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ORIGINAL RESEARCH ARTICLE

Vitamin D_3 among neonates born after in vitro fertilization compared with neonates from the general population

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Abstract

Introduction: Sufficient levels of vitamin D have been associated with higher chances for both clinical pregnancy and live birth among women undergoing assisted reproductive techniques, whereas low levels of maternal vitamin D have been associated with preeclampsia and late miscarriage. In Denmark, subgroups at risk for low vitamin D levels, including neonates and toddlers, are recommended to use supplementation. The aim was to study the level of vitamin D_3 among neonates born after in vitro fertilization compared with neonates from the general population.

Material and methods: In this cohort study a random sample of 1326 neonates representing the general population and 1200 neonates conceived by in vitro fertilization born in Denmark from 1995 to 2002 were identified from registries covering the whole Danish population. Information on use of assisted reproduction was collected from the Danish In Vitro Fertilization register, ICD-10 code: DZ358F. 25-Hydroxyvitamin D was measured from dried blood spots routinely collected by heel prick 48–72 h after birth and corrected according to the hematocrit fraction for capillary blood of neonates. Linear regression analysis was performed, both crude and adjusted, for predefined putative confounders, identified through directed acyclic graphs.

Results: Vitamin D_3 analysis could be performed from a total of 1105 neonates from the general population and 1072 neonates conceived by in vitro fertilization that were subsequently included in the study. The median vitamin D_3 was 24.0 nmol/L (interquartile range [IQR] 14.1–39.3) and 33.0 nmol/L (IQR 21.3–48.8) among neonates from the general population and neonates conceived by in vitro fertilization, respectively. The adjusted mean difference between neonates from the general population and those conceived by in vitro fertilization was 6.1 nmol/L (95% confidence interval 4.1–8.1).

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ART, assisted reproductive technology; BMI, body mass index; DBSS, dried blood spot samples; DCRS, Danish Civil Registration System; ICD-10, International Classification of Diseases, 10th revision; IQR, interquartile range; IVF, in vitro fertilization; LLOQ, lower limit of quantification; PKU, phenylketonuria; STROBE, Strengthening the Reporting of Observational studies in Epidemiology.

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Conclusions: In this study, children born after in vitro fertilization have a higher vitamin D_3 than a random sample of neonates in Denmark.

KEYWORDS
in vitro fertilization, infertility, neonate, vitamin D

1 | INTRODUCTION

The global burden of infertility is estimated to affect more than 186 million people.[1] The variability in infertility rates is considerable between regions of the world, with the most affected populations reporting up to 30%.[2]

Besides the well-described role of vitamin D in calcium metabolism, the receptors and enzymes involved in the metabolism of vitamin D have been found in the ovaries and the endometrium, suggesting vitamin D to be involved in female fertility.[3] However, although there seems to be no association between vitamin D levels and chance of spontaneous pregnancy[4,5] the association between vitamin D and the chance of clinical pregnancy and live birth among women undergoing treatment with assisted reproductive technology (ART) is not clear.[6–11]

Clinical pregnancy rates are found to be higher among non-vitamin-D-deficient women.[6,7,9,11] Based on a study with donor oocytes showing an association between vitamin D status and chance of pregnancy in oocyte recipients, Rudick et al. suggests that the endometrium mediates in the vitamin D effect.[6] However, after stratifying on source of the oocyte to reduce the impact of oocyte quality, Chu et al. found no significant association among studies examining donor oocytes; although, because of the low number of participants, a type 2 error cannot be ruled out.[11]

In a meta-analysis, the chance of live birth is higher among women replete in vitamin D (odds ratio 1.33, 95% confidence interval [CI] 1.08–1.65).[11] This clear association is in accordance with results based on donor oocytes.[6] However, because of the low numbers of vitamin D replete women (16%) in an observational study the statistical significance was lost in adjusted analyses.[8]

Based on sensitivity analyses in their meta-analysis, Cozzolino et al. state that vitamin D levels do not influence clinical pregnancy rate, live birth rate, and ongoing pregnancy rate.[10] However, studies have shown that in early pregnancy, low concentrations of vitamin D have been associated with preeclampsia[12] and late miscarriage.[4]

