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ORIGINAL CONTRIBUTION

Protein-rich food intake and risk of spontaneous abortion: a prospective cohort study

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Abstract

Purpose Diet quality is increasingly recognized as important for human reproductive capacity. We studied the association between intake of protein-rich foods and risk of spontaneous abortion (SAB).

Methods During 2013–2020, we recruited pregnancy planners from the United States and Canada (Pregnancy Study Online; PRESTO) and Denmark (SnartForaeldre.dk; SF). Participants completed a baseline questionnaire and a validated cohort-specific food frequency questionnaire. We estimated preconception intake of red meat, poultry, processed meat, seafood, eggs, plant-based proteins, and dairy from individual foods and mixed recipes. We included 4,246 PRESTO and 2,953 SF participants who reported a pregnancy during the study. Data on SAB were derived from questionnaires and population registries. We used Cox proportional hazards regression to estimate hazard ratios (HRs) and 95% confidence intervals (CI), representing the effect of substituting one type of protein-rich food for another.

Results SAB risk was 23% in PRESTO and 16% in SF. In PRESTO, substitution of seafood with other protein-rich foods was associated with higher SAB risk [for example, the HR for replacing 100 g of seafood/week with 100 g of red meat was 1.10 (95% CI 1.00, 1.20)]. In contrast, in SF, substituting seafood with other protein-rich foods was associated with lower SAB risk [HR for replacing 100 g of seafood/week with 100 g of red meat was 0.89 (95% CI 0.82, 0.98)]. Other protein-rich food substitutions were not meaningfully associated with SAB risk.

Conclusions Preconception intake of protein-rich foods was largely unrelated to SAB risk, with the exception of seafood, which was associated with higher risk of SAB in Denmark, but a lower risk in North America.

Keywords Meat · Preconception cohort · Pregnancy loss · Protein · Seafood · Spontaneous abortion

Introduction

Spontaneous abortion (SAB) is the natural death of an embryo or fetus before it is able to survive independently [1]. Estimates of the proportion of clinically-recognized pregnancies that end in SAB range from 11 to 22% [2–5], but the true incidence of post-implantation SAB is higher (> 30%) [6]. According to estimates from a nationally-representative study, incidence of SAB in the United States increased by 1% per year between 1990 and 2011 [2], but it is unclear whether this is due to improved sensitivity of home pregnancy tests over time or truly higher risk of loss. The consequences of SAB are both physical and psychological, and SAB is a risk factor for future obstetric complications and long-term health [7]. Therefore, identification of modifiable risk factors to reduce incidence of SAB is an important public health goal.

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Diet quality is increasingly recognized as an important determinant of human reproductive capacity [8]. Most research on diet and reproduction has focused on dietary patterns. For example, adherence to a Mediterranean diet has been associated with lower risk of SAB among couples undergoing in vitro fertilization [9–11]. While studies of dietary patterns are informative in documenting that overall diet quality is relevant for SAB, it is unclear from these studies which specific components of the diet are most relevant to the outcome. A recent focus in nutritional epidemiology emphasizes the utility of examining diet via substitution models, wherein increased intake of a particular food comes at the expense of another food, while total energy intake remains stable [12]. Analysis of food substitution can provide informative data on food choice and dietary composition for individuals, and can inform public health policy.

Protein-rich foods vary widely in nutritional composition and environmental contamination and, unsurprisingly, different types of protein-rich foods show varying associations with chronic disease risk. For example, seafood intake is related to lower risk of cardiovascular disease [13], whereas red meat intake is associated with higher risk [14]. There has been limited study of intake of protein-rich foods and reproductive outcomes. In the Environment and Reproductive Health (EARTH) study, a cohort study of 357 couples undergoing fertility treatment at a Massachusetts hospital, higher seafood intake (when eaten in place of other protein-rich foods) was associated with improved fertility treatment outcomes [15]. Greater red meat intake was also associated with improved treatment outcomes, whereas egg intake was associated with poorer outcomes.

Because the etiology of SAB may differ for pregnancies conceived spontaneously relative to those conceived with the use of fertility treatment [16], it is important to study the association between diet and SAB in a cohort of couples conceiving spontaneously. Herein, we examined the association between intake of protein-rich foods and SAB risk in two interrelated preconception cohorts of pregnancy planners residing in North America and Denmark.

Subjects and methods

Study design

Data were derived from two web-based prospective preconception cohort studies: Pregnancy Study Online (PRESTO) in the United States and Canada [17], and SnartForældre.dk (SF) in Denmark [18]. Eligible women were aged 21–45 years (PRESTO) or 18–45 years (SF) and attempting to conceive without fertility treatment. Participants completed a baseline questionnaire on socio-demographics, lifestyle, and reproductive and medical factors, as well as bimonthly follow-up questionnaires for up to 12 months to identify pregnancies. Ten days after enrollment, women were invited to complete a validated food frequency questionnaire (FFQ) in each cohort: the National Cancer Institute's Diet History Questionnaire II (DHQ II) [19] in PRESTO and an FFQ designed specifically for SF (SF-FFQ) [20]. Women who reported a conception on their follow-up questionnaire were asked to complete an early pregnancy questionnaire. In PRESTO, women completed a late pregnancy questionnaire at approximately 32 weeks' gestation. In SF, women provided their Civil Personal Registration (CPR) number, a ten-digit unique identifier assigned to all residents of Denmark, permitting linkage to population registries used to identify pregnancy outcomes [21].

From June 2013 through June 2020, 11,978 women completed the PRESTO baseline questionnaire. We restricted our sample to women who reported a conception during 12 months of follow-up ($n = 6325$). We excluded 1,764 women who did not complete the FFQ (28% did not complete), 96 women with reported energy intake < 600 or > 3800 kcal/day and 219 women who completed the FFQ at least six weeks into their pregnancies or after experiencing an SAB (to reduce the potential for reverse causation). Our analytic sample included 4246 women (Fig. 1). This includes pregnancies conceived spontaneously and via assisted reproductive technologies.

From August 2011 through September 2020, 7587 eligible women completed the SF baseline questionnaire. We excluded 2388 women who did not complete the FFQ (23% did not complete among those who were invited). We restricted to women who reported a pregnancy on a questionnaire during follow-up ($n = 3,085$), and excluded 78 women with reported energy intake < 600 or $> 3,800$ kcal/day and 54 women who completed their FFQ at least six weeks into their pregnancies or after experiencing an SAB (to reduce the potential for reverse causation). Our analytic sample included 2953 women (Fig. 1). This number includes pregnancies conceived spontaneously and via assisted reproductive technologies.

The institutional review board at the Boston University Medical Campus approved the study protocol. SF is registered at Aarhus University to comply with Danish law on data protection. All participants provided online informed consent.

