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The association between *in utero* exposure to maternal psychological stress and female reproductive function in adolescence: A prospective cohort study

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ABSTRACT

Background: Experimental studies suggest that prenatal stress affects reproductive function in female offspring, but human evidence is sparse and inconsistent. In this present study, we aim to investigate whether maternal psychological stress, quantified as stressful life events during pregnancy, affect reproductive function in the female offspring.
Method: In a large population-based pregnancy cohort study (The Raine Study) continuously followed from prenatal life through to adolescence we examined the association between the number of maternal stressful life events in both early and late gestation and subsequent ovarian and uterine function in 228 female adolescent offspring. Mothers prospectively reported stressful life events during pregnancy at 18 and 34 weeks using a standardized 10-point questionnaire. Female offspring (n = 228) age 14–16 years underwent gynecological examination including transabdominal abdominal ultrasound (TAUS) to measure uterine volume and ovarian AFC. Plasma samples on day 2–6 of the spontaneous menstrual cycle measured circulating AMH and inhibin B. Multivariate linear regression analysis was used to examine the associations between maternal stressful life events and reproductive function in female offspring. Adolescents taking hormonal contraception were excluded.
Results: Most adolescents (145/228, 64%) were exposed to at least one stressful life event in early gestation and around half (125/228, 55%) were exposed to at least one in later gestation. Exposure to one or more maternal stressful life events in late gestation was associated with a greater uterine volume (β = 0.13, 95% CI 0.04; 0.23) and higher ovarian AFC (β = 0.19, 95% CI 0.02; 0.35) at age 14–16 years. No associations between maternal stressful events in late gestation and reproductive function were identified. No associations between stressful life events in early or late gestation and circulating AMH or Inhibin B were observed.
Conclusion: Maternal psychological stress in late, but not early gestation was associated with a significantly greater uterine volume and ovarian antral follicle count (AFC) in adolescent offspring but did not affect ovarian production of antimullerian hormone (AMH) or Inhibin B. These findings suggest that female reproductive function is influenced by prenatal exposure to stress.

1. Introduction

Infertility, defined as the absence of pregnancy after a year of unprotected regular intercourse, affects around 15% of couples trying to conceive, and female factors contribute to around 50% of these cases [1]. Despite substantial progress in the treatment of infertility, there is still

limited knowledge about causative mechanisms. Burgeoning evidence from experimental and human studies suggests that female reproductive function is regulated by early life events including exposure to psychological stress. The global burden of disease from stress related conditions is second only to ischemic heart disease [2,3]. Thus, understanding the potential role of prenatal psychological stress in long-term reproductive health is of growing global public health importance.

The mechanisms underlying the association between prenatal stress and reproductive function are not fully understood, but the female offspring of mice exposed to stressors such as restraint in pregnancy demonstrate altered reproductive behaviors including play, courtship, parental and social behaviors compared to unexposed female offspring [4,5]. However, it is uncertain whether maternal stress directly affects female reproductive function [4,6,7]. In experimental studies of male reproduction, maternal stress (restraint, crowding and low temperatures) is more consistent and associated with reduced testicular weight, delayed puberty, lower testosterone, abnormal reproductive behaviors and reduced fertility [7,8].

The published evidence on maternal stress and reproductive function in humans is limited and only two previous studies have considered the association between maternal psychological stress and reproductive function in female offspring. In the first, the adolescent daughters of Finnish women who were pregnant during the Chernobyl explosion had higher circulating cortisol levels compared to those born one year earlier [9], but these effects could have been distorted by effects of radiation. In the other study, authors considered a general population and reported that bereavement (death of a spouse or close family member) was associated with decreased fertility in female offspring [10]. However, that study was limited by the uncertainty of the timing of the stressful life event, only considered one stressful event and the outcome was ascertained indirectly.

The aim of this study was to measure the associations between prenatal exposure to stressful life events and direct measures of female reproductive function in adolescence, and to determine the differential effects of early or late gestational exposures using a prospective longitudinal design. Maternal stressful life events were measured in early gestation (18 weeks) and late gestation (34 weeks). Reproductive function in adolescence was measured from circulating antimullerian hormone (AMH) and Inhibin B and from uterine volume and ovarian antral follicle count (AFC) measured using transabdominal ultrasound scan on day 2–6 of the spontaneous menstrual cycle.

