# Supplementary material

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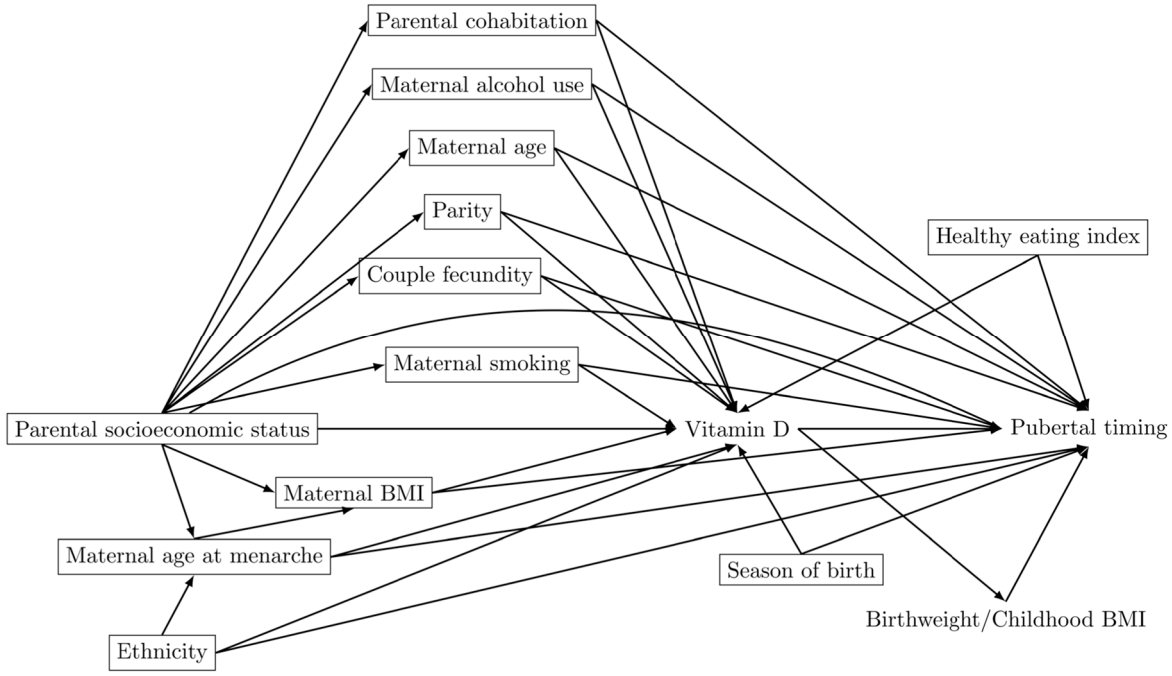
**Supplementary Figure S1: Directed acyclic graph illustrating the assumed causal framework of the study on maternal intake of vitamin D supplements in mid-pregnancy and pubertal timing in the children.**




Abbreviations: BMI, body mass index

Boxes indicate conditioning. Ethnicity was adjusted for by design, since the Danish National Birth Cohort consisted of primarily Caucasians. Healthy eating index was adjusted for in a sensitivity analysis. The potential mediation of birthweight and childhood BMI was investigated in sensitivity analyses.

**Supplementary Table S1: Maternal intake of vitamin D according to participation in the Puberty Cohort**

Maternal intake of vitamin D supplements according to participation in the Puberty Cohort for 17,392 participants with information from the food frequency questionnaire in mid-pregnancy, Denmark, 2000 – 2021

| | Participants | Non-participants |
|---|---|---|
| | n = 12,991 | n = 4,401 |
| Mean vitamin D supplements in µg/day (SD) | 7.6 (0.04) | 7.9 (0.08) |
| Mean vitamin D from foods in µg/day (SD) | 3.6 (0.02) | 3.5 (0.04) |
| Prevalence of early pregnancy vitamin D supplement intake | 10.6% | 10.2% |

Abbreviations: SD, Standard deviation

**Supplementary Table S2: Maternal intake of vitamin D supplements in mid-pregnancy according to maternal early pregnancy supplement intake**

Distribution of maternal intake of vitamin D supplements (µg/day) in mid-pregnancy according to maternal early pregnancy supplement intake among 12,991 children from the Puberty Cohort, Denmark, 2000 – 2021

| Early pregnancy vitamin intake | Mean maternal intake of vitamin D supplements in µg/day (SD) in mid-pregnancy |
|---|---|
| Vitamin D with/without calcium | 9.1 (6.1) |
| Multivitamin | 7.9 (4.1) |
| Other vitamin | 5.4 (4.6) |
| No vitamin | 6.4 (4.7) |