Assessment of diet

In both cohorts, intake of protein-rich foods was assessed using the nutrient composition of individual foods and mixed recipes reported on the cohort-specific FFQs. All questions were asked with respect to the previous year. We estimated intake of red meat, poultry, processed meat, seafood, eggs, plant-based proteins (nuts, seeds, legumes, and

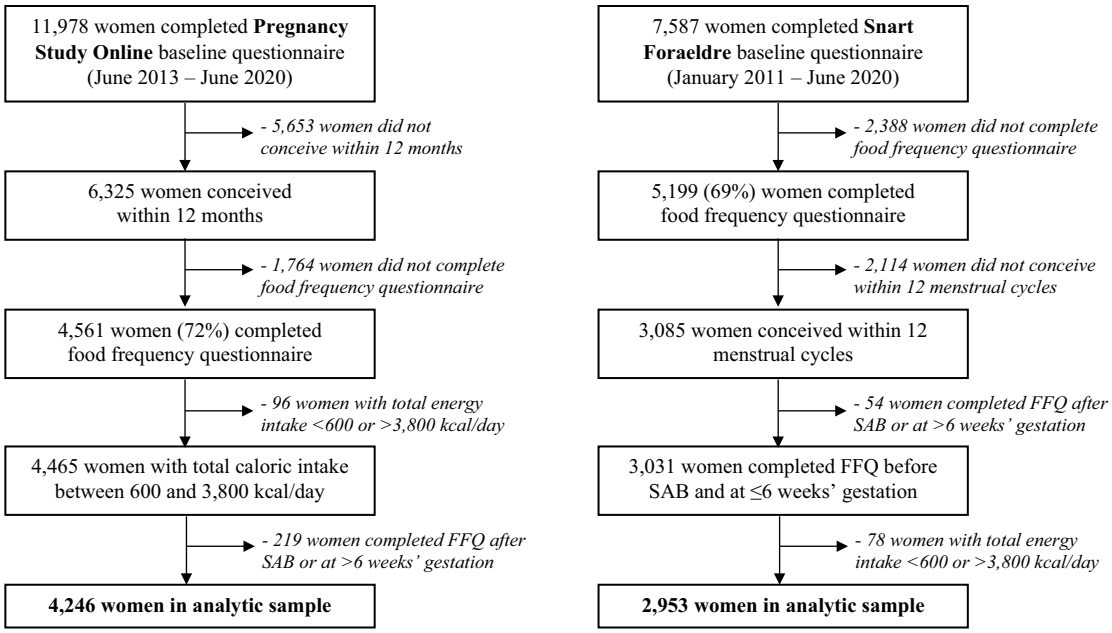


Fig. 1 Flow chart showing the exclusion criteria and final analytic sample for Pregnancy Study Online (left) and SnartForaeldre.dk (right)

soy), and dairy (milk, cheese, and yogurt). In PRESTO, the deattenuated correlation coefficients comparing data from the DHQ with repeated daily food diaries ranged from 0.45 (eggs) to 0.79 (red meat) [22], and was 0.60 for total protein [19]. In SF, when comparing the SF-FFQ data with a 4-day food diary, deattenuated correlation coefficients ranged from 0.49 (high-fat dairy) to 0.75 (seafood) and was 0.56 for total protein [20].

Assessment of spontaneous abortion

In both cohorts, we used data from self-administered questionnaires to identify SAB, defined as a pregnancy loss before 20 weeks' gestation. On follow-up questionnaires, we asked women the date of their last menstrual period (LMP), if they were currently pregnant, and if they had experienced any pregnancy losses since their last questionnaire. Currently pregnant women were directed to the early pregnancy questionnaire, where we asked for the date of first positive pregnancy test, method of pregnancy confirmation, and pregnancy due date.

In PRESTO, we obtained additional information on pregnancy loss via the late pregnancy questionnaire, which was sent to participants at 32 weeks' gestation. On this questionnaire, we asked women if they were still pregnant, and if not, why (response options: "I had a miscarriage or a chemical pregnancy", "I had an induced or therapeutic abortion", "I had an ectopic pregnancy", "I had a blighted ovum", "I had a stillbirth", and "I already had my baby". We defined SAB as miscarriage, chemical pregnancy, or blighted ovum. If women reported a pregnancy loss on any questionnaire, we asked how long the pregnancy lasted and the date of the loss. We attempted to identify outcome information on women who were lost to follow-up by searching on social media, for online baby registries, or by contacting women via email or telephone. If we were able to contact a participant, we asked if she was pregnant and if she had experienced any intervening pregnancy loss, including the date of the loss and weeks' gestation at the time of the loss. Finally, we identified women who did not experience SAB by linking participant data with birth registries in select states (California, Florida, Massachusetts, Michigan, Ohio, Pennsylvania, and Texas) [23]. If we found a live birth in the registry that corresponded with an LMP date during the study period and the participant was lost to follow-up, we assumed that the woman had a study-related birth.

In SF, we linked participant CPR numbers to the Danish National Patient Registry (DNPR) and the Danish Medical Birth Registry (DMBR) [23]. The DNPR provides information on inpatient and outpatient diagnoses and services, including labor and delivery, SAB, induced abortion, and weeks' gestation at pregnancy loss. The DMBR contains information about live births and stillbirths after 22 weeks' gestation. We used International Classification of Disease 10th edition codes O03, O020, O021 and O022 for SAB, O04-O06 for induced abortions, O00 for ectopic pregnancies, and O01 for molar pregnancies. Women who were only registered with code O08 (complications after abortion, type unspecified) were excluded because we could not ascertain the pregnancy outcome or gestational age at loss.

The positive predictive value of SAB diagnosis in the DNRP is 97.4% [24]. Registry data were available through the end of 2018; women with self-reported conceptions after July 30, 2018 (20 weeks before December 31, 2018) were censored at their weeks' gestation at last contact. When we had data from both the registry and self-report, we prioritized the registry data.

Assessment of covariates

We collected covariate information on the baseline questionnaire, including socio-demographic characteristics (age, race/ethnicity, education, income), lifestyle factors (caffeine, alcohol, smoking, sugar-sweetened soda, body mass index, physical activity, multivitamin or supplement intake), and reproductive history (parity, last method of contraception).

Statistical analysis

We conducted a time-to-event analysis with gestational weeks as the time scale. We used Cox proportional hazards models to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). We first fit restricted cubic splines to assess the shape of the association between intake of protein-rich foods and SAB, estimating the effect of increasing intake of a particular type of protein while holding all other types of protein and total energy intake constant (in other words, substituting a particular type of protein for fats and carbohydrates). We then estimated the effect of increasing one type of protein-rich food at the expense of another, holding total protein intake and total energy intake constant.

We began follow-up from the weeks' gestation at first positive pregnancy test (when available), or 4 weeks' gestation, otherwise. We ended follow-up at (a) the weeks' gestation of SAB, induced abortion, or ectopic pregnancy for women who experienced these outcomes, (b) the weeks' gestation at last contact for women who were lost to follow-up during their pregnancy, or (c) 20 weeks' gestation for women who did not experience a pregnancy loss.