2. Methods

2.1. Design prospective longitudinal cohort

2.1.1. The Raine Study

The Raine Study, formed from a pregnancy cohort study, was designed to measure the relationships between early life events and subsequent health and behavior. The study recruited almost 3000 women in between their 16th and 20th gestational week in the period from May 1989 to November 1991 (average recruitment at gestational week 18). The 2868 children (including 1414 girls) born to 2804 mothers (Generation 1) were retained to form The Raine Study, previously described in Ref. [11,11]. The present study included 228 young unselected Generation 2 adolescent girls who participated in a study of early life determinants of age at menarche, ovarian reserve and polycystic ovarian syndrome [12–14]. In brief, eligibility criteria included English speaking pregnant women (between 16 and 20 weeks gestation) living in Western Australia and not intending to move, expecting to deliver at King Edward Memorial Hospital, Perth, WA. The cohort was assessed at recruitment and throughout gestation, and the offspring were assessed at birth and then at 2–3 year intervals [15]. At each follow-up extensive clinical and health/lifestyle questionnaire data (diet, physical activity, drug, smoking, alcohol, prescription medicines, and sexual debut) were collected [15]. In brief, adolescent girls who were at least 6 months post-menarche

(n = 723, average age 15 years) were invited to participate. Two hundred and forty girls agreed to participate. Twelve were excluded due to oral contraceptive use which may affect measures of AMH leaving 228 girls in the present data set, Fig. 1.

The study visit was scheduled for day 2–6 of the spontaneous menstrual cycle by asking the girls to phone the study coordinator on day one of menstruation, ensuring that subjects with both regular and irregular cycles were sampled during the early follicular phase. All visits were timed between 1530 and 1630 h to account for diurnal variation in ovarian hormone production. Height and weight were measured and girls were asked to report their menstrual pattern using standardized definitions of regular and irregular menstruation [16].

Blood samples were drawn from an antecubital vein, centrifuged and plasma stored at −80C (never thawed and refrozen) until the assays reported in this study.

2.1.2. Exposure variable

Maternal psychological stress was quantified by measurement of stressful Life Events. Data were collected at 18- and 34-weeks' gestation using a 10-item questionnaire based on the 67-item Stressful Life Event Inventory developed by Tennant and Andrews for an Australian population [17,18]. In brief, the complete Tennant inventory was derived from established scales [19,20] and includes a wide range of stressful life events. Dimensions include health concerns/loss and bereavement/family conflicts and social relations/friends and relatives/educational concerns/job security/moving to a new house/finance and legal issues. In order to apply the Tennant inventory to stressful life events in pregnant women, The Raine study created a 10-item inventory which included at least one item from each dimension. To allow women to include stressful events that were not included in the 10-item scale, an option to report "other problems" was created for pregnant women recruited to the Raine Study. Stressful life event included death of a close relative, death of a close friend, separation or divorce, marital problems, problems with children, own involuntary job loss, partner's involuntary job loss, money problems, pregnancy concerns, residential moves and other events (Table 1). At 18 gestational weeks, mothers were asked to record stressful life events experienced since confirmation of their pregnancy and at 34 gestational weeks they were asked about events in the preceding four months in order to ensure that the same event was not reported twice. The response to each of each items in the questionnaire was recorded as "yes/no" once, in accordance with previous studies to maximize recall [21]. Separate continuous variables including the total number of maternal stressful life event reported at weeks 18 and 34 were created weighting each event equally [22–27]. A categorical (yes/no) variable was also developed for each gestational time point.

2.1.3. Outcome variables

Measurement of uterine volume and ovarian AFC. Transabdominal ultrasound (TAUS) was used to measure uterine volume and AFC on day 2–6 of the spontaneous menstrual cycle with a confirmed full bladder using a 5-2 MHz transducer (IU22; Philips Medical Systems, Bothell, WA) and standardized measurement protocol [13]. All ultrasound examinations were performed by one of two gynecological ultra-sonographers and images were evaluated and scored by one expert (JED).

