NPS) real-time reverse-transcriptase-polymerase-chain-reaction (rRT-PCR) test or by rapid antigen-detection tests (Panbio™ COVID-19 Ag Rapid Test Device) [26], during pregnancy, labor or immediately after childbirth, who were able to provide consent to participate in the study, from six maternity units, five in Spain (Hospital Universitario de Torrejón and Hospital Universitario Príncipe de Asturias in Madrid, Hospital Universitario Vall d'Hebrón in Barcelona, Hospital Clínico Universitario San Cecilio in Granada and Hospital Clínico Universitario Virgen de la Arrixaca in Murcia) and one in Hong Kong SAR, China (The Chinese University of Hong Kong COVID-19 collaborative network), from March 2020 to March 2021. Eligibility criteria were: confirmed SARS-CoV2 infection, over 18 years old, and fluent in the investigator's language. Additionally, for suspected cases of COVID-19 where rRT-PCR was negative, if the symptoms had started within seven days of testing, the rRT-PCR was repeated 24 h after the first test. If the symptoms had started beyond seven days of testing, a serology test (ELISA) was performed [27] and women with positive results by either test were also offered participation.

Breast milk samples were collected from the six maternity units. All participants were unvaccinated against SARS-CoV-2, and it was their first SARS-CoV-2 infection.

The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement was used for reporting the results (Additional file. Table 1s).

Participants had one sample of colostrum (between the day of birth and day 4 postpartum) collected and stored at -80ºC. Maternal blood for serological analysis was also collected simultaneously; serum was separated and stored at -80ºC. One sample of fore mature milk (from day 7 to 6 weeks postpartum) was also collected and stored whenever possible.

Clinical data, including maternal age, body mass index (BMI) at the beginning of pregnancy, gestational age at the time of SARS-CoV-2 infection, and disease severity, were recorded for every participant, pseudo-anonymized,

and entered into a secured common database. The COVID-19 severity was classified as asymptomatic, mild (when no hospitalization was required), and severe (when the diagnosis of pneumonia was established and hospitalization was needed) [28]. Gestational age was determined by first trimester sonographic assessment of fetal crown-rump length [29] or conception date in vitro fertilization pregnancy.

Biological sample collection and analysis

Breast milk (from 0.1 to 1.0 mL) was collected by manual expression with strict contact precautions to avoid contamination (facial mask and hand cleaning). Blood samples were collected in serum sep clot activator 8 mL tubes, centrifuged for five minutes at 3500 g, and then serum was collected. Both serum and breast milk samples were divided into 0.5 mL aliquots (when possible) in separate Eppendorf tubes, labeled with a unique patient identifier, and stored at -80ºC until subsequent analysis. Stored samples from Barcelona were analyzed locally at the end of the recruitment period. Samples from all other sites were sent without any further processing overnight on dry ice to Synlab Diagnósticos Globales Laboratory in Madrid every month from Spanish sites and in a single batch after rt-RT-PCR testing was performed locally at the end of the recruitment period from Hong Kong.

Breast milk samples were thawed at the laboratory and centrifuged at 800 g for 15 min. Fat was removed, and the supernatant was transferred to a new tube. Centrifugation was repeated twice to ensure the removal of all cells and fat [22]. Skimmed acellular milk was then tested against SARS-CoV-2 specific immunoglobulin M (IgM), immunoglobulin A (IgA), and immunoglobulin G (IgG) reactive to the receptor binding domain (RBD) of the SARS-CoV-2 spike protein 1 (protS1) [22]. As previously reported, serum samples were thawed and tested against SARS-CoV-2 specific antibodies. All equipment and reagents used for analyses are CE (Conformité Européenne) marked (Additional file. Table 2s).

Immuno-analyses

- Determination of IgA and IgG antibodies was performed by the ELISA method (Enzyme-Linked Immunosorbent Assay), providing semiquantitative serology results against the S1 domain of the spike protein of SARS-CoV-2 in serum samples (Anti-SARS-CoV-2 ELISA IgG and Anti-SARS-CoV-2 ELISA IgA, Euroimmunn Medizinische Labordiagnostika AG, Lubeck, Germany) [30, 31]. Semiquantitative results were calculated as extinction of the control patient sample/extinction of calibrator (further details on this type of analysis are provided in Table 2). IgA and IgG were considered positive, indeterminate, and negative when results were > 1.1, 0.8 to 1.1 and < 0.8, respectively, as recommended by the manufacturer.
- IgM determination was performed with chemiluminescence microparticle immunoassays, using spike protein-specific (Abbott test, SARS-CoV-2 IgM Abbott, Abbott Ireland Diagnostics Division Finisklin, Ireland) [32], providing semiquantitative (extinction of the control patient sample/extinction of calibrator). IgM was considered positive, indeterminate, and negative when results were > 1.1, 0.9 to 1.1 and < 0.9, respectively, as recommended by the manufacturer.

