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1 **SARS-COV-2 IgG antibody response in pregnant women at delivery.**

2

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54

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56 CVF has obtained a grand from Ferring pharmaceuticals for the COVIPREG study.

57

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65

1 **SARS-COV-2 IgG antibody response in pregnant women at delivery.**

2

3 **ABSTRACT**

4

5 **Background**

6 The prevalence of COVID-19 infection during pregnancy is not known. COVIPREG is a
7 prospective French multicenter study to assess the seroprevalence at the time of delivery
8 and the maternal and neonatal impact of COVID-19 infection during pregnancy. In order to
9 study factors associated with poor outcomes after COVID-19 Infection during pregnancy and
10 adapt the sample size of the study, a preliminary assessment of the prevalence of SARS-CoV-
11 2 IgG was planned after 500 inclusions in a one perinatal center of Paris area.

12

13 **Objectives**

14 To assess the prevalence of SARS-CoV-2 IgG antibody response in pregnant women at the
15 time of delivery during the COVID-19 pandemia.

16

17 **Study design**

18 A prospective observational study at Cochin hospital (Level III maternity). Patients admitted
19 for delivery were offered to participate to the study. Each patient participating to the study
20 was tested for anti-SARS-CoV-2-IgG antibodies using a commercially available ELISA.

21

22 **Results**

23 Among the 529 patients included in the COVIPREG study between April 29 and June 26, 529
24 were assessed for SARS-CoV-2 IgG antibody response and 25 had a positive test, ie 4.7%
25 with a confidence interval at 95% [3.0%-6.9%].

26

27 **Conclusions**

28 Four months after the beginning of the infection in Paris, the seroprevalence of SARS-CoV-2
29 IgG in pregnant women at the time of delivery is low. Studies evaluating the impact of
30 COVID-19 infection during pregnancy should take this information in account in order to
31 adapt the sample size.

32

33

34

35 **Keywords**

36 Pregnancy, COVID-19, SARS-CoV-2, seroprevalence

37

38 INTRODUCTION

39 SARS-CoV-2 is an emerging human coronavirus responsible for the disease called COVID-19
40 (1). These viruses are responsible for infections of the respiratory tract, gastrointestinal
41 system and nervous system (1,2). Human-to-human transmission occurs mainly directly, by
42 the respiratory route or indirectly via aerosols. Manual indirect transmission is possible.
43 Contagiousness precedes symptoms (4). The incubation period is around 5 days (between 2
44 and 14 days) (4).

45 Following the rapid spread of SARS-CoV-2 since December 2019, and in view of the severity
46 of the infection, the WHO declared COVID-19 as a global public health emergency on
47 January 30, 2020 and as a global pandemic from March 11, 2020. By September 15th, there
48 were more than 29 million infections worldwide and over 930,000 deaths. The majority of
49 people infected with SARS-CoV-2 are asymptomatic or present mild symptoms of rhinitis,
50 cough, fever and dyspnea (2). Digestive signs, anosmia, ageusia may also be present.
51 However, more severe symptoms have been described as pneumonia or acute respiratory
52 distress syndrome (ARDS) (3). The overall estimate of the mortality rate (including
53 asymptomatic and symptomatic people) seems to be around 5% (5).

54 In pregnant women, the symptoms appear to be the same as those in the general
55 population. During the third trimester patients are considered to be at risk of more severe
56 symptoms such as pneumonia or ARDS like other at-risk populations (6, 7). Concerning the
57 risks for the fetus and the newborn very limited information is available at the moment.
58 Fetal infection after transplacental passage of the virus has been described but appears to
59 be very rare (8). Neonatal infection is rare, and the majority of these infected newborns are
60 asymptomatic even if few severe cases were described (9).

61 The antibody response after SARS-CoV-2 infection has recently been described. In
62 symptomatic patients, antibodies against SARS-CoV-2 have been reported to be detected
63 from a few days to 3 weeks after onset of symptoms (10,11), with the median of 6 days (12).
64 High levels of neutralizing antibodies are induced about 10 days after onset of the disease
65 (13). The presence of SARS-CoV-2 IgG antibodies is indicative of current or previous
66 infection by SARS-CoV-2, however the extent and duration of protection conferred by SARS-
67 CoV-2 IgG antibodies remain unknown (12,14).

