SARS-CoV-2 in first trimester pregnancy: a cohort study

Running title: SARS-CoV-2 in first trimester pregnancy

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Abstract

Study question: Does maternal infection with SARS-CoV-2 in first trimester pregnancy have an impact on the fetal development as measured by nuchal translucency thickness and pregnancy loss?

Summary answer: Nuchal translucency thickness at the first trimester scan was not significantly different in pregnant women with versus without SARS-CoV-2 infection in early pregnancy and there was no significant increased risk of pregnancy loss in women with SARS-CoV-2 infection in the first trimester.

What is known already: Pregnant women are more vulnerable to viral infections. Previous coronavirus epidemics have been associated with increased maternal morbidity, mortality and adverse obstetric outcomes. Currently, no evidence exists regarding possible effects of SARS-CoV-2 in first trimester pregnancies.

Study design, size, duration: Cohort study of 1,019 women with a double test taken between Feb. 17 and Apr. 23, 2020, as a part of the combined first trimester risk assessment, and 36 women with a first trimester pregnancy loss between Apr. 14 and May 21, 2020, prior to the double test. The study period was during the first SARS-CoV-2 epidemic wave in Denmark.

Participants/materials, setting, methods: Cohort 1 included pregnant women with a double test taken within the study period. The excess serum from each double test was analyzed for SARS-

CoV-2 antibodies. Results were correlated to the nuchal translucency thickness and the number of pregnancy losses before or at the time of the first trimester scan. Cohort 2 included women with a pregnancy loss before the gestational age for double test sample. Serum from a blood test taken the day the pregnancy loss was identified was analyzed for SARS-CoV-2 antibodies. The study was conducted at a public university hospital serving approximately 12% of pregnant women and births in Denmark. All participants in the study provided written informed consent.

Main results and the role of chance: Eighteen (1.8%) women had SARS-CoV-2 antibodies in the serum from the double test suggestive of SARS-CoV-2 infection in early pregnancy. There was no significant difference in nuchal translucency thickness for women testing positive for previous SARS-CoV-2 infection (n=18) versus negative (n=994) (p=0.62). There was no significant increased risk of pregnancy loss for women with positive antibodies (n=1) (OR 3.4, 0.08-24.3 95% CI, p=0.27). None of the women had been hospitalized due to SARS-CoV-2 infection. None of the women with pregnancy loss prior to the double test (Cohort 2) had SARS-CoV-2 antibodies.

Limitations, reasons for caution: These results may only apply to similar populations and to patients who do not require hospitalization due to SARS-CoV-2 infection. A limitation of the study is that only 1.8% of the study population had SARS-CoV-2 antibodies suggestive of previous infection.

Wider implication of the findings: Maternal SARS-CoV-2 infection had no effect on the nuchal translucency thickness and there was no significant increased risk of pregnancy loss for women with SARS-CoV-2 infection in first trimester pregnancy. Evidence concerning Covid-19 in pregnancy

is still limited. These data indicate that infection with SARS-CoV-2 in not hospitalized women does not pose a significant threat in first trimester pregnancies. Follow up studies are needed to establish any risk to a fetus exposed to maternal SARS-CoV-2 infection.

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Introduction

The first case of Coronavirus disease 2019 (Covid-19) was reported in Wuhan, China, in December 2019 and within a few months it developed into a worldwide pandemic (Johns Hopkins University of Medicine, 2020). Covid-19 is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). As of October 11, 2020, more than 37.1 million people worldwide were infected resulting in 1,070,355 deaths (WHO, 2020).

Pregnant women are more vulnerable to viral infections and therefore represent a potential risk group for severe outcomes in relation to viral infections (Silasi *et al.*, 2015). Especially, they have an increased risk of severe pneumonia following infections with respiratory pathogens (Liu *et al.*, 2020). The increased susceptibility during first trimester pregnancy may be due to a pro-inflammatory state (Liu *et al.*, 2020).