rRT-PCR-SARS-CoV-2 testing

Whenever available, a second colostrum aliquot was tested for SARS-CoV-2 by rRT-PCR to assess the presence of the virus in the sample. In the Spanish samples, viral RNA was extracted with Chemagic Viral DNA/RNA Kit using the Chemagic 360 with integrated dispense, which includes lyophilized Poly(A) RNA, lyophilized Proteinase K, and a lysis/binding buffer, and were analyzed with Euroinmune Kit (ORF1ab an N targets) and TaqMan™ 2019-nCov Assay kitv2 Thermofisher (s,ORF1ab and N targets). In the Hong Kong samples, viral RNA was extracted using RNeasy® Mini Kit (QIAGEN), and the detection of SARS-CoV-2 RNA was performed with the FDA-authorized CDC 2019-Novel Coronavirus (2019 nCoV) Real-Time RT-PCR Diagnostic Panel (EUA 200001). The N gene (N1 and N2) was assayed, with the human RNase P (RP) as an endogenous reference control. In all cases, samples containing organic or inorganic contaminants interfering with the PCR amplification process were considered inhibited (these samples contained organic or inorganic contaminants that interfered with the PCR amplification process).

For this study, we included all women with available colostrum; additional samples or analyses were not mandatory for inclusion. Given the limited volume of colostrum and serum collected, not all tests could be carried out in all cases. For some laboratory analyses that failed at the first attempt, repeat testing was not possible. Besides, many women did not return to the clinic after birth due to the lockdown. Therefore, we could not collect mature milk in these cases.

Statistical analysis

Descriptive data were expressed as median and interquartile range (IQR) and in proportions (absolute and relative frequencies). Cohen's Kappa was used to assess

the colostrum and serum concordance. The kappa statistic was calculated without weighting; very good levels of agreement were considered when it is > 0.80, good 0.80–0.60, moderate 0.60–0.40, poor 0.40–0.20 and very poor < 0.20 [33]. Univariable logistic regression analysis was performed to assess if the presence of immunoglobulins in colostrum was associated with the presence of immunoglobulins in maternal serum, the severity of maternal symptoms, or the time passed from infection. Odds ratio (OR) and 95% confidence interval (CI) were calculated [34]. Lastly, the McNemar test was used to evaluate how each immunoglobulin type evolved in all paired colostrum-mature milk samples; this test reports *p* values based on the chi squared distribution with 1 degree of freedom. The level of significance was set at 0.05.

The statistical software R version 4.1.2 (Vienna, Austria) was used for all data analyses [35].

Results

A total of 246 pregnant women with confirmed SARS-CoV2 infection during pregnancy or childbirth were eligible and were approached with information about the study. After exclusions, 191 women agreed to participate (4 were underaged, 7 were unable to provide consent, and 44 were not interested in participating). Among those, 187 had colostrum available for analysis (Figs. 1, 2). Of these, 38 (20.3%), 65 (34.8%), and 84 (44.9%) women acquired the infection in the first (< 14 weeks), second (14–28^{+6}), and third trimester (> 28^{+6}) of pregnancy, respectively. Among the cases with third-trimester infection, 29 (34.5%) had active SARS-CoV-2 infection at childbirth (rRT-PCR-SARS-CoV-2 positive at birth). Pregnancy and disease characteristics are shown in Table 1.

The colostrum and blood samples were collected between the day of birth and day 4 postpartum (median = 1; IQR 0 to 1). Mature milk samples were collected after day 7 postpartum (median = 39 days, IQR 25 to 44). Sample availability and serological status are displayed in Table 2.

Out of the 187 samples collected, only 162 yielded results for IgA, 163 for IgG, and 76 for IgM due to technical issues such as limited volume and assay failures (Table 3). IgA, IgG, and IgM were present in 129/162 (79.6%), 5/163 (3.1%), and 15/76 (19.7%) colostrum samples, respectively. All immunoglobulin-positive colostrum samples tested positive for IgA, except for one sample that only tested positive for IgG (IgA and IgM negative). Another tested positive for IgM and IgG, but there was insufficient sample for the detection of IgA. None of the samples had all 3 immunoglobulins detected.

Seventy-six colostrum samples were tested for rRT-PCR-SARS-CoV-2, including 29 with active disease at birth. 73 tested negative, and 3 were inhibited (these samples contained organic or inorganic contaminants that interfered with the PCR amplification process).

Association between colostrum and serum

One hundred eighteen women had at least one serology result. Association between the colostrum and serum measured with Cohen's Kappa was 0.09 (CI 95% -0.11 to 0.30) for IgA; 0.06 (CI 95% -0.01 to 0.12) for IgG, and 0.29 (CI 95% 0.03 to 0.54) for IgM (Table 4).

Factors related to colostrum positivity

There were no statistically significant differences between the immunoglobulin status in colostrum and the severity of the symptoms nor the time interval from the disease, either as a continuous variable or considering only active disease at birth vs. no active disease at birth (Table 5).