TAUS with a full urinary bladder to facilitate visualization was conducted, with uterine length (L), width (W) and height (H) recorded and volume estimated using the ellipsoid formula $[\pi/6*(LxWxH)]$ [28–30]. AFC was determined by counting the number and diameter of follicles [14]. Antral follicles were defined as follicles <10 mm in diameter, including all follicles between 2 and 9 mm diameter. If a follicle ≥10 mm was seen the ultrasound was repeated in the early follicular phase of the next cycle.

Circulating concentrations of AMH and Inhibin B were secondary outcomes and were measured in plasma samples from day 2–6 of the menstrual cycle. AMH was measured using a commercially available two-site ELISA kit (A16507; Immunotech-Beckman Coulter, Prague, Czech
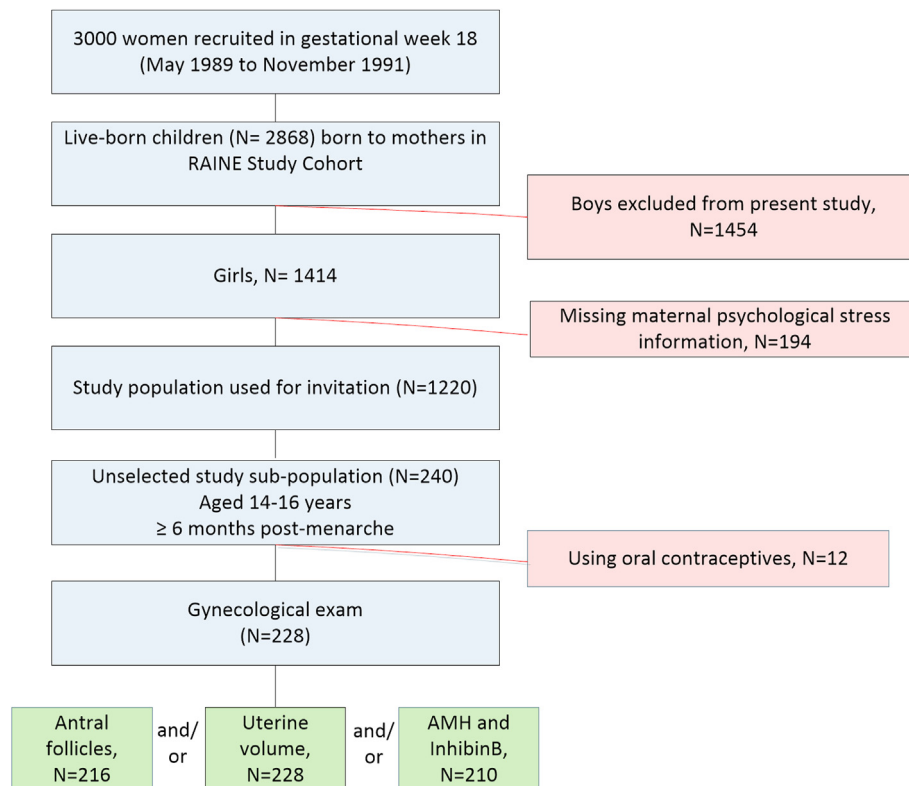
Fig. 1. Study population and exclusions.

Table 1
Type, number and timing of stressful life events experienced by the 228 women at each measured time point[a], during pregnancy, 1989–1991.

Stressful Life events	Early gestation	Late gestation
	N (%)[b]	N (%)[b]
Number of individual events reported		
Death of a relative	8 (3.5)	9 (4.0)
Death of a friend	6 (2.6)	4 (1.8)
Your own job loss (not voluntary)	4 (1.8)	2 (0.9)
Your partner's job loss (not voluntary)	8 (3.5)	7 (3.1)
Pregnancy concerns	69 (30.3)	46 (20.2)
Separation or divorce	2 (0.9)	7 (3.1)
Marital problems	8 (3.5)	15 (6.6)
Problems with your children	19 (8.3)	17 (7.5)
Money problems	56 (24.6)	43 (18.9)
Residential move	33 (14.5)	30 (13.2)
Other problems	35 (15.4)	28 (12.3)
Total Stressful life events (N)	*248*	*208*
Number of women reporting Stressful life events[c]		
None (0)	83 (36.4)	103 (45.2)
1	79 (34.6)	69 (30.3)
>1	66 (29.0)	56 (24.5)

[a] The questionnaire at early gestation related to the period since becoming pregnant, and on the late gestation questionnaire, the women were asked whether any of the stressful life events had been experienced during the past 4 months, ensuring that the same event was not counted twice.

