STROBE Checklist for Cohort Studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	4-5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-10
Bias	9	Describe any efforts to address potential sources of bias	11
Study size	10	Explain how the study size was arrived at	10-11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10-12
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10-12
		(*b*) Describe any methods used to examine subgroups and interactions	n/a
		(*c*) Explain how missing data were addressed	12
		(*d*) If applicable, explain how loss to follow-up was addressed	n/a
		(*e*) Describe any sensitivity analyses	10-12
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study— e.g., numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	13
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders	13, 29-31
		(b) Indicate number of participants with missing data for each variable of interest	Suppl. p.5
		(c) Summarise follow-up time (e.g., average and total amount)	n/a

Outcome data		15* Report numbers of outcome events or summary measures over time	n/a
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included	32-34
		(*b*) Report category boundaries when continuous variables were categorized	8-9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done— e.g., analyses of subgroups and interactions, and sensitivity analyses	Suppl p.6-8
Discussion			
Key results	18	Summarise key results with reference to study objectives	15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	18-19
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15-19
Generalisability	21	Discuss the generalisability (external validity) of the study results	18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	n/a

Calculation of Gestational Weeks at Birth

Supplementary Table 1 | BC Perinatal Data Registry algorithm used to calculate gestational age at birth in completed weeks.

Criteria	Source Used
LMP is recorded and there is no ultrasound.	LMP
LMP is recorded, there is no early ultrasound, but clinical exam of baby gives a GA at least 3 weeks different than LMP.	Clinical exam
LMP is recorded and equal to GA – in weeks – from ultrasound at <14 weeks.	LMP
If estimates are not equal.	Ultrasound
LMP is recorded and within 1 week of GA – in weeks – from ultrasound at 14-20 weeks.	LMP
If difference is more than 1 week.	Ultrasound
LMP is recorded and within 2 weeks of GA – in weeks – from ultrasound at 21-24 weeks.	LMP
If difference is more than 2 weeks.	Ultrasound
LMP is not recorded but GA from ultrasound <25 weeks is recorded.	Ultrasound
LMP and early ultrasound are not recorded.	Clinical exam
LMP, early ultrasound, and newborn clinical exam are not recorded.	Chart
If all are missing or out of range.	GA is missing

Abbreviations: LMP, last menstrual period; GA, gestational age. Only LMP estimates between 15 and 45 weeks and ultrasound estimates between 17 and 43 weeks are considered valid for the purposes of the calculation.

Multiple Imputation

Because of the high proportion of missing data for maternal height, pre-pregnancy weight and weight at delivery, multiple imputation was conducted as a sensitivity analysis for the regression models in which pre-pregnancy BMI and gestational weight gain were covariates or predictors. Maternal height, pre-pregnancy weight and weight at delivery were missing data for 13%, 17% and 33% of pregnant women, respectively. Maternal pre-pregnancy BMI and gestational weight gain were missing data for 21% and 39% of pregnant women, respectively. Therefore, 40 imputed datasets, consistent with the variable with the highest proportion of missing data, were created based on the rule of thumb in which the number of imputations performed is similar to the percentage of missing cases (27). The type of models used to conduct the imputations are as follows: linear regression for normally distributed continuous variables, predictive mean matching for skewed continuous variables, logistic regression for binary categorical variables and ordinal logistic regression for ordinal categorical variables. All covariate and outcome variables, as well as gestational weeks at maternal sample collection, newborn age at DBS sample collection and newborn DBS MMA concentration, were included in the multiple imputation model (**Supplementary Table 2**). Covariates without missing data were not imputed but were included in the imputation of missing data for the other variables. Pre-pregnancy BMI was passively computed from the imputed datasets using maternal height and pre-pregnancy weight. Gestational weight gain was passively computed from maternal pre-pregnancy weight and delivery weight. Imputation diagnostics, including assessment of the distribution of imputed values (e.g. graphically using boxplots, numerically using descriptive statistics) were done with the multiply imputed data to assess to the plausibility of the imputed values. The Stata "mibeta.pkg" package was used to conduct the linear regression analyses, as it additionally calculated the unadjusted and adjusted R^2. Assumptions of linear regression were checked for the first 5 imputed datasets to estimate that assumptions were approximately met.