Traditionally, serum vitamin D levels among adults are divided into sufficient (≥30 ng/mL; c.75 nmol/L); insufficient (≥20–29 ng/mL; c.50–74 nmol/L); or deficient (<20 ng/mL; c.50 nmol/L).[13,14] There is still no consensus on the optimum serum levels of vitamin D in relation to fertility; however, based on bone health, experts recommend a vitamin D level of at least 20 ng/mL (c.50 nmol/L) to meet the needs of at least 97.5% of the North American population.[15] In 2018, Randev et al. reported that the global prevalence of vitamin D deficiency varies from 6% to 68% when the cut-off is set at 20 ng/mL.[14]

> **Key message**
>
> Neonates born after in vitro fertilization have a higher vitamin D_3 than neonates from the general population.

The primary source for vitamin D is sunlight exposure; whereas a minor part is attained through diet and supplementation.[13] Due to differences in skin pigmentation, the synthesis of vitamin D through sun light exposure may vary according to not only ethnicity, but also genetic variation in vitamin D modulating genes, concealing clothing, outdoor activities and season, which can affect the vitamin D status.[16] In the northern countries, seasonal variation in vitamin D levels has been suggested to partly explain seasonal variations in fertility, where the number of births is higher during the spring; accordingly, most women conceive in the summer or fall after the vitamin D depots have been loaded by sunlight exposure.[3] In accordance with this Hasan et al. found preconception vitamin D sufficiency to be associated with a clinically confirmed pregnancy outcome following in vitro fertilization (IVF) treatment.[17]

As regards the sources of vitamin D for the fetus, placental transfer from the mother is the only vitamin D supply during pregnancy.[18] A study with 125 mother–infant dyads described insufficient vitamin D supplementation during pregnancy as a predominant factor of neonatal vitamin D deficiency (odds ratio 7.0, 95% CI 2.7–20.0).[19]

Considering the lack of association between vitamin D levels and chance of spontaneous pregnancy and the concurrent debate of a possible association with clinical pregnancy rate and live birth rate in ART, and considering that all ART pregnancies are wanted pregnancies, we hypothesized that women undergoing ART would be more likely to follow the recommendations of the health authorities to take vitamin D supplements during pregnancy and hence achieve higher mean levels of pregnancy, resulting in higher levels in their offspring.

With this study we aimed to examine the level of vitamin D_3 among neonates born after IVF compared with neonates from the general population.

2 | MATERIAL AND METHODS

2.1 | Study design

This cohort study compared neonatal vitamin D levels among neonates who were conceived by IVF treatment and neonates from the general population by using Danish registers and biobanks. The study was reported in accordance with the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) statement.[20]

2.2 | Data sources

The study population was identified using nationwide registers. All individuals residing in Denmark are registered in the Danish Civil Registration System (DCRS) with a unique 10-digit number enabling linkage to other national registers in Denmark.[21] Children conceived by IVF were identified using the Danish IVF register, a nationwide registry containing information on all IVF treatments carried out at public and private fertility clinics since 1994.[22] The DCRS, the Danish Medical Birth Register, and Statistics Denmark provided information on covariates.[21,23]

Routine newborn screening performed 48–72h after birth for congenital disorders via capillary blood samples taken by heel prick (so called phenylketonuria [PKU] tests) was routinely implemented from 1981. After screening, the samples are stored as dried blood spot samples (DBSS) at the Biological Specimen Bank for Neonatal Screening in the Danish National Biobank at the Statens Serum Institute at −20°C. Neonatal 25-hydroxyvitamin D (25(OH)D) concentrations were measured from punches of DBSS.

2.3 | Study population

The cohort of neonates representing the general population was a random sub-sample of all individuals born in Denmark between 1995 and 2002, identified using the DCRS ($n=1326$). Neonates conceived by IVF treatment were randomly sampled from the IVF register (ICD-10 code: DZ358F) where each birth cohort between 1995 and 2002 contributed with 150 children ($n=1200$) (Figure 1). The specific time period was chosen to optimize the data completeness. Using the possibility of linkage between registers and biobanks, DBSS were retrieved and analyzed for vitamin D concentrations.