[b] Percentages for each event are calculated based on total number of mothers, some mothers report more than one stressful life event, thus for early gestatio the total number of events is 248.

[c] Categories based on the unweighted number of the experienced stressful life events.

Republic). Plasma pools with means of 12 and 77 pmol/L were used to compare the assay characteristics among the seven assays used for this study. The intraassay coefficients of variation were less than 5% and the interassay coefficients of variation were less than 9%. The assay sensitivity was 1 pmol/L. Inhibin B concentrations were determined with an ELISA kit (Active DSL-10 –84100; Beckman, Western, Houston, TX). Inhibin B sensitivity was 7 pg/mL and the inter-and intraassay coefficients of variation were 9.2 and 7.0%, respectively [13,31,32].

2.1.4. Statistical approach

Descriptive data for exposures, co-variates and outcomes were computed. Continuous data were summarized using medians and interquartile ranges (IQRs) and categorical data were summarized using frequency distributions.

Estimations of the linear association between exposure to maternal psychological stress (stressful life events) and female reproductive function were performed using analysis in crude (no adjustments) and adjusted multivariate linear regression models. Main outcomes were uterine volume and AFC, secondary outcomes were circulating AMH and Inhibin B. All continuous outcomes were transformed [cubic root (Inhibin B), natural logarithm (uterine volume, AFC, AMH)] before statistical analyses to meet linear regression model assumptions of normal distribution and constant variance (homoscedasticity) in residuals (assessed using normal probability plots of the residuals, and the Shapiro-Wilk test). The main multivariate model was adjusted for potential confounders including maternal [body mass index (BMI, continuous), age (continuous), parity (dichotomized 0 or $\geq$ 1) and socio-economic status (SES) quantified as total household annual income: dichotomized to reflect a minimum income level (<\$24,000 p.a. or $\geq$ \$24,000 p.a.) according to the Australian Government guidelines at the time of the pregnancies (1989–1991)]. Gynecological age (time since menarche) and age at the time of participation (offspring) were included in the adjusted models to improve precision. Finally, offspring height was added to the model assessing association with uterine volume.

All associations were assessed by separately examining the effects of early gestational stressful life event exposures (reported at 18 weeks) and late gestational exposure (reported at 34 weeks). The exposures in each time window were included in separate models as categorically coded

explanatory variables (none (reference) versus $\geq$ 1 event). Models addressing effects of stressful life event in late gestation (34 weeks) were mutually adjusted for events reported in early gestation (at 18 weeks). Linear trend was assessed in all models by entering the stress strata into the model as a continuous explanatory variable.

The potential mediating effects of maternal smoking and pre-eclampsia on the association between gestational stressful life event exposure and offspring reproductive function (uterine volume, AFC, AMH and Inhibin B) were investigated separately in additional models. Finally, the effect modification of adolescent BMI (<25 kg/m^2/$\geq$25 kg/m^2) on the association between prenatal exposure to stressful life event and reproductive function (uterine volume, AFC, AMH and Inhibin B) was assessed by introducing interaction terms into the model and performing a log-likelihood test.

The results of the linear regression analyses are presented as β-coefficient effect estimates and 95% confidence intervals (CI) to indicate the pattern and magnitude of associations between the reproductive outcomes measured. To ease interpretation the estimated marginal means derived from the linear regression analyses are also presented as back-transformed values according to stressful life event strata. Data were analyzed using STATA software (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP.). A p-value < 0.05 was considered statistically significant.

Research was conducted in accordance with principles of the Declaration of Helsinki. The Raine Study was approved by the University of Western Australia Human Research Ethics Committee and written informed consent was obtained from all participants prior to enrollment. The study is reported according to the STROBE (Strengthening the Reporting of Observational Studies) guidelines and checklist.

