ORIGINAL ARTICLE: ENVIRONMENT

Chances of live birth after exposure to vitamin D–fortified margarine in women with fertility problems: results from a Danish population-based cohort study

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Objective: To study the association between extra vitamin D from a mandatory margarine fortification program and chance of live birth among infertile women.
Design: Nationwide cohort study.
Setting: Not applicable.
Patient(s): The study population consisted of 16,212 women diagnosed with infertility from June 1, 1980, to August 31, 1991.
Interventions(s): We took advantage of the mandatory vitamin D fortification program of margarine in Denmark that was abruptly stopped on May 31, 1985. The termination of the vitamin D fortification served as a cutoff point to separate the study population into various exposure groups.
Main Outcome Measure(s): Odds ratios and 95% confidence intervals for the association between vitamin D exposure status and chance of a live birth within 12, 15, and 18 months after first infertility diagnosis.
Result(s): Women who were diagnosed with infertility during the vitamin D–exposed period had an increased chance of a live birth compared with women diagnosed with infertility during the nonexposed period. For women diagnosed with infertility during the wash-out period, the chance of a live birth was also increased, but somewhat lower. Similar estimates were obtained with longer follow-up, in women with anovulatory infertility, and little seasonal variation was observed when calendar period of conception was applied.
Conclusion(s): Our findings suggest that infertile women exposed to extra vitamin D from a margarine fortification program had an increased chance of live birth compared with women not exposed to extra vitamin D from fortification. (Fertil Steril® 2019;■:■–■. ©2019 by American Society for Reproductive Medicine.)
Key Words: Vitamin D, infertility, live birth, cohort study, Denmark

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Received April 23, 2019; revised September 10, 2019; accepted September 13, 2019.
A.J. has nothing to disclose. M.L.N. has nothing to disclose. S.G. has nothing to disclose. S.K.K. has nothing to disclose. B.L.H. has nothing to disclose. U.S.K. reports personal fees from MSD for teaching of nurses on legal issues, outside of the submitted work.
Supported by a grant to B.L.H. by the Danish Council for Strategic Research (11-116213) and by internal funding from the Danish Cancer Society. The funding bodies were not involved in the study design or data collection, analyses, interpretation of the results, the decision to submit the manuscript for publication, or writing the manuscript.
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Fertility and Sterility® Vol. ■, No. ■, ■ 2019 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2019.09.017

Infertility, defined as failure to conceive spontaneously within 12 months of regular unprotected intercourse, affects one in six couples worldwide (1). During the past decades, a steady increase in the number of women receiving fertility treatment has been observed worldwide (2). In Denmark, which has one of the highest proportions of children conceived after fertility treatment in the Western world, 9% of all children were conceived after fertility treatment in 2017 (3).

A number of factors, including advanced age, pelvic inflammatory disease, endometriosis, smoking, alcohol, and obesity are known to adversely affect the chance of pregnancy, regardless of mode of conception (4, 5). However, little is known about the role of dietary components, except for specific groups of women such as those with polycystic ovary syndrome (PCOS) (6, 7). One dietary component that has received attention is vitamin D, with studies showing that up to 50% of all women in the reproductive age group suffer from vitamin D deficiency (8, 9). Considering that vitamin D deficiency has been associated with factors related to infertility including PCOS, endometriosis, and the presence of fibroids, it is reasonable to hypothesize that vitamin D may be more associated with chance of pregnancy among infertile women than among healthy women of fertile age (10). However, the current literature is somewhat conflicting. To our knowledge, four studies have assessed the association between vitamin D levels and chance of pregnancy and live birth in otherwise healthy women, of which two studies observed that higher vitamin D levels were associated with an increased likelihood of pregnancy and livebirth (11, 12) and the two other studies showed no apparent associations (13, 14). Most, but not all studies focusing on women undergoing treatment with the use of assisted reproductive technologies (ART) have shown that a high serum vitamin D level seems to be associated with an increased chance of pregnancy and live birth (15, 16).

