Original Research Article
Transmission of anti-SARS-CoV-2 antibodies to newborns by vaccination of pregnant women.

ABSTRACT

Aims: Vaccination in pregnant women is already performed for viruses and bacteria, ~~with~~ which hasadvantages ~~to~~ forthe mother and the newborn. Besides a general rejection of ~~Covid~~COVID-19 vaccinesin this population, these are considered safe, effective and may prevent severe disease in pregnant women. Neutralizing ~~antibodies~~ antibodiytiter measured in vaccinated pregnant is highly predictive of immune protection and can have a protective effect on the newborn**.** This study aimed to confirm the transfer of the antibodies across the placenta and to testif other factors impact the ~~antibodies~~ antibodiy'stiter levels in the newborn circulation.

Study design and method~~ology~~: We collected 24 samples of umbilical cord blood ~~of~~ fromwomen ~~who were~~ vaccinated during pregnancy or in the three months before ~~the~~ pregnancy and analyzed the data of those pregnancies.

~~Place and Duration of Study:~~This cohort study was developed in our hospital~~,~~ between March 2021 and May 2022.

Results: It was possible to confirm the passage of anti-Spike antibodies to the newborn. When comparing the IgG anti-S titers with the timing of vaccination, the analysis suggests that vaccination during the second or third trimester of pregnancy is more effective than before or in early pregnancy (p=0,005). As for the vaccine used (p=0,23) and the mode of delivery (p=0,48), there were no statistical differences.

Conclusion: We confirm the passage of antibodies during the pregnancy, which appears to be more effective during the second and third trimester of gestation. Despite lack of evidence for how long and how effectively this passive immunity can protect the neonate from infection, vaccination of pregnant women should be considered.

Comment [M11]: Replace the term aims with background

Comment [M12]: Place and duration of study are part of study design and method

Comment [M13]: Pay attention to the use of periods and commas in the data analysis results

1. INTRODUCTION

In December 2019, uncommon cases of pneumonia of unknown etiology were reported in Wuhan (China)~~, and w~~Within a few months, the coronavirus disease 2019 (COVID-19), caused by acute respiratory syndrome coronavirus 2 (SARS-CoV-2), spread rapidly all over the world. This viral infection can cause a mild to severe illness, depending on how immune system encounters the virus~~effectively the virus is encountered by the immune system~~[1].

Early on it, was clear that the development of a vaccine would be essential to control the disease, and in less than a year after it was declared ~~a~~ pandemic disease by WHO (World Health Organization)disease, the first vaccines against SARS-CoV-2 were already being distributed around the world.

Vaccination in pregnant women is not a new topic as it is already being done for other viruses, like influenza, or bacteria, like Bordetella pertussis. These strategies can have some advantages for the mother, like protection from a severe form of the disease, as well for the newborn, with passive protection from the antibodies produced by pregnant women and transferred ~~for~~ tothe fetus throughout the pregnancy.

For COVID-19, some of the first effective vaccines were produced with a theoretically safe technology for pregnant women.~~As for the COVID-19, some of the first effective vaccines were produced with a technology that theoretically was safe in pregnant women~~. Both the vaccines based on adenovirus vectors, like OxfordAstraZeneca ChAdOx1-S and the vaccines based on messenger RNA, like Pfizer-BioNTech BNT162b2, are secure and pose no risk of pregnant women or fetus infection and so are thought to be safe in this population [2-5].

However, it should be remembered~~kept in mind~~ that pregnant women were excluded from initial clinical trials for COVID-19 vaccines, limiting the recommendations ~~that can be made to~~for this specific population. This led to an initial rejection of vaccination by the obstetric population, despite current data suggesting that pregnant women may be at increased risk for severe illness, admission to an intensive care unit, mechanical ventilation, and even death when compared with nonpregnant women [6,7]. They are also more likely to deliver pre-term newborns, which have higher probability to be admitted to the neonatal intensive care unit [6]. This is highlighted by the Centers for Disease Control and Prevention (CDC), which included pregnancy as a risk factor for severe COVID-19 illness [8].