3. Results

Of 228 recruited females, 145 (64%) were exposed to one or more stressful life event in early gestation (reported at week 18) and of these 66 (29%) were exposed to two or more stressful life events. Exposure in late gestation were less common and around half (125/228, 55%) were exposed to at least one stressful life event (Table 1).

Person-related characteristics and untransformed uterine volume and markers of ovarian function were stratified according to number of individual gestational stressful life events in early and late gestation (none and $\geq$1) (Table 2). Most mothers (n = 208, 91.2%) were Caucasian. In both gestational period's mothers reporting stressful life events had lower mean incomes, were more likely to have existing children and less likely to have pre-eclampsia in the index pregnancy. BMI and age at birth were similar, whilst smoking was slightly more prevalent in mothers reporting more than one stressful life event (Table 2).

The median age of adolescent girls at the time of survey was 15.1 years and median age at menarche was 12.8 years, consistent with other published studies of similar populations [12,33,34]. Likewise, median uterine volume was 36.2 cm^3 and median AFC by was 11 (size, 2–9 mm), both values are consistent with previously published studies of uterine volume [35,36] and AFC [37] in this age group. Adolescents exposed to prenatal stressful life events in late gestation had significantly larger uterine volume and ovarian AFC, whilst no significant differences were observed according to exposure to stressful life events in early gestation. Adolescent size [at birth (length and weight) and adolescence (BMI and height)] were similar regardless of exposures to prenatal stressful life events (Table 2).

Exposure to one or more maternal stressful life events in late gestation was associated with a greater uterine volume (β = 0.11, 95% CI 0.02;

Table 2
Person-related characteristics and untransformed outcomes of the 228 participants and mothers - stratified by *in utero* psychological stress exposure (number of stressful life events reported in early and late gestation).

	All exposures	Early gestation		P-value*	Late gestation		P-value*
		None	≥1		None	≥1	
		Median (25th; 75th ‰)/N (%)					
Characteristics of Mothers (N)[a]	*228*	*83*	*145*		*103*	*125*	
Age at time of child's birth (years)	30 (26; 34)	30 (28; 34)	30 (26; 34)	0.39	30 (27; 34)	29 (25; 34)	0.49
Nulliparous	104 (45.6)	45 (54.2)	59 (40.7)	0.05	48 (46.6)	56 (44.8)	0.79
Body Mass Index (BMI, kg/m^2)	21.5 (20.0; 23.7)	21.8 (20.3; 23.5)	21.3 (20.0; 23.7)	0.49	21.5 (20.2; 23.5)	21.3 (19.8; 23.9)	0.94
Low socio-economic status[b]	77 (33.8)	17 (20.5)	60 (41.4)	0.001	29 (28.2)	48 (38.4)	0.10
Smoker	48 (21.1)	16 (19.3)	32 (22.1)	0.62	21 (20.4)	27 (21.6)	0.82
Pre-eclampsia	42 (18.4)	19 (22.9)	23 (15.9)	0.19	25 (24.3)	17 (13.6)	0.04
Caucasian	208 (91.6)	77 (92.8)	131 (91.0)	0.64	96 (94.1)	112 (89.6)	0.22
Person-related characteristics of adolescent girls at birth[c]							
Pre-term (<37 weeks)	20 (8.8)	6 (7.2)	14 (9.7)	0.53	11 (10.7)	9 (7.2)	0.36
Head circumference at birth (cm)	34.5 (33.0; 35.0)	35 (33; 36)	34.0 (33.0; 35.0)	0.66	34.0 (33.0; 35.0)	34.5 (33.3; 35.5)	0.30
Birth length (cm)	49 (48; 50)	49 (48; 50)	49.0 (47.5; 50.0)	0.66	49 (47; 50)	49.0 (48.0; 50.0)	0.49
Birth weight (g)	3328 (3040; 3645)	3330 (3040; 3565)	3325 (3050; 3685)	0.79	3315 (2860; 3625)	3330 (3085; 3660)	0.31
Gynecological exam and interview[c]							
Age at time of inclusion (years)	15.1 (14.9; 15.4)	15.1 (14.9; 15.4)	15.1 (15.0; 15.5)	0.53	15.1 (14.9; 15.4)	15.1 (15.0; 15.4)	0.35
Height (cm)	162 (159; 167)	162 (159; 165)	163 (159; 167)	0.85	162 (159; 166)	163 (158; 167)	0.96
Body Mass Index (BMI, kg/m^2)	21.0 (19.3; 23.6)	21.0 (19.1; 22.9)	21.0 (19.3; 23.9)	0.66	20.6 (19.0; 22.7)	21.2 (19.5; 24.3)	0.03
Puberty							
Age at menarche	12.8 (12.1; 13.3)	12.8 (12.2; 13.4)	12.7 (12.1; 13.2)	0.64	12.7 (12.1; 13.4)	12.8 (12.1; 13.2)	0.70
Time since menarche (months)	29.0 (21.8; 37.1)	28.8 (20.4; 36.7)	29.0 (22.3; 37.5)	0.53	29.3 (21.0; 36.6)	28.9 (22.4; 37.3)	0.65
Uterine volume (cm^3)	36.2 (29.9; 48.5)	34.6 (29.5; 46.4)	37.0 (30.2; 48.6)	0.30	33.9 (28.9; 44.2)	39.9 (32.3; 50.4)	0.003
Markers of ovarian reserve							
Antral follicle count (sizes 2–9 mm)[d]	11 (7; 14)	11 (8; 14)	11 (7; 15)	0.73	10 (7; 14)	12 (8; 16)	0.03
Anti-Mullerian Hormone, pmol/L[e]	24.9 (17.7; 36.2)	24.0 (17.1; 33.8)	26.1 (19.1; 37.2)	0.28	25.0 (16.8; 38.3)	24.4 (18.0; 34.1)	0.79
Inhibin B pg/mL[e]	50.8 (34.9; 65.8)	54.0 (39.7; 65.6)	49.7 (29.5; 67.0)	0.45	50.8 (36.3; 68.9)	50.9 (31.0; 65.4)	0.86

