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Hormonal imbalance in umbilical vein cord blood of pregnant women with endometriosis: a propensity score-matched analysis

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Abstract

Background Pregnancy involves a fine-tuned hormonal interplay between the fetus, placenta, and mother, which shapes long-term developmental outcomes. Endometriosis has been hypothesized to originate in utero due to altered fetal exposure to sex steroids. This study investigates differences in umbilical cord estradiol and androgen levels in female fetuses born to women with and without endometriosis, exploring a potential role of altered in utero hormone exposure in the intergenerational transmission of endometriosis risk.

Methods This is a case-control study nested within a cohort of women delivering singleton females at our institution between June 2022 and June 2023. Cases were women with endometriosis (diagnosed via imaging or surgery before pregnancy) and controls were women without endometriosis. Analyses were performed before and after propensity-score matching (PSM) at a 1:3 ratio to control for maternal age, gestational age, and delivery mode. Umbilical cord blood was collected at delivery, and hormonal levels of corticosteroids, progestins, androgens, and estradiol (E2) were measured using liquid chromatography-tandem mass spectrometry. Estradiol-to-androgens ratios were calculated, with 95% confidence intervals (CIs) determined using the bootstrapping method.

Results The total cohort included 160 women, 17 (10.6%) of whom had endometriosis. After 1:3 matching, 51 controls without endometriosis were included. Women with endometriosis had higher E2 levels compared to controls, both before PSM (6.8 [4.3–9.1] mcg/L vs. 3.6 [1.4–8.6] mcg/L, $p=0.03$) and after PSM (2.4 [1.1–6.6] mcg/L, $p=0.002$). Estradiol-to-androgens ratios indicated a higher-estrogen and lower-androgens hormonal status in endometriosis, with higher E2-to-testosterone ($p<0.001$), E2-to-androstenedione ($p=0.001$), E2-to-dehydroepiandrosterone (DHEA) ($p<0.001$), and E2-to-DHEA sulfate (DHEAS) ratios ($p<0.001$) compared to controls. After PSM, the E2-to-testosterone, E2-to-androstenedione, E2-to-DHEA, and E2-to-DHEAS ratios were 4.38 (95% CI: 2.28–6.49), 3.28 (95% CI: 1.29–5.28), 2.52 (95% CI: 1.32–3.72), and 4.57 (95% CI: 1.86–7.27) times higher, respectively.

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Conclusions This is the first study showing that female newborns of women with endometriosis are exposed to higher in utero estradiol compared to controls. This high estrogen low androgens environment may influence fetal programming of reproductive traits and might drive the in utero susceptibility to endometriosis.

Keywords Endometriosis, Pregnancy, Umbilical blood cord, In utero exposure, Estrogen, Estradiol-to-Androgen, Estrogenic imprinting

Background

Pregnancy orchestrates a unique hormonal symphony, driven by the metabolic and endocrine dynamics of the feto-placental unit [1]. Such hormonal interplay is crucial throughout pregnancy, from implantation to delivery. Moreover, its effects seem to reach far beyond fetal growth and in utero life, with lasting consequences that shape newborns' developmental trajectories well into adulthood. According to the Developmental Origins of Health and Disease (DOHaD) concept, the in utero environment plays a key role in both shaping physiological traits and determining future disease risks [2].

The hormonal microenvironment to which the fetus is exposed in utero results from complex steroidogenesis pathways. Unlike conventional hormone production within a single organ, it arises from a finely tuned and dynamic interplay between the fetus, the placenta, and the mother [3]. In this dialogue, the mother provides the placenta—and ultimately the fetus—with steroid precursors while also contributing to hormone clearance [4]. Thus, fetal endocrine exposure depends largely on maternal steroids availability for placental steroidogenesis.

This context frames our research question. Endometriosis, affecting approximately 1 in 10 women of reproductive age [5]. Several authors propose that endometriosis originates in utero as a result of exposure of female fetuses to high estrogen and low androgen concentrations [6]. However, only indirect indicators of such hyper-estrogenic intrauterine milieu, such as the anogenital distance, have been evaluated up to now [7–9]. Prospective studies storing umbilical cord blood at birth and then correlating steroids levels to the later onset of endometriosis are not available and would be extremely demanding to set and run. A surrogate possibility to test this hypothesis could be the evaluation of these hormones in the umbilical cord blood of the offspring of women with endometriosis. This could indirectly confirm the relevance of the hormonal intrauterine milieu in the pathogenesis of the disease and would support the possibility of a non-genetic vertical transmission of the disease.