2.4 | Assessment of vitamin D concentrations

The outcome of interest, neonatal vitamin D levels, was measured in 3.2-mm punches from DBSS using a modified version of a liquid chromatography tandem-mass spectrometry method at the Statens Serum Institute research laboratory.[24] Vitamin D concentrations were assessed by measuring 25(OH)D_2 and 25(OH)D_3. For 25(OH)D_2 and 25(OH)D_3 the lower limits of quantification (LLOQ) were 3 and 4nmol/L, respectively. Measures of 25(OH)D_2 were excluded from the analysis because 97% of the measures were below LLOQ, whereas all measures of 25(OH)D_3 were included in the analysis, including those below LLOQ (10.84%). Measures below LLOQ are considered uncertain and to accommodate the measures of 25(OH)D_3 below LLOQ, they were substituted with the value 2, corresponding to half the value of the quantification limit. The 25(OH)D_3 concentrations were corrected to reflect concentrations equivalent to serum concentrations using the formula: serum 25(OH)D_3 nmol/L = DBSSs 25(OH)D_3 nmol/L $\times$ 1/(1–0.61), where 0.61 is the hematocrit fraction for capillary blood.[25] The performing laboratory participates in the Vitamin D External Quality Assessment Scheme.[26]

2.5 | Covariates

The following confounders were selected a priori using a directed acyclic graph (Figure S1); season of birth (spring, summer, fall, winter), maternal ethnicity (European, non-European), maternal age (continuous, years), parity (nulliparous, multiparous), maternal education (basic/short [basic school 8th–10th class, short general upper–secondary education, short-cycle higher education or vocational education and training], medium [medium-cycle higher education or bachelor's], and long [long-cycle education and PhD]), maternal smoking (smoker/non-smoker), and maternal body mass index (BMI).

In addition, information on the following covariates was obtained to describe the population; sex (female, male), birthweight (continuous, grams), gestational age (preterm <37, term ≥37 weeks), maternal marital status (not married/ divorced/ widowed, married/registered partner), and embryo transfer (fresh, frozen).

Season of birth and sex were retrieved from DCRS. Birthweight, gestational age, parity, maternal age, and smoking were obtained from Danish Medical Birth Registry; maternal education, marital status and ethnicity were from Statistics Denmark; and embryo transfer was from the IVF register. Data on maternal BMI were not available, so were not included in the final model.

2.6 | Statistical analyses

General characteristics of both neonates conceived by IVF and neonates from the general population are presented as numbers (n) and percentages (%) for categorical variables, mean and standard deviations, or median and interquartile range (IQR) for continuous variables.

Examination of missing data included reasons for missing data; missing data patterns and the availability of auxiliary variables suggested that the analysis would benefit from multiple imputation.[27] Missingness was not associated with the outcome after conditioning on the covariates from the main analysis. Based on our knowledge of the data, we found it plausible that missing data were missing at random.