*Kruskal-Wallis for continuous and chi-square test for categorical values.

[a] Based on 228 mothers reported in their index pregnancy.

[b] Average annual family income level per annum below $24,000 reflecting the minimum income level in 1989–1991, according to the Australian Government guideline.

[c] Based on 228 adolescent girls that underwent transabdominal ultrasound of the uterus.

[d] The sum of ovarian follicles from both ovaries reported, based on 216 adolescent girls with ovarian antral follicle count.

[e] Based on 210 adolescent girls that provided a blood sample for analysis.

0.02) and greater ovarian AFC (β = 0.17, 95% CI 0.01; 0.32) in crude regression analyses. These associations persisted after adjustment in the multivariate linear regression analyses for uterine volume (β = 0.13, 95% CI 0.04; 0.23) and AFC (β = 0.19, 95% CI 0.02; 0.35). A linear trend effect for these positive associations was also detected (p-value < 0.04) (Table 3). The back-transformed mean uterine volume and ovarian AFC in adjusted models following exposure to late gestation stressful life events were 40.7 cm³ and 11, respectively (supplementary table S1), which corresponds to a 13% increase in uterine volume and an 18% increase in AFC in girls exposed to at least one event compared to girls with no exposure to late gestation stressful life events. No associations were found between number and/or timing of stressful life events and ovarian function (AMH and Inhibin B) (Table 3).

We detected no mediating effects on any of the observed effects (results not shown) by either maternal smoking or pre-eclampsia, nor was any effect modification of adolescent BMI (p-value for interaction, > 0.41) detected (results not shown).

4. Discussion

This is the first human study to measure the prospective relationships

Table 3
Beta-coefficient (β) effect estimates and 95% confidence intervals (CIs) from the linear regression analyses of the transformed outcome variables (uterine volume and markers of ovarian reserve) in adolescent (generation 2) girls aged 15 years as a function of number of stressful life events in EARLY and LATE GESTATIONAL periods. Girls with no exposure to maternal stressful life events served as reference population versus girls with exposure to at least one maternal stressful life event.

