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1 **Vitamin D status during pregnancy and in cord blood in a large prospective French**
2 **cohort.**

3
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57 **Abstract:**

58 **Background & Aims:** Vitamin D status during pregnancy and in newborns has never been
59 studied in France. This study aims at determining the vitamin D status during the first and
60 third trimesters of pregnancy (T1, T3) and in cord blood (CB) in the middle-north of France.

61 **Methods:** We conducted a prospective cohort study in five French centers (latitude 47.22 to
62 48.86°N). Serum 25(OH)-vitamin D (25(OH)D) concentrations were measured using a
63 radioimmunoassay during T1, T3 and in CB. According to the French guidelines, pregnant
64 women received cholecalciferol, 100,000 IU, in the seventh month.

65 **Results:** Between April 2012 and July 2014, 2832 women were included, of whom 2803 were
66 analyzed (mean±SD age: 31.5±5.0 years; phototypes 5-6: 21.8%). Three and 88.6% of
67 participants received supplementation during the month before inclusion and in the seventh
68 month, respectively. At T1, T3, and CB, mean 25(OH)D concentrations were 21.9±10.4,
69 31.8±11.5, and 17.0±7.2 ng/mL, respectively, and 25(OH)D was <20 ng/mL in 46.5%, 14.0%,
70 and 68.5%, respectively. At T1, body mass index ≥ 25 kg/m², dark phototypes, sampling
71 outside summer, and no supplementation before inclusion were independently associated with
72 vitamin D insufficiency (25(OH)D<20ng/mL). Women who received cholecalciferol
73 supplementation in month 7 had higher 25(OH)D at T3 than non-supplemented women
74 (32.5±11.4 *versus* 25.8±11.4 ng/mL, p=<0.001) and marginally higher 25(OH)D in CB
75 (17.2±7.2 *versus* 15.5±7.1 ng/mL, p=0.004).

76 **Conclusions:** Despite the recommended supplementation, vitamin D insufficiency is frequent
77 during pregnancy and in newborns in France.

78

79 **Keywords:** Vitamin D; Pregnancy; Newborns; Epidemiology; Supplementation

80

81

82 **INTRODUCTION**

83 Vitamin D is a prohormone with effects beyond the prevention of rickets/osteomalacia. In
84 addition to its protective effect against bone demineralization, vitamin D sufficiency is
85 associated with a reduced risk of many chronic diseases including type 2 diabetes mellitus,
86 cardiovascular diseases, cancers, and auto-immune and infectious diseases (1). During
87 pregnancy, poor vitamin D status is associated with pregnancy complications such as pre-
88 eclampsia (2,3), gestational diabetes mellitus (4), and increased risk of caesarean section (5)
89 and of preterm birth (6,7). It is also associated with an increased risk of wheezing and asthma
90 (8,9), of respiratory tract infections (10) and of low bone mass (11,12) in newborns and
91 children.

92 The assessment of vitamin D status is based on the measurement of the serum concentration
93 of 25(OH)-vitamin D (25(OH)D). During pregnancy, free 25(OH)D might reflect better the
94 vitamin D status than total 25(OH)D due to the rise of vitamin D protein levels (13). Although
95 there is a consensus to define vitamin D deficiency as serum 25(OH)D below 10 ng/mL (25
96 nmol/L), the definition of vitamin D insufficiency is less consensual. Whereas the US Institute
97 of Medicine (IOM) defines vitamin D insufficiency as serum 25(OH)D concentrations below
98 20 ng/mL (50 nmol/L) in the general population (14), the Endocrine Society considers
99 25(OH)D levels below 30 ng/mL (75 nmol/L) to be inadequate in chronically ill patients (15).

100 With low contemporary sun exposure and/or use of sunscreens, the relative contribution of the
101 solar source to total basal input of vitamin D seems to be at best of 25%, the remaining
102 coming from food sources (16). Regardless of the threshold for vitamin D insufficiency or
103 inadequacy, prevalence of low serum 25(OH)D concentrations is high in most countries,
104 including France, in all age groups (17).

105 Vitamin D status during pregnancy or in cord blood has been evaluated in studies conducted
106 in North America (18,19) and in many European countries, mainly northern Europe, although

107 few studies have measured 25(OH)D throughout the pregnancy or in cord blood in large
108 cohorts (20–22). Considering i) the high prevalence of vitamin D deficiency or insufficiency
109 during pregnancy reported in most of these studies, ii) the potential deleterious consequences
110 of low 25(OH)D circulating levels on health of both mother and child, and iii) the absence of
111 uniform guidelines for vitamin D supplementation in pregnant women, there is an urgent need
112 to assess vitamin D status in large populations of pregnant women and newborns and, for each
113 country, to systematically evaluate recommendations for vitamin D supplementation during
114 pregnancy. To our best knowledge, such a study has never been conducted in France. That's
115 why we felt important to gather national data given the particularity of the French
116 recommendation for vitamin D supplementation during pregnancy (23) and the lack of food
117 fortification in France.

118 The aims of the present study were to determine the vitamin D status and its evolution in a
119 large cohort of pregnant women living in France by analysis in the first and third trimesters
120 and in cord blood. We sought to assess the determinants of vitamin D status at each time point
121 and to study the impact of the French recommendations regarding vitamin D supplementation
122 during pregnancy. To answer these questions, we used the prospective observational FEPED
123 cohort study including pregnant women first seen during the first trimester in five centers of
124 the middle-north of France.

125

126 **PATIENTS AND METHODS**

127 *Study protocol*

128 The FEPED study was initially designed to investigate the association of vitamin D status
129 during pregnancy with pre-eclampsia in six centers (five French and one Belgian). Written
130 informed consent was obtained from each patient before inclusion in the study. The protocol
131 was conducted in accordance with the Declaration of Helsinki and was approved by a local

132 independent Ethics Committee (2011/13NICB). It is registered with the ClinicalTrials.gov
133 (identifier NCT01648842). Samples were stored in the Perinat Collection (ANR-10-EQPX-
134 0010).