Thus, our research question was whether a difference exists in estradiol and androgen concentrations on blood collected from the umbilical cord of female fetuses born from women with and without a history of endometriosis. If demonstrated, such a difference in the overall fetal sex steroid profile would constitute direct evidence in favor of a pro-estrogenic feto-placental-maternal endocrine interactions during gestation, potentially contributing to early in utero programming of offspring.

Methods

Population

This is a case-control study nested within a larger cohort study of women who delivered at our institution between June 2022 and June 2023. The study was conducted with the approval of the Ethics Committee of our institution (ENDO-2021-12371933).

The inclusion criteria for this case-control analysis were: (i) delivery of a singleton female; (ii) gestational age at delivery > 32 weeks; (iii) availability of an umbilical cord blood sample at the time of delivery; (iv) absence of severe pre-existing health conditions; (v) for endometriosis cases, a diagnosis confirmed by surgery or imaging before pregnancy; and (vi) for controls, a history of regular menstrual cycles and no previous diagnosis of endometriosis.

Women who did not meet the inclusion criteria, had insufficient umbilical vein cord blood sampling for hormone measurement, or did not provide informed consent were excluded. Maternal, pregnancy, delivery, and neonatal characteristics at birth were recorded in an electronic database at the time of delivery. The study was conducted and reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [10].

The samples were taken from the umbilical vein, after clamping the cord, following delivery of the newborn. The samples were immediately centrifuged and the serum stored at − 80 °C before all measurements are performed together.

Outcomes

The primary objective was to assess whether women with endometriosis exhibit different umbilical cord blood hormone levels and estradiol-to-androgen ratios compared to controls. Hormonal levels in umbilical cord blood were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays. The full panel of quantified steroid hormones included corticosteroids (aldosterone, cortisol, cortisone, 11-deoxycortisol, corticosterone, 11-deoxycorticosterone), progestins (17-OH progesterone, progesterone), androgens (testosterone,

androstenedione, dehydroepiandrosterone [DHEA], DHEA sulfate [DHEAS]), and 17β-estradiol (E2).

The secondary objective was to investigate potential correlations between hormonal levels and neonatal size outcomes. Weight/Length Ratio (WLR) and corresponding centiles were calculated according to the INTERGROWTH-21st standards [11].

Statistical analysis

Propensity-score matching (PSM) was applied to control for biological factors known to influence umbilical cord blood hormone levels, including maternal age at delivery (years), gestational age at birth (days), and mode of delivery (elective cesarean section [CS], vaginal birth [VB], or emergent/urgent CS during labor) [12]. Logistic regression scores were computed, and nearest-neighbor matching with a 1:3 ratio was applied to match endometriosis cases with controls. All analyses were performed both before and after PSM.

The Shapiro-Wilk test was used to assess the normality of distribution for continuous variables. Normally distributed variables were reported as mean ± standard deviation (SD), while non-normally distributed variables were expressed as median and interquartile range (IQR). Categorical variables were presented as absolute values and percentages (%). Group comparisons were performed using independent t-tests, Kruskal-Wallis tests, Pearson's chi-square test, or Fisher's exact test, as appropriate. The ratios of medians (incremental factors) for estradiol-to-androgen levels between endometriosis cases and controls were calculated, with 95% confidence intervals (CIs) obtained using the bootstrapping method. Bootstrapping was performed with 1,000 and 5,000 replications to improve the precision and robustness of the estimates (non-normally distributed variables and small sample sizes). Correlations between umbilical cord blood hormone levels and neonatal growth metrics were assessed using Spearman's rho, with a sensitivity analysis performed on uncomplicated pregnancies.

Statistical analyses were performed using STATA version 18 (StataCorp LLC, 2024, College Station, TX, USA).

Results

Baseline characteristics

Among the 160 women who delivered singleton female infants and met the inclusion criteria, 17 (10.6%) had endometriosis. Specifically, 7 women (41.2%) had a history of ovarian endometriosis, 7 (41.2%) had deep endometriotic lesions, 2 (11.8%) had superficial endometriosis detected during laparoscopy, and 1 (5.9%) had both deep and ovarian endometriosis.