~~It is therefore~~ Therefore, it isof great interest to study the maternal immune response to SARS-CoV-2 vaccination during pregnancy, as well as the extent to which maternal antibodies cross the placenta, to predict the degree of passive protection acquired by the newborn, and factors that can modulate the effectiveness of that process.

Since neutralizing antibody titer is already established to be highly predictive of immune protection~~Since is already established that neutralizing antibodies titer is highly predictive of immune protection~~ [9], this study aims primarily to document and confirm the presence of antibodies anti-SARS-CoV-2 in the umbilical cord blood of newborns of mothers vaccinated during pregnancy.

2. METHOD~~OLOGY~~

This cohort study was developed in our hospital, between March 2021 and May 2022. ~~To e~~Every woman~~,~~ who was vaccinated at least with one dose during the current pregnancy or in the three months before pregnancy, without known previous infection with SARS-CoV-2, and who were admitted to the delivery room in labor, was given the opportunity to participate in the study.

At admission, samples of posterior oropharyngeal and nasopharyngeal tissue were obtained to exclude asymptomatic SARS-CoV-2 infection, and positive results were excluded from this study.~~Samples of posterior oropharyngeal and nasopharyngeal to exclude asymptomatic SARS-CoV-2 infection were obtained at admission, and positive results were excluded from this study.~~After consent, an umbilical cord blood sample was collected at the time of delivery, regardless of the mode of delivery and the gestational age

After consent, was collected an umbilical cord blood sample at the time of the delivery, regardless of the mode of delivery and the gestational age.

Then, the blood samples were tested to determine the circulating IgG anti-S protein level by Chemiluminescence.

The primary goal of this study was to evaluate the transfer of the antibodies across the placenta. Additionally, there was an evaluation to determine if the gestational age at vaccination, mode of delivery or type of vaccine used, was important to the titer of circulating antibodies in the newborn circulation

Comment [M14]: state the informed consent and ethical clearance more clearly

Comment [M15]: state the parameters as variables analyzed, and explain the statistical tests used

Comment [M16]: Primary golas just similar with aims of this study, author should write ini more clearly in introduction

3. RESULTS

~~In total were~~We collected 24 samples between May 2021 and March 2022 (Table 1). The characteristics of the population studied are resumed in Table 2.

Table 1. Description of the cases included in the cohort

	Mother Age (years)	Complications during pregnancy	Gestational age at birth (weeks)	Mode of Delivery	Gestational age at vaccination (weeks)		Vaccine administered	IgG anti-S spike
					1st dose	2nd dose		
1	36	None	40+2	Vaginal	20+5	23+5	Pfizer BNT 162b2	703 BAU/mL
2	27	None	38+4	Vaginal	28+6	31+6	Pfizer BNT 162b2	>2080 BAU/mL
3	28	None	39+0	Cesarean	PC	3	Pfizer BNT 162b2	98.6 BAU/mL
4	32	None	39+1	Vaginal	1	4	Pfizer BNT 162b2	1500 BAU/mL
5	31	None	40+6	Cesarean	30+3	33+3	Pfizer BNT 162b2	>2080 BAU/mL
6	36	None	40+2	Vaginal	30+5	33+5	Pfizer BNT 162b2	>2080 BAU/mL
7	38	None	39+1	Vaginal	32+4	35+4	Pfizer BNT 162b2	676 BAU/mL
8	33	None	39+1	Vaginal	PC	PC	Pfizer BNT 162b2	259 BAU/mL
9	31	None	39+4	Vaginal	PC	PC	Pfizer BNT 162b2	163BAU/mL
10	37	None	40+0	Vaginal	23+1	26+1	Pfizer BNT 162b2	335 BAU/mL
11	31	None	38+3	Vaginal	20+5	23+5	Pfizer BNT 162b2	704 BAU/mL
12	37	None	37+3	Vaginal	24+5	27+6	Pfizer BNT 162b2	>2080 BAU/mL
13	43	Small intestine GIST	39+0	Vaginal	23+6	27+6	Pfizer BNT 162b2	430 BAU/mL
14	39	None	38+6	Cesarean	PC	PC	Pfizer BNT 162b2	59.40 BAU/mL
15	37	None	39+2	Cesarean	23+0	26+0	Pfizer BNT 162b2	765 BAU/mL
16	31	None	38+4	Cesarean	PC	PC	Pfizer BNT 162b2	260 BAU/mL
17	31	None	38+0	Cesarean	23+0	26+0	Pfizer BNT 162b2	784 BAU/mL
18	29	None	39+5	Vaginal	PC	NA	ChAdOx1 S recombinant	6.33 BAU/mL
19	28	None	39+0	Cesarean	22+0	25+0	Pfizer BNT 162b2	1080 BAU/mL
20	28	Pre-eclampsia at term	37+1	Cesarean	27+4	30+0	Pfizer BNT 162b2	1110 BAU/mL
21	33	None	40+0	Vaginal	PC	23+2	ChAdOx1 S recombinant	698 BAU/mL
22	32	Positive aneuploidy screening	39+2	Cesarean	22+3	25+3	Pfizer BNT 162b2	1060 BAU/mL
23	32	None	40+3	Vaginal	30+5	33+6	Pfizer BNT 162b2	1880 BAU/mL
24	30	None	39+5	Vaginal	26+6	29+6	Pfizer BNT 162b2	2050 BAU/mL