Vitamin D is synthesized in the skin and is activated primarily by exposure to ultraviolet radiation from the sun, whereas oral intake of certain foods and supplementations can serve as additional sources of vitamin D (17). In Denmark and other countries at northern latitudes, vitamin D deficiency is common because there is virtually no cutaneous synthesis of vitamin D during the winter season (October to March) (18). One strategy to avoid vitamin D deficiency is fortification of common food products that are ingested by the entire population (19, 20). Between 1962 and 1985, it was mandatory in Denmark to fortify all margarine products with vitamin D: 1.25 μg vitamin D_2 or D_3 per 100 g of margarine, corresponding to $\sim$50 IU/100 g of margarine (21). It has been calculated that this fortification averaged 13% of the daily vitamin D intake among Danish adults during the fortification period (22).

In the present study, we took advantage of this unique societal experiment and assessed the association between vitamin D fortification and chance of live birth among infertile women in a well described Danish cohort. This was done by comparing the chance of a live birth among women diagnosed with infertility before fortification ended with women diagnosed with infertility after fortification ended. We hypothesized that women diagnosed with infertility after the vitamin D fortification period ended may have been at higher risk of vitamin D deficiency and therefore had a lower likelihood of live births compared with women diagnosed with infertility during the vitamin D fortification period.

MATERIALS AND METHODS

Study Design, Study Population, and Ascertainment of Exposure Status

The study population was based on data from the Danish Infertility Cohort, established in 1997 and described in detail elsewhere (23, 24). In brief, the cohort comprised all women referred to public and private fertility clinics for fertility problems in the years 1963–1998 and identified from local computerized systems. In addition, all women with an infertility diagnosis (International Classification of Diseases, 8th Revision, code 628 and International Classification of Diseases, 10th Revision, code N97, excluding N97.4: female infertility due to male factor) recorded in the National Patient Registry in the years 1977–2012 and all women with recorded female infertility in the Danish In Vitro Fertilization (IVF) Registry in the years 1994–2012 were included. At the time of analysis, the Danish Infertility Cohort included information on 131,692 women with infertility registered from September 1, 1963, to December 31, 2012. Basic information for all women in the cohort included initial date of infertility evaluation, cause(s) of infertility, and personal identification number.

In the present study, we took advantage of the mandatory vitamin D fortification of margarine that was abruptly terminated on May 31, 1985 (21). The termination of the vitamin D fortification served as a cutoff point to separate the study population into three separate exposure groups: 1) the vitamin D–exposed group (all women who had their primary infertility diagnosis in the fortification period from June 1, 1980, to May 31, 1985 ($n = 6,313$); 2) a "wash-out" period group comprising all women with a primary infertility diagnosis from June 1, 1985, to August 31, 1986 ($n = 1,404$); and 3) a vitamin D–nonexposed group consisting of all women diagnosed with primary infertility from September 1, 1986, to August 31, 1991 ($n = 8,495$). The year 1980 was chosen as the initial cutoff year for inclusion in the cohort owing to a differential temporal registration of fertility status in the Danish Infertility Cohort: From 1963 to 1976, women with fertility problems were identified from local computerized systems at public and private fertility centers only. Only from 1977 onward was information on women with infertility diagnoses from the nationwide Danish National Patient Registry also available. Furthermore, because the coverage of the Danish National Patient Registry was most likely not optimal in the first few years of its existence (25), we chose 1980 as the initial cutoff year of inclusion to minimize the number of women with misclassified fertility status. During the entire study period from 1980 to 1991, clinical examinations for fertility problems and all types of fertility treatments were offered free of charge to all Danish couples who did not have a child together. Furthermore, private clinics offered self-paid fertility treatment for both primary and secondary infertility to couples.

Follow-Up for Reproductive Status (Live Birth)

The Danish Civil Registration System was established in 1968, and since then, all citizens have been registered with a unique personal identification number encoding date of birth and sex (26). Using this personal identification number as key identifier, we linked the study cohort to the Danish Medical Birth Registry, which contains computerized information about all births in Denmark since January 1, 1973 (27), to obtain information on any live birth for each woman in the cohort within a follow-up period of 12 months after the initial infertility diagnosis date.

Statistical Analysis

A multivariable logistic regression model was applied to estimate odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for the association between vitamin D exposure status and the chance of live birth within 12 months after first infertility diagnosis. All analyses were adjusted for the woman's age at primary infertility diagnosis.

