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A large series of molecular and serological specimens to evaluate mother-to-child SARS-CoV-2 transmission: a prospective study from the Italian Obstetric Surveillance System

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ABSTRACT

Objectives: To assay the presence of the SARS-CoV-2 genome in vaginal, rectal, and placental swabs among pregnant women and in newborn nasopharyngeal swabs and to investigate the immunological response and maternal antibiotic transfer through the umbilical cord blood and milk of unvaccinated mothers.

Methods: Vaginal, rectal, and placental specimens, maternal and neonatal serum, and milk were collected from a wide cohort of pregnant Italian women with confirmed SARS-CoV-2 infection admitted to the hospital between February 25, 2020 and June 30, 2021. Samples were tested in selected reference laboratories according to a shared interlaboratory protocol.

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Introduction

The ongoing SARS-CoV-2 pandemic is characterized by multiple epidemiological waves associated with different viral strains with diverse transmissibility and lethality. The previous highly pathogenic SARS-CoV-1 and middle east respiratory syndrome coronavirus were associated with poor obstetric outcomes (Schwartz and Graham, 2020). Given the importance of understanding COVID-19's impact on pregnant women, fetuses, and newborns, the Italian Obstetric Surveillance System (ItOSS), coordinated by the Istituto Superiore di Sanità (Italian National Institute of Health-ISS), launched a nationwide population-based, prospective cohort study aimed at analyzing cases of SARS-CoV-2 infection in pregnant women with the purpose to provide useful indications to guide decision makers and support clinical practice (Donati et al., 2021; 2022). The study network involved obstetric referral hospitals across the whole country, enrolling women with a diagnosis of SARS-CoV-2 infection at any gestational age, at birth, or postpartum. In addition to clinical data, maternal, adnexal, and newborn biological specimens were collected. The presence and significance of SARS-CoV-2 RNA in nonrespiratory specimens is quite a controversial subject: many studies evidenced that the viral genome can be found in various biological materials (i.e., feces, placenta, urine) (Bwire et al., 2021; Wang et al., 2020). However, data supporting virus replication competency are lacking, making effective virus transmission through these routes unlikely (Albert et al., 2021; Holm-Jacobsen et al., 2022; Krogstad et al., 2022). Antibodies were tested in maternal serum, cord blood, and milk to investigate the evolution of maternal immune response and the transmission of protective immunoglobulins (Igs) that represents the first barrier against infections in newborns (Edlou et al., 2020; Maishe et al., 2022; Zhu et al., 2021).

Anti-SARS-CoV-2 antibodies were demonstrated in maternal serum, cord blood, and milk, with differences in terms of class (IgG, IgM, and IgA) and persistence, especially in the difference of dynamics in mothers and newborns (Boelig et al., 2022; Peng et al., 2020; Rathberger et al., 2021). This study aimed to assay the presence of viral genome in vaginal, rectal, placental swabs, and newborn nasopharyngeal swabs (NPSs) and to investigate the immunological response and maternal antibody transfer through the umbilical cord blood and milk of unvaccinated mothers.

Methods

Study population

As part of a national population-based, prospective cohort study, the study population of the present project comprises a nonrepresentative sample of unvaccinated women with current or previous confirmed SARS-CoV-2 infection, admitted to the hospital during pregnancy or at birth between February 25, 2020 and June 30, 2021 in four Italian regions (Lombardy, Emilia-Romagna, Tuscany, and Campania) and the autonomous province of Trento, covering 42.5% of national births in 2020 (Directorate-general of digitalization, of health informative system and of statistics, Italian Ministry of Health, 2020). In Italy, the vaccination campaign started in January 2021; although, the vaccination for pregnant women was only recommended starting on September 2021 (Italian National Institute of Health, 2021). For this reason, we are quite confident that the vaccinated women in our sample are absent or negligible.

Confirmed SARS-CoV-2 infection was defined as the detection of viral RNA on reverse transcriptase-polymerase chain reaction (RT-PCR) testing of an NPS for mothers and newborns.

For each case, an identification number was generated. Reference clinicians of the 109 maternity units (Appendix 1) received a link to the LimeSurvey GmbH platform, where they entered information about maternal sociodemographic characteristics, medical and obstetric history, disease management, mode of delivery, and maternal and perinatal outcomes.

Sample collection and analyses

Biological materials (vaginal specimen and maternal serum during pregnancy and at birth, rectal and placental specimen at birth, neonatal serum and milk at birth) were collected only among women who gave written informed consent to participate in the study and were sent to the reference laboratories. Each participating region identified a reference laboratory with expert microbiologists in the perinatal field. Trained reference microbiologists evaluated the adequacy of the samples, described the molecular or serological assay used and the assay results, and uploaded this information in a dedicated online form that was previously revised and tested by a group of national experts.