Baseline characteristics of women with endometriosis and controls before and after PSM are summarized in Table 1.

Before matching, women with endometriosis had a higher incidence of placenta previa (11.8% vs. 0.7%, $p = 0.03$) and some differences in delivery mode, with more elective CS (47.1% vs. 21.7%) and fewer VB (47.1% vs. 70.6%; $p = 0.07$). Mode of conception was similar both before and after matching, with assisted reproductive technologies used in 11.8% of endometriosis cases vs. 9.1% ($p = 0.66$) and 9.8% ($p = 1.00$) of controls. Other maternal, pregnancy, delivery, and neonatal characteristics were also comparable between groups, both before and after PSM.

As a result of PSM, the matched cohort showed a well-balanced distribution of factors known to influence the outcome measure, including maternal age (35.8 ± 3.5 vs. 35.7 ± 3.6 years; $p = 0.91$), gestational age at delivery (275.2 ± 12.8 vs. 277.7 ± 8.7 days; $p = 0.37$), and mode of delivery (elective CS: 41.2%, VB: 51.0%, emergent/urgent CS: 7.8%; $p = 0.91$).

Umbilical blood cord hormonal levels and estrogen-to-androgens ratio

Umbilical vein cord blood steroid levels are presented in Table 2. Among androgens, dehydroepiandrosterone (DHEA) levels were lower in endometriosis cases compared to controls (before matching: 2.1 [1.6–3.2] vs. 3.3 [2.4–4.9], $p = 0.008$; after matching: 2.1 [1.6–3.2] vs. 3.2 [2.4–3.9], $p = 0.05$).

Estradiol levels were significantly higher in endometriosis cases both before PSM (6.8 [4.3–9.1] mcg/L vs. 3.6 [1.4–8.6] mcg/L, $p = 0.03$) and after PSM (6.8 [4.3–9.1] mcg/L vs. 2.4 [1.1–6.6] mcg/L, $p = 0.002$). Notably, 88.2% of women with endometriosis had E2 levels above the cohort average, compared to 37.2% of matched controls ($p < 0.001$).

Estradiol-to-androgen ratios confirmed a higher-estrogen, lower-androgen profile in endometriosis, with significantly elevated ratios for estradiol-to-testosterone ($p < 0.001$), estradiol-to-androstenedione ($p = 0.001$), estradiol-to-DHEA ($p < 0.001$), and estradiol-to-DHEAS ($p < 0.001$) (Table 3). The E2-to-testosterone, E2-to-androstenedione, E2-to-DHEA, and E2-to-DHEAS ratios were 4.38 (95% CI: 2.28–6.49), 3.28 (95% CI: 1.29–5.28), 2.52 (95% CI: 1.32–3.72), and 4.57 (95% CI: 1.86–7.27) times higher, respectively, in women with endometriosis compared to matched controls, as determined through bootstrapping with 1,000 replications (Table 4).

Corticosteroid and progestin levels were generally similar between groups, except for higher 11-deoxycortisol levels in endometriosis cases after PSM (9.4 [7.3–13.5] vs. 5.5 [3.7–8.9] mcg/L; $p = 0.002$).

Correlations between hormonal levels and newborn size

The correlation matrix of umbilical vein cord blood hormone levels with newborn size variables in women with

Table 1 Baseline characteristics of endometriosis and controls before matching ($n=160$) and after matching ($n=68$)