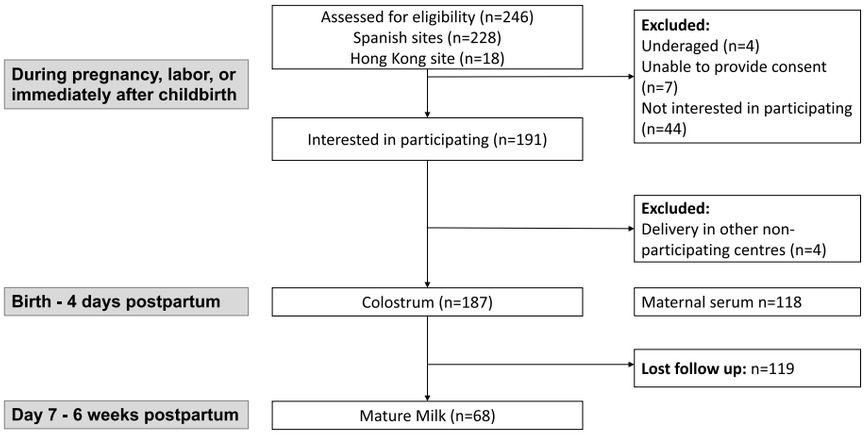

Fig. 1 Sample flow chart. STROBE layout

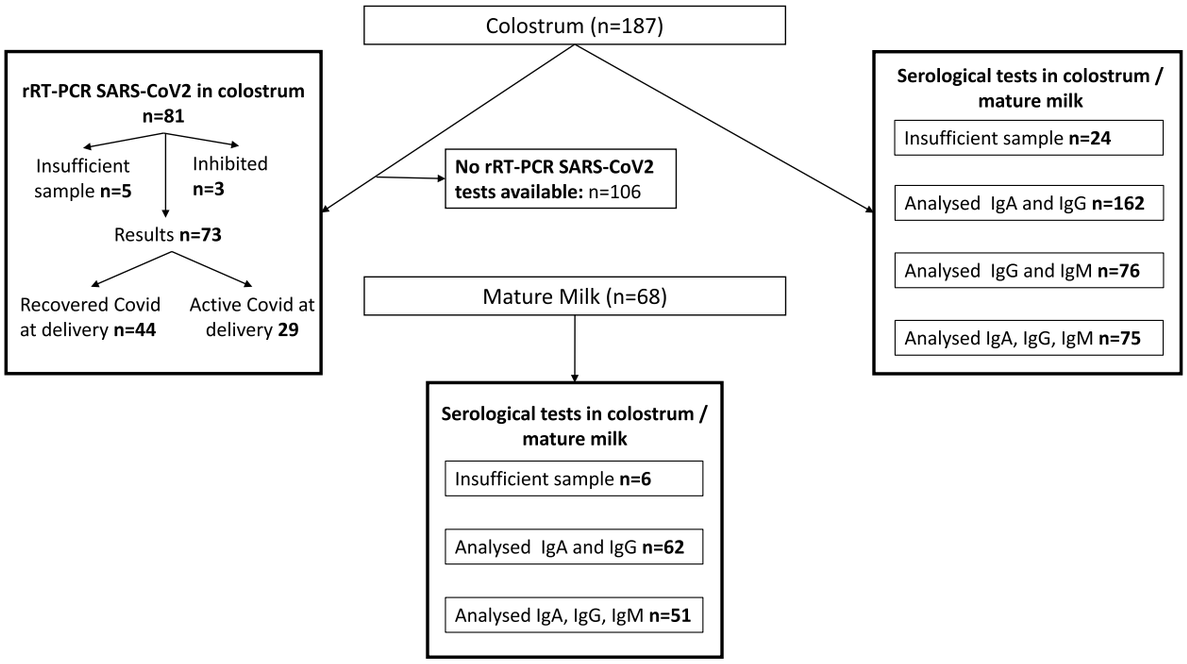

Fig. 2 Sample analysis Flow chart

Antibody evolution from colostrum to mature milk

In mature milk samples, IgG was positive in 2/62 (3.23%) (two women with active disease at birth that tested negative in colostrum); IgA was positive in 17/62 (27.42%) (32 women that tested positive in colostrum but negative in mature milk; *p*-value for the difference between IgA in mature milk vs. IgA in colostrum < 0.001, McNemar's chi-squared statistic = 29.032); and IgM was positive in 0/51 (6 of 51 were positive in colostrum).