Supplementary Table 2 | List of multiple imputation model variables.

	% Missing (*n*)[1]	Imputation Method
Imputed Variables		
Maternal height	13 (94)	Predictive Mean Matching
Pre-pregnancy weight	17 (120)	Predictive Mean Matching
Maternal weight at delivery	33 (228)	Predictive Mean Matching
Maternal total B_{12} (first trimester)	7 (52)	Predictive Mean Matching
Maternal total B_{12} (second trimester)	.9 (6)	Predictive Mean Matching
Maternal holoTC (first trimester)	9 (63)	Predictive Mean Matching
Maternal holoTC (second trimester)	12 (84)	Predictive Mean Matching
Maternal MMA (first trimester)	9 (62)	Predictive Mean Matching
Maternal MMA (second trimester)	7 (47)	Predictive Mean Matching
Maternal tHcy (first trimester)	15 (108)	Predictive Mean Matching
Maternal tHcy (second trimester)	20 (138)	Predictive Mean Matching
Gestational weeks at first trimester maternal sample collection	2 (16)	Predictive Mean Matching
Gestational weeks at second trimester maternal sample collection	2 (16)	Predictive Mean Matching
Parity	1 (8)	Logistic Regression
Gestational diabetes	1 (8)	Logistic Regression
Hypertensive disorder of pregnancy	1 (8)	Logistic Regression
Neighbourhood income	1 (9)	Ordinal Logistic Regression
Neighbourhood education	1 (9)	Predictive Mean Matching
Birth weight z-score	.1 (1)	Linear Regression
Head circumference z-score	2 (11)	Linear Regression
Gestational age at birth	.1 (1)	Predictive Mean Matching
Newborn dried blood spot MMA	14 (98)	Predictive Mean Matching
Age at dried blood spot sample collection	3 (24)	Predictive Mean Matching
Not Imputed		
Maternal age	None	N/A
Maternal ethnicity	None	N/A
Newborn sex	None	N/A
Passive Variables		
Pre-pregnancy BMI	21 (145)	N/A
Gestational weight gain	39 (271)	N/A

[1]Total *n*=700 (excluding stillbirths, *n*=9).

Supplementary Table 3 | Association between maternal vitamin B_{12} status and birth weight z-score in Canadian mother-newborn dyads using multiply imputed data.

	Unadjusted Model				Adjusted Model†			
Maternal B_{12} Biomarker	*n*	β (95% CI)	*P*	R^2	*n*	β (95% CI)	*P*	R^2
First Trimester								
HoloTC‡	700	0.0116 (-0.0198, 0.0430)	0.47	0.0011	700	-0.00359 (-0.0342, 0.0270)	0.82	0.115
Total B_{12}	700	0.000885 (-0.00570, 0.00747)	0.79	0.0002	700	-0.00306 (-0.00977, 0.00366)	0.37	0.117
MMA	700	-0.00319 (-0.0116, 0.00523)	0.46	0.0011	700	0.00177 (-0.00640, 0.00994)	0.67	0.116
tHcy	700	0.00121 (-0.0691, 0.0715)	0.97	0.0002	700	0.0258 (-0.0428, 0.0944)	0.46	0.117
Second Trimester								
HoloTC‡	700	-0.00231 (-0.0317, 0.0270)	0.88	0.0012	700	-0.00548 (-0.0338, 0.0228)	0.70	0.116
Total B_{12}	700	0.00198 (-0.00490, 0.00886)	0.57	0.0005	700	-0.00181 (-0.00875, 0.00513)	0.61	0.116
MMA	700	-0.00402 (-0.0125, 0.00445)	0.35	0.0014	700	0.00153 (-0.00674, 0.00980)	0.72	0.116
tHcy	700	-0.00778 (-0.0732, 0.0576)	0.82	0.0003	700	0.00684 (-0.0568, 0.0705)	0.83	0.116