135

136 For the purpose of the present epidemiological study, which aimed to determine the 25(OH)D
137 status of pregnant women living in France and in their newborns (in cord blood), we did not
138 include women recruited in Bruxelles (Belgium). The patients included in this study were
139 recruited between April 2012 and July 2014 in five French maternity departments ensuring
140 the obstetrical follow-up from the first trimester of pregnancy until delivery. Four of these
141 departments are located in Paris area (Béclère, Bicêtre, Cochin, and Trousseau University
142 hospitals, latitude 48.86°N) and one is located in Nantes in the mid-western part of France
143 (latitude 47.22°N). Patients were told about the study by the obstetrician or the midwife
144 during the first consultation for pregnancy follow-up if the following inclusion and exclusion
145 criteria were fulfilled. Inclusion criteria were: age ≥ 18 years, single pregnancy, gestational
146 age from 10 to < 15 weeks of amenorrhea (WA), corresponding to 8 to < 13 gestational weeks
147 (GW), at inclusion, and healthcare coverage. Exclusion criteria were conditions for which
148 vitamin D level could have been modified or vitamin D supplementation during the third
149 trimester could have been contra-indicated or inefficient, including serum calcium levels
150 > 2.65 mmol/L or other known pathologies of mineral metabolism, constitutive bone disease,
151 history of urinary stones, lithium treatment, or intestinal malabsorption, and conditions
152 susceptible to interfere with the diagnosis of pre-eclampsia, including uncontrolled
153 hypertension ($> 140/90$ mm Hg from the beginning of pregnancy) and renal insufficiency
154 (serum creatinine > 120 $\mu\text{mol/L}$). For all included patients, a blood sample was collected for
155 25(OH)D measurement between 11 and 14 WA, or between 9 and 12 GW, (the first
156 trimester, T1, sample), during the third trimester (between 28 WA, or 26 GW, and delivery,

157 the T3 sample) and from cord blood (CB) within the framework of the research protocol. The
158 patients were not required to fast before blood sample collection. Vitamin D (100,000 IU of
159 cholecalciferol) was prescribed to all women in the seventh month of pregnancy as a routine
160 procedure in agreement with the French guidelines (23). At obstetrics clinic from 28 GW the
161 patient was asked whether and when she took the vitamin D supplementation and the date was
162 recorded in the patient file. Follow-up outcomes were recorded such as outcome at birth (live
163 birth, per partum demise, termination of pregnancy), gestational age at delivery, birth weight,
164 vitamin D status in the first, third trimester and cord blood.

165 We defined vitamin D deficiency, insufficiency, inadequacy, and sufficiency as serum
166 25(OH)D concentrations of <10 ng/mL, <20 ng/mL, <30 ng/mL, and ≥ 30 ng/mL,
167 respectively. The phototype of each subject was determined according to the Fitzpatrick skin
168 type classification (24). Pre-pregnancy body mass index (BMI) calculated from height and
169 pre-pregnancy weight was classified using the WHO cut-off for overweight (<25 or ≥ 25
170 kg/m²) (25).

171 ***Biological analysis***

172 All blood samples were centrifuged and stored locally at -20°C and were subsequently
173 transferred monthly to the Department of Physiology of Necker University Hospital (Paris,
174 France) for centralized 25(OH)D measurement from serum. 25(OH)D was measured with the
175 DiaSorin radioimmunoassay (RIA). The Necker Hospital Physiology Laboratory participates
176 in the DEQAS proficiency control with excellent results. A value of 4 ng/mL, corresponding
177 to the limit of quantification that we determined in our laboratory, was assigned to any
178 undetectable concentration.

179 ***Statistical analyses***

180 All statistical analyses were undertaken using R 2.11.1 software. Statistical tests were two-
181 sided and p values less than 0.05 were considered statistically significant. Baseline

182 characteristics of women were described as means \pm standard deviations for quantitative
183 variables and frequencies (%) for qualitative variables.

184 Prevalence of vitamin D insufficiency and inadequacy (serum 25(OH)D concentrations <20
185 ng/ml and <30 ng/ml respectively) were estimated on the available samples at each time (T1,
186 T3, and CB) along with their corresponding 95% Wald CI.

187 Associations between characteristics of women and 25(OH)D insufficiency were investigated
188 using chi-squared test (or Fisher's test when it was appropriate) for qualitative factors and
189 Student's t test for quantitative parameters. Pearson's r and p value of its test were computed
190 to examine correlations between continuous variables. Multiple logistic regression models
191 were performed to assess determinants of 25(OH)D insufficiency at T1 and T3 and in CB.
192 Initial models included all significant factors identified by univariate analysis ($p < 0.05$). A
193 backward selection procedure based on likelihood ratio tests was used. Odds ratios, 95% CI,
194 and p values of determinants in the final models were computed.

195

196

197 **RESULTS**

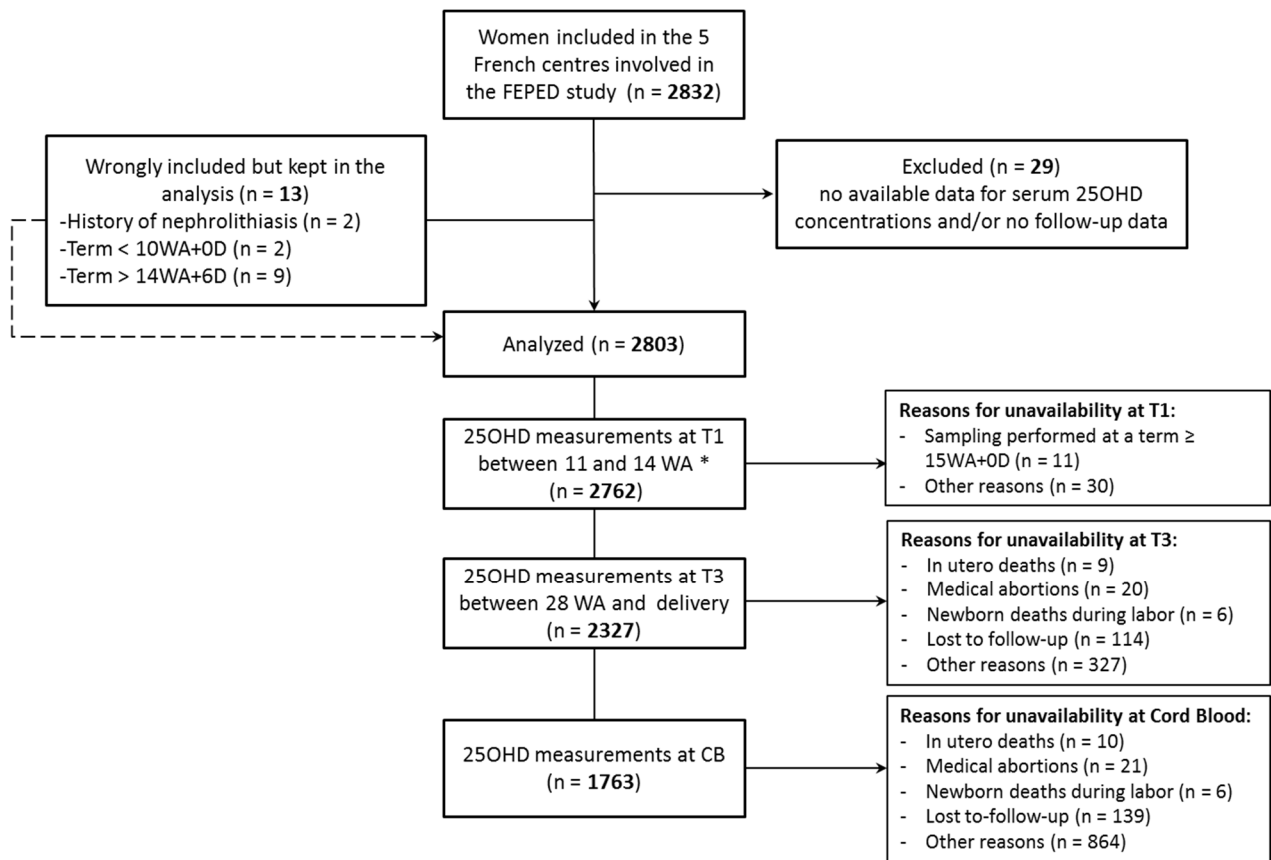
198 *Description of the study and of the study population*