Table 1 Maternal, pregnancy, and disease characteristics according to time of infection and disease activity

	Infection at the First trimester (*N* = 38)	Infection at the Second trimester (*N* = 65)	Infection at the Third trimester (*N* = 57)	No active disease (*N* = 158)	Active disease at birth (*N* = 31)	Overall (*N* = 189)
Maternal age (years)	33.0 [30.3, 37.0]	34.0 [30.0, 37.0]	31.0 [28.0, 36.0]	33.0 [29.0, 37.0]	33.0 [28.5, 37.0]	33.0 [29.0, 37.0]
Height (cm)	165 [159, 170]	162 [159, 168]	161 [156, 168]	163 [158, 169]	161 [156, 166]	163 [158, 168]
Weight (kg)	65.0 [56.3, 74.8]	69.0 [58.0, 76.0]	62.5 [54.0, 70.3]	65.0 [56.5, 74.0]	60.0 [54.4, 74.0]	64.5 [56.0, 74.0]
Missing	0 (0%)	0 (0%)	1 (1.8%)	1 (0.6%)	0 (0%)	1 (0.5%)
Body mass index (kg/m^2)	24.2 [21.5, 27.5]	24.9 [21.5, 28.7]	23.4 [21.7, 25.8]	24.1 [21.5, 27.6]	24.7 [21.0, 28.7]	24.2 [21.5, 28.0]
COVID-19 Diagnosis with						
Antigens	9 (23.7%)	13 (20.0%)	7 (12.3%)	29 (18.4%)	3 (9.68%)	32 (16.9%)
RRT-PCR	20 (52.6%)	38 (58.5%)	38 (66.7%)	94 (59.5%)	26 (83.9%)	120 (63.5%)
Serology	9 (23.7%)	14 (21.5%)	12 (21.1%)	35 (22.2%)	2 (6.45%)	37 (19.6%)
Gestational age at diagnosis of COVID-19 (days)	65.5 [50.5, 82.8]	162 [132, 184]	240 [226, 254]	176 [105, 226]	268 [265, 280]	195 [115, 248]
COVID-19 symptoms						
Asymptomatic	6 (15.8%)	8 (12.3%)	17 (29.8%)	30 (19.0%)	17 (54.8%)	47 (24.9%)
Mild	31 (81.6%)	50 (76.9%)	33 (57.9%)	113 (71.5%)	12 (38.7%)	125 (66.1%)
Pneumonia	1 (2.63%)	7 (10.8%)	7 (12.3%)	15 (9.49%)	2 (6.45%)	17 (8.99%)
Gestational age at birth (days)	279 [270, 284]	277 [272, 283]	278 [274, 283]	278 [273, 284]	275 [268, 286]	278 [272, 284]

Results are presented as median (interquartile range) or as n (%) as appropriate

Table 2 Number of colostrum, mature milk, and maternal blood samples and proportions with anti-SARS-CoV2 virus or antibodies detected by semiquantitative analysis (reported as a ratio)

Analysis	Colostrum	Mature milk	Maternal blood at birth
IgA			
Indeterminate (0·8 to 1·1)	4 (2.14%)	0 (0%)	15 (12.5%)
Negative (< 0·8)	29 (15.5%)	51 (75.0%)	42 (35.0%)
Positive (> 1·1)	129 (69.0%)	15 (22.1%)	62 (51.7%)
Insufficient sample	25 (13.4%)	2 (2.94%)	1 (0.833%)
Missing	2 (1.1%)	121 (64.0%)	69 (36.5%)
Total simples analysed	187	68	120
IgG			
Indeterminate (0·8 to 1·1)	3 (1.60%)	0 (0%)	9 (6.47%)
Negative (< 0·8)	155 (82.9%)	63 (92.6%)	61 (43.9%)
Positive (> 1·1)	5 (2.67%)	3 (4.41%)	68 (48.9%)
Insufficient sample	24 (12.8%)	2 (2.94%)	1 (0.719%)
Missing	2 (1.1%)	121 (64.0%)	50 (26.5%)
Total simples analysed	187	68	139
IgM			
Indeterminate (0·9 to 1·1)	0 (0%)	0 (0%)	4 (4.26%)
Negative (< 0·9)	61 (60.4%)	52 (96.3%)	59 (62.8%)
Positive (> 1·1)	15 (14.9%)	0 (0%)	30 (31.9%)
Insufficient sample	25 (24.8%)	2 (3.70%)	1 (1.06%)
Missing	88 (46.6%)	135 (71.4%)	95 (50.3%)
Total simples analysed	101	54	94
rRT-PCR			
Negative	73 (90.1%)		
Positive	0 (0%)		
Inhibited	3 (3.70%)		
Insufficient sample	5 (6.17%)		
Missing	108 (57.1%)		
Total simples analysed	81		

A semiquantitative method was used. For this analysis, the ratio between the extinction of the control or patient sample and the extinction of the calibrator is calculated according to the following formula: Extinction of the control or patient sample / Extinction of calibrator

Extinction refers to the Optical Density or Absorbance at 450nm Wavelength

The extinction of the calibrator defines the upper limit of the reference range of non-infected persons (cut-off or threshold) recommended by the manufacturer. Values above the indicated cut-off are considered positive and those below negative

IgA Specific anti-SARS-CoV2 immunoglobulin A, *IgG* Specific anti-SARS-CoV2 immunoglobulin G, *IgM* Specific anti-SARS-CoV2 immunoglobulin M), rRT-PCR Real-time reverse-transcriptase-polymerase-chain-reaction