		Before matching		After matching	
Characteristics	**Endometriosis ($n=17$)**	**Controls ($n=143$)**	***p*-value**	**Controls ($n=51$)**	***p*-value**
Pre-pregnancy					
Age at delivery (years)*	35.8 ± 3.5	34.7 ± 4.1	0.31	35.7 ± 3.6	0.91
Nulliparity	14 (82.4%)	95 (66.4%)	0.27	27 (52.9%)	0.05
BMI before pregnancy (kg/m^2)	20.1 [18.9–24.4][a]	21.4 [19.6–23.5]	0.63	21.8 [20.1–23.5]	0.48
Pregnancy					
Mode of conception			0.66		1.00
Spontaneous	15 (88.2%)	130 (90.9%)		46 (90.2%)	
ART	2 (11.8%)	13 (9.1%)		5 (9.8%)	
BMI at the end of pregnancy (kg/m^2)	26.0 [22.9–29.7][a]	25.8 [24.1–28.1][b]	1.00	26.2 [24.3–28.3]	0.72
Excessive weight gain during pregnancy[c]	1 (6.3%)[a]	19 (13.5%)[b]	0.69	10 (19.6%)	0.27
Pregnancy complications					
Placenta previa	2 (11.8%)	1 (0.7%)	**0.03**	1 (2.0%)	0.15
Gestational diabetes	1 (5.8%)	20 (14%)	0.70	4 (7.8%)	1.00
Hypertension	3 (17.6%)	9 (6.3%)	0.12	4 (7.8%)	0.36
Chronic hypertension	1 (5.8%)	2 (1.4%)	0.29	1 (2.0%)	0.44
Gestational hypertension	0 (0%)	4 (2.8%)	1.00	2 (3.9%)	1.00
Pre-eclampsia	2 (11.8%)	3 (2.1%)	0.08	1 (2.0%)	0.15
Induction of fetal lung maturation	2 (11.8%)	5 (3.5%)	0.16	1 (2.0%)	0.15
Delivery					
GA at delivery (days)*	275.2 ± 12.8	276.4 ± 8.5	0.60	277.7 ± 8.7	0.37
Mode of delivery*			0.07		0.91
Elective CS	8 (47.05%)	31 (21.7%)		21 (41.2%)	
Vaginal birth	8 (47.05%)	101 (70.6%)		26 (51.0%)	
Emergent/Urgent CS during labor	1 (5.9%)	11 (7.7%)		4 (7.8%)	
Intrapartum fever	1 (5.8%)	16 (11.3%)	0.70	5 (9.8%)	1.00
Newborn size					
Newborn length (cm)	49.5 ± 1.5[a]	49.6 ± 2.3[d]	0.80	49.9 ± 1.9[b]	0.47
Newborn weight (kg)	3.3 ± 0.4	3.2 ± 0.4	0.35	3.2 ± 0.4	0.22
Newborn WLR (kg/cm)	6.4 ± 0.7[a]	6.5 ± 0.7[d]	0.43	6.6 ± 0.7[b]	0.60
Newborn WLR centile[e]	44.4 [35.7–51.8][a]	47.4 [30.2–76.3][d]	0.70	41.2 [32.2–75.2][b]	0.90

Bold *p*-values indicate statistical significance

Data reported as mean ± SD (standard deviation) or median [interquartile range: 25th–75th] or n (%)

WLR Weight/Length Ratio, *GA* gestational age, *ART* assisted reproductive techniques, *BMI* body mass index, *CS* Cesarean Section

*Variables included for propensity scores calculation and matching (nearest neighbor)

[a]One missing observation

[b]Two missing observations

[c]Defined according to the Institute of Medicine (IOM)/National Research Council (NRC) guidelines

[d]Three missing observations

[e]Calculated according to GA at delivery using the INTERGROWTH-21st calculator for WLR at birth

endometriosis and matched controls is presented in Supplementary Table 1. In women with endometriosis, but not in controls, corticosteroids were positively correlated with newborn weight, length, and WLR. Notably, 11-deoxycortisol showed a strong correlation with newborn weight ($r=0.80$; $p<0.001$) and WLR ($r=0.73$; $p=0.002$), even when the analysis was restricted to uncomplicated pregnancies (weight: $r=0.88$; $p=0.002$; WLR: $r=0.87$; $p=0.002$) (Supplementary Fig. 1).

Discussion

Main results

The findings of this study suggest, for the first time, that female newborns of women with endometriosis experience a distinct hormonal environment in utero, characterized by significantly higher E2 levels. A consistent increase in all E2-to-androgen ratios was observed, with E2-to-testosterone and E2-to-DHEAS ratios exceeding four times the levels found in controls. While this heightened estrogenic in-utero exposure does not appear to directly affect newborn size, it is likely to have

Table 2 Umbilical cord blood steroidal levels of endometriosis and controls before and after matching