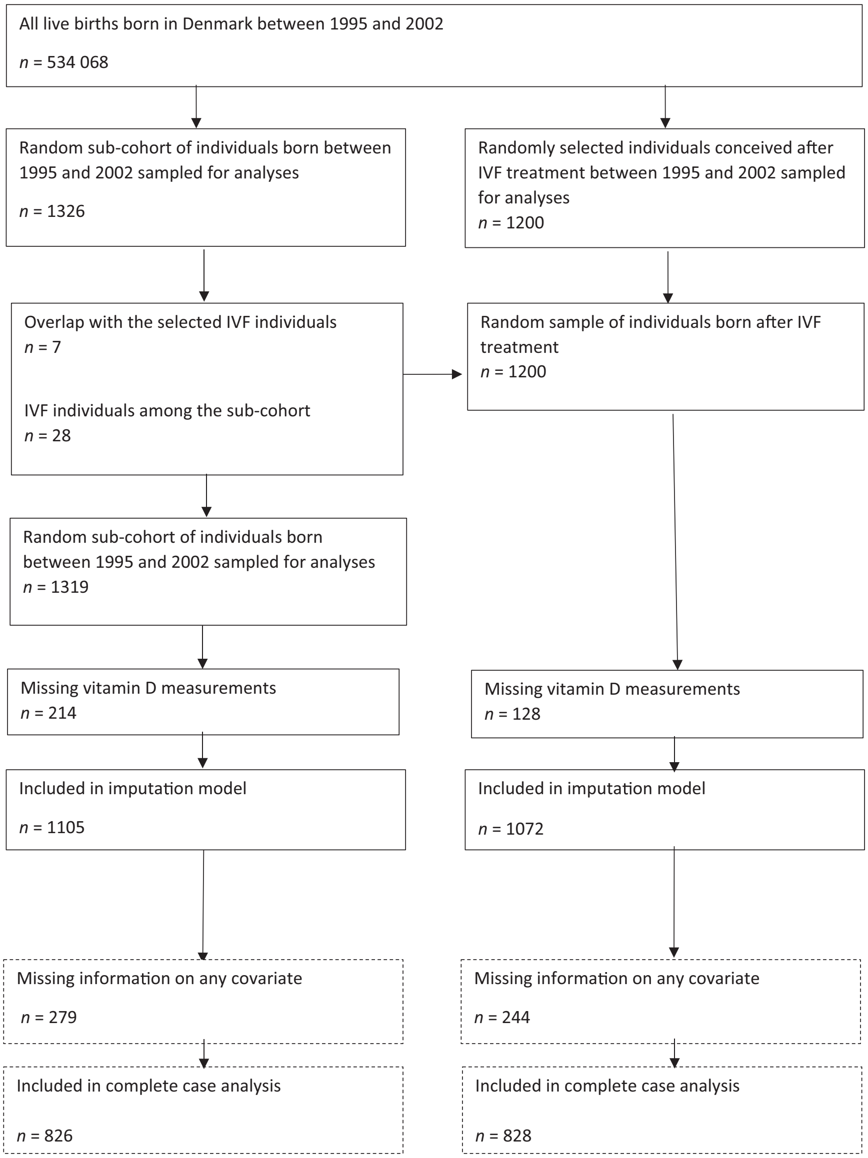

FIGURE 1 Flowchart of the included participants.

We included seven auxiliary variables with information from the national registers on the father (age, education, origin), household (number of children), and neonate (abdominal circumference, placental weight, birthweight) as they were associated with missingness or with the incomplete variables. We did not impute data on the outcome variable, vitamin D levels, but all variables from the analysis were included in the imputation model.[28] Multiple imputation by chained equations was performed, as we included both continuous and categorical variables in the model. We performed 25 imputations based on the rule of thumb that the number of imputations should be at least equal to the percentage of missing data (24%).[29]

We used a linear regression model to investigate the association between IVF treatment and neonatal vitamin D levels. First, we ran a crude analysis. Second, we conducted adjusted analysis based on the imputed data set (primary analysis) and on complete cases (sensitivity analysis). Results from the regression analyses are reported as means with 95% CI. We further conducted stratified analyses by gestational age and parity and an adjusted analysis restricted to the population of European origin. A graphical model check was performed, and minor concerns of the model assumptions were noted. We conducted a regression with robust standard errors as a sensitivity analysis, which did not change the conclusions from our main analysis. The imputed values were checked and compared with the observed values. All imputed values were plausible and resembled the observed values.

All statistical analyses were carried out with SAS Enterprise Guide 7.15.

3 | RESULTS

The flow of participants is shown in Figure 1. In the randomly selected sample from the general population, 35 neonates were conceived by IVF treatment, of which seven were in the randomly selected IVF sample. Dried blood spot samples were not found, analyses failed, or insufficient material was available for analysis and caused missing vitamin D_3 measurements in 342 neonates. After imputation on covariates these individuals were excluded leaving 1072 neonates conceived by IVF and 1105 neonates from the general population for the main analysis.

General characteristics of 1072 IVF-conceived neonates and 1105 neonates from the general population are presented in Table 1. The median vitamin D_3 level was higher among IVF-conceived neonates than neonates from the general population with median vitamin D_3 levels at 33.0 nmol/L (IQR 21.3–48.8 nmol/L) and 24.0 nmol/L (IQR 14.1–39.3 nmol/L) in the two groups, respectively.