Table 3 Colostrum serology results according to type of immunoglobulin

IgA (187)	IgG (187)	IgM (101)	Number of samples
Positive	Positive	Negative	2
		No result	2
	Negative	Positive	14
		Negative	39
		No result	70
	Indeterminate	No result	2
Negative	Positive	Negative	1
	Negative	Negative	17
		No result	10
	Indeterminate	No result	1
Indeterminate	Negative	Negative	2
		No result	2
No result	Negative	Positive	1

IgA Specific anti-SARS-CoV2 immunoglobulin A, *IgG* Specific anti-SARS-CoV2 immunoglobulin G, IgM Specific anti-SARS-CoV2 immunoglobulin M). No result: when no test result was obtained for that immunoglobulin analysis

Table 4 IgM, IgA and IgG results for colostrum and delivery serum samples

		Antibody results at delivery		
		Negative	**Positive**	**Total**
Antibody results in colostrum	**IgM**			
	Negative	41	11	52
	Positive	6	7	13
	Total	47	18	65
	IgA			
	Negative	8	9	17
	Positive	23	43	66
	Total	31	52	83
	IgG			
	Negative	57	50	107
	Positive	0	3	3
	Sum	57	53	110

Discussion

Main findings

The study has demonstrated that, firstly, all human breast milk tested for rRT-PCR SARS-CoV-2 are negative; secondly, antibodies against SARS-CoV-2 present in the colostrum do not seem to vary significantly in relation to the time when the infection has occurred during pregnancy or with regard to their presence in the maternal blood; and thirdly, IgA is the predominant immunoglobulin found in human breast milk and its concentrations are significantly lower in the mature milk compared with colostrum.

Study strengths and limitations

To our knowledge, this is the largest series of colostrum samples from women with SARS-CoV-2 infection during pregnancy or at the time of birth (MEDLINE via Pubmed search (September 2023): ((Human breast milk[MeSH Terms]) AND ("COVID-19" [MeSH Terms])) AND

Table 5 Factors related to colostrum positivity. Results from three univariable logistic regression models to identify significant predictors of immunoglobulin positivity in colostrum among a) symptoms, b) interval from disease to sample, and c) active disease at birth

Predictors of antibody positivity in colostrum	IgA (*n* = 158) Odds Ratio (95% Confidence interval)	IgG (*n* = 160) Odds Ratio (95% Confidence interval)	IgM (*n* = 76) Odds Ratio (95% Confidence interval)
Asymptomatic (*n* = 40, 42, 13)	Reference	Reference	Reference
Mild (*n* = 102, 104, 62)	1.27 (0.50, 3.04)	1.22 (0.15, 25.01)	0.80 (0.21, 3.97)
Severe (*n* = 16, 14, 1)	4.35 (0.72, 84.10)	3.15 (0.12; 83.62)	NA
Interval from disease to sample – colostrum (days)	1.00 (0.99, 1.00)	1.00 (0.98, 1.01)	1.00 (0.99, 1.00)
No active disease (*n* = 133, 136, 67)	Reference	Reference	Reference
Active disease (*n* = 25, 24, 9)	0.51 (0.20, 1.44)	1.43 (0.07, 10.25)	1.19 (0.16, 5.63)

Ig Immunoglobulin, Adjusted analyses were not possible due to small numbers. Numbers between parentheses are referring to the sample size of each stratum. For example, in the first line "(*n* = 40, 42, 13)" means that in the IgA model there were 40 asymptomatic women, in the IgG model 42 and in the IgM model 13

("antibodies" [MeSH Terms]), and where all three types of antibodies, as well as rRT-PCR-SARS-CoV-2, were tested. We also collected paired colostrum and mature milk samples and studied the serological status of the mother at the time of milk sampling, which allowed us to investigate the immunoglobulin association between the colostrum, mature milk, and maternal blood. Additionally, the protocol for collecting, handling, and storing samples was defined early and implemented in all centers [22]. Furthermore, we included 16 pregnant women with severe disease in the study, allowing us to investigate possible associations between the presence of immunoglobulins in colostrum and the severity of the disease.

The main limitations relate to the small sample size and the technical difficulties that further reduced the sample, which may have prevented us from recognizing other possible associations or significant findings. However, technical factors equally affect all samples, making it unlikely to be a source of bias. Besides, this study was conducted at the peak of the pandemic outbreak when vaccination was not a confounder, so the findings are still of great value. A second important limitation is that there is a wide range of gestational age at sampling, and the timing of colostrum and serum sample collection varied between days 0 to 4 postpartum, which may be responsible for physiological changes in immunoglobulin concentration. Nonetheless, we believe this also provides a better understanding of what happens during pregnancy and postpartum. Of note, there were fewer obese women and pregnancies ending in preterm birth than expected among infected COVID-19 pregnancies. However, this might be because most patients were recruited in non-tertiary referral centers, where the most severe cases were centralized.