68 The COVIPREG study was designed to study the COVID-19 seroprevalence in pregnant
69 women at the time of delivery, the consequences of the infection for the women and their

70 newborns and the risk factors of severe disease (NCT04355234). It is a prospective study
71 conducted in 10 perinatal centers of Paris area in France. Since no data was available
72 regarding COVID-19 seroprevalence in pregnant women, a preliminary analysis was planned
73 in the study protocol after 500 inclusions in order to adjust the sample size of the study to
74 achieve the objectives. We present the results of this intermediate analysis.

75

76 **2. METHODS**

77 2.1. Patients populations

78 One perinatal center of Paris area, Cochin Hospital (Port-Royal Maternity, Paris, France) was
79 considered for this preliminary analysis. Pregnant women were prospectively included in the
80 study at the time of delivery between April 29, 2020 and June 26, 2020. All patients
81 admitted to the delivery ward after 15 weeks of gestation (WG) were offered the possibility
82 to participate to the study independently of the gestational age and pregnancy
83 complications. Patients were not referred to our center specifically for COVID-19.

84 Exclusion criteria were age<18 years, refusal to participate to the study, patient unable to
85 read or understand the study documents. After informed consent of the patient maternal
86 blood was collected and serum samples were stored at -20°C for COVID-19 Elisa. All data
87 (clinical, laboratory, ultrasonographic) for the mother and the newborns were prospectively
88 collected.

89 The COVIPREG study was approved by an institutional review board, CPP SUD
90 MEDITERRANEE (N° 2020-A00924-35) on April 23, 2020.

91

92 2.2 Serology testing for SARS-CoV-2 specific IgG

93 Serum samples were tested using the SARS-CoV-2 IgG Architect assay (Abbott, Sligo, Ireland)
94 according to the manufacturer's instructions. The SARS-CoV-2 IgG assay is a
95 chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of
96 IgG antibodies to SARS-CoV-2 in human serum and plasma. The resulting chemiluminescent
97 reaction was measured as a relative light unit (RLU). The presence or absence of IgG
98 antibodies to SARS-CoV-2 in the sample (S) is determined by comparing the
99 chemiluminescent RLU in the reaction to the calibrator RLU (C) and expressed as the S/C
100 index. The results were considered negative if the IgG index was < 1.4, positive if ≥1.4. This

101 commercial chemiluminescent immunoassay is validated for clinical use by the FDA with
102 high analytical and clinical performances (15, 16).

103

104

105 2.3 Data analysis

106 Statistical analysis was performed by a dedicated statistician using the SAS 9.4 software.

107 The main objective of this preliminary analysis was to estimate the seroprevalence of COVID
108 19 infection and its 95% confidence interval in the selected sample.

109 We analyzed maternal characteristics (age, BMI, geographical origin, multiple pregnancy)
110 and the outcome of the pregnancy (term of delivery, mode of delivery and vital status of the
111 child) in positive and negative women. The distribution parameters were mean and
112 standard deviation for quantitative data or number and frequency for qualitative data. For
113 these comparisons, the appropriate tests will be used: Khi-2 or Fisher test.

114

115 **RESULTS**

116 During the study period 529 patients were included in the study and were offered a SARS-
117 CoV-2 IgG testing. Among those, 25 had a positive serology, ie 4.7% with a confidence
118 interval at 95% [3.0%-6.7%]. None of the patients had symptoms of COVID-19 infection at
119 the time of inclusion. The characteristics of the patients are summarized in table 1. Mean
120 maternal age was 33.7 (+/- 4.8) and mean gestational age at delivery was 38.9 (+/- 3.3).
121 Forty-one patients delivered preterm (7.7%). There were 12 fetal losses, 9 were termination
122 of pregnancy for genetic abnormalities or fetal malformations and 3 were stillbirths. These
123 patients were pregnant during peak of COVID-19 outbreak in Paris.