We selected potential confounders a priori based on a directed acyclic graph (Online Resource 1). Because we measured diet during preconception, we examined covariates collected at baseline rather than early pregnancy, to ensure that covariates were not on the causal pathway between diet and SAB. We adjusted for total energy intake, age (< 25, 25–29, 30–34, 35–39, ≥ 40 years), education (≤ 12, 13–15, 16, ≥ 17 years), household income (< $50,000, $50,000-$99,999, $100,000-$149,999, ≥ $150,000 USD/year or < 25,000, 25,000–39,999, 40,000–79,999, ≥ 80,000 DKK/month), pre-pregnancy BMI (< 25, 25–29, 30–34, ≥ 35 kg/m^2), physical activity (< 10, 10–19, 20–39, ≥ 40 MET-hours/week), smoking history (never, former, current occasional, current regular), alcohol intake (drinks/week), multivitamin or folic acid intake, parity, individual components of diet quality scores (Healthy Eating Index-2010 [HEI-2010] score in PRESTO [25] and Danish Dietary Guidelines Index [DDGI] score in SF [26]), and caffeine intake. In PRESTO, we additionally adjusted for race/ethnicity (non-Hispanic white, non-Hispanic Black, non-Hispanic Asian, non-Hispanic other race, Hispanic), which we conceptualized as a proxy for exposure to structural racism which determines access to healthy foods and societal resources. Additional adjustment for census tract-level median household income (available for women whose addresses could be geocoded) and residence in a USDA-defined food desert (an area in which it is difficult to access healthy food; available for U.S. women) did not meaningfully change measures of association; therefore, these variables were left out of final models.

We stratified final models by BMI (< 25 vs. ≥ 25 kg/m^2), with the hypothesis that associations might be stronger among overweight women who are higher risk of SAB. In addition, because the causes of early and late losses may differ [27], we compared the overall results with models restricted to < 8 weeks' gestation. We conducted sensitivity analyses restricting to women who conceived within 90 days of completing their FFQ and women who had been trying to conceive for < 3 menstrual cycles at study entry (to minimize the possibility of reverse causation).

We used multiple imputation to account for missing data on covariates and outcome using fully conditional specification methods [28]. In PRESTO, gestational weeks at pregnancy loss were missing for 5% of women; covariate missingness ranged from < 1% (physical activity) to 3% (income). In SF, gestational weeks at pregnancy loss were missing for < 1% of women; covariate missingness ranged from 1% (BMI and education) to 5% (income). We generated twenty imputation data sets and statistically combined estimates and standard errors across data sets.

Results

Most participants in both cohorts identified as non-Hispanic white, had ≥ 16 years of education and had an annual household income of more than $75,000 USD/year or 40,000 DKK/month. Mean body mass index was 26.5 kg/m^2 (SD = 6.4) in PRESTO and 24.1 kg/m^2 (SD = 4.8) in SF. Forty-nine percent of PRESTO women had been pregnant before and 32% had a previous live birth. In SF, 55% of women had been previously pregnant and 39% had a previous live birth.

The distribution of intake of each type of protein-rich food is shown in Online Resource 2. Participant responses to FFQ items related to intake of protein rich foods is shown in Online Resource 3 (PRESTO) and Online Resource 4 (SF). In PRESTO, consumption of specific types of protein-rich

food was strongly related to sociodemographic characteristics and factors related to a healthy lifestyle (Table 1). For instance, red meat intake was inversely associated with age, census tract median household income, personal household income, education, physical activity, daily multivitamin intake, and HEI-2010 score, and positively associated with living in a food desert, BMI, cigarette smoking, caffeine intake, and parity. Opposite associations were observed for seafood intake in PRESTO (e.g., seafood intake was positively associated with education and inversely associated with BMI). In SF, correlations between intake of protein-rich foods and sociodemographic or lifestyle factors tended to be weak (Table 2).

The proportion of women who experienced a SAB was 23% in PRESTO and 16% in SF, estimated using life-table methods to account for censoring; however, gestational week-specific estimates of SAB risk were similar across cohorts (Fig. 2). Median gestational weeks at loss was 6 in both cohorts (interquartile range: 5–9 weeks). In PRESTO, 67% of SABs were reported on a follow-up or early pregnancy questionnaire, 32% were reported on the late pregnancy or postpartum questionnaires, and 1% were identified based on discrepancies between LMP dates and infant birth dates in the birth registry or were self-reported by the participant via email or telephone. The follow-up and early pregnancy questionnaires were more likely to identify early losses (< 8 weeks' gestation), whereas the late pregnancy and postpartum questionnaires were more likely to identify later losses. In SF, 63% of SABs were identified via questionnaire only, 22% were found in the registry only (SABs occurring after completion of the early pregnancy questionnaire), and 15% were identified via questionnaire and registry. The distribution of the timing of loss for losses reported on the follow-up or early pregnancy questionnaires in SF were similar to that in PRESTO. Late losses (≥ 8 weeks' gestation) were more likely to be identified in the registry than reported on the follow-up or early pregnancy questionnaires.

Associations between intake of protein-rich foods and SAB varied across cohorts (Online Resource 5). Restricted cubic splines (Fig. 3) showed roughly linear associations for most protein-rich foods, with the exception of egg intake in SF. In PRESTO, increasing intakes of red meat and eggs were associated with slightly higher hazard of SAB, whereas increasing intake of seafood was associated with lower

Table 1 Distribution of baseline characteristics by preconception weekly intake of red meat and seafood, Pregnancy Study Online

Characteristic[a]	Red meat intake (g/wk)				Seafood intake (g/wk)			
	< 100	100–199	200–399	≥ 400	< 50	50–99	100–199	≥ 200
Number	1131	1486	1290	339	1891	1046	893	416
Age (year), mean	30.2	30.0	29.9	29.4	29.5	30.3	30.4	30.5
Race/ethnicity, %								
Non-Hispanic white	86.8	88.7	88.5	83.4	89.2	86.7	87.1	85.3
Non-Hispanic Black	2.3	1.7	1.1	0.9	1.6	1.6	1.9	1.3
Non-Hispanic Asian	1.8	1.5	1.5	2.1	1.2	1.5	1.7	3.3
Non-Hispanic mixed/other race	3.7	3.5	3.9	4.5	3.2	3.6	4.0	5.2
Hispanic	5.5	4.7	5.0	9.1	4.8	6.5	5.4	4.9
≤ 12 year education, %	14.0	18.0	20.3	33.4	22.7	17.5	14.8	14.8
Annual household income < 50,000 U.S. dollars, %	11.0	14.3	14.2	20.9	15.9	13.8	10.0	12.4
Census tract median household income (U.S. dollars)[b]	67,000	67,400	65,700	60,500	64,600	66,900	67,600	69,200
Census tract defined as food desert, %[c]	7.4	8.0	9.8	12.5	10.0	8.3	7.2	6.0
Body mass index (kg/m^2), mean	25.3	26.3	27.4	28.9	27.1	26.3	26.0	25.9
Physical activity (metabolic equivalent of task-h/week), mean	38.1	35.1	34.0	31.8	32.9	35.0	37.9	41.5
Current regular cigarette smoker, %	2.3	2.8	4.6	7.2	4.5	3.0	3.5	1.6
Alcohol intake (drinks/week), mean	3.1	3.2	3.2	3.3	2.6	3.5	3.6	3.8
Caffeine intake (mg/day), mean	119	123	133	145	117	130	140	134
Daily multivitamin/folic acid intake, %	88.1	86.5	85.6	79.9	84.4	85.9	88.7	89.5
% Energy from protein, mean	14.7	15.7	16.4	17.3	15.2	15.8	16.4	17.3
Healthy Eating Index 2010, mean	69.8	66.8	64.4	62.1	64.0	67.0	69.1	70.9
Total energy intake (kcal/day), mean	1340	1480	1700	2140	1450	1570	1690	1834
Parous, %	26.4	31.1	35.3	40.9	34.6	33.2	29.5	23.0

[a]Characteristics are standardized to the age distribution of the cohort

[b]Restricted to participants whose addresses could be geocoded

[c]Restricted to participants from the U.S.