	Early gestation		Late gestation[c]	
	β (95% CI)	p-value (linear trend)[d]	β (95% CI)	p-value (linear trend)[d]
Ovarian reserve				
Mean uterine volume (cm³), natural logarithm				
Crude model	0.05 (−0.05; 0.14)	0.31	**0.11 (0.02; 0.20)**	**0.01**
Adjusted model[a,b]	0.04 (−0.06; 0.14)	0.42	**0.13 (0.04; 0.23)**	**0.01**
Anti-Mullerian Hormone, AMH (pmol/L), natural logarithm				
Crude model	0.09 (−0.08; 0.26)	0.28	0.01 (−0.16; 0.18)	0.91
Adjusted model[a]	0.04 (−0.14; 0.22)	0.70	−0.01 (−0.18; 0.17)	0.95
Inhibin B (pg/mL), cubic root				
Crude model	−0.09 (−0.29; 0.11)	0.36	0.05 (−0.14; 0.26)	0.59
Adjusted model[a]	−0.08 (−0.30; 0.14)	0.46	0.03 (−0.18; 0.23)	0.82
Ovarian antral follicle count, natural logarithm				
Crude mode	−0.01 (−0.17; 0.14)	0.88	**0.17 (0.01; 0.32)**	**0.04**
Adjusted model[a]	−0.01 (−0.18; 0.16)	0.89	**0.19 (0.02; 0.35)**	**0.03**

[a] Model adjusted for maternal [age, pre-pregnancy Body Mass Index (BMI), socioeconomic status [total household annual income: dichotomized to reflect a minimum income level (<$24,000 p.a. or ≥ $24,000 p.a.) according to the Australian Government guidelines at the time (1989–1991)] and parity (dichotomized 0 or ≥ 1)] and the daughter's own (years) and time since menarche (months).
[b] Adjusted for the daughter's own height at the time of participation.
[c] Models in LATE GESTATION adjusted for stressful life events reported in EARLY GESTATION.
[d] P-value for linear trend, assessed by entering the stress strata into the multivariate linear regression model as a continuous explanatory variable.

between direct measures of prenatal exposure to psychological stress during early and late gestation and subsequent reproductive function using established direct measures in a large, unselected population of adolescents. Surprisingly, we found that exposure to one or more stressful life events in late gestation was associated with a significantly larger uterine volume and higher ovarian AFC, suggestive of enhanced reproductive function. No associations between early gestational stressful life events and reproductive function were observed and there was no deleterious association between prenatal stressful life events and markers of ovarian function (AMH, Inhibin B). Together, these findings do not confirm an association between maternal psychological stressors and female reproductive dysfunction via prenatal programming. The mechanisms underlying this association are uncertain. The uterus is an essential reproductive organ in mammals that develops from the Müllerian ducts during prenatal life. Despite the importance of the uterus for fertility and the health of women and their offspring, relatively little is known about the early life events regulating prenatal differentiation and development of the Müllerian ducts into the uterus [38]. Once formed, the maximum period of uterine growth is during late gestation (weeks 25 to 31) [39]. Our findings suggest that exposure late gestational psychological stress may affect this growth period leading to greater uterine volumes in adolescence. The clinical significance of this finding is uncertain, but adequate uterine volume is important for successful pregnancy outcomes and both reduced (<30 mL) and increased (≥70 mL) uterine volume are associated with adverse pregnancy outcomes in adult women including lower live birth rates [40].

Primordial follicles which constitute the ovarian reserve are formed during prenatal life, predominantly during the second trimester of gestation, and numbers are fixed by the time of birth [41,42]. The ovarian reserve makes a substantial contribution to female fertility and regulating age at menopause [43], therefore understanding prenatal factors regulating the ovarian reserve is of considerable importance to human health. There are no established markers for measuring ovarian reserve in adolescence, but in adult women both AMH and AFC are widely utilized biomarkers in clinical practice and epidemiological studies [44–47]. In this present study, adolescents exposed to late gestation stressful life events had higher AFC compared to the non-exposed population. This is suggestive of higher ovarian reserve in the exposed population, but this finding could not be confirmed without histological examination of the ovaries to count primordial follicles. The primordial follicle count is thought to peak at around 26 weeks gestation and the mechanisms regulating the subsequent dramatic loss in primordial follicles are poorly understood [41]. Although reproductive markers change during puberty, increased AFC is one of the criteria used for diagnosing polycystic ovary morphology (PCOM) together with enlarged ovarian volume [48]. Being diagnosed with PCOM constitutes a risk for being diagnosed with PCOS according to the Rotterdam criteria [12] which in later life can cause fertility issues and increase risk of cardiovascular diseases [49] and type 2 diabetes [50].

