SUPPLEMENTAL LABORATORY PROCEDURE:

***Procedures***

The blood sample was collected by direct venipuncture in a coagulation activator serum tube. They were centrifuged at 3,500 rpm for 10 minutes.

The serum was frozen at -20ºC to subsequently analyze the IgM against the RBD antigen receptor of the S1 protein (SARS-CoV-2 Spike RBD IgM)).

The milk extraction was carried out in the hospital setting, preferably in the morning, at least one hour after the last feeding, by means of mechanical extraction (SPECTRA S1®) and disposable extraction systems, Beldico® with 1μm filter and non-return valve. The objective volume of the extraction was 20-30 mL collected in food standard Polypropylene (PP) pot. The samples were immediately sent to the laboratory and after centrifugation at 3,500 rpm for 15 minutes, the supernatant was removed with a Pasteur pipette, repeating the same procedure twice. Once skimmed, they were frozen at -20ºC for later analysis.

The presence of antibodies in serum was analyzed using appropriate assay kits according to the manufacturers ’instructions.

The cut-off values for HM testing were calculated from the control milk samples as follows: mean + 2xSD. Thus, the cut off was 0.12 BAU/mL and for IgG and 0.37 S/P ratio forIgA, respectively. For IgM assessment in serum and HM, 1 S/P ratio was used as cut off, following the manufacturer's instruction.

***Laboratory procedures***

**Anti-SARS-CoV-2 N IgG:** The SARS-CoV-2 IgG Architect Abbott® assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of IgM antibodies to SARS-CoV-2 in human serum and plasma.

The assays results are reported as a numerical Index value (ratio of the chemiluminescent signal between the samples and a calibrator) that give a qualitative result of “Positive” or “Negative”.

The SARS-CoV-2 IgG detect immunoglobulin class G (IgG) antibodies to the nucleocapsid protein of SARS-CoV-2 from patients with signs and symptoms of infection who are suspected of corona coronavirus virus disease (COVID-19) or in serum of subjects that may have been infected by SARS-CoV-2.The presence of antibodies was analyzed using appropriate assay kits according to the manufacturers’ instructions on the automated Abbott ARCHITECT *i*2000SR instrument.

**Anti-SARS-CoV-2 S1 IgM:** It´s CMIA used for the qualitative detection of IgM antibodies against the SARS virus- CoV-2 (SARS-CoV-2 IgM Architect Abbott®).

The presence of antibodies was analyzed using appropriate assay kits according to the manufacturers’ instructions on the automated Abbott ARCHITECT *i*2000SR instrument.

Results are reported as an Index (ratio of the chemiluminescent signal between the samples and a calibrator), with values >1.4 indicating a positive result.

IgM antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion. SARS-CoV-2 antibody negative samples collected 15 days or more post symptom onset should be reflexed to a test that detects and reports SARS-CoV-2 IgG.

**Anti-SARS-CoV-2 RBD-S1 IgG:** The SARS-CoV-2 IgG II Quant assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative and quantitative determination of IgG antibodies to SARS-CoV-2 in human serum and plasma on the Alinity and ARCHITECT i Systems. The SARS-CoV-2 IgG II Quant assay is to be used as an aid in the diagnosis of SARS-CoV-2 infection in conjunction with clinical presentation and other laboratory tests. The assay is also to be used as an aid in evaluating immune status of individuals with quantitative measurement of IgG antibodies against the spike receptor-binding domain (RBD) of SARS-CoV-2.

The spike glycoprotein (S-protein), has a pivotal role in viral pathogenesis, mediating binding to target cells through the interaction between its receptor-binding domain (RBD) and the human angiotensin converting enzyme 2 (ACE2) receptor. The S-protein has been found to be highly immunogenic, and the RBD is possibly considered the main target in the effort to elicit potent neutralizing antibodies.