124 Twenty-one patients had a SARS-CoV-2 RT-PCR test during pregnancy because of COVID-19
125 symptoms. The SARS-CoV-2 RT-PCR was negative for 11, and all these patients had a
126 negative serology at delivery. The SARS-CoV-2 RT-PCR was positive for 10 and 8 patients had
127 a positive serology whereas 2 had a negative serology at delivery. For one of these 2
128 patients the IgG index was in the grey zone at 0.8 (grey zone between 0.8 and 1.4 according
129 to the manufacturer). This patient had symptoms of COVID 19 (anosmia) and a positive PCR
130 test two months before delivery. The second patient had a PCR test in a private lab because
131 of moderate fever (37.8°C) without any other symptom. The PCR test was not checked in
132 our hospital. The IgG index was at 0.0.

133 One of the patients with a positive serology had a termination of pregnancy at 17WG for
134 severe fetal malformation (ventriculomegaly, spine malformation and cleft feet) without
135 abnormalities found at the genetic tests. SARS-CoV-2 PCR was negative on fetal tissues.
136 Maternal and neonatal characteristics were compared between the two groups of patients
137 with positive and negative SARS-CoV-2 serology. No significant difference was found
138 between the two groups concerning maternal age, maternal BMI, geographic origin, twin
139 pregnancy, prematurity rate, mode of delivery nor the rate of fetal loss (Table 3).

140

141 **DISCUSSION**

142 France is a country that was severely touched by COVID-19 outbreak in the first trimester of
143 2020. First cases were reported in France on January 24th, 2020 and general lockdown was
144 declared by the French government for two months between March 17th and May 11th. At
145 the present time, September 15, France totalizes 381,500 cases and 30,900 deaths due to
146 COVID-19 infection. The study was performed in the Paris area which was particularly
147 affected by the disease and totalized 40% of national deaths.

148 The current study shows that, three months after the first cases of COVID-19 infection,
149 seroprevalence of SARS-CoV-2 in pregnant women at the time of delivery in our institution
150 is quite low (4.5%). This seroprevalence is lower than the prevalence of 10.6% measured in
151 the general population of the Paris area between April 6th and April 12th (17) and shouldn't
152 be attributed to a low performance of the test. The test used is approved by the FDA and
153 has been associated to high analytical performances with a sensitivity between 92.7 and
154 97.3% and a specificity >99% (18,19). This low seroprevalence is possibly explained by the
155 fact that pregnant women were more accurate in accepting and applying national
156 recommendations to limit the spread of the virus and the risk to be infected (social
157 distancing, use of surgical masks, use of hydro-alcoholic solutions) compared to non-
158 pregnant individuals. Moreover, according to national recommendations, pregnant women
159 were advised to stop professional activities after 28 WG to limit the risk of maternal
160 infection during the third trimester. Therefore, most of the patients of our cohort didn't
161 have any professional nor social contacts even after the end of the lockdown.

162 In our study, two patients with a positive SARS-Cov-2 PCR had a negative serology. The first
163 had clinical symptoms of COVID-19 (anosmia) and an IgG ratio of 0.8 in the grey zone of the
164 test. Therefore, this case can be considered as a false negative result of the serology. For the

165 second patient, the PCR was a screening test performed in a private lab and the details of
166 the results couldn't obtained. The only symptoms were a temperature at 37.8°C. The
167 patients delivered 6 weeks later with a negative serology (IgG index at 0.0). The sample was
168 tested with another immunoassay (Euroimmune) and was also negative. Therefore, this
169 case was possibly a false positive test of the PCR.

170 The result of our preliminary study is consistent with the recent report of SARS-CoV-2
171 seroprevalence among parturient women in Philadelphia (20). Among 1293 delivering
172 women in two centers of Philadelphia between April 4th and June 3rd 2020, 6.2% had
173 positive SARS-CoV-2 specific IgG and/or IgM. Another recent study performed in 3 university
174 hospitals in Barcelona reported a higher seroprevalence of 14% among 502 parturients. This
175 higher seroprevalence may reflect the critical situation of COVID-19 outbreak in Spain but
176 could also be related to the fact that the authors tested for anti-SARS-CoV-2 IgG, IgM, and
177 IgA antibodies (21).