199 The flow chart of the study protocol is shown in Figure 1. None of the patients were included
200 twice during the study. Among the 2658 women with available data regarding pregnancy
201 outcomes, pregnancies were terminated as follows: 2621 (98.6%) live births, six (0.2%)
202 newborn deaths during labor, 10 (0.4%) in utero deaths, and 21 (0.8%) medical abortions. The
203 mean delivery term was 37.5 ± 1.8 GW.

204

205 **Figure 1:** Flow chart of the study protocol. 25(OH)D: 25(OH)-vitamin D, D: day, T1: first
206 trimester, T3: third trimester, CB: cord blood, GW: gestational weeks. *25(OH)D

207 measurements performed for women at a term ≥ 13 GW+0D at the time of T1 sampling were
 208 excluded from the analysis.



209

210

211 Characteristics of the cohort are shown in Table 1. Among the 2779 women with available
 212 data regarding ethnical origin, 1550 (55.8%) patients originated from Continental France, 63
 213 (2.3%) from northern Europe, 122 (4.4%) from southern Europe, 420 (15.1%) from northern
 214 Africa, 292 (10.5%) from Sub-Saharan Africa, 116 (4.2%) from French West Indies, 105
 215 (3.8%) from Asia, and 111 (4%) from other countries. Of note, most women received vitamin
 216 D after inclusion in agreement with the French national guidelines.

217

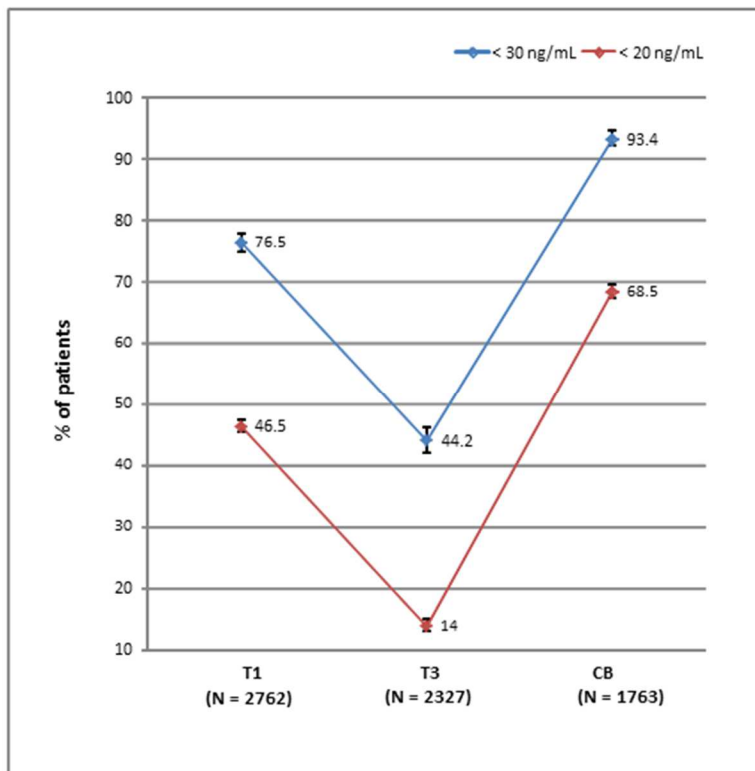
218 ***Evolution of vitamin D status during pregnancy and in cord blood***

219 Mean serum 25(OH)D concentrations for all women with available samples at each time point
 220 are presented in Table 2. Vitamin D deficiency (25(OH)D <10 ng/mL) was present in more

221 than 10% of cases during the first trimester and a cord blood but was nearly absent during the
222 third trimester. As shown in Figure 2, around half of the women had vitamin D insufficiency
223 (25(OH)D <20 ng/mL) during the first trimester but only 14% had vitamin D insufficiency
224 during the third trimester. Vitamin D inadequacy (25(OH)D <30 ng/mL) was found in three-
225 quarters of women during the first trimester and was present in nearly half of women during
226 the third trimester. Of note, vitamin D insufficiency or inadequacy was highly prevalent in
227 newborns based on our cord blood analyses.

228

229 **Figure 2.** Prevalence of 25(OH)-vitamin D (25(OH)D) insufficiency (serum 25(OH) D <20
230 ng/mL, red line) and inadequacy (serum 25(OH)D <30 ng/mL, blue line) at the first and third
231 trimesters (T1 and T3) and in cord blood (CB) (with 95% confidence interval). N= number of
232 patients for whom serum 25(OH)D measurements were performed at T1, T2 and T3.