For each of the collected samples, the adequacy was assessed as follows: (i) sufficient amount of biological material according to the requirements of the assays and the absence of hemolysis for vaginal, rectal, and placental swabs; (ii) sufficient amount of maternal and neonatal serum according to the requirements of assays and the absence of hemolysis and clots; and (iii) >0.5 ml of milk and the absence of lipid clots.

Samples were tested in the different participating laboratories, adapting a restricted number of assays according to a shared interlaboratory protocol to maximize harmonization within the network. Supplementary Tables S1 and S2 summarize the main characteristics of the molecular and serological assays. Nucleic acid extraction and amplification by real-time PCR were performed using various platforms in association with Table S1 assays, as hereafter reported: QIASymphony® SP with QIASymphony® DSP Virus/Pathogen Kit (QIAGEN GmbH, Hilden, Germany).
plus RealStar SARS-CoV-2 RT-PCR kit 1.0 (E gene, S gene), Abbott RealTime SARS-CoV-2 on Abbott m2000 RealTime System (Abbott Laboratories, Chicago, IL, USA), NUCLEISENS® EASYMAG® (bioMérieux SA, Marcy l’Etoile, France) plus CDC 2019-Novel coronavirus (2019-ncov) real-time RT-PCR Diagnostic Panel; MagNA Pure System (Roche Diagnostics International, Rotkreuz, Switzerland) plus CDC 2019-Novel coronavirus real-time RT-PCR Diagnostic Panel, and GenefinderTM Plus RealAmp Kit on ELITE InGenius® system (ELITechGroup, Puteaux, France). Samples were considered positive when all genes were detected with a cycle threshold value <35; conversely, a cycle threshold between 35 and 38 and/or the partial detection of targets was defined as weak positive. Negative results were defined as absence of amplification or a cycle threshold >38. Antibody detection was performed using chemiluminescence enzyme immunosassays, chemiluminescent magnetic microparticle immunoassay, electrochemiluminescence immunosassay analyzer, or enzyme-linked immunosassay methods, using nucleocapside and/or surface proteins as capturing antigens (Table S2).

Outcomes
Outcome measures included the results of molecular assays for viral genome detection adapted for vaginal, rectal, and placental specimens and results of serological assays for IgM and IgG detection adapted for maternal and neonatal sera and maternal milk.

Covariates
Covariates include sociodemographic, obstetric, and medical characteristics that could act as potential risk factors: age (<30, 30-34, and ≥35 years), citizenship (Italian, not Italian), previous co-morbidities (at least one of the following: asthma requiring medical treatment, cardiovascular diseases, diabetes, HIV/AIDS, hypertension, lung diseases, other pathologies), obesity (defined as a body mass index >30 kg/m²), COVID-19 pneumonia, gestational age at delivery (<37 weeks, ≥37 weeks), gestational age at SARS-CoV-2 diagnosis (<14 weeks, 14-27 weeks, ≥28 weeks), and the interval between the date of diagnosis and the date of sample collection or delivery (<14 days, 15-30 days, 31-60 days, >60 days). Maternal and perinatal outcomes were also investigated in the study.

Statistical analysis
Frequency distributions by sociodemographic, obstetric characteristics, and maternal and neonatal outcomes were calculated for women for whom biological materials were collected. For each type of collected specimen and serological sample, the frequency distributions of the cases by adequacy, type of assay, and results were calculated. Missing values were excluded when their proportion was lower than 5%; otherwise, they were included in the frequency distributions. For maternal serum collected during pregnancy and the maternal and neonatal serum collected at birth, the association of the SARS-CoV-2 IgG detection with women's characteristics and the time interval from infection diagnosis was assessed through logistic regression models directed to estimate mutually adjusted odds ratios (ORs) and 95% confidence interval (CI). Listwise deletion was used to handle missing data in the models.

Results
Study population
Biological materials were collected from 1086 unvaccinated women with current or previous confirmed SARS-CoV-2 infection enrolled in the ItOSS cohort during pregnancy or at birth between February 25, 2020 and June 30, 2021. Table 1 describes their sociodemographic and obstetric characteristics and maternal and neonatal outcomes. Most women were Italian (82.6%) and enrolled during the third trimester of pregnancy (73.0%); previous co-morbidities and body mass index >30 kg/m² were present in 137 (13.0%) and 127 (11.9%) of the patients, respectively, with the occurrence of 14 severe morbidities and one maternal death. Vaginal deliveries were 73.0%, and cesarean section (CS) due to COVID-19 occurred in 1.2%. We recorded 99.8% live births and 6.0% preterm births.