Table 2 Distribution of baseline characteristics by preconception weekly intake of red meat and seafood, SnartForaeldre.dk

Characteristic[a]	Red meat intake (g/week)				Seafood intake (g/week)			
	< 100	100–199	200–399	≥ 400	< 50	50–99	100–199	≥ 200
Number	158	171	1087	1537	361	594	1217	566
Age (years), mean	29.7	29.7	29.7	29.6	29.0	29.6	29.6	30.0
≤ 12 years of education, %	6.1	5.5	3.3	4.8	8.9	2.9	4.1	3.7
Monthly household income < 25,000 Danish kroner, %	9.2	13.3	10.1	8.6	8.3	10.8	9.4	9.3
Body mass index (kg/m^2), mean	23.2	22.9	23.7	25.0	25.3	24.4	24.0	24.1
Physical activity (metabolic equivalent of task-hours/week), mean	75.1	67.2	58.9	63.5	66.9	59.4	58.0	69.3
Current regular cigarette smoker, %	3.7	2.8	5.0	6.2	7.8	6.4	5.4	3.6
Alcohol intake (drinks/week), mean	2.9	2.9	2.6	2.6	2.7	2.5	2.6	2.7
Caffeine intake (mg/day), mean	206	189	191	176	166	174	188	192
Daily multivitamin/folic acid intake, %	74.7	71.9	74.0	70.8	69.3	70.8	72.1	74.8
% Energy from protein, mean	13.2	14.8	16.1	16.8	15.4	16.3	16.3	16.8
Danish Dietary Guidelines Index, mean	4.0	4.5	4.4	3.9	3.5	3.8	4.1	4.6
Total energy intake (kcal/day), mean	1555	1758	1823	2019	1598	1782	1895	2170
Parous, %	33.3	28.1	34.6	39.5	33.9	35.9	37.6	36.4

[a]Characteristics are standardized to the age distribution of the cohort

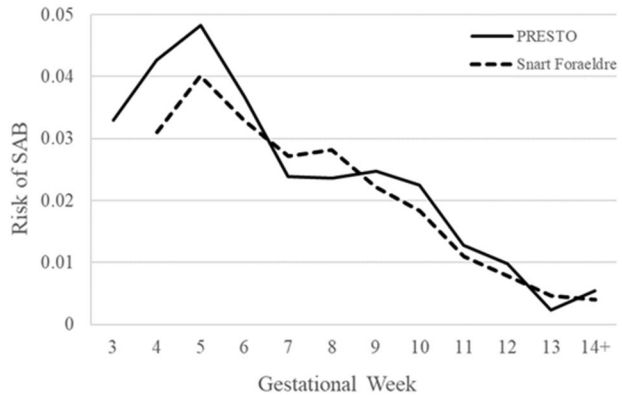


Fig. 2 Risk of SAB by gestational week, Pregnancy Study Online (solid line) and SnartForaeldre.dk (dashed line)

hazard of SAB. On the other hand, in SF, processed meat and seafood were associated with higher hazard, whereas egg and dairy intakes were associated with slightly lower hazard of SAB.

In PRESTO, substitution of seafood with other types of protein-rich foods was generally associated with higher risk of SAB (Fig. 4). For example, replacing 100 g of seafood/week with 100 g of red meat was associated with 1.10 times the hazard of SAB (95% CI: 1.00, 1.20). In contrast, in SF, substituting seafood with other types of protein-rich foods was associated with lower SAB risk. Replacing 100 g of seafood/week with 100 g of red meat was associated with 0.89 times the hazard of SAB (95% CI 0.82, 0.98). In PRESTO, egg intake was associated with slightly higher risk of SAB when substituted for other protein sources, whereas the opposite was true in SF. The hazard of SAB did not change substantially when substituting other types of protein-rich foods with each other.

Associations between seafood intake and SAB in PRESTO were stronger when we restricted to women who conceived within 90 days of completing the FFQ; the association between seafood intake and SAB in SF was approximately null when we made the same restriction (Online Resource 6). Associations did not differ appreciably in either cohort when restricting to women who had been attempting to conceive for < 3 cycles at study entry (Online Resource 6).

The inverse association between seafood intake and SAB risk in PRESTO was stronger among women with BMI < 25 kg/m^2, compared with BMI ≥ 25 kg/m^2 (Online Resource 7). Other associations in PRESTO and all associations in SF were similar across strata of BMI (Online Resource 7). In both cohorts, results were similar when restricted to early SAB (Online Resource 8).

Discussion

In parallel analyses of two preconception cohort studies from North America and Denmark, associations between preconception intake of protein-rich foods and SAB risk varied by cohort and type of food. Intake of seafood was associated with increased risk of SAB in Denmark, but lower risk of SAB in North America. Egg intake was