For pregnant women, previous coronavirus epidemics such as middle east respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) have been associated with increased maternal morbidity, mortality and adverse obstetric outcomes (Schwartz and Graham, 2020). Only a few documented cases of SARS in pregnant women have been reported. A case study from Hong Kong of seven first trimester cases showed a pregnancy loss rate of 57% in women infected with SARS (Wong *et al.*, 2004). Only 11 confirmed cases with MERS infection during pregnancy have been documented worldwide showing a maternal- and infant fatality rate of 27% (Assiri *et al.*, 2016; Schwartz and Graham, 2020).

Vertical maternal-fetal transmission with serious fetal consequences may occur in relation to maternal infection with TORCH agents (Toxoplasma, Other, Rubella, Cytomegalovirus, Herpes) and Zika virus (Silasi *et al.*, 2015; Alvarado and Schwartz, 2017; Schwartz, 2020). As the fetal organs develop during the first trimester of pregnancy, maternal infections at this stage may be more severe compared to later gestational ages (Silasi *et al.*, 2015; Alvarado and Schwartz, 2017). Parvovirus B19 infection in first trimester pregnancy, even in asymptomatic women, has in case reports been associated with an increased nuchal translucency thickness (edema) (Smulian *et al.*, 1998; Grubman *et al.*, 2019; Markenson *et al.*, 2000;) and may be harmful for the fetus. However, Sebire *et al.* found that increased fetal nuchal translucency in the first trimester was not associated with infection with toxoplasmosis, rubella virus, cytomegalovirus, herpes virus or parvovirus B19 (Sebire *et al.*, 1997). Vertical transmission in relation to SARS and MERS has not yet been documented (Schwartz, 2020) and needs to be investigated.

Evidence concerning Covid-19 in pregnancy is still limited and serological testing for SARS-CoV-2 antibodies has only been reported in few studies of pregnant women in the third trimester of pregnancy (Flannery *et al.*, 2020; Zeng *et al.*, 2020) and measured in one study of 138 pregnant women attending for first trimester screening (Cosma *et al.*, 2020). One study from Wuhan, China, reported very good maternal, fetal, and neonatal outcomes of seven pregnant women infected in late pregnancy and stresses that the effect of SARS-CoV-2 in earlier stages of pregnancy is unknown (Yu *et al.*, 2020). A cohort study in the UK with 427 pregnant women admitted to hospital with PCR confirmed SARS-CoV-2 infection asks for serological studies of Covid-19 in first trimester pregnancies (Knight *et al.*, 2020). Thus, there is a general paucity of data on which to base public health policies for pregnant women and risks associated with SARS-CoV-2 infection.

In this study, we used unselected serological testing in more than 1,000 women to identify if SARS-CoV-2 infection in early pregnancy has an impact on the nuchal translucency thickness and pregnancy loss.

Materials and methods

All pregnant women in Denmark are offered a combined first trimester risk assessment (performed at gestational age 11-14) as part of the public antenatal and obstetric health care service, free of charge. More than 90% of the women accept (Danish Fetal Medicine Database, Annual Report 2018). The risk assessment includes a double test (blood sample for pregnancy-associated plasma protein A (PAPP-A) and free beta human chorionic gonadotropin (β-hCG)) and a nuchal translucency measurement with ultrasonography. PAPP-A and free β-hCG values are converted to MoM-values (Multiples of the Median). One MoM corresponds to the median value at the specific gestational age for a normal material of patients. The excess serum from the double test is stored at minus 80 degrees Celsius at the hospital.

Participants

The Department of Obstetrics and Gynecology at Copenhagen University Hospital Hvidovre is the largest obstetric department in Denmark serving approximately 12% of pregnant women and births in Denmark. The geographical admission area of the hospital is Copenhagen South and West. All pregnant women, who had a double test performed at Hvidovre Hospital from February 17, 2020 to April 23, 2020 were invited to participate in the study. The women were contacted electronically with written information about the study. If they agreed to participate an informed

consent form was signed and the women were included in the study (Cohort 1). We included women who consented up until May 28, 2020.