SARS-CoV-2 RNA detection
The presence of SARS-CoV-2 RNA was tested in vaginal specimens collected during pregnancy (N = 459) and at birth (N = 545) (Table 2). Moreover, rectal (N = 497) and placental (N = 538) specimens were tested at birth. Over 90% of the collected specimens were evaluated as adequate by the regional reference laboratories, and the following analyses refer only to these adequate specimens. QIAGEN QIASymphony + RealStar SARS-CoV-2 RT-PCR kit 1.0 (E gene, S gene) was the most frequently used method for viral genome detection, followed by Abbott real-time SARS-CoV-2 (SARS-CoV-2 RNA-dependent RNA polymerase, SARS-CoV-2 N, and IC), ROCHE MagNa Pure + CDC 2019-Novel coronavirus real-time RT-PCR Diagnostic Panel (N gene) and GeneFinderTM COVID-19 Plus RealAmp Kit (RNA-dependent RNA polymerase gene, E gene, and N gene) for all types of specimens (Table S3). Vaginal specimens collected during pregnancy were positive for SARS-CoV-2 RNA in three (0.7%) cases (Table 2). At birth, vaginal and placental specimens were positive in nine (1.7%) and five (1.0%) cases, and rectal samples in 39 (8.4%) cases. Of 968 newborns, eight (0.8%) had a positive NPS, seven within 24 hours of birth, and one after 24 hours from birth. None of the babies born from women with a positive rectal swab had a positive NPS. Among babies who tested positive, five were delivered vaginally and three by CS.

Maternal immune response and antibody transfer
Antibody response to SARS-CoV-2 infection was detected among maternal serological samples collected during pregnancy (N = 422), among maternal (N = 555) and neonatal serological samples (N = 628) collected at birth, and maternal milk (N = 183) (Table 3). Almost nine in 10 maternal and neonatal serological samples at birth and more than 90% of maternal samples collected during pregnancy were evaluated as adequate, whereas maternal milk was classified as adequate in 77.0% of the cases. PANTEC-SARS-CoV-2 IgG/IgM was the most frequently used assay for both IgG and IgM detection, followed by Elecsys Anti-SARS-CoV-2, LIAISON SARS-CoV-2 S1/S2 IgC, and IgG SARS-CoV-2 of Abbott for IgG detection and IgM SARS-CoV-2 of Abbott for IgM detection (Table S4).

IgG was tested in all adequate samples, whereas IgM was only in some (62.6%) of them. Among analyzed maternal sera, 45.2% of those collected during pregnancy and 39.7% of those collected at birth tested positive for IgG, whereas 50.5% tested positive among neonates. IgG was detected in 3.6% of the milk samples (Table 3). Positivity for IgM was detected for 1.9% of analyzed maternal sera during pregnancy and 6.1% at birth, 1.5% of neonatal serum samples, and 7.5% of milk samples.

Figure 1 describes the distribution of the IgG detected among women and newborns at different time intervals from the date of SARS-CoV-2 diagnosis. The trend was similar for peripartum maternal and neonatal sera, peaking at over 70% of antibody posi-
tivity between 31 and 60 days from infection diagnosis, followed by a decrease after 2 months from the positive NPS; more pronounced among women than newborns. Antibodies detected in the serum of women during pregnancy showed a slightly different curve shape, peaking earlier, between 15 and 30 days from infection (71.6%) and decreasing after the 1st month from the positive SARS-CoV-2 test.

Table 4 shows the adjusted ORs to SARS-CoV-2 IgG detection among women and newborns. The serum positivity was strongly associated with the time interval from the date of diagnosis, confirming the higher occurrence of positivity in the 2nd