The crude linear regression showed a higher level of vitamin D_3 among the IVF-conceived neonates (8.8 nmol/L, 95% CI 7.0–10.6 nmol/L) (Table 2). The adjusted analysis based on the imputed data set also showed higher mean levels of vitamin D_3 among the IVF-conceived neonates compared with the neonates from the general population. IVF-conceived neonates had 6.1 nmol/L (95% CI 4.1–8.1 nmol/L) higher mean level of vitamin D_3 (Table 2). As expected, neonates born in the fall had higher levels of vitamin D_3 compared with neonates born in other seasons. Similarly, neonates born to mothers of European origin and non-smokers as well as firstborn neonates had higher levels of vitamin D_3 (data not shown). Complete case analyses showed similar results (see Table S1). Adjusted analysis restricted to the population of European origin also showed similar results (data not shown).

Stratified analyses by gestational age showed that IVF-conceived neonates had a higher mean vitamin D_3 irrespective of being born premature or at term (Table 3). Stratified analyses on parity likewise showed that vitamin D_3 levels of IVF-conceived neonates were higher irrespective of the mother being primiparous or multiparous (Table 3).

4 | DISCUSSION

We found a higher level of vitamin D_3 among neonates conceived by IVF compared with neonates from the general population, even after controlling for putative confounders; the adjusted difference was 6.1 nmol/L (95% CI 4.1–8.1 nmol/L).

Neonatal vitamin D levels have been shown to be closely related to levels of the mother.[30] During pregnancy, the fetal vitamin D levels are dependent on maternal supply. In the present study, the higher vitamin D_3 level among IVF-conceived neonates compared with neonates from the general population could potentially reflect a difference in underlying maternal status and imaginably suggest a higher motivation for a generally healthy lifestyle among women achieving pregnancy and childbirth after fertility treatment. Couples undergoing fertility treatment are information seeking and motivated to change lifestyle in order to increase their chances of becoming parents.[31,32] Furthermore, previous studies have shown that women undergoing ART with higher levels of vitamin D have a better chance for achieving a clinical pregnancy[6,7] and live birth.[8,9] If this association is limited to women undergoing ART, this may partly explain the higher level of vitamin D_3 among the IVF-conceived neonates than the neonates from the general population. Under the assumption that the proportion of pregnancy planners is higher among mothers in the IVF group than in the group representing the general population, the mothers using IVF may, to a greater extent, adhere to national recommendations including taking pre-pregnancy vitamin supplementation and vitamin D supplementation during pregnancy and enabling higher vitamin D levels among the IVF-conceived neonates, as also suggested by the seasonal data where differences are seen only in the dark months. Vitamin D is considered an essential micronutrient because of the risk of disease across all ages in case of vitamin D deficiency.[33] Without vitamin D supplements the established level during fetal life has been shown to drop to rickets-level during the first 6 weeks after birth.[30] This decrease in vitamin D underlines the importance of measuring vitamin D shortly after birth, which was successfully done in this cohort. The Danish Health Authorities have recommended vitamin D supplementation during pregnancy since the late 1990s.[33] Infants are recommended to receive a supplement of vitamin D from 2 weeks after birth until 4 years of age. The routine PKU screening is performed 48–72 h after birth; so, no significant external supply of vitamin D influences the blood samples.

Previous studies on vitamin D levels based on DBSS in Denmark have examined the association between vitamin D levels and clinical outcomes. A low level of vitamin D has been found to be associated with impaired development of the lungs and immune system, because high neonatal vitamin D levels were found to reduce the risk of childhood asthma.[34] Neonatal vitamin D status has also been associated with neurological development, and individuals with low vitamin D levels were found to have slightly lower cognitive ability.[35] High neonatal vitamin D levels were found to be associated with higher risk of childhood epilepsy.[36] Both high and low quintiles of vitamin D levels were found to be associated with mental disorder in later life, manifested as schizophrenia,[37] whereas no increased odds were seen for development of myopia among male individuals with a low vitamin D level compared with the remaining quintiles of vitamin D.[38]

Hence, from a public health point of view, our findings may suggest that children conceived through ART treatment, may have lower risk of the above long-term conditions associated with low vitamin D status at birth.

At the same time, however, children born after ART treatment may be at increased risk of some somatic disorders, e.g. asthma and epilepsy.[39,40]

This current study may be a first step to understanding any underlying mechanisms behind these associations. If children born following ART have higher levels of vitamin D than newborns representing the background population, this would potentially be a mechanism behind the associations described for higher rates of epilepsy but would clearly not be an explanation for any associations with asthma.