178 Many studies are ongoing in different countries to evaluate the consequences (for the
179 mothers, the fetuses and the newborns) of COVID-19 infection during pregnancy. All these
180 studies were designed in emergency with very little information to calculate the number of
181 patients to include in order to achieve sufficient statistical power. The low prevalence we
182 report in this manuscript should help study-investigators to adapt the sample size of their
183 study while the inclusions are ongoing.

184 This low prevalence will certainly increase with time as SARS-CoV-2 is still actively circulating
185 in France. The COVIPREG study is ongoing and we will be able to evaluate the evolution of
186 the SARS-CoV-2 seroprevalence over time.

187

188 **CONCLUSION**

189 These results suggest that the seroprevalence of SARS-CoV-2 IgG in pregnant women at the
190 time of delivery is quite low (4.7%) approximately 2 to 3 months after beginning of outbreak
191 in our region. Studies evaluating the impact of COVID-19 infection during pregnancy should
192 take in account this information in order to adapt their sample size.

193

194

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263

264 **Tables**

265

266

267

268 **Table 1** : Maternal and neonatal characteristics of the 529 patients.

269 Data shown as mean (standard deviation) or number (%)

270

	Pregnant women at the time of delivery N=529
Maternal age	33.7 (4.7)
BMI	22.4 (3.7)
Multiple pregnancy	16 (3.0%)
SARS-CoV-2 RT PCR during pregnancy	21 (4%)
Positive SARS-CoV-2 RT PCR during pregnancy	10/21 (47.6%)
Gestational age at delivery	38.9 (3.3)
Preterm delivery	41 (7.7%)
Cesarean section rate	81 (15.3%)
Newborn status	
- Alive	517 (97.7%)
- Stillbirth	12 (2.3%)

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278 **Table 2** : Description of the 12 fetal losses

	n (%)
Gestational age at delivery	
16-17 WG	4 (33.4)
18-21 WG	3 (25.0)
22-26 WG	2 (16.7)
27-30 WG	3 (25.0)
Cause of fetal loss	
TOP	9 (75.0)
IUFD	3 (25.0)
SARS-CoV-2 IgG at delivery	
Positive	1 (8.3)
Negative	11 (91.7)

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280 WG : weeks of gestation, IUFD : intra-uterine fetal death, TOP : termination of pregnancy

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299 **Table 3** : Characteristics of the 529 mothers and their newborns according to the anti-SARS-
 300 Cov-2 IgG testing

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	Anti-SARS-Cov-2 IgG					p-value
	Positive (n = 25)		Negative (n = 504)			
	n/N	(%)	n/N	(%)		
Maternal age						0.90
	< 35 y	15/25	(60.00)	309/504	(61.31)	
	≥ 35 y	10/25	(40.00)	195/504	(38.69)	
BMI						1.00
	< 25	21/25	(84.00)	408/504	(80.95)	
	≥ 25	4/25	(16.00)	96/504	(19.05)	
Geographic origin						0.17
	Africa	7/23	(30.43)	90/496	(18.15)	
	Other	16/23	(69.57)	406/496	(81.85)	
Multiple pregnancy						1.00
	No	25/25	(100.00)	488/504	(96.83)	
	Yes	0/25	(0.00)	16/504	(3.17)	
Gestational age at delivery						0.71
	< 37 WG	1/25	(4.00)	40/504	(7.94)	
	≥ 37 WG	24/25	(96.00)	464/504	(92.06)	
Mode of delivery						0.40
	Vaginal	23/25	(92.00)	425/504	(84.33)	
	Cesarean	2/25	(8.00)	79/504	(15.67)	
Neonatal status						0.44
	Alive	24/25	(96.00)	493/504	(97.82)	
	Stillbirth	1/25	(4.00)	11/504	(2.18)	

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