		Before matching		After matching	
Hormonal levels	**Endometriosis (*n* = 17)**	**Controls (*n* = 143)**	***p*-value**	**Controls (*n* = 51)**	***p*-value**
Corticosteroids					
Aldosterone (mcg/L)	0.3 [0.3–0.5]	0.3 [0.2–0.5]	0.34	0.3 [0.2–0.5]	0.28
Cortisol (mcg/L)	26.6 [24.0–44.6]	38.6 [29.7–67.5]	0.10	35.9 [29.7–73.2]	0.15
Cortisone (mcg/L)	94.1 [65.9–150.3]	144.8 [96.5–181.2]	**0.02**	125.3 [75.4–167.0]	0.95
11-Deoxycortisol (mcg/L)	9.4 [7.3–13.5]	7.4 [4.3–11.3]	0.06	5.5 [3.7–8.9]	**0.002**
Corticosterone (mcg/L)	2.6 [1.5–3.1]	2.9 [1.5–4.7]	0.34	2.4 [1.2–3.9]	0.94
11-Deoxycorticosterone (mcg/L)	0.8 [0.6–1.3]	0.9 [0.6–1.4]	0.70	0.7 [0.5–1.2]	0.40
Progestins					
17-OH Progesterone (mcg/L)	25.2 [20.2–33.3]	28.1 [17.8–41.3]	0.68	18.9 [14.3–29.5]	0.08
Progesterone (mcg/L)	530.8 [406.8–651.8]	591.7 [447.4-718.3]	0.38	524.1 [382.6–635.4]	0.50
Androgens					
Testosterone (mcg/L)	0.05 [0.03–0.08]	0.07 [0.04–0.11]	0.15	0.07 [0.03–0.12]	0.34
Androstenedione (mcg/L)	0.7 [0.6–0.8]	0.8 [0.6–1.1]	0.07	0.7 [0.5–0.9]	0.68
DHEAS (mcg/L)	1,715.0 [978.5–2,510.1]	1,879.1 [1,215.3–3,078.1]	0.27	1,547.1 [1,236.2–2,313.9]	0.61
DHEA (mcg/L)	2.1 [1.6–3.2]	3.3 [2.4–4.9]	**0.008**	3.2 [2.4–3.9]	0.05
Estrogens					
E2 (mcg/L)	6.8 [4.3–9.1]	3.6 [1.4–8.6]	**0.03**	2.4 [1.1–6.6]	**0.002**

Bold *p*-values indicate statistical significance

Data reported as median [interquartile range: 25th–75th]

DHEA Dehydroepiandrosterone, *DHEAS* Dehydroepiandrosterone Sulphate, *E2* estradiol

complex physiological effects with potential long-term consequences.

The development of the hypothalamic-pituitary-ovarian (HPO) axis begins in utero and is regulated by various factors, particularly prenatal hormonal exposures [13]. Early programming of the reproductive axis by in utero endocrine influences plays a crucial role in pubertal development and reproductive health later in life, by shaping hormonal regulation and susceptibility to dysregulation later in life [14].

Since endometriosis symptoms typically emerge during adolescence or early adulthood, its pathophysiological origins are thought to occur earlier in life, potentially even in utero [5]. Evidence suggests that daughters of mothers with endometriosis are twice as likely to develop the disease [15]. However, despite epidemiological support for heritability, no definitive causal genetic mutation has been so far identified. Our findings of increased prenatal estrogen exposure in the offspring of women with endometriosis may support the hypothesis of intergenerational disease transmission, potentially driven by in utero estrogenic imprinting and epigenetic regulation. Notably, prenatal exposure to diethylstilbestrol (DES)—a synthetic estrogen with high affinity for estrogen receptors alpha and beta (ERα and ERβ) [16]—has been strongly associated with an increased risk of estrogen-sensitive diseases later in life, including endometriosis [17, 18].

From a biological perspective, increased in utero estrogen exposure in female offspring of women with endometriosis may act as an initial "estrogenic hit," predisposing them to endometriosis disease development in adolescence or early adulthood [19]. However, from an evolutionary perspective, elevated prenatal estrogen exposure may not necessarily have been a detrimental factor [20].