233

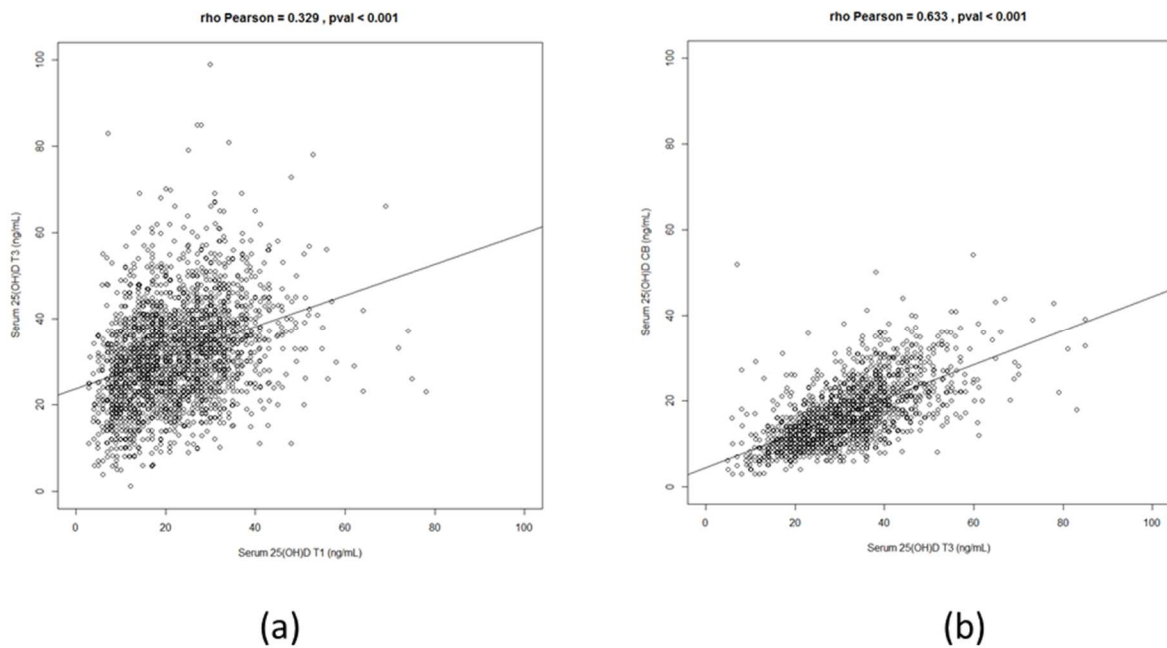
234

235 Serum 25(OH)D significantly increased between the first and the third trimesters among the
236 2289 women with serum 25(OH)D measurements available at these two visits (22.2 ± 10.5
237 ng/mL versus $31.8 \pm 11.5 \text{ ng/mL}$, respectively, with a mean difference of $9.5 \pm 12.8 \text{ ng/mL}$,
238 $p < 0.001$). Serum 25(OH)D significantly decreased between the third trimester and cord blood
239 among the 1606 women with serum 25(OH)D measurements available at these two visits
240 ($31.7 \pm 11.5 \text{ ng/mL}$ versus $17.1 \pm 7.3 \text{ ng/mL}$ respectively, with a mean difference of -14.7 ± 8.9
241 ng/mL , $p < 0.001$). Figure 3a shows that serum 25(OH)D during the third trimester positively
242 correlates with serum 25(OH)D during the first trimester. Figure 3b shows that the positive
243 correlation between serum 25(OH)D during the third trimester and in cord blood is even
244 stronger.

245

246 **Figure 3. a:** Correlation between serum 25(OH)-vitamin D (25(OH)D) at the first trimester
247 (T1) and at the third trimesters (T3) for the 2289 women with serum 25(OH)D measurements
248 at T1 and at T3. **b:** Correlation between serum 25(OH)D at T3 and in cord blood (CB) for the
249 1606 women with serum 25(OH)D measurements at T3 and at CB.

250



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255 ***Determinants of vitamin D status during pregnancy and in newborns***

256 Univariate analysis of determinants of vitamin D insufficiency (25(OH)D <20 ng/mL) during
257 pregnancy and in cord blood is summarized in Table 3. Table 4 shows 25(OH)D
258 concentrations and categories among women who received or did not receive the
259 recommended supplementation during the seventh month of pregnancy. Vitamin D
260 supplementation during the seventh month did not significantly influence the presence of
261 vitamin D insufficiency in cord blood (Table 3). However, serum 25(OH)D was slightly but
262 significantly higher in cord blood in case of vitamin D supplementation during the seventh
263 month of pregnancy (Table 4). Moreover, there was less vitamin D deficiency (25(OH)D <10
264 ng/mL) in cord blood in supplemented women (Table 4)).

265 Supplementation had a significant impact on serum 25(OH)D concentration during the third
266 trimester since there was virtually no vitamin D deficiency during the third trimester in
267 supplemented women (Table 4). Of note, 11.6% and 41.8% of the supplemented women had
268 vitamin D below 20 or 30 ng/mL, respectively, during the third trimester.

269 Weight gain during pregnancy was not associated with vitamin D insufficiency during the
270 third trimester (+8.7±5.0 kg in the 308 women with serum 25(OH)D <20 ng/mL *versus*
271 +8.5±4.3 kg in the 1908 women with serum 25(OH)D ≥ 20 ng/mL, p= 0.54).

272 Table 5 shows variables independently associated with serum 25(OH)D concentration <20
273 ng/mL during pregnancy and in cord blood. In multivariate analysis, overweight before
274 pregnancy, dark phototype, sampling during fall, winter, or spring, and absence of vitamin D
275 supplementation at the very beginning of pregnancy were independently associated with
276 vitamin D insufficiency in the first trimester. In the third trimester, age below 35 years, parity
277 ≥1, dark phototype, sampling during fall, winter, or spring, absence of vitamin D
278 supplementation during pregnancy, and presence of vitamin D insufficiency or deficiency in

279 the first trimester were independently associated with vitamin D insufficiency. Vitamin D
280 insufficiency in newborns was independently associated with overweight before pregnancy,
281 sampling during fall, winter, or spring, and presence of vitamin D insufficiency or deficiency
282 in the third trimester.

283

284 **DISCUSSION**

285 We report the first large scale population cohort study describing serum 25(OH)D status
286 during pregnancy and in cord blood in French women. Given the disparities in results from
287 vitamin D studies conducted during pregnancy world-wide, we will compare our results to
288 those from other European studies.