Unadjusted R^2 reported for univariable model and adjusted R^2 reported for multivariable models. β coefficients represent change in birth weight z-score with each 10-unit increase in maternal serum holoTC, total B_{12} and MMA concentration and 1-unit increase in tHcy.

†All multivariable models were adjusted for maternal age, maternal ethnicity, parity, GWG, pre-pregnancy BMI, hypertensive disorder of pregnancy, gestational diabetes, neighbourhood income and neighbourhood education.

‡Discontinuous regression.

Supplementary Table 4 | Association between maternal vitamin B_{12} status and head circumference z-score in Canadian mother-newborn dyads using multiply imputed data.

	Unadjusted Model				Adjusted Model†			
Maternal B_{12} Biomarker	*n*	β (95% CI)	*P*	R^2	*n*	β (95% CI)	*P*	R^2
First Trimester								
HoloTC‡	700	-0.00548 (-0.0427, 0.0317)	0.77	0.0034	700	-0.00649 (-0.0439, 0.0310)	0.73	0.048
Total B_{12}	700	-0.00396 (-0.0118, 0.00386)	0.32	0.0015	700	-0.00272 (-0.0111, 0.00564)	0.52	0.049
MMA	700	-0.000977 (-0.0109, 0.00895)	0.85	0.0002	700	-0.000521 (-0.0105, 0.00945)	0.92	0.049
tHcy	700	0.00207 (-0.0813, 0.0854)	0.96	0.0002	700	0.0165 (-0.0682, 0.101)	0.70	0.049
Second Trimester								
HoloTC‡	700	-0.0258 (-0.0600, 0.00833)	0.14	0.0040	700	-0.0220 (-0.0564, 0.0124)	0.21	0.050
Total B_{12}	700	-0.00706 (-0.0153, 0.00114)	0.09	0.0041	700	-0.00640 (-0.0150, 0.00218)	0.14	0.052
MMA	700	0.00209 (-0.00789, 0.0121)	0.68	0.0003	700	0.00386 (-0.00627, 0.0140)	0.46	0.050
tHcy	700	0.0669 (-0.0125, 0.146)	0.10	0.0051	700	0.0728 (-0.00613, 0.152)	0.07	0.054

Unadjusted R^2 reported for univariable model and adjusted R^2 reported for multivariable models. β coefficients represent change in head circumference z-score with each 10-unit increase in maternal serum holoTC, total B_{12} and MMA concentration and 1-unit increase in tHcy.
†All multivariable models were adjusted for maternal age, maternal ethnicity, parity, GWG, pre-pregnancy BMI, hypertensive disorder of pregnancy, gestational diabetes, neighbourhood income and neighbourhood education.
‡Discontinuous regression.

Supplementary Table 5 | Association between first trimester maternal vitamin B_{12} status and gestational age at birth in weeks in Canadian mother-newborn dyads using multiply imputed data.