Abbreviations: PC, Preconception; NA, not applicable; GITS, Gastrointestinal Stromal Tumor.

Table 2. Median characteristics of the population studied

Characteristics of the population studied	Median
Age (years)	

Comment [M17]: Authors should not write down complete data from all subjects, but summarize it in the form of mean+/-mean for age, dose and anti-S spike IgG levels. The author should write the complication, mode of delivery and vaccine administered variables of subject in the form of number and presentage

Comment [M18]: display data with mean +/- standard deviation or median (min;max)

Separate data for percentage data and median/average data

Gestational age at birth (week)	39 + 1 (37+1; 40+6)	
Mode of delivery	Vacuum delivery	3 (12,5%)
	Euthocic delivery	12 (50%)
	Cesarian delivery	9 (37,5%)
IgG anti spike	Positive result	23 (95,8%)
	Negative result	1 (4,2%)

Pregnant women were on average 33 years and all pregnancies went to term, with delivery between 37 weeks $^{+ 1 day}$ and 40 weeks $^{+ 6 days}$. The mode of delivery was a vaginal delivery in 15 cases (62,5%), including 3 vacuum deliveries, and a cesarean delivery in 9 cases (37,5%).

Most pregnancies included in this study were uneventful. The exceptions were one pregnant woman who developed pre-eclampsia at term, one who was diagnosed with small intestine GIST, and another one with a positive aneuploidy screening (who refused amniocentesis but had an infant without apparent structural anomalies).

In 2 cases, the ChAdOx1 S recombinant vaccine was administered, and in the other 22 cases, the BNT162b2 vaccine was administered.~~In 2 cases the vaccine administrated was the ChAdOx1 S recombinant vaccine and in the other 22 cases was administered the BNT162b2 vaccine~~.

In 95,5% of cases the result was above the cut-off to be considered positive (33.6 BAU/mL). So, there was only one negative case, in which the woman was vaccinated with the ChAdOx1 S recombinant vaccine, some days after the last menstrual period, having rejected the second dose, due to a pregnancy positive test.

As shown in Table 3, in one-third of the cases the first dose of the vaccine was administered before conception, and the same for 15,4% of the second dose. The vaccines were administered during different gestational ages in different pregnant women: only 2 doses were administered in the first trimester (none as a first dose); 21 in the second trimester (11 as a first dose); and 12 in the third trimester (5 as a first dose). The latest dose was given at 35 weeks $^{+ 4 days}$, nonetheless, had a delay of 25 days between the second dose and the delivery.