289 As highlighted in table 6, during early or mid-pregnancy in countries of northern Europe,
290 serum 25(OH)D levels are relatively low with a mean or median value around 20 to 22
291 ng/mL, as in our study (20,21,26–28). In the two studies with serum 25(OH)D values above
292 30 ng/mL despite the high latitudes (Finland and northwest of England) (29,30), a large
293 proportion of women received vitamin D supplementation (95% in the Finland study and 73%
294 in England study). Moreover, there are vitamin D food fortification policies in Finland (31);
295 thus, in the Finnish study the mean vitamin D intake was 15.7 µg/day (29). Mean vitamin D
296 intake for the French general population is 2.3 µg/day (32), a value that can be extrapolated to
297 French pregnant women before the third trimester in the absence of vitamin D
298 supplementation and of food fortification policies in France. Low vitamin D levels are also
299 frequently found in pregnant women in countries from southern Europe despite theoretically
300 higher and more efficient UVB radiation (33). The close values between northern and
301 southern European countries may be explained by high consumption of fatty fish in northern
302 European countries and by vitamin D food fortification policies in some countries (31,33), but
303 also by less voluntary sun exposure and by darker phototype in southern Europe.

304 We observed a clear increase in serum 25(OH)D concentrations between the first and third
305 trimester. Our results must be interpreted taking into account the vitamin D supplementation
306 currently recommended in France (100,000 IU of cholecalciferol during the seventh month)
307 (23). Consequently, 25(OH)D levels were overall higher in our study during the third
308 trimester than levels reported in most European studies (table 6) (26,27,33,34).

309 We observed a dramatic decrease in 25(OH)D levels between the third trimester and sampling
310 of cord blood. As observed in previous studies, 25(OH)D levels were approximately two-fold
311 lower in cord blood than in mother's serum during pregnancy (20–22,30,35). In our study, as
312 in these previous studies, sampling in the mother was performed several weeks before
313 delivery. By contrast, in two studies with maternal sampling performed at delivery, exactly at
314 the same time as cord blood sampling, 25(OH)D concentrations in mothers were similar to the
315 ones found in newborns (34,36). This finding suggests that the decrease in serum 25(OH)D
316 observed between the second or third trimesters and cord blood may be due to a rapid
317 decrease in serum 25(OH)D concentrations between sampling and delivery. Possible
318 explanations for this rapid decrease in 25(OH)D levels during the last 4 weeks of pregnancy
319 (mean delay between the third trimester and cord blood samplings in our study) in the absence
320 of further supplementation could be reduced outdoor activity and sun exposure combined with
321 an increase in fat mass at the end of pregnancy. Another explanation could be the rapid
322 decrease in serum 25(OH)D concentrations after the single administration of 100,000 IU of
323 vitamin D₃, as recently described (37). Another hypothesis to explain the discrepancy between
324 25(OH)D levels in mothers during the second or third trimesters and in newborns could be
325 that 3-epi-25(OH)D₃, an isoform not detected by the current immunoassays, may be present at
326 high concentrations in cord blood. However, this theory was ruled out by a study showing that
327 the proportion of 25(OH)D as 3-epi-25(OH)D₃ was only 11.2% in cord blood (22) and by
328 another study reporting similar levels of 3-epi-25(OH)D₃ in mothers at delivery and in

329 newborns (36). We would like to emphasize that our study demonstrates that this high dose
330 of cholecalciferol given once, 100 000 IU theoretically corresponding to 1100 IU daily during
331 3 months (23), is clearly insufficient to obtain serum 25(OH)D levels above 20 ng/mL in most
332 newborns and is also insufficient to completely prevent vitamin D deficiency.

333 Finally, we analyzed the determinants of vitamin D insufficiency (25(OH)D <20 ng/mL) at
334 each time point. Most studies found, as we did, that season of sampling was strongly
335 associated with 25(OH)D status during pregnancy, with maximal concentrations reached
336 during summer (20,26–28,30,33,38). As in our study, dark phototype was also reported to be
337 independently associated with maternal vitamin D status (26,27,30,33,39). As in other
338 European studies (20,26–28,30,33), we found that vitamin D supplementation during
339 pregnancy was a strong independent determinant of vitamin D status. Whereas some studies
340 found no association between BMI and vitamin D status in pregnant women (27,34), others
341 found, like us, a negative association (40,41) .

342 Few European studies have described the determinants of vitamin status in cord blood. A
343 study from Scotland (20) and a study from Ireland (22), found, like us, that seasonal variation
344 and maternal 25(OH)D status were independently associated with 25(OH)D levels in cord
345 blood. Whereas the study from Scotland (20) found, as we did, that vitamin D
346 supplementation during pregnancy did not influence vitamin D insufficiency in cord blood,
347 two studies (22,29), reported a positive association between antenatal vitamin D supplements
348 and vitamin D concentrations in cord blood. Finally, unlike us, others did not find that
349 maternal BMI was an independent determinant of 25(OH)D levels in cord blood (20,22,29).

350 We must acknowledge that our study has some limitations. First, although the DiaSorin RIA
351 used to measure 25(OH)D concentration in our study is a “historic” 25(OH)D assay that has
352 been used in many studies, it does not allow a strict comparison with the previously published
353 data on vitamin D status in pregnant women due to a certain degree of inter-method

354 variability. Such a comparison is, however, important to develop evidence-based international
355 guidelines for vitamin D supplementation during pregnancy. A way to achieve this goal
356 would have been to collaborate with a laboratory that uses a CDC-certified chromatography
357 tandem-mass spectrometry (LC-MS/MS) method in order to apply the VDSP protocols for
358 standardizing existing 25(OH)D data from national surveys around the world (42). However,
359 when the present study was designed (in 2009), the new international and the standard LC-
360 MS/MS reference method for measuring 25(OH)D were not published so such a collaboration
361 was not possible. Moreover, we did not assess the concentration of the biologically active free
362 25(OH)D. Of note, Patients were recruited only in centers from the middle-north of France, so
363 we cannot extrapolate our results to the whole French territory. Finally, some data susceptible
364 to modify serum 25(OH)D concentration including dietary habits, sun exposure, use of
365 sunscreen and outdoor activity were not recorded in the present study.

366 Our study also has several strengths. To our best knowledge, it is the first study to evaluate
367 the vitamin D status of a French cohort during pregnancy and in cord blood and this study is
368 the largest European study regarding this issue. We also evaluated the effects of the
369 supplementation recommended by current French guidelines (23), which is of high
370 importance to improve the care of pregnant women and newborns.