To evaluate the robustness of the main result, a number of sensitivity analyses were performed. First, we performed an analysis using a more calendar-period-restricted study population. In this analysis, the exposed group comprised all women diagnosed with primary infertility from February 1, 1984, to May 31, 1985 (n = 1,696) only, and the unexposed group comprised all women diagnosed with primary infertility from September 1, 1986, to December 31, 1987 (n = 1,721) only. This analysis was performed to obtain a study group that was influenced by the marked increase in the number of IVF treatments in Denmark in the late 1980s (28). Second, we aimed to perform an analysis stratified for cause of infertility. However, throughout the study period, the vast majority of women were not registered with a specific cause of infertility, and most specific causes of infertility, including, e.g., endometriosis and tubal factor, were registered for only a very small number of women, which hampered any meaningful stratified analyses. Accordingly, only the association between vitamin D exposure status and the chance of live birth for women with anovulation was estimated. Third, we evaluated whether the timing of vitamin D fortification in relation to the time of year of conception affected the association between vitamin D exposure status and the chance of a live birth. For this purpose, we used the fact that vitamin D is virtually not synthesized during the dark winter months in Denmark from October to March. We hypothesized that the effect of vitamin D fortification on the chance of live birth may be more pronounced among women with conception dates in the winter months and consequently performed a sensitivity analysis stratified on date of conception in respectively a "dark" (winter: October 1–March 31) or a "light" (summer: April 1–September 30) period. Date of conception was calculated by subtracting 266 days from the child's date of birth as registered in the Medical Birth Registry. The 266 days was based on a pregnancy lasting for an average 280 days minus the average expected time between the last menstrual period and ovulation (14 days into the natural menstrual cycle). For all of the above-mentioned analyses, we also assessed the chance of live birth 15 and 18 months after first infertility diagnosis according to vitamin D exposure. Finally, to further investigate whether the observed decrease in the chance of live birth during the study period could be attributable to changes in vitamin D fortification, we used a linear regression model to estimate the beta coefficients (slopes) for the three birth cohorts with different exposures, using cutoff points at the end of the fortification (exposure) and wash-out periods. If no changes (i.e., virtually similar slopes in the exposure, wash-out, and non-exposure periods) were observed, it would suggest that the chance of a live birth has decreased steadily and was not affected by exposure to vitamin D fortification, whereas different slopes would suggest that changes in the chance of a live birth could be attributable to changes in vitamin D fortification (29). Level of statistical significance was set at 0.05 for all analyses. All analyses were conducted with the use of Stata 14.0 software.

Ethical Approval

The study was approved by the Local Ethical Committees for Copenhagen and Frederiksberg municipalities (J.nr. 01-298/96) and approved and registered in the local archive list DCS-DCRC-2100 of the Danish Cancer Society Research Center (J.nr. 2019-DCRC-0020).

RESULTS

Baseline characteristics for the study population are presented in Table 1. The entire study population comprised 16,212 women. A total of 6,313 women (39%) had their primary infertility diagnosis in the vitamin D fortification period from June 1, 1980, to May 31, 1985, 1,404 women (9%) had their infertility diagnosis in the 15-month wash-out period after the termination of the vitamin D fortification on May 31, 1985, and 8,495 women (52%) had their primary infertility diagnosis in the vitamin D–unexposed period from September 1, 1986, to August 31, 1991. The mean age at first infertility diagnosis was slightly lower in the exposed group compared with the wash-out period and the nonexposed period. A higher proportion of women in the exposed group had a live birth (15%) compared with women in the wash-out period (12%) and women in the nonexposed period (9%). Finally, the proportion of live births among women with conception during the dark period from October to March was consistently higher than during the light period from April to September in all three vitamin D exposure periods.