	Unadjusted Model				Adjusted Model†			
Maternal B_{12} Biomarker	*n*	β (95% CI)	*P*	R^2	*n*	β (95% CI)	*P*	R^2
First Trimester								
HoloTC‡	700	0.00797 (-0.0465, 0.0624)	0.77	0.0040	700	-0.0178 (-0.0718, 0.0363)	0.52	0.066
Total B_{12}	700	0.00276 (-0.00868, 0.0142)	0.64	0.0003	700	-0.00881 (-0.0209, 0.00327)	0.15	0.070
MMA	700	-0.00741 (-0.0220, 0.00719)	0.32	0.0017	700	-0.00259 (-0.0176, 0.00124)	0.73	0.067
tHcy	700	0.0596 (-0.0643, 0.183)	0.35	0.0016	700	0.0717 (-0.106, 0.141)	0.79	0.067
Second Trimester								
HoloTC‡	700	0.0356 (-0.0151, 0.0836)	0.17	0.0032	700	0.00859 (-0.0421, 0.0593)	0.74	0.068
Total B_{12}	700	0.00416 (-0.00797, 0.0163)	0.50	0.0007	700	-0.00821 (-0.0208, 0.00435)	0.20	0.069
MMA	700	-0.00142 (-0.0162, 0.0133)	0.85	0.0001	700	0.00352 (-0.0112, 0.0182)	0.64	0.067
tHcy	700	0.00927(-0.107, 0.126)	0.88	0.0003	700	-0.0136 (-0.130, 0.103)	0.82	0.067

Unadjusted R^2 reported for univariable model and adjusted R^2 reported for multivariable models. β coefficients represent change in gestational age at birth (weeks) with each 10-unit increase in maternal serum holoTC, total B_{12} and MMA concentration and 1-unit increase in tHcy.

†All multivariable models were adjusted for maternal age, maternal ethnicity, parity, GWG, pre-pregnancy BMI, hypertensive disorder of pregnancy, gestational diabetes, neighbourhood income, neighbourhood education and newborn sex.

‡Discontinuous regression.

Supplementary Table 6 | List of study variables.

Variable	Unit	Description
Exposure Variables		
Serum Total B_{12}	pmol/L	Maternal direct B_{12} biomarker.
Serum HoloTC	pmol/L	Maternal direct B_{12} biomarker.
Serum MMA	nmol/L	Maternal functional B_{12} biomarker.
Serum tHcy	μmol/L	Maternal functional B_{12} biomarker.
Outcome Variables		
Gestational Age at Birth	weeks	Gestational age at birth calculated by last normal menstrual period, first ultrasound, newborn examination and maternal chart.
Birth Weight	grams	Weight of the newborn at birth.
Birth Weight z-Score	z-score	Birth weight standardized for gestational age at birth and sex using the Kramer charts.
Birth Head Circumference	centimetres	Head circumference of the newborn at birth.
Birth Head Circumference z-Score	z-score	Birth head circumference of the newborn standardized for gestational age at birth and sex using the Barbier charts.
Newborn MMA	pmol per 8-mm punch	Newborn dried blood spot MMA.
Confounding Variables		
Maternal Ethnicity	South Asian; European	Self-identified maternal ethnicity.
Maternal Age	years	Maternal age at the time of prenatal genetic screening.
Neighbourhood Income	income quintile	Income quintile based on forward sortation area median family income of mother's resident forward sortation area relative to all British Columbia forward sortation areas.
Neighbourhood Education	proportion	Proportion of individuals in mother's resident forward sortation area who have less than a secondary degree or equivalent.
Parity	nulliparous; multiparous	Indicates whether mother has previously delivered a pregnancy that reached 20 weeks gestation or 500 grams birth weight.
Pre-Pregnancy BMI	kilograms/ metres^2	Maternal body mass index based on pre-pregnancy weight and height.