| Table 1 | Women’s characteristics (N = 1086). |
|---------|-----------------------------------|
| Maternal characteristics | N = 1086 |
| | n | % |
| **Age** (13 missing) | | |
| <30 years | 338 | 31.5 |
| 30-34 years | 381 | 35.5 |
| ≥35 years | 354 | 33.0 |
| **Citizenship** (6 missing) | | |
| Italian | 892 | 82.6 |
| Not Italian | 188 | 17.4 |
| **COVID-19 disease severity** (10 missing) | | |
| Mild | 1031 | 95.8 |
| Moderate | 29 | 2.7 |
| Severe | 16 | 1.5 |
| **Gestational trimester at SARS-CoV-2 diagnosis** (13 missing) | | |
| I (<14 weeks) | 103 | 9.6 |
| II (14-27 weeks) | 187 | 17.4 |
| III (≥28 weeks) | 783 | 73.0 |
| **Previous comorbidities** (35 missing) | | |
| No | 914 | 87.0 |
| Yes | 137 | 13.0 |
| **Body mass index ≥30 Kg/m²** (18 missing) | | |
| No | 941 | 88.1 |
| Yes | 127 | 11.9 |
| **Maternal outcomes** | | |
| Severe morbidity | 14 | 1.3 |
| Death | 1 | 0.1 |
| **Mode of delivery** (3 missing) | | |
| Vaginal | 694 | 73.0 |
| Elective CS | 121 | 12.7 |
| Urgent/emergency CS due to maternal/fetal indication | 125 | 13.1 |
| Urgent/emergency CS due to COVID-19 | 11 | 1.2 |
| **Gestational age at birth** (6 missing) | | |
| <31 weeks | 10 | 1.1 |
| 32-36 weeks | 47 | 5.0 |
| ≥37 weeks | 891 | 94.0 |
| **Perinatal outcomes** | | |
| Stillbirths | 2 | 0.2 |
| Livebirths | 966 | 99.8 |
| Neonatal deaths | 2 | 0.2 |
| SARS-CoV-2-positive swab at birth | 8 | 0.8 |

a Mild: absence of COVID-19 pneumonia; Moderate: COVID-19 pneumonia requiring at most oxygen therapy; Severe: COVID-19 pneumonia requiring mechanical ventilatory support and/or intensive care unit admission.

b Mode of delivery and gestational age at delivery were calculated among 954 women who gave birth.

BMI: body mass index; CS: Caesarean section; COVID-19: Coronavirus Disease 2019; NICU: neonatal intensive care unit; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

| Table 2 | Adequacy of samples and results of molecular assays for viral genome detection. |
|---------|--------------------------------------------------------------------------|
| Results | Vaginal specimen during pregnancy (N = 450) | Vaginal specimen at birth (N = 545) | Rectal specimen at birth (N = 497) | Placental specimen at birth (N = 538) |
| | n | % | n | % | n | % | n | % |
| Adequate samples | 449 | 97.8 | 527 | 96.7 | 467 | 94.0 | 527 | 98.0 |
| Weakly positive | 1 | 0.2 | 5 | 1.0 | 21 | 4.5 | 3 | 0.6 |
| Positive | 1 | 0.4 | 4 | 0.8 | 18 | 3.6 | 2 | 0.4 |
| Negative | 443 | 98.9 | 516 | 98.1 | 425 | 91.0 | 516 | 98.5 |
| Indeterminate | 2 | 0.4 | 1 | 0.2 | 3 | 0.6 | 3 | 0.6 |
| Missing | 1 | 0.2 | 0 | 0.0 | 3 | 0.6 | 3 | 0.6 |

a Sufficient amount of biological material according to the assay requirements and absence of hemolysis for vaginal, rectal, and placental swabs.
month after the diagnosis for maternal serum at birth (OR 7.14; 95% CI: 3.21-15.88) and neonatal serum (OR 11.98; 95% CI: 6.00-23.89) and between 15 and 30 days after the diagnosis for maternal serum collected during pregnancy (OR 4.81; 95% CI: 2.40-9.63). Among the other variables included in the three models, a statistically significant association of the detection of antibodies was found for the diagnosis of SARS-CoV-2 infection during the Alpha variant period for maternal serum collected during pregnancy (OR 2.13; 95% CI: 1.10-4.13) and for the infection diagnosis during the second pregnancy trimester among the newborns' sera (OR 2.61; 95% CI: 1.08-6.32).

Discussion

The current study reports the molecular and serological characterization of 1086 unvaccinated pregnant women who were SARS-CoV-2 positive in the framework of the ItOSS national surveillance study (Donati et al., 2021; 2022). To the best of our knowledge, our population is the largest for which data on biological samples are available; in addition, the extended monitoring protocol implemented, with the collection of various relevant specimens at diagnosis, delivery, and lactation, allowed the evaluation of possible mother-to-child routes of transmission. As for virological samples, a low rate was found for all specimens at any time of collection: vaginal swabs during pregnancy and at birth as well as placentals were negative in more than 98% of women, whereas in rectal samples, the percentage decreased to 91.0%. Scarce data on vaginal samples are available in the literature: previous reports analyzed up to 45 patients, usually at only one time point, whereas our findings are based on a very large population, especially considering that more than 500 subjects were tested at delivery, when the virus at vaginal level could represent a source of infection (Fenizia et al., 2021; Mattar et al., 2020; Milbak et al., 2021). The presence of viral RNA was rare, thus opposing the role of this route of transmission (Fenizia et al., 2021; Mattar et al., 2020; Milbak et al., 2021).