From April 14 to May 21, 2020, women referred with a pregnancy loss before the time of the double test, were also invited to participate. If a woman with a pregnancy loss wanted to participate, a blood sample was drawn, and baseline characteristics were collected by cross-referencing medical files (Cohort 2).

A questionnaire concerning symptoms of Covid-19 during the pregnancy, smoking habits, body mass index (BMI), influenza vaccination in 2019/2020 and comorbidity was completed by all participating women.

Antibody analysis

The stored excess serum from the double tests and the blood samples from women with pregnancy loss, 30 µl serum from each sample, were analyzed for antibodies (IgM and IgG) against SARS-CoV-2 as an indicator of previous SARS-CoV-2 infection (Infantino *et al.*, 2020b). Antibodies may be present from day four following the first symptoms (Xiang *et al.*, 2020) and the median seroconversion time is day-12 for IgM and day-14 for IgG (Zhao *et al.*, 2020).

Samples were analyzed using YHLO's iFlash 1800 and SARS-CoV-2 IgM/IgG kits (Chemiluminescence immunoassay; YHLO Biotechnology, Shenzhen, China). IgM antibody values <8.0 AU/ml were considered a negative test result and values ≥8.0 AU/ml were considered a positive result. IgG antibody levels <10.0 AU/ml were considered a negative test result and IgG values ≥10.0 AU/ml were considered a positive result. If IgM and IgG or IgG-only was positive, the patient was classified with previous SARS-CoV-2 infection. Patients with IgM-only antibodies were considered to be negative for infection unless there was a positive IgG follow up sample.

According to a recent study the sensitivity (95% CI) was 42.0% (34.4%-50.0%) for IgM and 94.0% (89.0%-96.8%) for IgG and the specificity was 99.7% (98.8%-99.9%) for IgM and 99.3% (98.3%-99.7%) for IgG (Harritshøj *et al.*, 2020).

Statistical analysis

Data and figures were analyzed and produced using R, an open source statistical software (the R foundation, www.r-project.org). Comparisons of nuchal translucency thickness, free β-hCG, and PAPP-A between women with and without SARS-CoV-2 antibodies were performed using the Wilcoxon Rank Sum test. Multivariable modelling of the effect of Covid-19 infection in first trimester pregnancy on nuchal translucency thickness was performed using an ordinal regression model, taking maternal age and gestational age into account. Differences in reported Covid-19 symptom frequency were analyzed using Fishers exact test. A p-value < 0.05 was considered statistically significant.

Ethical approval

The study was approved by Knowledge Centre on Data Protection Compliance, The Capital Region of Denmark (P-2020-255) and by the Scientific Ethics Committee of the Capital Region of Denmark (journal number H-20022647). All participants in the study provided written informed consent.

Results

A total of 1,356 double tests were performed from 1,356 pregnant women during the study period. Of the 1,356 women, 1,019 (75.1%) provided informed consent to participate (Cohort 1).

Additionally, 36 women with an early pregnancy loss prior to the time of the double test were included (Cohort 2). The overview of the study is illustrated in Figure 1.

The median gestational age was 11 weeks and 0 days (11+0) at the double test and 13+0 at first trimester scan. The median gestational age among the 36 women with early pregnancy loss was 8+1. The characteristics of the two cohorts are presented in Table I.

The total number of women with SARS-CoV-2 antibodies (positive IgM and IgG values or IgG-only) in Cohort 1 was 18 (1.8%). Two women were IgM and IgG positive, and 16 women were IgG-only positive. Eight women in cohort 1 were IgM-only positive (IgM values 8.3-15.1 AU/ml). Five of these had a follow up blood sample 54-77 days after the double test sample, none developed IgG ≥10.0 AU/ml. None of the 36 women from Cohort 2 had positive SARS-CoV-2 antibodies.