Table 2 presents the ORs for a live birth within 12 months after a primary infertility diagnosis, due to all causes or to anovulation only, according to vitamin D fortification exposure period. For the total study period (1980–1991), women who were diagnosed with infertility due to all causes during the vitamin D–exposed period had a higher chance of a live birth compared with women diagnosed with infertility during the nonexposed period (OR 1.87, 95% CI 1.68–2.08). For women diagnosed with infertility due to all causes during the wash-out period, the chance of a live birth was somewhat lower,

TABLE 1

Characteristics of the study population (n = 16,212) according to exposure to vitamin D fortification.[a]

	Vitamin D fortification period					
	Exposed period (June 1, 1980–May 31, 1985)		"Wash-out" period (June 1, 1985–August 31, 1986)		Nonexposed period (September 1, 1986–August 31, 1991)	
Characteristic	**n**	**%**	**n**	**%**	**n**	**%**
Total no. of women	6,313	38.9[b]	1,404	8.7[b]	8,495	52.4[b]
Age at first infertility diagnosis (y), median (interquartile range)	28.1 (25.4–31.0)		28.9 (26.0–31.6)		29.4 (26.8–32.4)	
Live birth within 12 months after an infertility diagnosis						
Yes	917	14.5[c]	174	12.4[c]	737	8.7[c]
No	5,396	85.5[c]	1,230	87.6[c]	7,758	91.3[c]
Period of conception						
October 1–March 31 ("dark period")	561	61.2[d]	114	65.5[d]	484	65.7[d]
April 1–September 30 ("light period")	356	38.8[d]	60	34.5[d]	253	34.3[d]

[a] The mandatory vitamin D fortification of margarine in Denmark was abruptly terminated on May 31, 1985.
[b] Percentages of total study population.
[c] Percentages within exposure groups.
[d] Out of all live births in the exposure group.

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but still higher compared with women with infertility in the nonexposed period (OR 1.52, 95% CI 1.27–1.81). For the restricted study period (1984–1987), this risk pattern remained, although the ORs for both the wash-out and the exposure periods were, as expected, somewhat attenuated compared with the total study period (Table 2). We also assessed the secular trends in the chance of a live birth within 12 months after a primary infertility diagnosis during the study period. The data suggested a stable trend of chance of a live birth during the exposed period (beta coefficient −0.0512), a steep trend of decrease during the wash-out period (beta coefficient −1.7085), and a small trend of decrease during the nonexposed period (beta coefficient −0.3708; Fig. 1).

TABLE 2

Adjusted odd ratios (ORs) and 95% confidence intervals (CIs) for the association between vitamin D fortification[a] and the chance of a live birth within 12 months after a woman's primary infertility diagnosis.

		Primary infertility diagnosis due to all causes			Primary infertility diagnosis due to anovulation		
Study period	**Vitamin D exposure period**	**Total no. of women**	**No. of women with a live birth**	**OR[b] (95% CI)**	**Total no. of women**	**No. of women with a live birth**	**OR[b] (95% CI)**
Total (June 1, 1980–August 31, 1991)	Nonexposed (September 1, 1986–August 31, 1991)	8,495	737	1 (reference)	325	37	1 (reference)
	Wash-out (June 1, 1985–August 31, 1986)	1,404	174	1.52 (1.27–1.81)	320	46	1.28 (0.80–2.03)
	Exposed (June 1, 1980–May 31, 1985)	6,313	917	1.87 (1.68–2.08)	1,378	231	1.58 (1.09–2.29)
Restricted (February 1, 1984–December 31, 1987)	Nonexposed (September 1, 1986–December 31, 1987)	1,721	176	1 (reference)	189	22	1 (reference)
	Wash-out (June 1, 1985–August 31, 1986)	1,404	174	1.24 (0.99–1.55)	320	46	1.23 (0.94–2.66)
	Exposed (February 1, 1984–May 31, 1985)	1,696	224	1.36 (1.10–1.68)	394	68	1.59 (0.94–2.67)

[a] The mandatory vitamin D fortification of margarine in Denmark was abruptly terminated on May 31, 1985.
[b] Adjusted for woman's age at infertility diagnosis.