Table 3

| Molecular assay | Maternal serum during pregnancy (N = 422) | Maternal serum at birth (N = 555) | Neonatal serum at birth (N = 628) | Maternal milk at birth (N = 183) |
|-----------------|------------------------------------------|----------------------------------|----------------------------------|---------------------------------|
|                 | n  | %  | n  | %  | n  | %  | n  | %  |
| Adequate samples | 408 | 96.7 | 493 | 88.8 | 564 | 89.8 | 141 | 77.0 |
| IgG detection   |    |     |     |     |     |     |     |     |
| Negative        | 211 | 53.0 | 279 | 58.0 | 256 | 47.3 | 125 | 92.6 |
| Positive        | 180 | 45.2 | 191 | 39.7 | 273 | 50.5 | 4   | 3.0  |
| Indeterminate   | 7   | 1.8  | 11  | 2.3  | 12  | 2.2  | 6   | 4.4  |
| Missing         | 10  |   23 | 12  | 23   | 12  | 23   | 6   | 6.5  |
| IgM detection   |    |     |     |     |     |     |     |     |
| Negative        | 252 | 97.7 | 247 | 93.9 | 337 | 97.7 | 118 | 88.1 |
| Positive        | 5   | 1.9  | 16  | 6.1  | 5   | 1.5  | 10  | 7.5  |
| Indeterminate   | 1   | 0.4  | 0   | 0.0  | 0   | 0.0  | 6   | 4.5  |
| Missing         | 1   | 0    | 0   | 0    | 0   | 0    | 7   | 7    |
| No detection of IgM | 149 | 230  | 222 | 0    |     |     |     |     |

* Sufficient amount of maternal and neonatal serum according to the assay requirements and absence of hemolysis and clots; >0.5 ml of milk and absence of lipid clots.

Figure 1. Temporal trend of immunoglobulin G detection.
SARS-CoV-2 was demonstrated to actively enter and replicate in the human placenta both in vitro and in vivo, even though it was not elucidated if this event has pathological outcomes: maternal immune cells infiltration was found in histological specimens, whereas the expression of inflammatory cytokines resulted negative (Argueta et al., 2022; Fahmi et al., 2021). Placental swabs collected in the current study showed a high negative rate (516/527, 98.5%), and similar results were obtained in smaller populations of other studies, supporting evidence against in utero infection. Edlow and Mattar reported no viral RNA in five swabs and 44 placental histological sections, respectively (Edlow et al., 2020; Mattar et al., 2020). The varying expression of SARS-CoV-2 entry molecules in placental cells during pregnancy is an important and interesting aspect. Argueta underlined how the receptor angiotensin-converting enzyme 2, as well as co-receptors neuropilin-1, transmembrane protease serine 2, cathepsin, and the proprotein convertase furin, are widely present in the first and second trimesters, whereas co-receptors significantly diminish in the third one (Argueta et al., 2022). Consequently, the time between infection and delivery may considerably influence the probability of detecting viral RNA in the placenta-derived materials.

Considering the SARS-CoV-2 presence in the stool and the strict proximity of the vagina and anus, peripartum maternal rectal swabs were screened. This specimen had the highest positive rate among those included in the protocol; although, it remained lower than 10% (39/467, 8.4%). As for other biological materials, only a few previous reports on small populations are available. Carosso et al. (2020) presented a case of a woman with positive stools and negative vaginal and placental swabs, delivering a healthy baby who was SARS-CoV-2-positive. Similarly, in two small cohorts of pregnant women, the rate of positive rectal samples was 27% (9/34) and 7.7% (1/13), respectively, with negative vaginal results (Fenizia et al., 2021; Wu et al., 2020). The significance of such outcomes remains unclear. As reviewed by Zhou et al. (2022), the presence of SARS-CoV-2 RNA in stools was broadly investigated and confirmed, also considering the presence of angiotensin-converting enzyme 2 in enteric tissues. Moreover, the authors found a statistical difference between patients with and without diarrhea and not according to disease severity, suggesting a possible role of gastrointestinal involvement. However, no data on the isolation of competent viruses from feces were found to prove the SARS-CoV-2 fecal-oral transmission definitively. Although the sensitivity of rectal swabs used in the current study compared with the stool ones could lead to misestimating the phenomenon, our results suggest a minor role of this route at birth. None of the newborns delivered by women with a positive fecal test (39/467) had a positive NPS at birth.