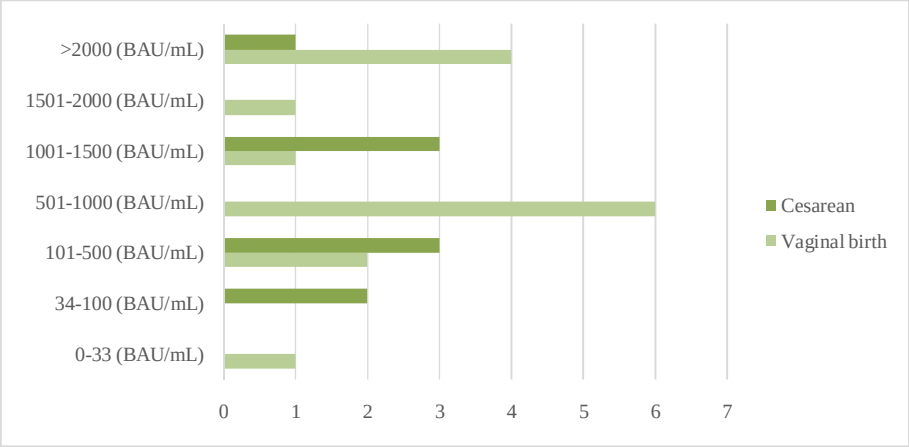
Table 3. Timing of vaccine doses

First Dose (n=24)	
Before pregnancy	8 (33,3%)
1º Trimester	0 (0%)
2º Trimester	11 (45,8%)
3º Trimester	5 (20,8%)
Second Dose (n=23)	
Before pregnancy	4 (15,4%)
1º Trimester	2 (8,7%)
2º Trimester	10 (43,5%)
3º Trimester	7 (30,4%)

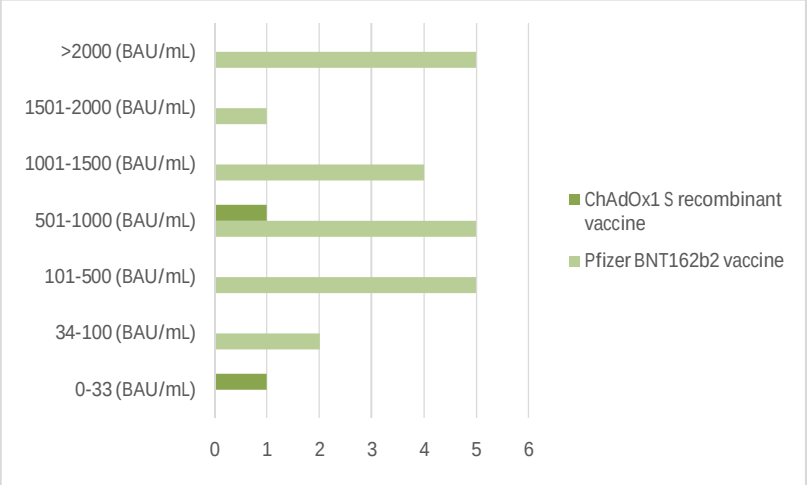
There was no statistical significance of the IgG anti-S titers (p=0,48) when compared the result of samples obtained after a vaginal delivery (Median of 1043 BAU/mL ± 801) and after cesarean delivery (Median of 811 BAU/mL ± 634) (Graphic 1). When comparing the vaccine used, there was no statistical difference (p=0,23) when pregnant women were vaccinated with the

ChAdOx1 S recombinant vaccine (Median of 352 BAU/mL ± 489) or with Pfizer BNT162b2 vaccine (Median of 1011 BAU/mL ± 739) (Graphic 2).