For Cohort 1 we subsequently compared the nuchal translucency thickness (measured at the first trimester scan) between women with SARS-CoV-2 antibodies reflecting previous infection versus no previous infection. Women, where the fetus was found to have a chromosome anomaly (trisomy), were excluded from the analysis of the nuchal translucency thickness. The median nuchal translucency thickness, free β-hCG, and PAPP-A levels as well as the MoM-values were not significantly different between women with negative versus positive levels of SARS-CoV-2 antibodies (Table II). Also, after accounting for maternal age and gestational week, positive antibodies (p=0.81) did not affect nuchal translucency thickness.

Table III displays pregnancy status for all 1,055 pregnancies (1,019 in Cohort 1 and 36 in Cohort 2) after the first trimester and according to SARS-CoV-2 antibodies. For the 1001 pregnancies in cohort 1, 15 women had a pregnancy loss between the double test and the nuchal translucency scan, three women were diagnosed with a missed abortion at the nuchal translucency scan (Figure 1), and one woman was lost to follow up after the double test. One woman with positive SARS-CoV-2 antibodies had a pregnancy loss and 17 women with ongoing pregnancies had positive SARS-CoV-2 antibodies. There was no significant increased risk of pregnancy loss in women with positive antibodies (OR=3.4, 0.08-24.3 95% CI, p=0.27).

Figure 2 illustrates Covid-19 symptoms reported by pregnant women with negative or positive SARS-CoV-2 antibodies. Significantly more women with positive antibodies reported Covid-19 symptoms in early pregnancy compared to women with negative antibodies (53% versus 26%) (OR=3.2, 95% CI 1.1-9.6, p=0.023). One woman with antibodies did not answer the questionnaire regarding symptoms of Covid-19. Among the 9 (53%) women with SARS-CoV-2 antibodies who reported symptoms of Covid-19 in early pregnancy four women reported only one symptom and five women reported two or more symptoms. Reported symptoms were: Ageusia and or anosmia (n=5), dry cough (n=4), extreme tiredness (n=3), fever (n=2), arthralgia (n=2), dyspnea (shortness of breath) (n=2), headache (n=1).

The cumulative frequency of pregnant women included after the double test (Cohort 1) and with positive SARS-CoV-2 antibodies during the study period is displayed in Supplementary Figure 1.

Discussion

We found that pregnant women with SARS-CoV-2 infection in the first trimester did not have a significantly different nuchal translucency thickness measured at their first trimester scan. Furthermore, there was no significant increased risk of pregnancy loss in women with SARS-CoV-2 infection in the first trimester before the time of the double test.

Of the 36 women with early pregnancy loss, before the double test was taken (Cohort 2), none had SARS-CoV-2 antibodies. It is well known that early pregnancy loss is predominantly related to intrinsic embryonic inborn errors (Ouyang *et al.*, 2016; Pylyp *et al.*, 2018). However, we included Cohort 2 to minimize a risk of bias that could potentially exist if there was a high number of women with SARS-CoV-2 antibodies in this cohort. Significantly more women with positive antibodies reported symptoms of Covid-19 compared to women without antibodies. None of the women had been hospitalized for Covid-19.

The first case of Covid-19 in Denmark was confirmed February 27, 2020. At the beginning of the epidemic in Denmark, it was only individuals requiring hospitalization who were tested for SARS-CoV-2 with a respiratory specimen. Citizens suspected of Covid-19 but not requiring admission were asked to remain at home and self-quarantine and were not tested. Only 53% of the women with SARS-CoV-2 antibodies in our study reported symptoms of Covid-19 in pregnancy. This corresponds well to a comparable study from Italy where 6 out of 14 (42.8%) first trimester pregnant women with SARS-CoV-2 antibodies referred previous symptoms of Covid-19 (Cosma *et al.*, 2020). Symptoms of Covid-19 are very similar to symptoms of other viral infections and 26% of the women without SARS-CoV-2 antibodies in our study reported similar symptoms. Therefore, serological testing in appointed risk groups, such as pregnant women, is a valuable tool to identify previous infections and to evaluate whether infection in pregnant women requires additional

vigilance during the pregnancy. Our findings suggest that pregnant women in their first trimester are not at increased risk of severe Covid-19 disease. This is similar to what has been reported for pregnant women in the third trimester in Wuhan, China (Chen *et al.*, 2020) and in the first trimester in Turin, Italy (Cosma *et al.*, 2020).