| Variable | IgG detection: positive vs negative |
|----------|----------------------------------|
|          | Model 1\* maternal serum during pregnancy (N = 262) | Model 2\* maternal serum at birth (N = 446) | Model 3\* neonatal serum at birth (N = 514) |
|          | OR      | 95% CI     | OR      | 95% CI     | OR      | 95% CI     |
| Period   |         |            |         |            |         |            |
| Wild-type (February 25, 2020-January 31, 2021) | 1 | 1 | 1 |
| Alpha variant (February 01, 2021-June 30, 2021) | 2.13 | 1.10 | 4.13 | 0.98 | 0.55 | 1.75 | 1.46 | 0.87 | 2.46 |
| Maternal age |         |            |         |            |         |            |
| <30 years | 1 | 1 | 1 |
| 30-34 years | 0.78 | 0.41 | 1.50 | 0.98 | 0.59 | 1.63 | 0.66 | 0.40 | 1.08 |
| ≥35 years | 0.88 | 0.45 | 1.71 | 1.08 | 0.64 | 1.80 | 0.67 | 0.41 | 1.11 |
| Citizenship |         |            |         |            |         |            |
| Italian | 1 | 1 | 1 |
| Not Italian | 1.11 | 0.51 | 2.43 | 1.47 | 0.87 | 2.48 | 1.19 | 0.72 | 1.98 |
| Gestational trimester at SARS-CoV-2 |         |            |         |            |         |            |
| diagnosis |         |            |         |            |         |            |
| I (<14 weeks) | 1 | 1 | 1 |
| II (14-27 weeks) | 0.83 | 0.39 | 1.78 | 1.23 | 0.33 | 4.56 | 2.61 | 1.08 | 6.32 |
| III (>28 weeks) | 0.80 | 0.37 | 1.73 | 0.59 | 0.12 | 2.78 | 2.46 | 0.90 | 6.72 |
| COVID-19 pneumonia |         |            |         |            |         |            |
| No | 1 | 1 | 1 |
| Yes | 1.30 | 0.16 | 10.74 | 1.06 | 0.38 | 2.94 | 0.97 | 0.35 | 2.68 |
| Body mass index ≥30 Kg/m² |         |            |         |            |         |            |
| No | 1 | 1 | 1 |
| Yes | 2.47 | 0.94 | 6.50 | 1.65 | 0.83 | 3.28 | 1.50 | 0.75 | 3.02 |
| Comorbidities |         |            |         |            |         |            |
| No | 1 | 1 | 1 |
| Yes | 0.67 | 0.32 | 1.44 | 0.98 | 0.53 | 1.82 | 1.66 | 0.90 | 3.06 |
| Gestational age at delivery |         |            |         |            |         |            |
| <37 weeks | 1 | 1 | 1 |
| ≥37 weeks | 1.15 | 0.47 | 2.81 | 1.74 | 0.68 | 4.47 |
| Interval between date of diagnosis and date of sample collection/date of delivery |         |            |         |            |         |            |
| ≤14 days | 1 | 1 | 1 |
| 15-30 days | 4.81 | 2.40 | 9.63 | 4.36 | 2.23 | 8.56 | 4.19 | 2.28 | 7.72 |
| 31-60 days | 2.84 | 1.25 | 6.46 | 7.14 | 3.21 | 15.88 | 11.98 | 6.00 | 23.89 |
| >60 days | 1.24 | 0.48 | 3.23 | 1.36 | 0.46 | 4.02 | 6.63 | 3.19 | 13.77 |

\* Seven cases with indeterminate result were excluded in model 1, 11 cases in model 2, 12 cases in model 3.

\* The date of sample collection was used for maternal serum collected during pregnancy; the date of delivery was used for maternal and neonatal serum collected at birth.