371 In conclusion, vitamin D insufficiency is highly prevalent at the beginning of pregnancy and
372 in cord blood in the middle-north of France. The supplementation with cholecalciferol
373 100,000 IU during the seventh month of pregnancy is insufficient to prevent vitamin D
374 insufficiency and deficiency in newborns and should therefore be reevaluated.

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380 Hospitalier Intercommunal de Créteil, for the management and storage of biospecimens. We
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382

383 **Statement of Authorship:**

384 MC interpreted the data and wrote the manuscript.

385 ABa and CE analyzed and interpreted the data.

386 ABe, CE and JCS conceived and designed the study.

387 JCS performed 25(OH)D measurements.

388 JT, VT, JG, MVS, HH, JJ, MG, JMJ, MCH, NW, and DM included patients.

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390 revising it critically for important intellectual content and to final approval of the version to be
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392 related to the accuracy or integrity of any part of the work are appropriately investigated and
393 resolved.

394

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396 JC. Souberbielle reports lecture fees and/or travel/hotel expenses from DiaSorin, Roche
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542

| | n | Mean ± SD or n (% of patients) |
|--|----------|---------------------------------------|
| Age at inclusion (years) | 2803 | 31.5±5.0 |
| Age ≥35 (years) | | 734 (26.2%) |
| Gestational age at inclusion (GW) | 2803 | 10.8±0.8 |
| BMI before the beginning of pregnancy (kg/m²) | 2773 | 23.5±4.6 |
| BMI ≥25 (kg/m ²) | | 861 (31.1%) |
| Pre gestational diabetes mellitus | 2778 | 103 (3.7%) |
| Season of conception | 2803 | |
| Summer | | 712 (25.4%) |
| Fall | | 692 (24.7%) |
| Winter | | 704 (25.1%) |
| Spring | | 695 (24.8%) |
| Phototype* | 2803 | |
| Types 1 to 4 | | 2191 (78.2%) |
| Types 5 and 6 | | 612 (21.8%) |
| Parity** | 2781 | |
| 0 | | 1349 (48.5%) |
| 1 | | 966 (34.7%) |
| >1 | | 466 (16.8%) |
| Smoking | | |
| Before the ongoing pregnancy | 2760 | 569 (20.6%) |
| Active at the beginning of pregnancy | 2743 | 317 (11.6%) |
| Active at inclusion | 2727 | 225 (8.2%) |
| Vitamin D supplementation during the month before inclusion | 2452 | 74 (3.0%) |
| Vitamin D supplementation in 7th month*** | 2592 | 2296 (88.6%) |

546

547

Table 1. Characteristics of the population.

548 BMI: body mass index, GW: gestational weeks, n: number of patients with available data, SD:
 549 standard deviation. *According to the Fitzpatrick phototyping scale, **excluding the ongoing
 550 pregnancy, *** vitamin D supplementation in 7th month of pregnancy consists of 100,000 IU
 551 cholecalciferol, according to the French guidelines.

552
 553

| | | 1st trimester | 3rd trimester | Cord blood |
|---|--------------------------------------|---------------------------------|---------------------------------|-------------------------|
| | n | 2762 | 2327 | 1763 |
| Gestational age (GW) | Mean ± SD [Min-Max] | 10.8±0.8 [6.6-12.9] | 33.4±2.4 [23.9-39.9] | 37.6±1.5 [25.4-40.7] |
| Serum 25(OH)D (ng/mL) | Mean ± SD [Min-Max] | 21.9±10.4 [3.0-78.0] | 31.8±11.5 [1.0-99.0] | 17.0±7.2 [3.0-54.0] |
| 25(OH)D categories n (%) | <10 ng/mL | 286 (10.4%) | 29 (1.2%) | 231 (13.1%) |
| | 10-20 ng/mL | 998 (36.1%) | 297 (12.8%) | 977 (55.4%) |
| | 20-30 ng/mL | 829 (30.0%) | 703 (30.2%) | 439 (24.9%) |
| | ≥30 ng/mL | 649 (23.5%) | 1298 (55.8%) | 116 (6.6%) |

554
 555 **Table 2.** Serum 25(OH)-vitamin D during pregnancy and at cord blood.

556 n: number of patients, SD: standard deviation, Min: minimum, Max: maximum, 25(OH)D:
 557 25(OH)-vitamin D, GW: gestational weeks.

558

| | 1 st trimester | | | 3 rd trimester | | | Cord blood | | |
|---|---------------------------|----------------|---------|---------------------------|----------------|---------|-----------------|---------------|---------|
| | 25(OH)D (ng/mL) | | P value | 25(OH)D (ng/mL) | | P value | 25(OH)D (ng/mL) | | P value |
| | <20 n=1284 | ≥20 n=1478 | | <20 n=326 | ≥20 n=2001 | | <20 n=1208 | ≥20 n=555 | |
| Age (years) | | | 0.029 | | | 0.017 | | | 0.18 |
| <35 | 973 (75.8) | 1066 (72.1) | | 256 (78.5) | 1445 (72.2) | | 913 (75.6) | 403 (72.6) | |
| ≥35 | 311 (24.2) | 412 (27.9) | | 70 (21.5) | 556 (27.8) | | 295 (24.4) | 152 (27.4) | |
| BMI (kg/m²) | | | <0.001 | | | 0.012 | | | <0.001 |
| <25 | 827 (65.3) | 1056 (72.0) | | 205 (63.3) | 1401 (70.2) | | 797 (66.4) | 420 (75.8) | |
| ≥25 | 439 (34.7) | 411 (28.0) | | 119 (36.7) | 594 (29.8) | | 403 (33.6) | 134 (24.2) | |
| Phototype* | | | <0.001 | | | <0.001 | | | <0.001 |
| 1 to 4 | 901 (70.2) | 1259 (85.2) | | 209 (64.1) | 1657 (82.8) | | 931 (77.1) | 483 (87.0) | |
| 5 and 6 | 383 (29.8) | 219 (14.8) | | 117 (35.9) | 344 (17.2) | | 277 (22.9) | 72 (13.0) | |
| Parity** | | | 0.026 | | | 0.002 | | | 0.062 |
| 0 | 587 (46.2) | 741 (50.4) | | 135 (41.5) | 1015 (50.7) | | 573 (47.5) | 290 (52.2) | |
| ≥1 | 684 (53.8) | 728 (49.6) | | 190 (58.5) | 985 (9.3) | | 634 (52.5) | 265 (47.8) | |
| Smoking*** | | | 0.41 | | | 0.64 | | | 1 |
| no | 1112 (89.0) | 1277 (87.9) | | 287 (89.4) | 1750 (88.5) | | 1050 (88.2) | 484 (88.2) | |
| yes | 138 (11.0) | 175 (12.1) | | 34 (10.6) | 227 (11.5) | | 141 (11.8) | 65 (11.8) | |
| Season**** | | | <0.001 | | | 0.014 | | | <0.001 |
| Summer | 160 (12.5) | 455 (30.8) | | 59 (18.2) | 529 (26.5) | | 245 (20.3) | 207 (37.3) | |
| Fall | 305 (23.7) | 416 (28.2) | | 103 (31.7) | 595 (29.7) | | 326 (27.0) | 154 (27.7) | |
| Winter | 394 (30.7) | 274 (18.5) | | 70 (21.5) | 366 (18.3) | | 284 (23.5) | 82 (14.8) | |
| Spring | 425 (31.1) | 333 (22.5) | | 93 (28.6) | 510 (25.5) | | 353 (29.2) | 112 (20.2) | |
| Vitamin D before inclusion[‡] | | | <0.001 | | | | | | |
| no | 1101 (99.1) | 1245 (95.2) | | NA | NA | | NA | NA | |