Interpretation

It is well known that breastfeeding protects babies against gastrointestinal and respiratory infections [36–39]. IgA represents around 90% of all immunoglobulins in human milk, and its concentration is higher in the colostrum, decreasing during the first year of lactation [15]. Due to its low degradation and absorption rate in the infant's gastrointestinal system, IgA is the most important immunoglobulin in human milk since it protects the infant against infections at the mucosa level [16, 40]. Recently, it has been demonstrated that specific IgG, IgM, and IgA anti-SARS-CoV-2 antibodies in breast milk neutralize the virus in vitro [11, 41–43]. Therefore, anti-SARS-CoV-2 IgA in human breast milk could also protect the infant against the SARS-CoV-2 infection locally in their gastrointestinal mucosa, similar to what happens with other viral infections [44, 45].

In our study, most colostrum samples tested positive for IgA, irrespective of the time of SARS-CoV-2 infection. A significant reduction in IgA positivity was found when evaluating longitudinal changes in the colostrum and the mature milk. This is similar to what happens in other viral infections [15]. Importantly, IgA was present even in the colostrum of mothers with a negative serological status at childbirth, contrary to what happened with IgG, which was more likely to be detected when IgG in serum was also present. A possible explanation for this could be related to the fact that IgA is secreted from the maternal Gastrointestinal Antigen Linfoid Tissue (GALT) system and transported into the maternal mammary glands, where they are incorporated into the breast milk, while IgG is mostly filtered from the maternal plasma, albeit at a lower concentration [46]. When the infant nurses, they receive these antibodies along with essential nutrients from the maternal milk, providing passive immunity and protection against infections until their

immune system matures [47, 48]. This system is responsible for secreting antibodies against common infections prevalent in maternal living area and, therefore, represent maternal memory [49]. This system also secretes IgM but at much lower concentrations.

In this study, 29 samples from women with active disease at childbirth were tested by rRT-PCR-SARS-CoV-2, and all were negative. Evidence suggesting the presence of SARS-CoV-2 in breast milk is conflicting [2–5, 8–10, 20], and it is possible that cross-contamination was responsible for the positive results [11]. Goad et al. investigated the presence of cell-specific expression of angiotensin-converting enzyme 2 (ACE2), proteases TMPRSS2, and cathepsins CTSB and CTSL in breast epithelium, and they did not find co-expression of ACE2/TMPRSS2 or ACE2/CTSB/L, which is essential for the entry of the virus into the cell. Therefore, they concluded that there was no risk of vertical transmission of SARS-CoV-2 in neonates through breastfeeding [50].

Clinical implications

This study confirms that SARS-CoV-2 is not detected in breast milk, even when active infection occurs at birth. Therefore, the possibility of vertical transmission while breastfeeding is extremely low. Furthermore, since antibodies are found in the colostrum irrespective of the time of infection, all women should be encouraged to breastfeed their infants, regardless of the time when the condition has occurred during the pregnancy, undertaking contact precautions when there is active disease. Nevertheless, since IgA concentrations drop significantly from the colostrum to mature milk, we could speculate that they might be even lower beyond six weeks postpartum, so public health measures should still be maintained to reduce the risk of the babies acquiring the infection.

Conclusions

Our study has provided further evidence that breastfeeding is safe during maternal SARS-CoV-2 infection as the virus has not been detected in human breast milk, and protective antibodies have been found instead. However, larger studies with longer follow-ups are still needed.

Abbreviations

ACE2	Angiotensin-converting enzyme 2
BMI	Body mass index
CI	Confidence interval
ELISA	Enzyme-linked immunosorbent assay
GALT	Gastrointestinal antigen Linfoide tissue
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IQR	Interquartile range
LMP	Last menstrual period
protS1	SARS-CoV-2 spike protein 1
RBD	Receptor binding domain
rRT-PCR	Real-time reverse-transcriptase-polymerase-chain-reaction

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13006-023-00605-w.

Additional file 1: Table S1. STROBE Statement-Checklist of items that should be included in reports of cohort studies.

Additional file 2: Table S2. Conformité Européene (CE) registration number of the reagents used for sample analyses.

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Authors' contributions

Conceptualization: IFB, LCP, MMG. Data curation: IFB, NR, JCS, BS, OOH, BWL, JLD, DSN, SV, LM, AS, RPT, VR, BS, MMG, LCP. Formal analysis: IFB, NR, VR, MMG. Investigation: IFB, NR, JCS, BS, OOH, BWL, JLD, DSN, SV, LM, AS, RPT, VR, BS, MMG, LCP. Methodology: IFB, MMG, LCP. Project administration: MMG, LCP. Supervision: MMG, LCP. Validation: IFB, NR, JCS, BS, OOH, BWL, JLD, DSN, SV, LM, AS, RPT, VR, BS, MMG, LCP. Writing – original draft: IFB, NR, MMG, LCP. Writing – review & editing: IFB, NR, JCS, BS, OOH, BWL, JLD, DSN, SV, LM, AS, RPT, VR, BS, MMG, LCP. Statistics: VR.