According to some developmental biologists, endometriosis represents an extreme reproductive phenotype associated with enhanced reproductive potential. This phenotype appears to be "programmed" in utero by increased estrogen and reduced androgen exposure [21, 22], a pattern also observed in our cohort. In ancestral populations, higher in utero estrogen levels likely facilitated earlier reproductive maturity, enabling

Table 3 Umbilical cord blood estradiol-to-androgens levels of endometriosis and controls before and after matching

		Before matching		After matching	
E2-to-androgens ratios	**Endometriosis (*n* = 17)**	**Controls (*n* = 143)**	***p*-value**	**Controls (*n* = 51)**	***p*-value**
E2-to-testosterone	171.7 [120.7–209.9]	47.6 [20.0–127.0]	**0.002**	39.2 [19.7–80.3]	**0.0004**
E2-to-androstenedione	10.6 [6.9–16.5]	4.2 [1.6–11.4]	**0.003**	3.2 [1.5–10.4]	**0.001**
E2-to-DHEA	3.4 [2.1–4.7]	1.0 [0.4–3.1]	**0.0005**	0.7 [0.4–2.8]	**0.0002**
E2-to-DHEAS	3×10^{-3} [3×10^{-3}–6×10^{-3}]	2×10^{-3} [1×10^{-3}–5×10^{-3}]	**0.002**	1×10^{-3} [1×10^{-3}–3×10^{-3}]	**0.0001**

Bold *p*-values indicate statistical significance

Data reported as median [interquartile range: 25th–75th]

E2 estradiol, *DHEA* Dehydroepiandrosterone, *DHEAS* Dehydroepiandrosterone Sulphate

Table 4 E2-to-androgens ratios in endometriosis versus controls: ratio of medians with bootstrapped 95% CIs

	Before matching				After matching			
E2-to-androgens ratios	**Ratio of medians**	**95% CIs (1,000)[a]**	**95% CIs (5,000)[a]**	***p*-value**	**Ratio of medians**	**95% CIs (1,000)[a]**	**95% CIs (5,000)[a]**	***p*-value**
E2-to-testosterone	3.61	1.95–5.27	1.96–5.25	**< 0.001**	4.38	2.28–6.49	2.31–6.46	**< 0.001**
E2-to-androstenedione	2.51	1.17–3.85	1.12–3.89	**< 0.001**	3.28	1.29–5.28	1.23–5.34	**0.001[b]**
E2-to-DHEA	3.46	1.44–5.48	1.50–5.42	**0.001**	4.57	1.86–7.27	1.88–7.25	**0.001**
E2-to-DHEAS	2.09	1.25–2.93	1.25–2.93	**< 0.001**	2.52	1.32–3.72	1.31–3.73	**< 0.001**

Bold *p*-values indicate statistical significance

E2 estradiol, *DHEA* Dehydroepiandrosterone, *DHEAS* Dehydroepiandrosterone Sulphate, *95% CI* 95% confidence intervals (calculated using bootstrapping)

[a]Normal-based 95% CIs calculated using bootstrapping with 1,000 and 5,000 replications to assess the robustness of the estimates

[b]p=0.002 with 5,000 bootstrapping replications

earlier conceptions, closely spaced pregnancies, and prolonged breastfeeding. Under such conditions, this hormonal environment would have been advantageous, and endometriosis would have remained rare, as women experienced fewer menstrual cycles over their lifetime. However, with rapid societal and reproductive transitions, this once-adaptive trait may have become maladaptive. Over the past century, the interval between menarche and first childbirth has increased nearly tenfold, while fertility rates and breastfeeding durations have declined. As a result, modern women undergo a significantly greater number of menstrual cycles, leading to prolonged estrogen exposure during reproductive years, repeated ovulations, and chronic menstrual-related inflammation all of which promoting ectopic lesion formation and disease pathogenesis [23]. Our findings align with this theory, but further verification is needed.

In addition to elevated estrogen levels, female offspring of women with endometriosis may experience broader alterations in steroidogenesis. Indeed, compared to matched controls, we found that women with endometriosis had significantly higher levels of 11-deoxycortisol in umbilical cord blood, which strongly correlated with newborn size—a pattern not observed in controls. Interpreting these findings requires consideration of estradiol's role in modulating placental steroid metabolism. Estradiol is known to enhance the activity of 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2), the placental enzyme responsible for converting cortisol into its inactive form, cortisone [24, 25]. In the context of endometriosis, elevated E2 levels could lead to increased placental cortisol inactivation, resulting in reduced fetal cortisol levels and an accumulation of its precursor, 11-deoxycortisol. Given cortisol's critical role in fetal development, the observed correlation between corticosteroid levels and newborn size measures appears biologically plausible. However, whether these corticosteroid changes arise as a secondary effect of elevated E2, result from altered placental steroidogenesis, or reflect an independent dysfunction of the feto-placental hypothalamic-pituitary-adrenal (HPA) axis in endometriosis pregnancies remains to be determined.