In general, the study population had normal BMI and the vast majority were non-smokers. Our results and conclusion may therefore not apply directly to populations with higher BMI, higher frequency of smoking and associated higher frequency of lifestyle diseases. People with lifestyle diseases and individuals who smoke are at higher risk of developing more severe Covid-19 disease if infected (Guo *et al.*, 2020; Mehra *et al.*, 2020; Petrakis *et al.*, 2020). Our study does not rule out the possibility that more severe Covid-19 disease might lead to a higher risk of adverse outcomes for the developing fetus.

The frequency of participants with previous SARS-CoV-2 infection in pregnancy was relatively low and steady over the study period (Supplementary Fig. 1). This is most likely a result of the extended measures implemented by the Danish government at an early stage of the epidemic to limit the transmission of the virus. Measures included closing the national borders, banning of group gatherings of more than ten people, closure of all educational facilities, implementing work-from-home measures for all non-critical government and state employees, and recommending that private employees also work from home where feasible. However, in addition to the general societal changes, the relatively low occurrence of SARS-CoV-2 antibodies among participants could also be due to pregnant women taking additional precautionary measures such as self-quarantine and limiting social contacts even before the implementation of official governmental restrictions.

As per May 23, 2020, it was estimated that the seroprevalence of people with SARS-CoV-2 antibodies in the Danish population was 1.1% (95%CI 0.5–1.8) (SSI, 2020a), but the admission area covered by Hvidovre Hospital had a higher than average incidence of SARS-CoV-2 infected individuals at the time (SSI, 2020b). It is therefore not surprising that the prevalence of SARS-CoV-2 positive antibodies in the study population is higher than 1.1%.

There is still uncertainty concerning the accuracy of the various tests for SARS-CoV-2 antibodies (Infantino *et al.*, 2020a). One study from China used a sandwich enzyme linked immunosorbent assay (Xiang *et al.*, 2020) and found the sensitivity and specificity to be 77.3%/100% for IgM and 83.3%/95% for IgG, respectively. We used iFlash 1800 with its IgM/IgG kit, which has previously shown highly accurate results (Infantino *et al.*, 2020b). We used the reference values for positive and negative test result suggested by Harritshøj et al. who tested the diagnostic accuracy of the SARS-CoV-2 antibody assays (Harritshøj *et al*, 2020). According to Harritshøj et al. YHLO iFlash assays showed acceptable IgG sensitivity and acceptable and high IgM and IgG specificity. It is very likely that more assays will be developed in the future and as reviewed by Infantino *et al*., more studies are needed to validate the serological assays, especially for use as screening tools for asymptomatic individuals (Infantino *et al.*, 2020a). Additionally, our study includes a risk of bias due to the low prevalence of positive samples in the study population (Christopher Sempos, 2020).

Despite a high participant rate, it is a potential limitation of the study that not all invited women participated in the study. By the end of May 28, 2020, a total of 337 women had not responded to our study invitation. It could potentially introduce selection bias if the none-respondents were different in terms of rates of positive SARS-CoV-2 antibodies and pregnancy loss. The blood

sample for antibody analyses from women in Cohort 2 were drawn at the day of pregnancy loss, and we found none with SARS-CoV-2 antibodies. This provides some certainty that we did not overlook a potential effect of SARS-CoV-2 at the earlier stage of pregnancy before the double test is taken and if any relationship exists it is possibly very low.