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Availability of data and materials

The data presented in this study are available on request from the corresponding author and conditioned to approval from the relevant Research Ethics Committees due to data protection regulations.

Declarations

Ethics approval and consent to participate
The study was approved by the Vall d'Hebron University Ethics Committee (PR(AMI)181/2020) and Hong Kong (no number provided) on March 27, 2020, and subsequently validated by each Local Research Ethics Committee at the participating centers. Verbal and written information about the study was provided to the woman by a medical team member during pregnancy, labor, or immediately after childbirth. Written informed consent was obtained from every participant.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Publisher's Note

RESUMEN DE RESULTADOS Y DISCUSIÓN

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Estudio 1: Aplicación de un nuevo protocolo para la asistencia obstétrica en el área de consultas durante la pandemia de COVID-19 en un hospital público de Madrid, España.

Los principales hallazgos de este estudio fueron, en primer lugar, que un protocolo preventivo puede implementarse de forma rápida adaptándose a la evolución del conocimiento científico; en segundo lugar, que la implementación de este nuevo protocolo en un servicio de consultas externas de obstetricia permitió transformar aproximadamente el 23% de las consultas presenciales en consultas telefónicas; y, en tercer lugar, que como resultado de las medidas preventivas adoptadas, solo un profesional sanitario se infectó durante el primer pico de la pandemia.

A pesar de que la tasa de seroprevalencia de la COVID-19 descrita a nivel nacional fue inferior al 5%, la comunidad sanitaria sufrió una prevalencia mucho mayor, de hasta un 20% (57–59). El municipio de Torrejón de Ardoz, donde se desarrolló este estudio, fue un “punto caliente” durante la primera ola de la pandemia y uno de los primeros lugares con casos de infección por SARS-CoV2 en España, con un 20% de la población general con presencia IgG frente al SARS-CoV2 (56). Esto puso de manifiesto la gravedad de la situación en esta área de la Comunidad de Madrid y la necesidad de tomar medidas para minimizar el riesgo de transmisión del virus.

Gracias a una rápida y precoz implementación de un nuevo protocolo de asistencia a la mujer embarazada, la reestructuración de citas de control de embarazo y medidas de seguridad establecidas en el área de consultas de Obstetricia del Hospital de Torrejón, el cuidado prenatal no se detuvo en ningún momento durante la pandemia, lo que no ocurrió en ningún otro servicio. Estos cambios incluyeron el reemplazo de visitas presenciales por telemáticas y el diseño de un sistema de filtro, en el que las gestantes pasaban por un triaje previo a la consulta para detectar casos sospechosos COVID-19 y adaptar su manejo en consecuencia. Todo ello permitió disminuir los contagios entre los profesionales sanitarios.

Estudio 2: Estudio PRECORSE: Seroprevalencia del coronavirus responsable del síndrome respiratorio agudo grave tipo 2 en el primer trimestre de embarazo durante la primera ola de la pandemia de COVID-19 y complicaciones subsiguientes del embarazo: un estudio de cohortes.

Este estudio demostró que la seroprevalencia de anticuerpos anti-SARS-CoV2 fue del 8,20% (n = 58) entre las 707 pacientes consecutivas que acudieron a su cita hospitalaria del primer trimestre para el control rutinario del embarazo en dos hospitales comarcales, situados en una de las zonas de mayor incidencia de Madrid durante el pico de la primera ola de la pandemia de COVID-19. Las primeras muestras de sangre en las que se detectaron anticuerpos contra el SARS-CoV2 correspondieron al periodo comprendido entre el 1 y el 15 de enero de 2020, lo que indica una circulación del virus mucho anterior al primer caso de COVID-19 oficialmente declarado en España el 31 de enero de 2020. Esto sugiere que la propagación del virus podría haber comenzado a finales de diciembre de 2019 y no a finales de enero de 2020. Se identificaron un total de 8 casos seropositivos antes de que la Organización Mundial de la Salud declarara oficialmente la pandemia. Además, las tasas de complicaciones del embarazo no se incrementaron de manera significativa en las pacientes asintomáticas o con síntomas leves.

La seroprevalencia encontrada en gestantes fue similar a la reportada en la región en población no gestante, no confirmando la mayor ni menor susceptibilidad de esta población al virus. Nuestro estudio no encontró diferencias significativas en la tasa de complicaciones obstétricas en función del estatus serológico, a pesar de la asociación descrita en la literatura de complicaciones como el aborto, anomalías fetales, parto prematuro, muerte fetal anteparto y alteraciones del crecimiento fetal, en mujeres que han padecido la infección por SARS-CoV2 durante el embarazo (15,28–32,66).

El conocimiento sobre la dinámica de seroconversión frente a la infección por SARS-CoV2 en población vulnerable, como la mujer gestante, puede proveer a la comunidad científica con información valiosa en caso de futuras pandemias (18), mejorando la asistencia a la gestante con serología positiva frente a SARS-CoV2 haciéndola más especializada y con mayor rigor científico.