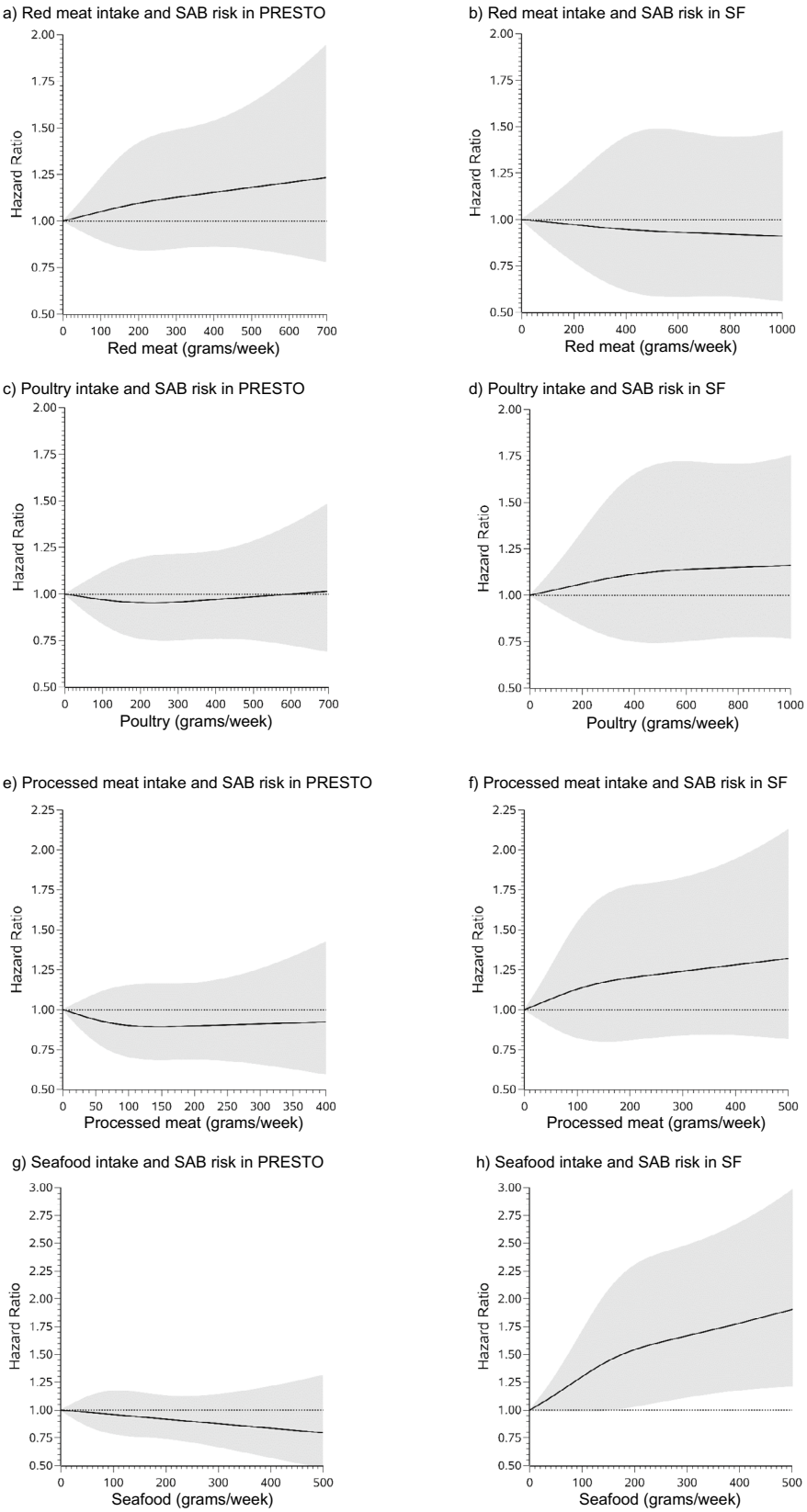
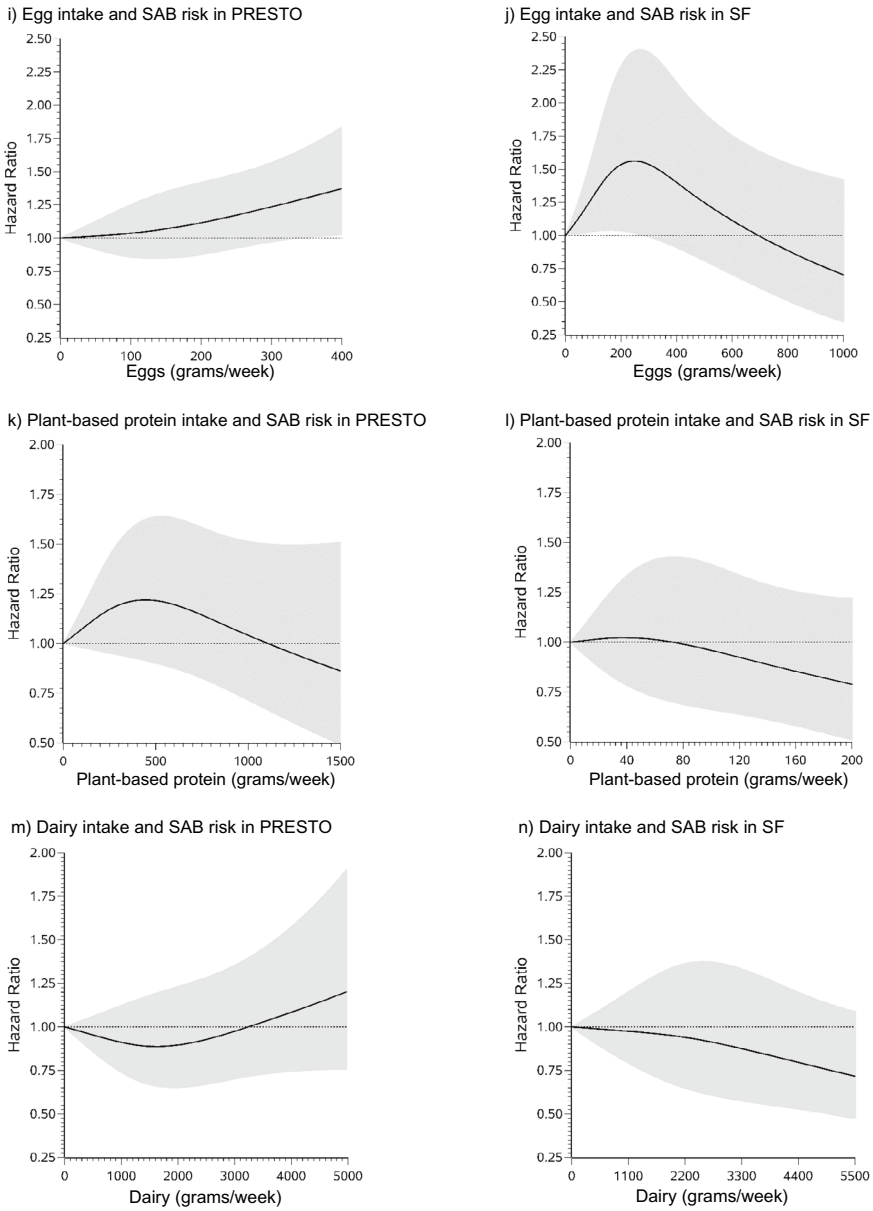


Fig. 3 Associations between intake of protein-rich foods and risk of spontaneous abortion, fit using restricted cubic splines, Pregnancy Study Online (left panels) and Snart Foraeldre (right panels). HRs represent the association between increasing intake of a specific protein-rich food while keeping intake of all other protein-rich foods and total energy intake constant. Each spline has three knots, located at the 10th, 50th, and 90th percentiles. The reference group for each spline is 0 g. Splines are adjusted for total energy intake, age, education, income, BMI, physical activity, smoking history, alcohol intake, multivitamin or folic acid intake, caffeine intake, parity, individual components of the HEI score or DDGI score unrelated to protein intake, and other protein-rich foods. PRESTO models are adjusted for race/ethnicity

Fig. 3 (continued)



associated with higher risk of SAB in North America, but lower risk of SAB in Denmark.

Most previous studies on intake of protein-rich foods and SAB have focused on dietary patterns, and have generally found that seafood intake, as part of a healthy diet, was associated with lower risk of SAB [9–11, 15, 29]. The one study that used substitution models to examine specific types of protein-rich foods in relation to SAB was EARTH, a cohort study of 357 couples undergoing in vitro fertilization at a Massachusetts hospital [15]. The authors reported that pre-treatment seafood intake was associated with greater odds of live birth. This finding is consistent with our results in PRESTO, but not SF.