BMI: body mass index; COVID-19: Coronavirus Disease 2019; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.
Milk represented a major subject of investigation in the post-partum period from both molecular and serological points of view. As demonstrated by several works, among which only one reported a single positive result, the detection of the viral genome is a rare event (Bäuerl et al., 2022; Pace et al., 2021; Young et al., 2022). A double meaning could be attributed to such evidence: an unlikely direct passage of SARS-CoV-2 from maternal tissues to milk during its production or a milk contamination occurring during breastfeeding without any role in pathogen transmission. Considering specific anti-SARS-CoV-2 antibodies, IgG and IgM were detected in a small proportion of milk samples, 4/141 (3.0%) and 10/141 (7.5%), respectively. Milk usually contains a high IgA concentration, representing the main class of secreted antibodies released by mammary tissue after plasma cells production. IgM follows the same process, albeit to a lesser extent, whereas IgG derives from maternal plasma; thus, they are rarely detected in milk (Hurley and Theil, 2011). Anti-SARS-CoV-2 antibody response seems to follow the same pattern because IgA were found to be highly expressed in multiple studies, and IgG and IgM showed a different profile and were not detected in some populations (Bäuerl et al., 2022; Low et al., 2022; Pullen et al., 2021). Such variability could be due to three main factors: (i) the capturing antigen used in each assay because of different affinity for different Ig classes and subclasses (Bäuerl et al., 2022; Low et al., 2022), (ii) the time-lapse between sample collection and diagnosis because IgA increases in few days, whereas IgG increase over time (Bäuerl et al., 2022; Young et al., 2022), and (iii) the disease severity, as shown by Pullen who found higher IgA and IgG concentrations in women experiencing a severe COVID-19 disease (Pullen et al., 2021). The great majority of women enrolled in the iOTSS series presented only mild symptoms (95.8%) and had a positive molecular test during the third gestational trimester (73.0%), supporting the detected low positive antibody rate. Moreover, because samples were tested on two platforms using assays detecting antibodies against whole N and whole N plus S proteins, an instrumental influence cannot be excluded. Moreover, the immune response against S antigen seems earlier than that against the N antigen, further contributing to the lower antibody positivity detected in the cohort (Tang et al., 2020).

The mother-to-child transmission was also evaluated by testing NPSs in newborns that were found positive in only eight of 968 (0.8%) subjects, of which seven were within 24 hours of birth and one after 24 hours from birth. Three of eight rectal and vaginal swabs at birth were available and negative, as was an additional rectal swab for another subject. The only one preterm delivery recorded (27th gestational week) resulted in a newborn admission to the intensive care unit, but it was not related to the maternal COVID-19 course, and the baby’s infection did not give rise to clinical complications. Serological data did not provide useful information, being available only for two dyads: in one, no evidence of antibodies was found, whereas in the other, IgG was detected in both maternal and cord blood. SARS-CoV-2 vertical transmission can occur in utero, intrapartum, or after birth (World Health Organization, 2021). The virus could reach the placenta through hemotogenous dissemination, an event usually correlated to severe disease course. In this case, vascular and placental damages have been frequently detected, enhancing the possibility of viral translocation to the fetus and of viral RNA presence in the fetal tissues (Halasa et al., 2022). Contamination by vaginal secretions, feces, or droplets could occur during labor, whereas postnatal exposure is likely from the mother or other infected caregivers (World Health Organization, 2021). Our newborn positive rate is in the range reported in previous studies (0-6.5%), an outcome that the high variability in terms of the number of assays performed (ranging from 31 to 3790) could have partially influenced (Malshe et al., 2022; Fenizia et al., 2021). The positive rate of vaginal (1.7%), placental (1.0%), and rectal swabs (8.4%), coupled with the absence of positive children related to these samples, suggest a limited significance of these sources of infection. In addition, considering the uncommon detection of viral RNA in amnion fluid and blood cord, the vertical transmission should be considered an event mostly linked to postnatal exposure (Edlow et al., 2020; Malshe et al., 2022; Mattar et al., 2020).

From a public health perspective, because the infection in newborns is not associated with an increased risk of adverse outcomes (Allotey et al., 2022), preventive actions, such as CS, separating mother from children at birth and not breastfeeding, appear unjustified (Giuliani et al., 2022).

The assessment of the presence of antibodies in women and neonates represented a key point of this study. The protective role of maternal lg in the newborn is well known, and according to a recent study, maternal vaccination was associated with a reduced risk of hospitalization for COVID-19 and critical illness among infants aged >6 months (Halasa et al., 2022). However, significant variations may occur, depending on several factors, such as gestational age at infection, timespan between infection and delivery, or immune response persistence over time. A recent review underlined how all anti-SARS-CoV-2 antibody classes peaked within 1 month after symptoms onset, with a rapid decay of IgA and IgM soon after infection resolution and a longer IgG persistence, although at a low level (Zuiani and Wesemann, 2022). In our cohort, we found a very low IgM positive rate in both maternal and cord serum, whereas the IgG ranged from 40% to 50%. Data stratification according to the time interval between diagnosis and sample collection showed that more than 20% of serum samples were IgG-positive within 7 days from diagnosis. The positive percentage maximized within 30 days during pregnancy and 60 days in maternal serum at delivery and in the neonatal population, with a subsequent decrease after these time limits. The early detection of IgG could be related to the unavailability of the symptom onset date: because many participants were enrolled through predelivery SARS-CoV-2 screening without any COVID-19 clinical evidence, the date of diagnosis was used as a proxy for the time of infection. However, it was demonstrated that NPSs can remain positive for weeks, during which the immune system can develop a complete response (Carmona et al., 2020). Previous studies reported a significant variability of immune response duration and Ig production, which also correlated to disease severity. In our series, less than 5.0% of women experienced moderate or severe COVID-19 disease, probably inducing a more rapid decrease in the antibody titers, as well as the limited detection of IgM (Demonbreun et al., 2021; Mariën et al., 2021).