| | | | | | | | | | |
|--|-------------|-------------|--|---------------|----------------|--------|----------------|---------------|--------|
| yes | 10 (0.9) | 63 (4.8) | | NA | NA | | NA | NA | |
| Vitamin D in 7th month^W | | | | | | <0.001 | | | 0.20 |
| no | | | | 74 (23.8) | 148 (7.6) | | 119 (10.2) | 44 (8.2) | |
| yes | | | | 237 (76.2) | 1802 (92.4) | | 1052 (89.8) | 493 (91.8) | |
| Previous 25(OH)D^{WY} | | | | | | <0.001 | | | <0.001 |
| ≥30 ng/mL | | | | 28 (8.7) | 530 (26.9) | | 436 (39.7) | 450 (88.7) | |
| 20-30 ng/mL | | | | 77 (24.1) | 624 (31.7) | | 456 (41.5) | 43 (8.5) | |
| 10-20 ng/mL | | | | 136 (42.5) | 664 (33.7) | | 190 (17.3) | 12 (2.4) | |
| <10 ng/mL | | | | 79 (24.7) | 151 (7.7) | | 17 (1.5) | 2 (0.4) | |

560

561 **Table 3.** Univariate analysis of the determinants of severe vitamin D insufficiency (defined as
562 serum 25(OH)-vitamin D <20 ng/mL) during pregnancy and in cord blood.

563 BMI: body mass index, 25(OH)D: serum 25(OH)-vitamin D concentration, NA: non-
564 applicable. *According to the Fitzpatrick phototyping scale,**excluding the ongoing
565 pregnancy,***active at the beginning of pregnancy, ****season of sampling, ¥vitamin D
566 supplementation during the month before inclusion, ¥¥vitamin D supplementation in 7th month
567 of pregnancy (cholecalciferol, 100,000 IU), ¥¥¥ serum 25(OH)D concentrations at the previous
568 visit (for the third trimester, the previous visit took place in the first trimester; for cord blood,
569 the previous visit took place in the third trimester. Results are shown as n (%).

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| | Supplementation* | 3 rd trimester | | P value | Cord blood | | p value |
|---------------------------------|---------------------|---------------------------|--------------|---------|------------|-------------|---------|
| | | No | Yes | | No | Yes | |
| | n | 222 | 2039 | | 163 | 1545 | |
| Serum 25(OH)D (ng/mL) | Mean ± SD | 25.8±11.1 | 32.5±11.4 | <0.001 | 15.5±7.1 | 17.2±7.22 | 0.004 |
| 25(OH)D categories n (%) | <10 ng/mL | 12 (5.4%) | 16 (0.8%) | <0.001 | 33 (20.2%) | 191 (12.4%) | 0.03 |
| | 10-20 ng/mL | 62 (27.9%) | 221 (10.8%) | | 86 (52.8%) | 861 (55.7%) | |
| | 20-30 ng/mL | 68 (30.6%) | 616 (30.2%) | | 37 (22.7%) | 388 (25.1%) | |
| | ≥30 ng/mL | 80 (36.1%) | 1186 (58.2%) | | 7 (4.3%) | 105 (6.8%) | |

577 **Table 4.** Serum 25(OH)-vitamin D at the third trimester and in cord blood for subjects who
578 were and who were not supplemented as recommended by the French guidelines in the 7th
579 month of pregnancy (cholecalciferol, 100,000 IU)*.

580 n: number of subjects, SD: standard deviation, 25(OH)D: 25(OH)-vitamin D.

| | 1 st trimester | | 3 rd trimester | | Cord blood | |
|---|---------------------------|---------|---------------------------|---------|------------------|---------|
| | OR [95% CI] | p value | OR [95% CI] | p value | OR [95% CI] | p value |
| Age (years) | | | | | | |
| <35 | | | 1.00 | | | |
| ≥35 | | | 0.66 [0.48-0.91] | 0.01 | | |
| BMI (kg/m²) | | | | | | |
| <25 | 1.00 | | | | 1.00 | |
| ≥25 | 1.33 [1.10-1.61] | 0.003 | | | 1.32 [1.00-1.73] | 0.049 |
| Phototype* | | | | | | |
| 1-4 | 1.00 | | 1.00 | | | |
| 5-6 | 2.70 [2.17-3.36] | <0.001 | 1.80 [1.34-2.40] | <0.001 | | |
| Parity** | | | | | | |
| 0 | | | 1 | | | |
| ≥1 | | | 1.47 [1.12-1.92] | 0.005 | | |
| Season*** | | | | | | |
| Summer | 1.00 | | 1.00 | | 1.00 | |
| Fall | 2.03 [1.57-2.63] | <0.001 | 1.86 [1.27-2.72] | 0.002 | 2.18 [1.59-2.99] | <0.001 |
| Winter | 4.43 [3.41-5.76] | <0.001 | 4.04 [2.62-6.23] | <0.001 | 3.15 [2.19-4.51] | <0.001 |
| Spring | 4.16 [3.22-5.38] | <0.001 | 2.24 [1.51-3.31] | <0.001 | 1.96 [1.39-2.75] | <0.001 |
| Vitamin D before inclusion[¥] | | | NA | NA | NA | NA |
| no | 1.00 | | NA | NA | NA | NA |
| yes | 0.16 [0.08-0.31] | <0.001 | NA | NA | NA | NA |