The conflicting findings across PRESTO and SF were unexpected, given that biologic effects of specific nutrients on SAB should not vary across these populations. Differences could reflect chance variation, rather than causal mechanisms. However, there are several other possible explanations for the conflicting findings. First, we used different FFQs in PRESTO and SF due to the stark differences in dietary patterns between North American and Danish women; this could have lead to exposure misclassification that varied in magnitude and direction across the two cohorts. For example, if there is heterogeneity in SAB risk across different types of seafood—in other words, if some seafood is related to higher risk of SAB and other seafood is related to lower risk of SAB—and our accuracy

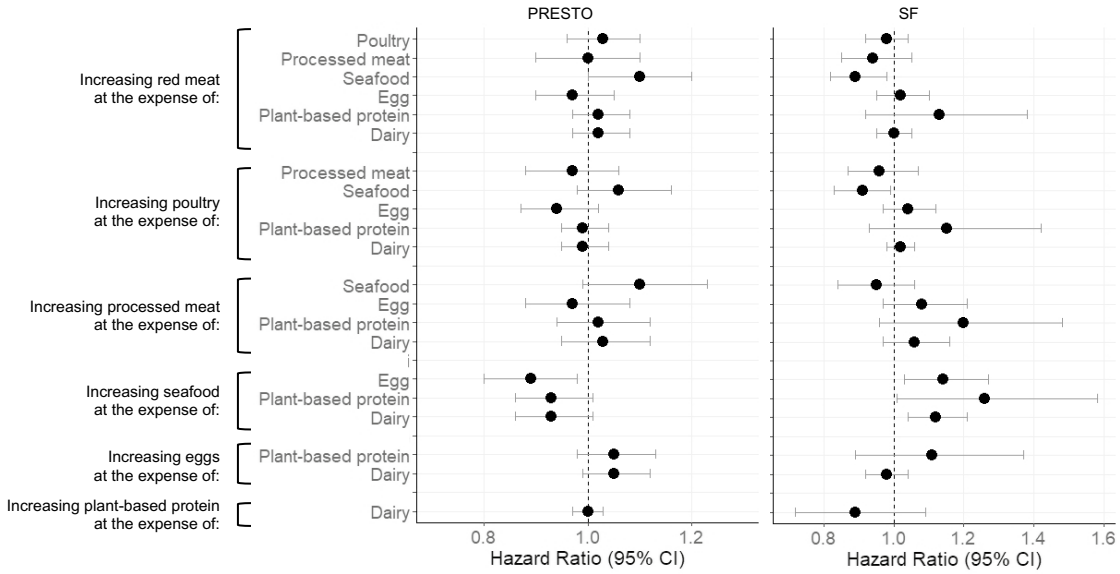


Fig. 4 Association between intake of protein-rich foods and spontaneous abortion (SAB) in PRESTO (left panel) and Snart Foraeldre (right panel). HRs estimate the association of substituting 100 g of one type of protein for 100 g of another type. Models are adjusted for total energy intake, age, education, income, BMI, physical activity, smoking history, alcohol intake, multivitamin or folic acid intake, caffeine intake, parity, individual components of the HEI score or DDGI score unrelated to protein intake, other protein-rich foods (excluding the one being cut out of the diet), and total intake of protein-rich foods. PRESTO models are adjusted for race/ethnicity

in measuring these specific types of seafood varied across cohorts, we could see differences in the direction of associations. Completeness of ascertainment may also vary by cohort: the PRESTO FFQ included 6 items on seafood, whereas the SF FFQ included 12 items on seafood.

Second, seafood is an important source of environmental contaminants, including mercury and polychlorinated biphenyls, which have been related to SAB in some [30, 31], but not all [32, 33] studies. Therefore, if the seafood consumed by the Danish population is more heavily contaminated than that consumed in North America, this could explain the higher SAB risk with increasing seafood intake in SF but not PRESTO. However, existing evidence on the extent of seafood contamination in Denmark does not suggest that the risks of increasing consumption of environmental contaminants outweighs the benefits [34]. In addition, we are unable to evaluate this hypothesis specifically in our study, as we did not collect detailed information on the specific types or sources of seafood, nor did we measure biomarkers of exposure to chemical contaminants.

Third, there may be unmeasured confounding by other aspects of the diet or healthy lifestyle that differed across cohorts in magnitude and direction. We attempted to reduce the likelihood of unmeasured confounding by adjusting for individual components of the dietary quality scores, but it is possible that these adjustments do not sufficiently control for unmeasured confounding by other dietary factors. Likewise, we adjusted for socioeconomic status and factors related to a healthy lifestyle, but found little difference in our unadjusted and adjusted models, indicating either that confounding by factors we included in our models was minimal, or that we did not have adequate measures of the confounders.

The main limitation of this study is likely exposure misclassification. We did not collect detailed information on type or source of seafood, as described above. We relied on FFQs to determine average intake over the year before enrollment in the study and collected dietary information only once during the preconception period. For some women, particularly those who took a long time to conceive, these data could have been collected many months before conception, and thus may be outside the relevant window of susceptibility for SAB. Although the FFQ was designed to assess typical dietary intake in the 1-year period before enrollment, it is possible that diet changes with increasing pregnancy attempt time [35], which could result in reverse causation. Therefore, we may not have captured periconceptional diet for all women. In our sensitivity analysis restricting to women who conceived within 90 days of completing their FFQ, the protective association of seafood intake on SAB risk was stronger in PRESTO than the main analysis, whereas the adverse association of seafood on SAB in SF was weaker than the main analysis. In addition, we did not repeat the FFQ in early pregnancy, and therefore we did not directly ascertain the association between early pregnancy seafood intake and SAB risk.

The extent of exposure misclassification may vary by participant characteristics. For instance, previous work shows that individuals with high BMI tend to underestimate their

caloric intake more so than individuals with low BMI [36]. If misclassification in our study is BMI-dependent, BMI may appear to modify the association between intake of protein-rich foods and SAB even in the absence of true effect modification [37].

Although preconception enrollment and early and frequent use of home pregnancy tests in these cohorts likely allowed us to capture a higher percentage of early SABs than in studies enrolling women with clinical pregnancies, nonetheless we almost certainly under-ascertained SAB. Without collecting daily urine specimens to measure human chorionic gonadotropin, which allows for identification of pregnancy soon after implantation, we could not identify losses that occurred before pregnancy was recognized. However, the median weeks' gestation at first positive pregnancy test was not associated with seafood intake; therefore, we suspect that any outcome misclassification due to imperfect sensitivity is likely non-differential.

In addition, we observed different incidence of SAB across the two cohorts. While this could reflect true differences in incidence, there may be methodologic explanations as well. Use of home pregnancy tests and timing of pregnancy detection was similar across the cohorts, so these variables are not a likely explanation for the observed differences. However, to capture losses that occurred after completion of the early pregnancy questionnaire, we relied on self-reported data from late pregnancy and postpartum questionnaires in PRESTO and registry data in SF. These differences in ascertainment could potentially account for the differences in incidence across cohorts. However, when we restricted our analysis to SAB that occurred before 8 weeks' gestation (for which ascertainment was more similar between the two cohorts), results were similar.