**Anti-SARS-CoV-2 S1 IgA:** Anti-SARS-CoV-2 ELISA (IgA) Euroimmun® is enzyme immunoassay (ELISA) provides semiquantitative in vitro determination of human antibodies of the immunoglobulin class IgA against SARS-CoV-2 in serum. The microplate wells are coated with recombinant structural protein (S1 domain) of SARS-CoV-2. The presence of antibodies in serum was analyzed using appropriate assay kits according to the manufacturers’ instructions on the DS2® system, an automated microplate technology. Results can be evaluated semiquantitatively by calculating a ratio of the extinction of the control or patient sample over the extinction of the calibrator.

|  |  |  |  |
| --- | --- | --- | --- |
| **Table 2 Characteristics of vaccinated participants according to duration of breastfeeding** | | | |
| **Characteristicsa** | **[0,23]b months (N=76)** | **(23,50]b months (N=24)** |  |
| Age-yr | 36(33-38) | 37.5(35-40) | *P=*.38 |
| Body‐mass indexc | 23.3 (20.8-25.7) | 22.2 (19.9-26.8) | *P=*.95 |
| **Infant feeding modality (%)** |  |  | *P=*0.23 |
| Exclusive breastfeeding | 28(37) | 0(0) | ***P <***.01 |
| Partial breastfeeding | 5(6) | 0(0) | ***P=***0.33 |
| Breastfeeding and complementary feeding | 43(57) | 24(100) | ***P***<.001 |
| **Child´s sex (%)** |  |  | ***P>***.99 |
| Male | 35(46) | 11(46) |  |
| Female | 41(54) | 13(54) |  |
| **Childbirth (%)** |  |  |  |
| Gestational age | 39.9 ± 1.1 | 39.3 ± 1.8 | P=.16 |
| Birth weight\* | 3255(2991-3486) | 3250(3043-3625) | *P=*.61 |
|  |  |  |  |

aMedian and IQR (interquartile range). b2 groups were divided according to the duration of lactation of 0-23 months, ≥24 months. cThe body‐mass index is the weight in kilograms divided by the square of the height in meters. This calculation was based on the weight and height measured at the time of screening.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Table 3 Inmunoglobuline of vaccinated participants according to duration of breastfeeding** | | | | |
|  | **[0,23] (N=76)** | **(23,50] (N=24)** |  | **Test** |
| **Anti-SARS-CoV-2 RBD-S1 IgG-HM** | 8(4.74-11.19) | 18.06(12.13-25.36) | *P* <.001 | Wilcoxon |
| **Anti-SARS-CoV-2 S1 IgM-HM** | 0.02(0.02-0.03) | 0.03 (0.02-0.04) | *P* =.01 | Wilcoxon |
| **Anti-SARS-CoV-2 S1 IgA-HM** | 1.05(0.56-1.69) | 2.67(1.71-5.89) | *P* <.001 | Wilcoxon |

aMedian and IQR (interquartile range). b2 groups were divided according to the duration of lactation of 0-23 months, ≥24 months. cThe body‐mass index is the weight in kilograms divided by the square of the height in meters. This calculation was based on the weight and height measured at the time of screening. The IgA e IgM value is expressed in a ratio of the extinction of participant vaccinated over the extinction of the calibrator. The Ig G is expressed in BAU/mL.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Table 4. Inmunoglobuline of vaccinated participants and control participants** | | | | |
|  | **Control group** | **vaccinated participants** |  | **Test** |
| **Anti-SARS-CoV-2 RBD-S1 IgG-Serum** | 0.41±0.37 | 3379.64±1639.46 | *P* <.001 | t. test |
| **Anti-SARS-CoV-2 RBD-S1 IgG-HM** | 0.02±0.05 | 12.19±11.74 | <0.001 | t.test |
| **Anti-SARS-CoV-2 S1 IgM-HM** | 0.01±0.00 | 0.04±0.08 | 0.003 | t.test |
| **Anti-SARS-CoV-2 S1 IgA-HM** | 0.21±0.08 | 1.73±1.59 | <0.001 | t.test |