SUPPLEMENTARY MATERIAL

Inefficient placental virus replication and absence of neonatal cell-specific immunity upon Sars-CoV-2 infection during pregnancy

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Supplementary Figure 1: Placental SARS-CoV-2 spike (SP) and nucleoprotein (NP) expression, assessed by ICH on placental specimen taken at delivery. All specimen were taken from one specific case of acute infection (case #3, see Supplementary Table 1 for details). Five different commercially available antibodies were used for ICH, as specified in Supplementary Table 2. The photomicrographs show very sporadic SARS-CoV-2 expression in this case, as expression could only be detected in one area of the syncytiotrophoblast, indicated by the arrow in each staining. Note the highly unspecific staining when using the antibody labelled as NP4. Bar represents 200 µm.
Supplementary Figure 2

Supplementary Figure 2: Comparison of SARS-CoV-2 cell-mediated immunity (CMI) in pregnant (maternal) and non-pregnant (non-preg) women. SARS-CoV-2-specific CMI was analysed using an interferon gamma (IFN-γ) release assay. Blood samples were treated with stimulants as indicated and IFN-γ was measured in supernatants. Absolute measurements (upper row) and calculated ratios to the negative control (lower row) are depicted as indicated.
**Supplementary Table 1:** Covid-19-specific details and pregnancy complications occurring within the studied patient cohort. Cases have been sorted by week of gestation at which SARS-CoV-2 infection was diagnosed.

| Case ID | Infection status (positive PCR, weeks gestation) | Time interval between infection and delivery (days) | Course of disease | Covid-19 after B1.1.7 emergence, causing at least 50% diseases (Feb 20, 2021) | Pregnancy complications | SARS-CoV-2 status at delivery (variant, if sequenced) | SARS-CoV-2 PCR Placenta | Maternal SARS-CoV-2 IgG antibody at birth (AU/ml) | SARS-CoV-2 mRNA in cord blood | Cord blood SARS-CoV-2 IgG antibody (AU/ml) | SARS-CoV-2 mRNA breast milk | SARS-CoV-2 mRNA neonatal nasopharyngeal swab |
|---------|---------------------------------|---------------------------------|------------------|-------------------------------------------------|----------------------|---------------------------------|------------------------|---------------------------------|-----------------|-----------------------------|-----------------------------|---------------------------------|
| 1       | 39+6 mild                       | 5                               | none             | positive                                          | negative             | negative                          | negative              | negative                        | negative         | negative                     | negative                     | negative                       |
| 2       | 39+1 asymptomatic               | 1                               | none             | positive                                          | negative             | negative                          | negative              | negative                        | negative         | negative                     | positive                     | negative                       |
| 3       | 38+5 mild                       | 1                               | none             | positive                                          | negative             | negative                          | negative              | negative                        | negative         | negative                     | negative                     | negative                       |
| 4       | 38+1 moderate                   | 11                              | none             | negative                                          | negative             | negative                          | negative              | negative                        | negative         | negative                     | negative                     | negative                       |
| 5       | 37+0 mild                       | 17                              | none             | positive                                          | negative             | negative                          | negative              | negative                        | negative         | negative                     | negative                     | not determined                |
| 6       | 36+4 mild                       | 4                               | yes (B1.1.7 sequenced) Twins (DC) Preterm birth | positive            | negative/ negative                  | negative             | negative/ negative                   | negative/ negative      | not determined                | not determined                | negative/ negative             |
| 7       | 36+3 mild                       | 0                               | yes (B1.1.7 sequenced) Twins (DC) Preterm birth | positive            | negative/ negative                  | negative             | negative/ negative                   | negative/ negative      | not determined                | not determined                | not determined                |
| 8       | 36+2 mild                       | 21                              | yes (not sequenced) Twins (DC) Preterm birth | negative            | negative                           | negative             | negative                           | negative                        | not determined                | not determined                | not determined                |
| 9       | 35+1 mild                       | 33                              | none             | negative                                          | negative             | 127.0                            | negative              | 71.6                           | negative                     | negative                     | negative                       |
| 10      | 34+6 moderate                   | 14                              | yes (B1.1.7 sequenced) Preterm birth | positive            | negative                           | 26.9                | negative                           | negative                        | not determined                | not determined                | not determined                |
| 11      | 33+6 moderate                   | 30                              | none             | negative                                          | negative             | 146.0                            | negative              | 92.2                           | negative                     | negative                     | not determined                |
| 12      | 33+4 severe                     | 13                              | yes (not sequenced) Preterm birth | positive            | negative                           | 37.6                | negative                           | negative                        | not determined                | not determined                | negative                       |
| 13      | 32+1 asymptomatic               | 31                              | PPROM Preterm birth | negative                                         | negative             | 20.9                             | negative                           | negative                        | not determined                | not determined                | not determined                |
| 14      | 30+5 mild                       | 69                              | none             | negative                                          | negative             | 22.6                             | negative                           | 35.8                           | not determined                | not determined                | not determined                |
| 15      | 29+6 mild                       | 73                              | none             | negative                                          | negative             | negative                           | negative                        | not determined                | not determined                | not determined                | not determined                |
| 16      | 29+3 mild                       | 75                              | none             | negative                                          | negative             | negative                           | negative                        | not determined                | not determined                | not determined                | not determined                |
| 17      | 28+1 mild                       | 97                              | none             | negative                                          | negative             | 39.4                             | negative                           | 78.7                           | not determined                | not determined                | not determined                |
| 18      | 28+0 mild                       | 79                              | none             | negative                                          | negative             | 57.7                             | negative                           | 61.4                           | not determined                | not determined                | not determined                |
| Week | GA | Status | BirthWeight | Outcome | Length of Stay | Status | Duration | Hemoglobin | Platelet Count | Other | Notes |
|------|----|--------|-------------|---------|---------------|--------|----------|------------|--------------|-------|-------|
| 19   | 27+6 | mild | 81 | none | negative | negative | 22.1 | negative | 31.2 | negative | not determined |
| 20   | 27+4 | mild | 84 | yes (B1.1.7 sequenced) | negative | negative | negative | negative | negative | negative | negative |
| 21   | 26+4 | moderate | 16 | positive | not determined | 3.6 | not determined | negative | negative | not determined |
| 22   | 26+3 | mild | 98 | none | negative | negative | not determined | negative | 28.0 | not determined | not determined |
| 23   | 24+2 | mild | 115 | none | negative | negative | not determined | negative | 13.7 | negative | not determined |
| 24   | 24+1 | mild | 113 | none | negative | negative | negative | negative | negative | negative | not determined |
| 25   | 23+3 | mild | 123 | none | negative | negative | negative | negative | negative | negative | not determined |
| 26   | 22+3 | mild | 114 | none | negative | negative | negative | negative | negative | negative | not determined |
| 27   | 21+1 | mild | 121 | none | negative | negative | negative | negative | negative | negative | not determined |
| 28   | 19+2 | mild | 109 | none | negative | negative | 62.7 | negative | 57.9 | negative | negative |
| 29   | 19+0 | asymptomatic | 142 | none | negative | negative | negative | not determined | negative | negative | not determined |
| 30   | 18+5 | mild | 152 | none | negative | negative | negative | negative | negative | negative | not determined |
| 31   | 18+4 | asymptomatic | 147 | none | negative | negative | negative | negative | negative | not determined | not determined |
| 32   | 16+4 | mild | 173 | none | negative | negative | 45.8 | negative | 57.6 | negative | negative |
| 33   | 14+4 | mild | 140 | none | negative | negative | 22.7 | negative | 27.5 | negative | not determined |
| 34   | 14+2 | mild | 152 | none | negative | negative | negative | negative | negative | negative | not determined |
| 35   | 13+1 | moderate | 143 | negative | negative | 49.1 | negative | 49.3 | negative | not determined |
| 36   | 12+5 | mild | 202 | none | negative | negative | 36.8 | negative | 65.9 | negative | not determined |
| 37   | 11+6 | moderate | 184 | none | negative | negative | 65.6 | negative | 78.9 | negative | not determined |
| 38   | 10+5 | mild | 185 | none | negative | negative | 30.2 | negative | 29.8/30.3 | negative | not determined |