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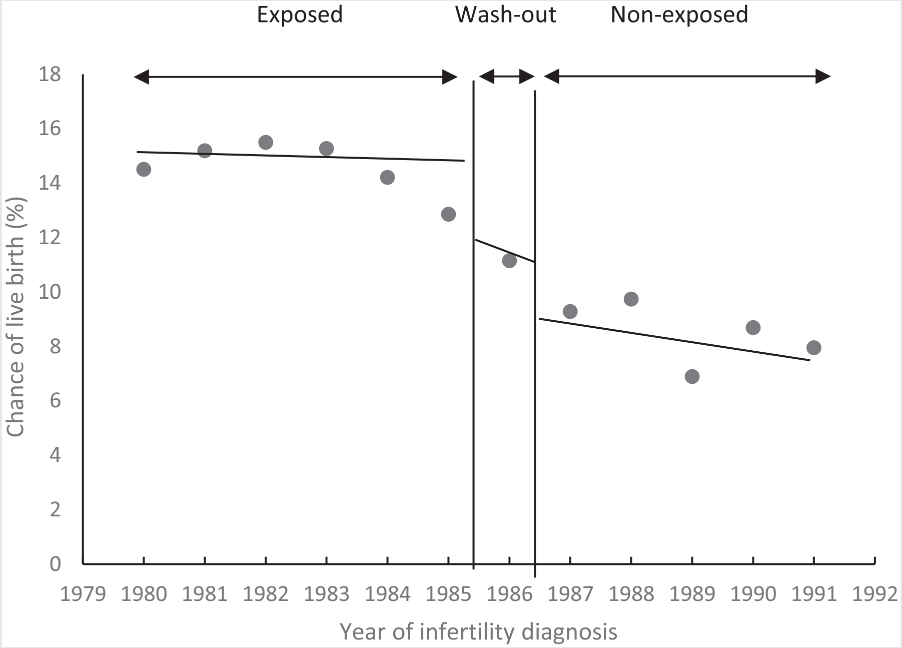
FIGURE 1



Secular trends in the chance of a live birth within 12 months after a primary infertility diagnosis during the study period, depicted by beta coefficients (slopes) for the three vitamin D exposure periods, using cutoff points at the end of the vitamin D fortification (exposure) and wash-out periods. Beta coefficients: vitamin D exposed period, −0.0512; wash-out period, −1.7085; non-exposed period, −0.3708.

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In general, we observed the same risk patterns for infertility due to anovulation as for infertility due to all causes, i.e., a drop in the ORs from the exposed to the wash-out exposure period. For the total study period, the OR for a live birth was higher among women diagnosed with anovulatory infertility in the exposed period compared with women diagnosed with infertility during the nonexposed period (OR 1.58, 95% CI 1.09–2.29), whereas the OR for a live birth was not different for women diagnosed with primary anovulatory infertility in the wash-out period compared with women diagnosed with infertility during the nonexposed period. For the restricted study period (1984–1987), the risk patterns were essentially similar (Table 2).

We also evaluated whether the timing of vitamin D fortification in relation to the time of year of conception affected the association between vitamin D exposure status and the chance of a live birth within 12 months after first infertility diagnosis (Table 3). In general, calendar period of conception (dark or light calendar period) did not markedly affect the association between vitamin D exposure and chance of live birth among women diagnosed with infertility due to all causes. The ORs were, however, generally slightly higher for women with date of conception in the light compared with the dark period.

For all the above analyses, we also assessed the chance of live birth 15 and 18 months after an infertility diagnosis. In general, the risk estimates were somewhat attenuated compared with the 12-month follow-up period, but the overall risk patterns were essentially similar (data not presented).

DISCUSSION

To our knowledge, this is the first study to investigate whether extra vitamin D from a nationwide mandatory fortification program influenced the chance of live birth among infertile women. By taking advantage of the unique societal intervention of the Danish national mandatory margarine vitamin D fortification program, which was abruptly terminated on May 31, 1985, results from our study indicated an 87% higher chance of live birth during a 12-month follow-up period among women with infertility exposed to extra vitamin D from fortification of margarine compared with women with infertility who were not exposed to extra vitamin D from fortification. Results from the intermediate wash-out period immediately after fortification of margarine ended showed a 52% higher chance of live birth. Similar, though slightly attenuated, estimates were obtained in analyses with a longer follow-up period up to 18 months and in analyses of women with anovulatory infertility only. Finally, calendar period of conception did not markedly affect the association between vitamin D exposure and chance of live birth among women with infertility.