In conclusion, we found little association between risk of SAB and intake of protein-rich foods, including red and processed meat, poultry, plant-based proteins, eggs and dairy. We found an association between preconception seafood intake and SAB risk that varied across cohorts, in opposite directions. The divergence of results across our two study cohorts reduces the plausibility that either of these associations is causal.

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Author contributions AKW analyzed the data and drafted the manuscript. SKW analyzed the SF data and reviewed the manuscript. ASDL contributed to exposure and outcome assessment in SF and reviewed the manuscript. EMM designed the study, led primary data collection in SF, contributed to outcome assessment, and reviewed the manuscript. TRW assisted with data analysis, contributed to exposure and outcome assessment in PRESTO, and reviewed the manuscript. ET contributed to exposure assessment in SF and reviewed the manuscript. KLT contributed to exposure assessment in PRESTO and reviewed the manuscript. KJR designed the study, contributed expertise to the statistical analysis, and reviewed the manuscript. LAW designed the study, led primary data collection in PRESTO, and reviewed the manuscript. EEH designed the study, led primary data collection in SF, and reviewed the manuscript. All authors approved the final version of the manuscript for submission.

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Declarations

Conflict of interest PRESTO has received in-kind donations from FertilityFriend.com, Kindara.com, Sandstone Diagnostics, and Swiss Precision Technologies for primary data collection. Dr. Wise serves as a consultant to AbbVie, Inc. for her work on uterine fibroids. The remaining authors have no conflicts of interest to report.

Availability of data Data are not available due to European Union privacy laws.

Code availability Code is available upon reasonable request to the corresponding author.

Ethics approval The institutional review board at the Boston University Medical Campus approved the study protocol. SF is registered at Aarhus University to comply with Danish law on data protection.

Consent to participate All participants provided informed consent.

References

1. (2012) The johns hopkins manual of gynecology and obstetrics (4th edition). Lippincott Williams & Wilkins
2. Rossen LM, Ahrens KA, Branum AM (2018) Trends in risk of pregnancy loss among US women, 1990–2011. Paediatr Perinatal Epidemiol 32(1):19–29. https://doi.org/10.1111/ppe.12417
3. Mukherjee S, Velez Edwards DR, Baird DD, Savitz DA, Hartmann KE (2013) Risk of miscarriage among black women and white women in a U.S. Prospective Cohort Study. Am J Epidemiol 177(11):1271–1278. https://doi.org/10.1093/aje/kws393
4. Ammon Avalos L, Galindo C, Li DK (2012) A systematic review to calculate background miscarriage rates using life table analysis. Birth Defects Res A Clin Mol Teratol 94(6):417–423. https://doi.org/10.1002/bdra.23014
5. Bruckner TA, Mortensen LH, Catalano RA (2016) Spontaneous pregnancy loss in denmark following economic downturns. Am J Epidemiol 183(8):701–708. https://doi.org/10.1093/aje/kww003
6. Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, Armstrong EG, Nisula BC (1988) Incidence of early loss of pregnancy. N Engl J Med 319(4):189–194
7. Quenby S, Gallos ID, Dhillon-Smith RK, Podesek M, Stephenson MD, Fisher J, Brosens JJ, Brewin J, Ramhorst R, Lucas ES, McCoy RC, Anderson R, Daher S, Regan L, Al-Memar M, Bourne T, MacIntyre DA, Rai R, Christiansen OB, Sugiura-Ogasawara M, Odendaal J, Devall AJ, Bennett PR, Petrou S, Coomarasamy A (2021) Miscarriage matters: the epidemiological, physical, psychological, and economic costs of early pregnancy loss. Lancet