Variable	Unit	Description
Pre-Pregnancy BMI Category	underweight; normal; overweight; obese	Maternal pre-pregnancy BMI category based on WHO guidelines.
Gestational Weight Gain	kilograms	Maternal weight gain during pregnancy based on weight on admission to hospital and pre-pregnancy weight.
Gestational Weight Gain Category	inadequate; adequate; excessive	Maternal gestational weight gain category based on Institute of Medicine guidelines.
Gestational Diabetes	yes; no	Indicates diagnosis of gestational diabetes.
Hypertensive Disorder of Pregnancy	yes; no	Indicates diagnosis of pre-existing or hypertension in pregnancy.
Predictors of Newborn B_{12} Status		
Mode Of Delivery	caesarean; vaginal	Method of delivery of newborn.
Newborn Feeding During Hospital Stay	breastmilk; breastmilk and formula	Type of feeding given to the newborn during the entire hospital stay, including discharge.
Newborn Antibiotic Use	yes; no	Indicates if antibiotics were given to newborn during hospital admission for delivery.
Maternal Antibiotic Use	yes; no	Indicates if antibiotics were given to mother during hospital admission for delivery.
Newborn Sex	male; female	Biological sex of the newborn.
Other Maternal Variables		
Number of Previous Preterm Deliveries	numeric	Total number of previous pregnancies delivered between 20 to 36 completed weeks gestation. Variable collected due to association with birth outcomes.
Prior Low Birthweight Baby	yes; no	Mother had at least one prior low birth weight baby (<2,500 g) at ≥20 weeks gestation. Variable collected due to association with birth outcomes.
Prior Macrosomic Baby	yes; no	Indicates if mother had at least one prior macrosomic baby (birth weight>4,000g). Variable collected due to association with birth outcomes.
History of Mental Illness	yes; no	Indicates history of mental illness (depression, previous postpartum depression, anxiety, bipolar disorder, other, or unknown type) prior to or during the

Variable	Unit	Description
		current pregnancy. Variable collected due to association with maternal B_{12} status.
Third Trimester Hemoglobin	numeric	Lowest hemoglobin value for the third trimester. Variable collected as an indicator of anemia during pregnancy.
Postpartum Hemoglobin	numeric	Value of postpartum hemoglobin test result during episode of care. Variable collected as an indicator of postpartum anemia.
Substance Use During Pregnancy	yes; no	Use of the following substances at any time during the current pregnancy: heroin/opioids, cocaine, methadone, solvents, or marijuana; OR care provider lists use of prescription, 'other', or unknown other drug as a risk to the pregnancy. Variable collected to verify exclusion criteria.
Alcohol Use During Pregnancy	yes; no	Alcohol use at any time during current pregnancy. Variable collected to verify exclusion criteria.
Smoking During Pregnancy	yes; no	Mother smoked tobacco products during current pregnancy. Variable collected to verify exclusion criteria.
No Selected Risks	yes; no	Indicates the mother did not have any of the specific risk factors collected in the PDR identified in the current pregnancy, past pregnancies, or in the mother's medical history. Variable collected as a descriptor of study sample.
In vitro Fertilization Used for Current Pregnancy	yes; no	Indicates mother had *in vitro* fertilization to achieve the current pregnancy. Variable collected to verify exclusion criteria.
Entonox (Nitronox)	yes; no	Indicates if entonox (nitronox) anaesthetic was given during labour. Variable collected due to potential interaction with B_{12} status.
Blood Transfusion	yes; no	Indicates if mother received whole or packed red blood cells during delivery admission. Variable collected due to association with maternal hematological status.
Other Newborn Variables		
Date of Birth	year/month	Variable collected to verify that newborn data is for the correct pregnancy/newborn.

Variable	Unit	Description
Intrauterine Growth Restriction	yes; no	Indicates risk of fetal growth restriction. Variable collected as an indicator of adverse birth outcome.
Apgar Score	<7; 7-10	Assessment of newborn muscle tone, heart rate, reflexes, skin colour and respiration at 5 minutes. Variable collected as an indicator of overall newborn health.
Birth Type	stillbirth; live birth	Indicates birth type for births at or after 20 weeks gestation or weighing at least 500 grams. Variable collected to verify exclusion criteria.
Discharged to	adoption; death/stillbirth; foster home; home; other hospital; unknown	Where the baby was discharged to, or the status of the baby at the time of discharge. Variable collected as a descriptor of study sample.
Total TPN Days	numeric	Total number of days (in whole numbers) the baby received any total parenteral nutrition (TPN). Variable collected to verify exclusion criteria.