A growing number of studies have examined the potential effect of vitamin D level on outcomes after ART. The majority of them have been included in two recent meta-analyses (15, 16). The most recent meta-analysis, by Chu et al. from 2018 (16), included the largest amount of studies and participants and showed that women replete in vitamin D were more likely to have a clinical pregnancy (OR 1.46, 95% CI 1.05–2.02; based on 11 studies with 2,700

TABLE 3

Adjusted odd ratios (ORs) and 95% confidence intervals (CIs) for the associations among vitamin D fortification,[a] calendar period of conception, and the chance of a live birth within 12 months after a woman's primary infertility diagnosis (all causes).

		Primary infertility diagnosis due to all causes		
Calendar period of conception	Vitamin D exposure period	Total no. of women	No. of women with a live birth	OR[b] (95% CI)
"Dark" calendar period (October 1–March 31)	Nonexposed (September 1, 1986–August 31, 1991)	8,242	484	1 (reference)
	Wash-out (June 1, 1985–August 31, 1986)	1,344	114	1.51 (1.22–1.87)
	Exposed (June 1, 1980–May 31, 1985)	5,957	561	1.73 (1.52–1.97)
"Light" calendar period (April 1–September 30)	Nonexposed (September 1, 1986–August 31, 1991)	8,011	253	1 (reference)
	Wash-out (June 1, 1985–August 31, 1986)	1,290	60	1.54 (1.15–2.06)
	Exposed (June 1, 1980–May 31, 1985)	5,752	356	2.15 (1.81–2.54)

[a] The mandatory vitamin D fortification of margarine in Denmark was abruptly terminated on May 31, 1985.
[b] Adjusted for woman's age at infertility diagnosis.

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participants) and a live birth (OR 1.33, 95% CI 1.08–1.65; based on 7 studies with 2,026 participants) compared with women deficient in vitamin D.

A single study has measured vitamin D serum levels in the Danish population over the same period as in the present study (30). That study, by Jacobsen et al. (30), assessed the association between neonatal vitamin D status and risk of type 1 diabetes and measured $25(OH)D_2$ and $25(OH)D_3$ levels in neonatal dried blood spots in 912 children with type 1 diabetes and 2,866 children without diabetes 1 born in Denmark from 1981 to 2002. It has been shown that vitamin D levels in newborns blood reflects the vitamin D status of their mothers (31), and the study by Jacobsen et al. (30) showed that the $25(OH)D_3$ concentrations increased slightly over the study period from $\sim$20 nmol/L in 1981 to $\sim$29 nmol/L in 2002 and that the increase appeared stronger until about 1985. However, the trends of increase were not statistically significantly different before and after the abandonment of the margarine fortification policy on May 31, 1985. It has been calculated that the fortification of margarine averaged 13% of the daily vitamin D intake among Danish adults during the fortification period (22). The amount of margarine consumed per week was stable during the exposed period from 1980 to 1985, being 308–322 g per person, equaling 3.85–3.99 μg vitamin D per person per day from fortified margarine (32). This may seem to be a small contribution, but in countries at northern latitudes, even small doses of additional vitamin D may be an important factor influencing the maintenance of sufficient vitamin D levels in humans because the cutaneous synthesis of vitamin D is practically absent in the winter season owing to very low exposure to ultraviolet light from the sun (17). Therefore, if vitamin D is associated with an increased chance of pregnancy, it is plausible that some seasonal variation could be expected. In fact, seasonal variation in conception rates, with higher rates observed in summer and early autumn, have been observed (33). Furthermore, recent studies have suggested that high vitamin D levels are associated with increased pregnancy rates in northern-latitude countries (34, 35), whereas such an association has been more difficult to find in southern-latitude countries (36, 37). In our subanalysis according to dark (October–March) and light (April–September) calendar periods, we attempted to account for the variation in vitamin D levels due to differences in sun exposure at different times of the year in a northern-latitude country like Denmark. Our a priori hypothesis was that a light period would eradicate any potential difference in vitamin D levels that the fortification of margarine brings, even if the fortification contributes only a part of total vitamin D intake. However, our results were essentially the same regardless of time of year of conception, and based on those results we therefore hypothesize that this may be due to the fact that the generally low sun exposure in Denmark made the fortification an important source of vitamin D regardless of the time of year.