Estudio 3: Cambios serológicos maternos debido a la COVID-19: Comparación entre la tasa de seroconversión en el primer y tercer trimestre del embarazo y las complicaciones obstétricas subsiguientes: Estudio de cohortes.

El principal hallazgo de este estudio es que, durante el primer pico de la pandemia de COVID-19, la tasa de seroconversión en el tercer trimestre (12,8%) fue el doble que en el primer trimestre (6,6%). Sin embargo, no se observaron diferencias en las complicaciones obstétricas o maternas entre ambos grupos. Además, demostramos que aproximadamente dos tercios (18 de 27) de las mujeres con serología positiva en el primer trimestre se mantuvieron positivas en el tercer trimestre. Este resultado destaca que la inmunidad adquirida de forma natural frente al SARS-CoV2 puede durar varios meses.

El testeo serológico frente al SARS-CoV2 al principio y al final de la gestación, en un grupo consecutivo de gestantes, ha permitido proporcionar una tasa de seroconversión frente a la enfermedad más precisa y así poder enriquecer nuestro conocimiento sobre la misma. Aunque las diferentes tasas de serologías positivas entre el primer y el tercer trimestre pudo estar ocasionado por los cambios inmunológicos que se van sucediendo a lo largo del embarazo (24–26), también podrían justificarse por la relajación en las medidas de aislamiento social tras la finalización de la primera ola de la pandemia. En cualquier caso, no encontramos diferencias significativas en la tasa de complicaciones obstétricas en función del momento de la infección, a diferencia de otros autores que sí reportaron una mayor tasa de aborto, muerte fetal anteparto o parto prematuro en gestantes que pasaron la infección durante su primer trimestre (27,34).

Nuestro estudio también puso de manifiesto la utilidad del almacenamiento masivo de muestras sanguíneas para investigaciones posteriores. En nuestro caso, el análisis serológico fue útil para proporcionarnos información valiosa en un momento crucial para la comunidad científica, aumentando nuestro conocimiento sobre el impacto de la infección sobre el embarazo.

Estudio 4: Anticuerpos específicos contra el SARS-CoV2 presentes en leche materna humana tras la infección por SARS-CoV2 durante el embarazo: estudio de cohortes prospectivo.

Este estudio demostró, en primer lugar, que todas las muestras de leche materna analizadas mediante rRT-PCR para SARS-CoV2 resultaron negativas, lo que hace poco probable la transmisión vertical por esta vía; en segundo lugar, los anticuerpos contra SARS-CoV2 presentes en el calostro no parecen variar de manera significativa en relación con el momento en que se produjo la infección durante el embarazo ni en función de su presencia en la sangre materna, poniendo de manifiesto su duración en el tiempo; y, en tercer lugar, la IgA es la inmunoglobulina predominante encontrada en la leche materna humana, y su concentración es significativamente mayor en calostro en comparación con la leche madura, y mayor en el calostro de mujeres que tuvieron neumonía durante su embarazo o en el momento del frente a los casos asintomáticos o con síntomas leves.

En el inicio de la pandemia, la lactancia materna se contraindicaba en las mujeres con COVID-19 por el presunto riesgo que podría generar en el recién nacido, incluso se les separaba de sus madres para protegerlos frente a la infección (67). Nuestro estudio no encontró virus SARS-CoV2 en la leche materna, aunque sí se hallaron anticuerpos frente al virus, tanto en calostro como en leche madura, especialmente tipo IgA. Por lo tanto, no solo la posibilidad de transmisión al recién nacido durante la lactancia es extremadamente baja, sino que toda mujer debería ser animada a dar de lactar a su recién nacido por la inmunización pasiva, más marcada en el caso del calostro.

CONCLUSIONES

CONCLUSIONES

1- La implementación rápida de un protocolo preventivo en un servicio obstétrico ambulatorio de hospital, que incluya un triaje, medidas higiénicas y preventivas, y reprogramación de citas de embarazo, permite la atención ininterrumpida a las gestantes y reduce el porcentaje de trabajadores de la salud afectados por el SARS-CoV2.

2- La tasa de seroprevalencia en las mujeres de nuestro estudio fue del 8% aproximadamente, similar a la de la población no gestante en el mismo momento epidemiológico.

3- La tasa de seroprevalencia en las mujeres de nuestro estudio fue significativamente mayor durante su tercer trimestre de embarazo que el primero.

4- No se observaron diferencias significativas en la tasa de complicaciones obstétricas al comparar las gestaciones con infección por SARS-CoV2 con las no infectadas, ni en función del estatus serológico en el primer trimestre o del momento en que se produjo la seroconversión.

5- Nuestro estudio ha aportado evidencia adicional de que la lactancia materna es segura durante la infección por SARS-CoV2 en la madre, ya que no se ha detectado la presencia del virus en la leche materna humana y, en cambio, se han identificado anticuerpos protectores.

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