The multivariate analysis performed on IgG detection showed a statistically significant effect of the time span between infection diagnosis and sample collection. A time span of over 15 days was associated with a higher occurrence of Ig detection among both maternal and fetal sera.

The newborns’ sera showed a higher IgG rate in the case of SARS-CoV-2 diagnosis during the second trimester of pregnancy. Although IgS cross the placenta using the neonatal Fc receptor, which are abundantly present in syncytiotrophoblast cells, altered glycosylation during the third trimester among pregnant women who are positive could negatively influence the transportation after the second trimester (Atyeo et al., 2021). The enhanced IgG-positive rate detected in maternal sera during pregnancy among women infected by the Alpha variant compared with the wild-type virus may be attributable to the link between infection severity and antibody production (Funk et al., 2021).

Study limitations include the subnational series; although, a lack of representativeness is unlikely, given that the participating regions covered all the country’s geographical areas and the unavailability of a control group of biological samples. Moreover, samples were tested on two platforms using whole N and whole
N plus S proteins; therefore, instrumental influence on the analyses cannot be excluded. The strengths of the current study include the large population enrolled in four regions, covering 42.5% of total births in Italy (Directorate-general of digitalization, of health informative system and of statistics, Italian Ministry of Health, 2020) and the availability of prospective epidemiological data describing sociodemographic and clinical characteristics of the women. No wider series was found in the literature. The confirmed SARS-CoV-2 infection through RT-PCR testing of NPS of both mothers and newborns and the selection of reference laboratories with expert microbiologists adapting a shared protocol are additional important strengths of the current study. Moreover, no vaccinated subjects were included in the present cohort, providing a homogeneous population consisting of only cases of natural infection.

In conclusion, the current study detected a very low SARS-CoV-2 RNA positive rate in multiple relevant biological specimens, underlying the unlikeliness of the vertical transmission in a large comprehensive Italian cohort of pregnant women with confirmed new COVID-19 infection. Such evidence is also supported by the limited number of severe COVID-19 disease in the maternal population that is recognized as a main factor influencing the possible in utero transmission, placental damage, and the need for harmful CS. Normal caregiving procedures like skin-to-skin, rooming-in, and breastfeeding, coupled with preventive measures, such as masks and hands hygiene, must be continued and encouraged, considering the low probability of effective transmission and the benefits of mother-child interaction. From the immunological point of view, the mothers’ humoral response and maternal antibody transfer throughout umbilical cord blood were detected in about half of the samples with peripartum maternal and neonatal sera, increasing above 70% positivity rate, considering an infection-to-delivery time span of 31–60 days. A broad cross-protection was consequently found, an important factor in the prevention of severe outcomes in newborns and in the promotion of vaccination during pregnancy.

Declaration of Competing Interest

The authors have no competing interests to declare.

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Ethical approval

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the ethical committees of Istituto Superiore di Sanità – The Italian National Institute of Health (reference number: 0010136) and of each participating center. A written informed consent form was signed by all subjects included in the study.

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Author contributions

Data curation, project administration, visualization, and writing—review and editing, ECD; data curation, formal analysis, methodology, and writing—review and editing, MAS; investigation, methodology, project administration, resources, validation, and writing—original draft, AM; investigation, methodology, project administration, resources, validation, and writing—review and editing, GP, AR, CV; conceptualization, methodology, supervision, validation, and writing - original draft, SD.

Author contributions among the ItOSS COVID-19 Working Group: resources, and writing—review and editing, IA, FB, MB, IC, LDC, PDR, AF, GG, SL, BP, LP, VS, SS, RT; investigation, resources, and writing—review and editing, GMA, SB, MC, MRG, FM, MFP, MP, AP, AT; project administration, and writing—review and editing, IC, ML, FM, EP, FT.

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Data sharing

The data used in this study are available on request from the corresponding author.

Supplementary materials

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