397(10285):1658–1667. https://doi.org/10.1016/S0140-6736(21)00682-6
8. Gaskins AJ, Chavarro JE (2018) Diet and fertility: a review. Am J Obstet Gynecol 218(4):379–389. https://doi.org/10.1016/j.ajog.2017.08.010
9. Karayiannis D, Kontogianni MD, Mendorou C, Mastrominas M, Yiannakouris N (2018) Adherence to the Mediterranean diet and IVF success rate among non-obese women attempting fertility. Hum Reprod 33(3):494–502. https://doi.org/10.1093/humrep/dey003
10. Vujkovic M, de Vries JH, Lindemans J, Macklon NS, van der Spek PJ, Steegers EA, Steegers-Theunissen RP (2010) The preconception Mediterranean dietary pattern in couples undergoing in vitro fertilization/intracytoplasmic sperm injection treatment increases the chance of pregnancy. Fertil Steril 94(6):2096–2101. https://doi.org/10.1016/j.fertnstert.2009.12.079
11. Twigt JM, Bolhuis ME, Steegers EA, Hammiche F, van Inzen WG, Laven JS, Steegers-Theunissen RP (2012) The preconception diet is associated with the chance of ongoing pregnancy in women undergoing IVF/ICSI treatment. Hum Reprod 27(8):2526–2531. https://doi.org/10.1093/humrep/des157
12. Ibsen DB, Laursen ASD, Wurtz AML, Dahm CC, Rimm EB, Parner ET, Overvad K, Jakobsen MU (2020) Food substitution models for nutritional epidemiology. Am J Clin Nutr. https://doi.org/10.1093/ajcn/nqaa315
13. Rimm EB, Appel LJ, Chiuve SE, Djousse L, Engler MB, Kris-Etherton PM, Mozaffarian D, Siscovick DS, Lichtenstein AH, American Heart Association Nutrition Committee of the Council on L, Cardiometabolic H, Council on E, Prevention, Council on Cardiovascular Disease in the Y, Council on C, Stroke N, Council on Clinical C (2018) Seafood long-chain n-3 polyunsaturated fatty acids and cardiovascular disease: a science advisory from the American heart association. Circulation 138(1):e35–e47. https://doi.org/10.1161/CIR.0000000000000574
14. Al-Shaar L, Satija A, Wang DD, Rimm EB, Smith-Warner SA, Stampfer MJ, Hu FB, Willett WC (2020) Red meat intake and risk of coronary heart disease among US men: prospective cohort study. BMJ 371:m4141. https://doi.org/10.1136/bmj.m4141
15. Nassan FL, Chiu YH, Vanegas JC, Gaskins AJ, Williams PL, Ford JB, Attaman J, Hauser R, Chavarro JE, Teaggm ES (2018) Intake of protein-rich foods in relation to outcomes of infertility treatment with assisted reproductive technologies. Am J Clin Nutr 108(5):1104–1112. https://doi.org/10.1093/ajcn/nqy185
16. Tamhankar VA, Liu B, Yan J, Li TC (2015) A comparison of pattern of pregnancy loss in women with infertility undergoing IVF and women with unexplained recurrent miscarriages who conceive spontaneously. Obstet Gynecol Int 2015:989454. https://doi.org/10.1155/2015/989454
17. Wise LA, Rothman KJ, Mikkelsen EM, Stanford JB, Wesselink AK, McKinnon C, Gruschow SM, Horgan CE, Wiley AS, Hahn KA, Sorensen HT, Hatch EE (2015) Design and conduct of an internet-based preconception cohort study in North America: pregnancy study online. Paediatr Perinat Epidemiol 29(4):360–371. https://doi.org/10.1111/ppe.12201
18. Wise LA, Wesselink AK, Mikkelsen EM, Cueto H, Hahn KA, Rothman KJ, Tucker KL, Sorensen HT, Hatch EE (2017) Dairy intake and fecundability in 2 preconception cohort studies. Am J Clin Nutr 105(1):100–110. https://doi.org/10.3945/ajcn.116.138404
19. Subar AF, Thompson FE, Kipnis V, Midthune D, Hurwitz P, McNutt S, McIntosh A, Rosenfeld S (2001) Comparative validation of the block, willett, and national cancer institute food frequency questionnaires : the eating at America's table study. Am J Epidemiol 154(12):1089–1099
20. Knudsen VK, Hatch EE, Cueto H, Tucker KL, Wise L, Christensen T, Mikkelsen EM (2016) Relative validity of a semi-quantitative, web-based FFQ used in the "Snart Foraeldre" cohort - a Danish study of diet and fertility. Public Health Nutr 19(6):1027–1034. https://doi.org/10.1017/S1368980015002189
21. Schmidt M, Pedersen L, Sorensen HT (2014) The Danish Civil Registration System as a tool in epidemiology. Eur J Epidemiol 29(8):541–549. https://doi.org/10.1007/s10654-014-9930-3
22. Millen AE, Midthune D, Thompson FE, Kipnis V, Subar AF (2006) The National Cancer Institute diet history questionnaire: validation of pyramid food servings. Am J Epidemiol 163(3):279–288. https://doi.org/10.1093/aje/kwj031
23. Wise LA, Wang TR, Wesselink AK, Willis SK, Chaiyasarikul A, Levinson JS, Rothman KJ, Hatch EE, Savitz DA (2021) Accuracy of self-reported birth outcomes relative to birth certificate data in an Internet-based prospective cohort study. Paediatr Perinat Epidemiol. https://doi.org/10.1111/ppe.12769
24. Bliddal M, Broe A, Pottegard A, Olsen J, Langhoff-Roos J (2018) The Danish medical birth register. Eur J Epidemiol 33(1):27–36. https://doi.org/10.1007/s10654-018-0356-1
25. Lohse SR, Farkas DK, Lohse N, Skouby SO, Nielsen FE, Lash TL, Ehrenstein V (2010) Validation of spontaneous abortion diagnoses in the Danish National Registry of Patients. Clin Epidemiol 2:247–250. https://doi.org/10.2147/CLEP.S13815
26. Guenther PM, Kirkpatrick SI, Reedy J, Krebs-Smith SM, Buckman DW, Dodd KW, Casavale KO, Carroll RJ (2014) The Healthy Eating Index-2010 is a valid and reliable measure of diet quality according to the 2010 Dietary Guidelines for Americans. J Nutr 144(3):399–407. https://doi.org/10.3945/jn.113.183079
27. Hansen CP, Overvad K, Tetens I, Tjonneland A, Parner ET, Jakobsen MU, Dahm CC (2018) Adherence to the Danish food-based dietary guidelines and risk of myocardial infarction: a cohort study. Public Health Nutr 21(7):1286–1296. https://doi.org/10.1017/S1368980017003822
28. Karmon AE, Toth TL, Chiu YH, Gaskins AJ, Tanrikut C, Wright DL, Hauser R, Chavarro JE, Earth Study T (2017) Male caffeine and alcohol intake in relation to semen parameters and in vitro fertilization outcomes among fertility patients. Andrology 5(2):354–361. https://doi.org/10.1111/andr.12310
29. Liu Y, De A (2015) Multiple imputation by fully conditional specification for dealing with missing data in a large epidemiologic study. Int J Stat Med Res 4(3):287–295. https://doi.org/10.6000/1929-6029.2015.04.03.7
30. Gaskins AJ, Nassan FL, Chiu YH, Arvizu M, Williams PL, Keller MG, Souter I, Hauser R, Chavarro JE, Team ES (2019) Dietary patterns and outcomes of assisted reproduction. Am J Obstet Gynecol 220(6):567 e561-567 e518. https://doi.org/10.1016/j.ajog.2019.02.004
31. Tsukimori K, Tokunaga S, Shibata S, Uchi H, Nakayama D, Ishimaru T, Nakano H, Wake N, Yoshimura T, Furue M (2008) Long-term effects of polychlorinated biphenyls and dioxins on pregnancy outcomes in women affected by the Yusho incident. Environ Health Perspect 116(5):626–630. https://doi.org/10.1289/ehp.10686
32. Sikorski R, Juszkiewicz T, Paszkowski T, Szprengier-Juszkiewicz T (1987) Women in dental surgeries: reproductive hazards in occupational exposure to metallic mercury. Int Arch Occup Environ Health 59(6):551–557. https://doi.org/10.1007/BF00377918
33. Small CM, Cheslack-Postava K, Terrell M, Blanck HM, Tolbert P, Rubin C, Henderson A, Marcus M (2007) Risk of spontaneous abortion among women exposed to polybrominated biphenyls. Environ Res 105(2):247–255. https://doi.org/10.1016/j.envres.2006.11.010
34. Buck Louis GM, Smarr MM, Sundaram R, Steuerwald AJ, Sapra KJ, Lu Z, Parsons PJ (2017) Low-level environmental metals and metalloids and incident pregnancy loss. Reprod Toxicol 69:68–74. https://doi.org/10.1016/j.reprotox.2017.01.011

35. Thomsen ST, Pires SM, Devleesschauwer B, Poulsen M, Fagt S, Ygil KH, Andersen R (2018) Investigating the risk-benefit balance of substituting red and processed meat with fish in a Danish diet. Food Chem Toxicol 120:50–63. https://doi.org/10.1016/j.fct.2018.06.063
36. Wesselink AK, Hatch EE, Rothman KJ, Willis SK, Orta OR, Wise LA (2020) Pesticide residue intake from fruits and vegetables and fecundability in a North American preconception cohort study. Environ Int 139:105693. https://doi.org/10.1016/j.envint.2020.105693
37. Ferrari P, Slimani N, Ciampi A, Trichopoulou A, Naska A, Lauria C, Veglia F, Bueno-de-Mesquita HB, Ocke MC, Brustad M, Braaten T, Jose Tormo M, Amiano P, Mattisson I, Johansson G, Welch A, Davey G, Overvad K, Tjonneland A, Clavel-Chapelon F, Thiebaut A, Linseisen J, Boeing H, Hemon B, Riboli E (2002) Evaluation of under- and overreporting of energy intake in the 24-h diet recalls in the European Prospective Investigation into Cancer and Nutrition (EPIC). Public Health Nutr 5(6B):1329–1345. https://doi.org/10.1079/PHN2002409
38. Greenland S (1980) The effect of misclassification in the presence of covariates. Am J Epidemiol 112(4):564–569. https://doi.org/10.1093/oxfordjournals.aje.a113025