In recent years, several biologic explanations for a potential influence of vitamin D on the chance of pregnancy and live birth have been proposed. Vitamin D receptors (VDRs) and enzymes involved in the metabolism of vitamin D have been shown in reproductive tissue, for example, in the endometrium (38) and ovaries (39). Furthermore, a positive effect of vitamin D on steroidogenesis in human ovarian cells has been suggested (39, 40) as well as on the secretion of hCG from the human syncytiotrophoblast (40). Furthermore, HOXA10 gene is expressed in response to sex steroids at the time of implantation in the human endometrium, and vitamin D may influence the endometrial receptivity by an autocrine pathway in gene transcription in endometrial stromal cells (41), thereby occupying a critical role in the matter of embryo implantation (42). HOXA10 expression has also been shown to be defective in several disorders related to infertility, e.g., adenomyosis (43). During pregnancy it has been suggested that vitamin D may influence the production of progesterone and estradiol in the human placenta (44). In contrast to the overall findings from our study on infertile women, the few recent studies on spontaneous chance of pregnancy and vitamin D levels have provided somewhat conflicting results. Two studies found higher vitamin D levels to be associated with an increased chance of pregnancy (11, 12), whereas two others suggested no marked association between serum vitamin D concentration and chance of pregnancy (13, 14). It may therefore be hypothesized that the above-mentioned biologic mechanisms linking vitamin D status to chance of pregnancy and live birth is of less importance among generally healthy women conceiving spontaneously, but may be more closely associated with reproductive success among women with infertility.

Our study has several strengths. In general, the nationwide design and large study size resulted in highly precise risk estimates, including in subgroup analyses. The study design mimicked randomization by using a fixed time point (initiation of margarine vitamin D fortification) to completely separate exposed from nonexposed infertile women. Fertility status was based on data from the Danish Infertility Cohort, with multiple sources to identify infertile women, thus minimizing the number of women with misclassification for this variable to a very low level. Finally, the analysis of secular trends in chance of a live birth within 12 months after a primary infertility diagnosis during the study period showed that the chances of a live birth differed markedly (i.e., different slopes) among the three periods (vitamin D fortification period, wash-out period, and nonexposed period). Thus, the different slopes suggest that the observed changes in the chance of a live birth could be attributable to changes in vitamin D fortification and that our findings may represent a true association between the exposure (vitamin D) and the outcome (live birth).

This study also has some limitations. First, we have no knowledge of what proportion of the study women received fertility treatment, because this information is not available in the Danish Registries before the Danish IVF Registry was established in 1994. However, the timing of the study period allowed us to largely avoid the potential influence on fertility of the introduction of IVF treatment in the late 1980s in Denmark. Second, even though we were able to perform analyses stratified for causes of infertility, we had no information on length of unwanted childlessness, which may affect the chance of a live birth, but that information is not available in the Danish registries. Third, we had no information on dietary patterns and potential supplement intake of vitamin D at the individual level in either of the study groups, nor did our data include important potential confounders such as tobacco smoking, body mass index, ethnicity, and socioeconomic status, leaving room for residual confounding (45). Fourth, besides vitamin D, margarine was also fortified with vitamins A and E in Denmark during the 1980s (22). The fortification with vitamin A continued during the entire study period, whereas vitamin E fortification was terminated at the same time as the vitamin D fortification (1985), which may slightly have confounded our results. Fifth, we did not directly measure blood levels of vitamin D in the affected women. Finally, it is possible that women and couples seeking fertility treatment during the study period may represent a self-selected part of the infertile population, but such self-selection is unlikely to be associated with use of vitamin D–containing products.

In conclusion, our results show that infertile women exposed to extra vitamin D from margarine owing to a population-based fortification policy had an increased chance of live birth within a 12–18-month follow-up period compared with infertile women not exposed to extra vitamin D, independently from the time of year of conception. Our results thus support our hypothesis that infertile women with sufficient vitamin D levels have an increased chance of live birth. Further studies on potential biologic mechanisms linking vitamin D to chance of live birth in infertile women are needed, as are studies to determine who may benefit from supplementation with vitamin D and to determine what the optimal dose is for those in need of vitamin D supplementation.

Acknowledgments: The authors gratefully acknowledge the assistance of Helle Hulegaard Sørensen for data management.

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