**Notes:**
- GA: gestational age in weeks and days.
- Status: condition status.
- BirthWeight: birth weight.
- Length of Stay: length of stay in days.
- Other: additional notes or conditions.
- Notes: additional comments or conditions.
| Week | GA | <br>mm | Status  | Diagnosis | Test 1 | Test 2 | Test 3 | Test 4 | Test 5 | Test 6 | Test 7 | Test 8 | Test 9 | Test 10 | Test 11 | Test 12 | Test 13 | Test 14 | Test 15 |
|------|----|-------|---------|-----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 39   | 7+5| 209   | asymptomatic | no | Placenta praevia | negative | negative | negative | negative | not determined | not determined | negative | negative | negative | not determined | negative | negative | not determined |
| 40   | 7+4| 199   | asymptomatic | no | PPROM Preterm birth | negative | negative | negative | not determined | negative | negative | not determined | negative | negative | not determined | negative | negative | not determined |
| 41   | 5+1| 253   | mild | no | none | negative | negative | 12.8 | negative | 22.0 | negative | not determined | negative | negative | not determined | negative | negative | not determined |
| 42   | 3+0| 261   | mild | no | none | negative | negative | 19.5 | negative | 27.1 | negative | not determined | negative | negative | not determined | negative | negative | not determined |

1. [https://www.hpi-hamburg.de/de/aktuelles/covid-19/daten-der-hamburg-surveillance-plattform/](https://www.hpi-hamburg.de/de/aktuelles/covid-19/daten-der-hamburg-surveillance-plattform/). Abbreviations used in Table: PPROM: Preterm premature rupture of membranes, DC: dichorionic; IUGR: intrauterine growth restriction, CCAM: congenital cystic adenomatoid malformation
## Supplementary Table 2: Anti-SARS-CoV-2 antibodies

| Antigen | Label* | Clone | Species         | Dilution | Order number   | Company       |
|---------|--------|-------|-----------------|----------|----------------|---------------|
| Spike   | SP1    | 1A9   | Mouse Mab       | 1:300    | #GTX632604     | GeneTex       |
| NP      | NP1    | 05    | Mouse Mab       | 1:300    | #40143-MM05    | Sino Biological |
| NP      | NP2    | 001   | Rabbit Mab      | 1:5000   | #40143-R001    | Sino Biological |
| NP      | NP3    | n/a   | Rabbit PolyAb   | 1:1000   | #40143-T62     | Sino Biological |
| NP      | NP4    | n/s   | Rabbit Mab      | 1:3000   | #A20021        | ABclonal      |

Abbreviations used: Mab: monoclonal antibody; PolyAb: polyclonal antibody; NP: nucleoprotein; n/a: not applicable; n/s: not specified. * refers to column label in Figure 1.