Graphic 1. Anti-Spike titers according to the mode of delivery



Graphic 2. Anti-Spike titers according to the type of vaccine used



As for the timing of vaccine doses (Graphic 3), there was a statistical difference (p=0,005) between women vaccinated in the second or third trimester (Median of 1211 BAU/mL ± 664) and women vaccinated before or very early in the gestation (Median of 163 BAU/mL ± 523). Even when comparing the titers of antibodies anti-S in the umbilical cord samples of women vaccinated in the 2nd trimester (Median of 864 BAU/mL ± 486) versus women vaccinated in the

3rd trimester (Median of 1708 BAU/mL ± 575), the results also favored a vaccination in the third trimester (p=0,005). The smaller p-value was obtained when comparing women vaccinated before the pregnancy and women vaccinated in the 3rd trimester of the pregnancy (p=0,0005).

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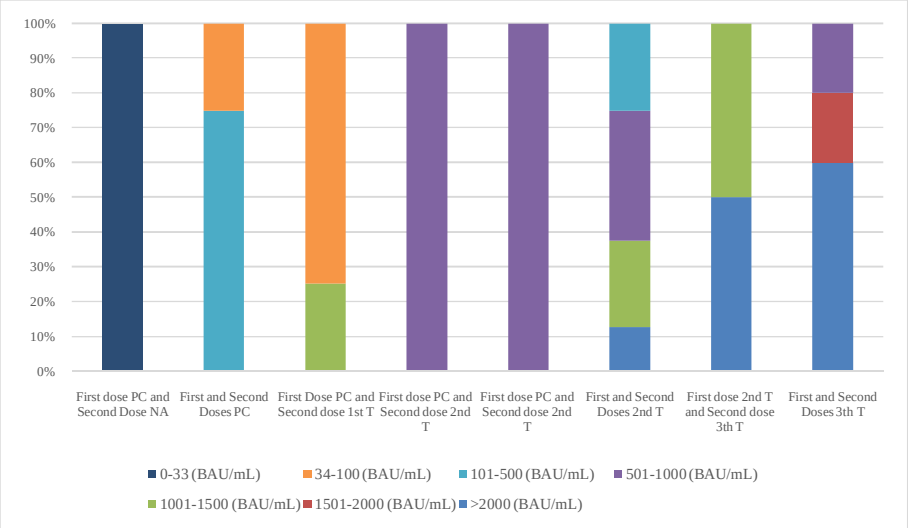
Graphic 3. Anti-Spike titers according to the timing of the vaccine doses



Abbreviations: PC, Preconcepcion; NA, not applicable; T, Trimester.

Table 4 resumes the statistical results described here.

Table 4. Effect of mode of delivery, the timing of vaccination, and vaccine used on IgG anti-Spike titers

Comment [M110]: The writing of table 4 is still difficult to understand, it should be improved so that it is easier for readers to understand.

Fix writing median (min, max)

Mode of delivery	
Vaginal delivery	Median of 1043 BAU/mL ± 801
Cesarean delivery	Median of 811 BAU/mL ± 634
p= 0,4671	
Timming of vaccination	
Second or third trimester	Median of 1211 BAU/mL ± 664
Before or very early on the gestation	Median of 163 BAU/mL ± 523
p= 0,0051	
Third Trimester	Median of 1708 BAU/mL ± 575
Before pregnancy	Median of 149 BAU/mL ± 115
p= 0,0005	
Third Trimester	Median of 1708 BAU/mL ± 575
Second trimester	Median of 864 BAU/mL ± 486
p= 0,0052	
During pregnancy	Median of 1168 BAU/mL ± 680
Before the pregnancy	Median of 149 BAU/mL ± 115
p= 0,0034	

Vaccine used	
Pfizer BNT 162b2 vaccine	Median of 1011 BAU/mL ± 739
ChAdOx1 S recombinant vaccine	Median of 352 BAU/mL ± 489
p= 0,2342	

4. DISCUSSION

It is established that ~~covid~~COVID-19 disease can cause severe disease in pregnant women and can be associated with worse obstetric outcomes, with a meta-analysis reporting a rate of adverse events of 27%, with an unusually high cesarean rate [10]. As already mentioned, neonates born from mothers with COVID~~covid~~-19 can also have severe comorbidities like respiratory distress syndrome, pneumonia, and other, secondary to iatrogenic preterm birth and maternal status[11]. Therefore, it is central to mention that vertical transmission can occur, and although the risk is quite low (estimated to be 8% or even lower)[10,12,13], it should not be ignored. Another consideration is the possibility of postnatal infection by respiratory transmission [14], emphasizing the importance of their previously acquired protection.