Conclusion

This study focused on possible signs of maternal SARS-CoV-2 infection in first trimester pregnancies. We found no significant different nuchal translucency thickness at the first trimester scan among pregnant women with previous SARS-CoV-2 infection in early pregnancy compared to women without previous SARS-CoV-2 infection. Furthermore, we found no significant increased risk of pregnancy loss in women with SARS-CoV-2 infection in the first trimester. Serological studies investigating the impact of SARS-CoV-2 on later stages of pregnancy are needed to develop clinical guidelines and recommendations for any possible restrictions for pregnant women in relation to SARS-CoV-2.

Data Availability

The data underlying this article cannot be shared for ethical/privacy reasons.

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Authors' roles

HSN, NlCF, HLJ and HW were main responsible for the study design and conception. NlCF, PE, KVRH, ERS, AMK, DW, LFO, LP, AZ, AHC, JRN, DB, SB, JOL, AI, JBR, DMS, JEF, ERH, CWJ, FSJ, HW, HLJ and HSN were responsible for data collection, accuracy of the data, data interpretation and revising the manuscript critically for important intellectual content. DW (primary), NlCF, PE and HSN were responsible for the statistical analysis and figures. NlCF, PE, HSN and DW were responsible for the literature search. NlCF was responsible for drafting the manuscript. All authors approved the final version to be published.

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Conflict of interest

Henriette Svarre Nielsen has received speaker´s fees from Ferring Pharmaceuticals, Merck Denmark A/S and Ibsa Nordic (outside the submitted work). Nina la Cour Freiesleben has received a grant from Gedeon Richter (outside the submitted work). Astrid Marie Kolte has received

speaker´s from Merck (outside the submitted work). The other authors did not report any potential conflicts of interest.

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Figure legends

Figure 1. Flowchart of the two cohorts

Figure 2. Incidence of self-reported coronavirus disease 2019 (Covid-19) symptoms for women with and without Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection in first trimester pregnancy.

Supplementary Figure 1. The cumulative frequency of pregnant women with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) antibodies during the study period

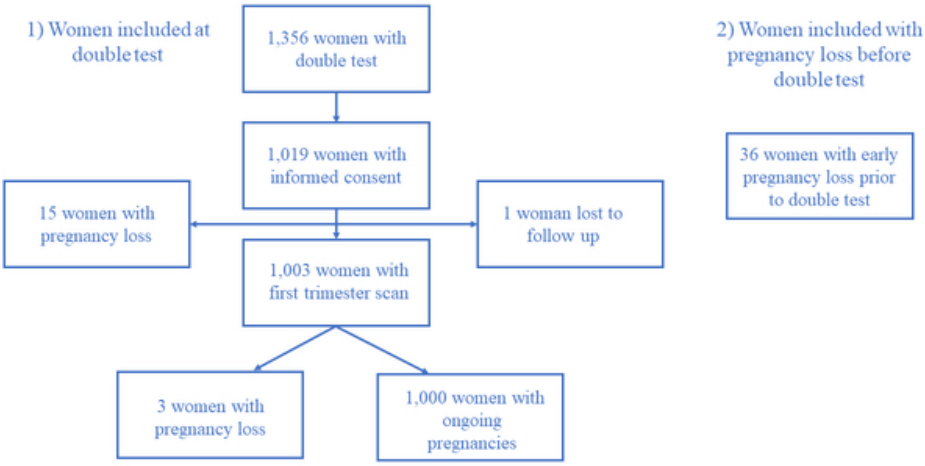

Figure 1. Flowchart of the two cohorts

54x30mm (300 x 300 DPI)