Future studies, including potential studies based on this cohort, could help to disentangle such mediation.

TABLE 1 General characteristics of study population.

	IVF-conceived neonates ($n = 1072$)				Neonates from the general population ($n = 1105$)			
	n	%	Mean (SD)	Median (IQR)	*n*	%	Mean (SD)	Median (IQR)
D_3 vitamin, nmol/L	1072			33.00 (21.3–48.8)	1105			24.0 (14.1–39.3)
Gender, male	574	53.5			575	52.0		
Season of birth								
Spring	196	18.3			288	26.1		
Summer	289	27.0			294	26.6		
Fall	259	24.2			268	24.3		
Winter	328	30.6			255	23.1		
Birthweight, g Missing $n = 35$	1066		3458.6 (618.5)		1076		3517.0 (608.0)	
Birthweight for gestational age								
SGA	139	13.0			122	11.0		
AGA	787	73.4			806	72.9		
LGA	117	10.9			128	11.6		
Missing $n = 78$								
Gestational age, weeks Missing $n = 56$	1050			40.0 (38.9–40.9)	1071			40.0 (39.0–41.0)
Gestational age								
$<37^{+0}$	75	7.0			46	4.2		
$\geq 37^{+0}$	975	91.0			1025	92.8		
Missing $n = 56$								
Embryo transfer								
Fresh	93	8.7						
Frozen	979	91.3						
Maternal origin, European	1036	96.6			982	88.9		
Missing $n = 8$								
Maternal age, years	1072		33.3 (3.8)		1092		29.5 (4.7)	
Missing $n = 13$								
Parity								
Primiparous	809	75.5			439	39.7		
Multiparous	247	23.0			637	57.7		
Missing $n = 54$								

TABLE 1 (Continued)

	IVF-conceived neonates ($n = 1072$)				Neonates from the general population ($n = 1105$)			
	n	%	Mean (SD)	Median (IQR)	*n*	%	Mean (SD)	Median (IQR)
Maternal education								
Basic/Short								
Medium	679	63.3			744	67.3		
Long	297	27.7			259	23.4		
Missing $n = 54$	86	8.0			58	5.3		
Maternal marital status Married/registered partner	802	74.8			647	58.6		
Missing $n = 22$								
Smoking in pregnancy	144	13.4			170	15.4		
Missing $n = 465$								

Note: AGA, appropriate for gestational age; IQR, interquartile range; IVF, in vitro fertilization; LGA, large for gestational age; SD, standard deviation; SGA, small for gestational age.

TABLE 2 Crude and adjusted mean differences (95% CI) of vitamin D levels (nmol/L) among 1072 IVF and 1105 neonates from the general population.

	Estimate (SE)	95% CI	*p* value
Crude analysis			
Neonate from the general population	*Reference*		
IVF-conceived neonate	8.8 (0.9)	(7.0–10.6)	<0.0001
Adjusted analysis[a]			
Neonate from the general population	*Reference*		
IVF-conceived neonate	6.1 (1.0)	(4.1–8.1)	<0.0001

Note: CI, confidence interval; IVF, in vitro fertilization; SE, standard error.

[a]Adjusted for season, maternal ethnicity, parity, maternal education, and maternal age.

There were several strengths in our study. First, the sample sizes minimized the risk of type II errors. All estimates of associations are given with 95% CI, enabling evaluation of uncertainty due to random error. Second, all samples were analyzed at the same national laboratory eliminating the risk of systematic errors between the groups, and the reported association may not be distorted in this regard. Third, the covariates used in the analyses were based on information from the Danish national registers, which are documented to deliver high-quality variables,[23,41] whereby misclassification is unlikely to distort the associations.

Participants in research studies generally tend to be healthier, married, and better educated than the background population. However, participation in the present study was not restricted to voluntary commitment because the study is register based. Hence, the results from our study are anticipated to be generalizable to the Danish population of neonates.