Production of IgG antibodies against SARS- CoV-2 virus occurs following natural infection, a process that also occurs with active immunization. Current data suggests that vaccine-induced immune responses are significantly greater than the response that occurs with natural infection [15,16].

Early in the final weeks of the first trimester of gestation, maternal IgG is transferred across the placenta to the developing fetus to protect them from infection as a neonate. This process is called passive immunization and it peaks in the last trimester, where the majority of IgG is transferred in the final 4 weeks of gestation. Although most IgG antibody crosses the placenta in the third trimester, the process is time-dependent, so immunization should be ideally done at least 6 or more weeks prior to delivery [17].

The COVID-19 vaccines that were early commercialized were based on messenger RNA technology that targeted the S protein (protein Spike). Therefore, the circulating level of IgG anti-S is a measure of the immune response to the vaccination [18]. Although thisresponse does not only relies on the titer of IgG anti-S antibodies (since there is also a cellular immune response), these are an objective measure of the reactivity of the immune system and a measure to predict an eventual passive protection of the newborn. As evidence supports, anti-SARS-CoV-2 antibodies titers are associated with a lower risk of symptomatic disease [16,19,20].

In agreement with other recent studies, it was observed that the anti-SARS-CoV-2 antibodies, produced by the pregnant woman's immune system in response to the vaccine, crossed the placenta and became present in the fetus's blood resulting in passive immunity, at least in the neonatal period [19,21,22].

The increasing levels of maternal IgG over time and the increasing placental IgG transfer ratio over time suggest that the timing between vaccination and delivery may be an important factor to consider in vaccination strategies in this population.

Although some studies suggest that a longer latency between vaccination and delivery correlates with higher anti-S IgG titers [21,23], there is already evidence that vaccination of pregnant women in the third trimester can be equally effective [24]. In fact, these vaccines induce a rapid stimulation of the immune system with antibody production as early as 5 days after the first dose, and transplacental transfer of passive immunity to the neonate as early as

16 days after the first vaccination dose [25,26]. As this study demonstrates, vaccination in the third trimester seems to correlate to higher titers of anti-Spike antibodies in the umbilical cord blood at the delivery.

This study also tried to prove if there was any correlation between the anti-S antibodies titers and the type of vaccine used or the mode of delivery. As expected, the mode of delivery did not seem to interfere with the anti-S titers in the umbilical cord as that passage occurs during gestation and not at the time of the delivery. Considering the type of vaccine used, although the only negative sample was from a pregnant woman vaccinated with the ChAdOx1 S recombinant vaccine, she received only one dose, instead of the usual two. Besides that, our sample was too small to draw a conclusion based on the vaccines used.

Despite the lack of evidence for how long this passive immunity can protect the neonate from infection and how effective is that protection, the confirmation of the passage of these antibodies through the placenta and the fact, already established, that pregnant women are a group of higher risk for severe disease when compared with the general population, may justify vaccination even during pregnancy.

5. CONCLUSION

Comment [M111]: The author needs to clarify the sentences in the conclusion

In conclusion, this procedure should be encouraged and any misconception about risks for the mother and the fetus should be corrected considering the evidence available. Although is needed more investigation to make recommendations on when the vaccination is more efficient, this work suggest that vaccination during the second or third trimester of gestation is effective.

CONSENT (WHEREEVER APPLICABLE)

All participants included in the study sign an informed consent stating their consent to participate in this study.

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

This study was approved by the ethics committee of our institution (with the approval code: Ref.ª 1166/CES/2021). The procedures used in this study are in line with the principles of the Declaration of Helsinki.

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