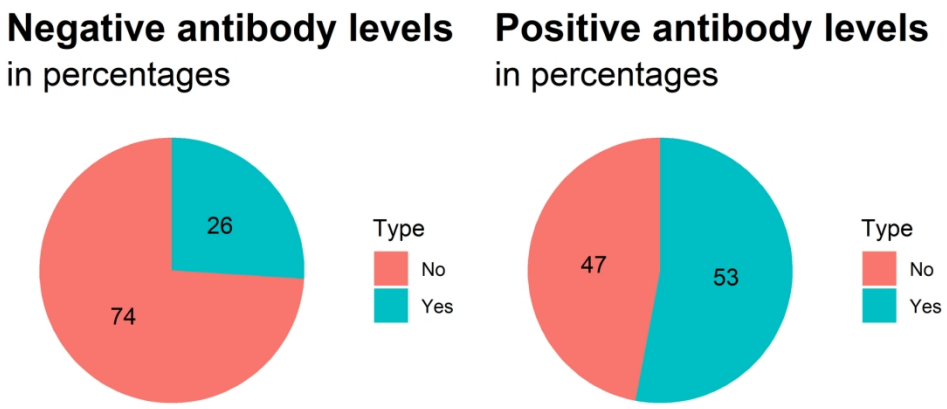

Figure 2. Incidence of self-reported Covid-19 symptoms for women with and without SARS-CoV-2 infection in first trimester pregnancy.

164x193mm (300 x 300 DPI)

Table I. Characteristics of the included women in the two cohorts

	Women included after double test n = 1,019 (Cohort 1)	Women with pregnancy loss before double test n= 36 (Cohort 2)
Age (y), *mean (SD)*	31.71 (4.52)	32.96 (5.22)
BMI (kg/m²), *mean (SD)*	23.92 (4.65)	25.33 (5.51)
Gestational week, *median*		
- *At blood sample for double test*	11+0	
- *At first trimester scan (nuchal translucency)*	13+0	
- *At pregnancy loss*		8+1
Smoking, *n (%)*		
- *Yes*	31 (3.0)	3 (8.3)
- *No*	953 (93.5)	31 (86.1)
- *Unknown*	35 (3.4)	2 (5.6)
***Asthma,** n (%)*		
- *Yes*	56 (5.5)	4 (11.1)
- *No*	942 (92.4)	31 (86.1)
- *Unknown*	21 (2.1)	1 (2.8)
Influenza vaccination 2019/2020, *n (%)*		
- *Yes*	99 (9.7)	5 (13.9)
- *No*	900 (88.3)	23 (63.9)
- *Unknown*	20 (2.0)	8 (22.2)
SARS-CoV-2 antibodies, *n (%)*		
- *Negative*	1001 (98.2)	36 (100)
- *Positive*	18 (1.8)	0

Table II. Primary outcomes for cohort 1 according to SARS-CoV-2 antibody status. Cohort 1 was included after the double test.

	Negative (n = 994)	**Positive** (n = 18)	***p*-value** *Positive versus negative*
Nuchal translucency thickness (mm), *median (quartiles)*	1.7 (1.5-2.0)	1.8 (1.5-2.0)	0.62
Free β-hCG (IU/L), *median (quartiles)*	51.9 (32.9-80.0)	53.8 (22.1-86.8)	0.63
Free β-hCG (MoM), *median (quartiles)*	1.0 (0.7-1.5)	1.1 (0.7-1.6)	0.81
PAPP-A (IU/L), *median (quartiles)*	1.7 (1.1-2.9)	1.3 (0.8-3.5)	0.64
PAPP-A (MoM), *median (quartiles)*	1.1 (0.8-1.7)	1.0 (0.6-1.5)	0.30

Information on nuchal translucency thickness was available for 982 of the included women and information on free β-hCG and PAPP-A for 1,012. MoM values were available for 978 of the included women.

Table III. Pregnancy status after the first trimester according to SARS-CoV-2 antibody status in the double test. The table includes both Cohort 1 and 2.

	Cohort 1		Cohort 2	
	Negative (n = 1000)	Positive (n = 18)	Negative (n = 36)	Positive (n = 0)
Ongoing pregnancy, *n*	983	17	0	0
Pregnancy loss, *n*	17	1	36	0

One woman with a negative test result was lost to follow up after double test.