However, some limitations need to be considered. In epidemiological research, missing data are unavoidable. Selecting the most appropriate analytical method to deal with missing data is crucial to provide unbiased estimates. Missingness was associated with the covariate maternal ethnicity and was not missing completely at random. Missingness was not associated with the outcome, why a complete case analysis would yield unbiased estimates. However, discarding data would result in loss of statistical power and precision. The primary analysis was based on multiple imputation including auxiliary variables to provide information on the missing variables and support the missing at random assumption.

Missingness on the outcome was primarily a result of lack of sample material, uncertain sample identity, or loss of the DBSS card, which are all anticipated to be unrelated to the value of the vitamin D level or covariates and thereby not introducing bias.

The measurement of vitamin D in DBSS analyzed by liquid chromatography tandem mass spectrometry has been found to accurately reflect plasma concentrations.[42] However, differences in the analytical methods used in recent decades complicates comparison across

TABLE 3 Adjusted[a] mean differences (95% CI) of vitamin D levels (nmol/L) among IVF-conceived neonates and neonates from the general population stratified by term and parity.

	Pre-term (<37 weeks)	Term (≥37 weeks)
General population neonates	Reference	Reference
IVF-conceived neonates	11.0 (3.3–18.6)	5.6 (3.5–7.6)
	Primiparous	Multiparous
General population neonates	Reference	Reference
IVF-conceived neonates	5.4 (2.8–8.1)	7.1 (4.1–10.1)

Note: CI, confidence interval; IVF, in vitro fertilization.

[a]Adjusted for season, maternal ethnicity, maternal age, maternal education, and maternal smoking and additionally for parity in the term analysis.

studies, since the 25(OH)D level is reported to vary considerably.[43] In the present study, all vitamin D measurements are completed at the same laboratory, hence technical factors like temperature, light, or repeated freeze–thawing are considered not to affect the validity of the difference in vitamin D concentration.[44] Furthermore, in the present study the results are limited to measurements of only one of the metabolites, D_3, because most of the D_2 measurements were below the LLOQ.

Important confounding factors were identified and included in our final model. Information on maternal BMI and socio-economic status were not available. Obesity is known to have a negative impact on vitamin D through several biological and social mechanisms, including preferences for selected food items low in vitamin D, low sun exposure and possible accumulation in adipose tissue.[45] Women with obesity generally have lower vitamin D levels compared with women with normal weight in early pregnancy.[46] Besides a prolonged time to pregnancy there is an increased risk of several pregnancy and birth-related complications among women with a BMI above the normal range, for example early miscarriage and stillbirth.[47–49]

In the Danish cohort study "Aarhus Birth Cohort" the proportion of women with overweight or obesity before pregnancy were found to be higher among women who became pregnant after fertility treatment compared with fertile women.[50] If this distribution of BMI is also present in our study, the adjusted estimated difference in vitamin D_3 concentration of 6.1 nmol/L (95% CI 4.1–8.1 nmol/L) in favor of the IVF-conceived neonates, may be underestimated.

5 | CONCLUSION

The vitamin D level is higher among neonates conceived by IVF compared with neonates from the general population. Future studies should investigate whether the difference in vitamin D levels at birth may influence long-term disease risk in children born after IVF treatment versus children from the general population.

AUTHOR CONTRIBUTIONS

Ulrik Schiøler Kesmodel: conceptualization, methodology, writing—review and editing, supervision. Berit Lilienthal Heitmann: conceptualization, methodology, investigation, writing—review and editing, supervision. Arieh Cohen: validation, formal analysis, investigation, resources. Ina Olmer Specht: methodology, software, validation, investigation, data curation, writing – review and editing. Fanney Thorsteinsdottir: methodology, software, validation, formal analysis, investigation, data curation, writing—review and editing. Karen Christina Walker: methodology, software, validation, formal analysis, investigation, data curation, writing original draft, visualization, project administration. Sofie Gry Pristed: methodology, writing original draft, project administration.

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CONFLICT OF INTEREST STATEMENT

The authors report no financial or personal interests.

ETHICS STATEMENT

The Ethical Committee of the Capital Region of Denmark granted permission to conduct the study (H-3-2011-126) on November 29, 2011 and the Danish Data Protection Agency provided permission to process data (2012-41-1156). The Danish Health Data Authority and Statistics Denmark granted permission to use register data. The steering committee for scientific use of the Biological Specimen Bank for Neonatal Screening granted permission to use the DBSS.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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