| | | | | | | |
|---|----|----|--------------------|--------|--------------------|--------|
| Vitamin D in 7th month^{¥¥} | NA | NA | | | | |
| no | | | 1.00 | | | |
| yes | | | 0.21 [0.15-0.29] | <0.001 | | |
| Previous 25(OH)D^{¥¥¥} | NA | NA | | | | |
| ≥30 | NA | NA | 1.00 | | 1.00 | |
| 20-30 | NA | NA | 2.83 [1.77-4.53] | <0.001 | 10.59 [7.49-14.98] | <0.001 |
| 10-20 | NA | NA | 4.71 [2.98-7.47] | <0.001 | 16.26 [8.89-29.74] | <0.001 |
| <10 | NA | NA | 13.84 [8.18-23.43] | <0.001 | 7.66 [1.73-33.90]§ | 0.007 |

581

582 **Table 5.** Multivariate analysis of the determinants of severe vitamin D insufficiency (defined
583 as serum 25(OH)-vitamin D<20 ng/mL) during pregnancy and in cord blood.

584 BMI: body mass index, 25(OH)D: serum 25(OH)-vitamin D concentration, NA: non-
585 applicable. *According to the Fitzpatrick phototyping scale,**excluding the ongoing
586 pregnancy, ***season at the time of sampling, ¥vitamin D supplementation during the month
587 before inclusion, ¥¥vitamin D supplementation in 7th month of pregnancy (cholecalciferol,
588 100,000 IU), ¥¥¥ serum 25(OH)D concentrations at the previous visit (for the third trimester,
589 the previous visit took place at the first trimester; for cord blood, the previous visit took place
590 at the third trimester), §among women with 25(OH)D below 10 ng/mL at the third trimester,
591 only two had 25(OH)D above 20 ng/mL in cord blood.

592

| Country | Latitude | n | Pregnancy | | Cord Blood | Method for 25(OH)D measurement |
|--|----------|-----------------------------|---------------------------------------|--|--|--------------------------------|
| | | | Timing of sampling | 25(OH)D concentrations (ng/mL), and by category when available (%) | 25(OH)D concentrations (ng/mL), and by category when available (%) | |
| Finland (29) | 60°N | 584 | T1 GW 6–13 | Mean ±SD 35.5±7.6 -1% <20 ng/mL | Mean ±SD 35.3 ± 8.8 -1% <20 ng/mL | CLIA |
| south-western Sweden Gravid study (27) | 57-58°N | 1985 | T1 before GW 17 | Mean ±SD 25.8±9.8 - 25% <20 ng/L - 10% <12 ng/mL | NO | LC-MS/MS |
| | | 1836 | T3 after GW 31 | Mean ±SD 29.8±13.8 | | |
| Scotland (20) | 57°N | 1205 (T1 and cord blood) | T2 GW 19 | Mean (95%CI) 16.0 (15.4-16.7) - 21.5% <10 ng/mL | Mean (95%CI) 8.7 (8.2-9.4) - 50 % <10 ng/mL | LC-MS/MS |
| Denmark (28) | 54-57°N | 1494 | T2 GW 25 | Mean ±SD 22.7±9.8 - 76.9% <.30 ng/ml - 42.3% < 20 ng/ml - 10.1% < 10 ng/ml | NO | LC-MS/MS |
| North West of England (30) | 53°N | - Mother: 608 - CB: 345 | T2/T3 26.9 GW (range 26.0-28.7) | Median (IQR) 30.6 (19.2–38.1) -27% <20 ng/mL -7% <10 ng/mL | Median (IQR) - 15.4 (9.8–22.4) - 65% <20 ng/mL - 26% <10 ng/mL | LC-MS/MS |
| Ireland SCOPE study (21) | 52°N | 1768 | T2 GW 15 (range, 14-16) | Mean ±SD 22.7±10.4 - 75% <30 ng/mL - 44% <20 ng/mL - 11% <10 ng/mL | NO | LC-MS/MS |
| Ireland SCOPE study (22) | 52°N | 1050 | | | Mean ±SD 14.0±7.2 - 80% <20 ng/mL - 35% (50% during winter) <10 ng/mL | LC-MS/MS |

| | | | | | | |
|---------------------|-------------|----------------------------|--|---|--|------|
| Belgium (26) | 49- 51°N | 640 | T1 | Median 20.4 | NO | RIA |
| | | 666 | T3 | Median 22.7 | | |
| | | 1311 | T1+ T3 (+5 patients at T2) | - 74.1% <30 ng/mL - 44.6% <20 ng/mL - 12.1% <10 ng/mL | | |
| Germany (34) | 47- 54°N | - Mother: 261 - CB: 328 | delivery or within 72 h post-partum | Median (IQR) 10.0 (5.0–18.2) -77% <20 ng/mL | Median (IQR) 13.6 (7.1–23.4) - 69% <20 ng/mL | CLIA |
| Germany (38) | 47- 54°N | 429 | between the 2 nd and 41 st GW (mean±SD: 23.8 ±11.5) | Mean ±SD 14.2± 8 | NO | CLIA |
| Switzerland (39) | 47°N | 204 | 7 GW | 63% <20 ng/mL | NO | CLIA |
| | | n=75 Vit ≥20 ng/ml | 7 GW | Mean (95%CI) 26.1 (24.8–27.4) | | |
| | | n=129 Vit <20 ng/ml | 7 GW | Mean (95%CI) 10.5 (9.7–11.5) | | |

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594 **Table 6:** Vitamin D status during pregnancy and in cord blood reported in studies from
595 northern Europe (countries with latitudes equal or higher than the one reported in the present
596 study: 47-49°N). Studies are presented according to the latitude of the country (higher to
597 lower latitudes). Results from the countries of southern Europe are not mentioned in this table
598 since it was the purpose of a review by Karras et al published in 2016 (33).

599 CLIA: chemiluminescence immunoassay, LC-MS/MS: chromatography tandem-mass
600 spectrometry, RIA: Radioimmunoassay, GW: gestational weeks, T1: first trimester, T2:
601 second trimester, T3: third trimester, CB: cord blood, SD: standard deviation, IQR:
602 interquartile range, CI: confidence interval, 25(OH)D: serum 25(OH)-vitamin D
603 concentration, n: number of patients.

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