Strengths of this study include detailed prospective data collected using standardized questionnaires measuring maternal stressful life events during both early and late gestation. This minimized the possibility of recall bias. Uterine volume was directly measured by an experienced gynecologist and biomarkers of ovarian function were measured using high sensitivity assays. Additional strengths include the prospectively collected measures of potential confounders, mediators and effect modifiers which minimized information and selection bias.

When assessing our results, we adjusted for several confounders but could not exclude the influence of unmeasured pre and postnatal factors on our outcomes of interest. It is not possible to account for all these factors, thus residual confounding or confounding by unmeasured variables cannot be ruled out. For example, most mothers reported at least one stressful life event in pregnancy, particularly those from more socially disadvantaged groups (as indicated by income level). Thus, common factors, unaccounted here, that impact both stressful life event, socio-economic status and reproductive outcomes may partially explain

our findings. In addition, women may vary in their response to stressful events and measures of perceived stress severity may have been more relevant.

Our sample size was moderate with over 63%/55% exposed to at least one maternal stressful life event in early/late gestation. A larger sample size would have conferred greater statistical power with less risk of false negative results (Type II error). We performed tests for our main outcomes independently and when applying the Bonferroni method of correction, the significance level of p-values would be adjusted to 0.0125, compared to the significance levels of 0.05 applied in this study, potentially implying that the associations with AFC may have been due to chance.

Transvaginal ultrasound may have provided a more accurate measure of uterine volume and ovarian AFC, particularly in obese women [51]. However, we found no correlation between BMI and uterine volume (P = 0.487, results not shown), nor did BMI modify the association between maternal stressful life events and uterine volume. We have previously shown that transabdominal ultrasound is equally sensitive to MRI in the measurement of uterine volume [35]. Median uterine volume in this study was 36.2 cm^3 (excluding endometrial volume) and median AFC was (2–9 mm) are consistent with previously published studies in this age group [35–37].

Participants were age 15.1 (25th-75th percentile: 14.9 to 15.5) years at the time of ultrasound which may have coincided with a period of uterine growth [52]. Normal variations in the trajectory of uterine growth may have accounted for the apparent variation in uterine volume due to gestational stressful life events and postnatal factors may have affected uterine volume in adolescence. Genetic factors may also contribute to uterine volume, but we had no information on maternal uterine volume. Similarly, adolescent uterine volume may depend on adolescent height, but this was addressed in our statistical analyses and did not affect the associations we detected.

4.1. Perspectives

Future studies should consider measuring maternal response to psychological stress in addition to stressful life events and potential mechanisms including HPA axis reactivity.

5. Conclusion

Exposure to maternal psychological stress, quantified as stressful life events in late gestation affect was associated with 13% greater uterine volume and 18% higher AFCs. Although, the clinical significance of greater uterine volume and higher AFC in adolescence is uncertain, the uterus is an essential organ of reproduction and these data add support to the hypothesis that prenatal psychological stress has effects on reproductive function in offspring.

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Disclosures

None.

Author contribution statement

EVB drafted the manuscript and TK performed data clean up and statistical analyses. AJ, RH, MH and DD contributed to the manuscript preparation. EVB, TK, MH, AJ and RH contributed to the concept and design for the study. DD prepared data for analyses and EVB contributed with data clean-up. DD, TK and EVB collaborated on the statistical strategy. RH provided all The Raine Study data. JD performed all TAUS. All authors contributed to critical interpretation of data and the final draft of the manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The following financial interests:

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cpnec.2020.100026.

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