Abbreviations: SD, Standard deviation

**Supplementary Text S1: Correlation between self-reported intake of vitamin D and plasma 25(OH)$D_3$**

It was possible to assess the correlation between self-reported intake of vitamin D and 25(OH)$D_3$ in The Fetal Programming of Semen Quality (FEPOS) cohort, which represents a sub population of the Danish National Birth Cohort (DNBC). The FEPOS is a nested cohort in the DNBC. There were no overlap between FEPOS and the Puberty Cohort.

The FEPOS cohort was established as a male-offspring cohort within the DNBC in 2017 (1). If the mothers had completed the first two questionnaires in the DNBC in addition to having a gestational blood sample stored in the biobank, sons aged 18 years and 9 months and living in the area of Aarhus or Copenhagen were eligible for invitation. Between March 2017 and December 2019, 5,697 adult sons born 1998 – 2000 were invited among the 21,623 eligible for participation in FEPOS. In total, 1,058 sons participated (19%). Further details are described in the cohort profile paper (2).

*Maternal 25(OH)$D_3$*

The plasma from the gestational blood samples obtained from the mothers of the sons included in FEPOS was stored at -80°C in the Danish National Biobank, Copenhagen, Denmark. As some cryotubes contained too little plasma for analysis, a total of 827 plasma samples was analysed for 25-hydroxyvitamin $D_3$ (25(OH)$D_3$) from 2019 – 2020.

Quantitative analysis of 25(OH)$D_3$ was performed using two-dimensional liquid chromatography tandem mass spectrometry (LC-MS/MS; QTRAP 6500+; AB Sciex, Framingham, MA, USA) at the Division of Occupational and Environmental Medicine, Lund University. The method is previous described in detail (3). Quality controls met given standards.

*Correlation between self-reported intake of vitamin D and 25(OH)$D_3$*

Ordinary least square linear regression models and Pearson's correlation coefficient with 95% confidence intervals (CIs) were used to assess the correlation between maternal 25(OH)$D_3$ level in plasma and self-reported information on i: intake of vitamin D from supplements; ii: intake of vitamin D from diet; and iii: total dietary intake of vitamin D. For a subset of the mothers having information on gestational 25(OH)$D_3$ level, we had self-reported information on intake of vitamin D from diet and from dietary supplementations (n = 598). This information

was derived from the food-frequency questionnaire (FFQ) provided in gestational week 25, as described in the main text.

A higher intake of vitamin D from supplements was associated with a higher maternal plasma level of 25(OH)$D_3$ of 0.79 (95% CI: 0.36; 1.23) nmol/L/µg/day. Pearson's correlation coefficient was 0.14 (95% CI: 0.06; 0.21). A higher intake of vitamin D from diet was not associated with a higher maternal plasma level of 25(OH)$D_3$ (0.0 (95% CI: -0.61; 0.52)). Pearson's correlation coefficient was 0.0 (95% CI: -0.08; 0.08). A higher total dietary intake of vitamin D from diet and supplement combined was associated with a higher maternal plasma level of 25(OH)$D_3$ of 0.46 (95% CI: 0.12; 0.80) nmol/L/µg/day. Pearson's correlation coefficient was 0.11 (95% CI: 0.03; 0.18).

Supplements with vitamin D may therefore correlate better with bioavailable vitamin D than vitamin D from diet as reported in the FFQ in this population.

**References**

1. Olsen J, Melbye M, Olsen SF, Sorensen TI, Aaby P, Andersen AM, et al. The Danish National Birth Cohort--its background, structure and aim. Scand J Public Health. 2001; 29:300-307.

2. Keglberg Hærvig K, Bonde JP, Ramlau-Hansen CH, Toft G, Hougaard KS, Specht IO, et al. Fetal Programming of Semen Quality (FEPOS) Cohort - A DNBC Male-Offspring Cohort. Clin Epidemiol 2020: 757-770.

3. Gaml-Sørensen A, Brix N, Hærvig KK, Lindh C, Tøttenborg SS, Hougaard KS, et al. Maternal vitamin D levels and male reproductive health: a population-based follow-up study. Eur J Epidemiol. 2023.