Future studies need to focus also on male offspring considering that two studies—employing different study designs—observed an increased risk of cryptorchidism in male infants born to affected mothers, although the risk was quite modest in one of the two [26, 27]. The early-life androgen-to-estrogen balance is well known to play a role in the physiological process of testicular descent [27].

Strengths and limitations

A key strength of this study is that it provides the first report of umbilical cord blood hormonal assessments in female singleton pregnancies of women with endometriosis. The consistency observed across all E2-to-androgen ratios—considered more informative for human development than absolute concentrations [12]—further reinforces the robustness of our findings. Another major strength is the use of LC-MS/MS assays for hormone quantification. This method is currently the preferred approach for measuring circulating hormones in cord blood due to its superior sensitivity and lower risk of cross-reactivity compared to radioimmunoassay (RIA) [28]. Additionally, the PSM analysis, adjusted for obstetric confounders, allowed us to account for biological factors known to influence umbilical cord blood assessments [29, 30].

The primary limitation of this study is its small sample size, which may restrict the generalizability of the findings. Steroidogenesis during pregnancy relies on the interdependence of multiple organ systems, none of which independently possess all the necessary enzymatic capabilities [31]. Instead, they function in a complementary manner. Given that steroid precursors cross the placental barrier, altered hormone metabolism could originate from maternal, fetal, or placental sources—particularly placental aromatization [32]. Therefore, simultaneous sampling of all three compartments will be essential to elucidate the underlying mechanisms. Notably, since the umbilical vein cord blood passes through

the placental circulation and might be affected by the maternal hormonal levels, there is a possibility that detected E2 levels may not faithfully reflect those in the fetus.

Furthermore, although umbilical cord blood is considered the most reliable indicator of direct feto-placental hormonal interactions [12], there is currently no gold standard method for measuring prenatal hormone exposure [33]. Longitudinal studies with extended follow-ups, starting from early pregnancy, are necessary to confirm the presence of a hyper-estrogenic hormonal imbalance during the early in utero development of female reproductive traits in the daughters of women with endometriosis.

Additionally, an important limitation of this study—and a key recommendation for future studies—is the need to complement E2 measurement with two other estrogens that play crucial roles during pregnancy: estrone (E1) and estriol (E3). Notably, E1 levels in umbilical cord blood have been found to be lower in female offspring of mothers with polycystic ovary syndrome (PCOS) [34], supporting the hypothesis that endometriosis and PCOS may represent evolutionary diametric disorders originating from in utero development [35].

Conclusions

This study is the first to measure umbilical cord blood hormone levels in women with endometriosis. Despite a limited sample size, the consistent and robust effects observed compared to controls strongly suggest increased in utero estrogen exposure in female offspring of affected women.

Our findings provide biological evidence supporting the hypothesis that endometriosis susceptibility originates from an imbalance of high estrogen and low testosterone during fetal development— a hormonal environment that "programs" female reproductive traits and may drive in utero susceptibility to endometriosis.

Abbreviations

PSM	propensity-score matching
E2	estradiol
CIs	confidence intervals
DHEA	dehydroepiandrosterone
DHEA-S	dehydroepiandrosterone suplhate
DOHaD	Developmental Origins of Health and Disease
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
LC-MS/MS	liquid chromatography-tandem mass spectrometry
WLR	Weight/Length Ratio
CS	cesarean section
VB	vaginal birth
IQR	interquartile range
SD	standard deviation
HPO	hypothalamic-pituitary-ovarian
DES	diethylstilbestrol
ER	estrogen receptor
11β-HSD2	11β-hydroxysteroid dehydrogenase 2
RIA	radioimmunoassay
HPA	hypothalamic-pituitary-adrenal
RIA	radioimmunoassay
E1	estrone
E3	estriol
PCOS	polycystic ovary syndrome

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12958-025-01467-z.

Supplementary Material 1

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None.

Author contributions

N.S.: Conceptualization, Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft. M.C.: Investigation, Validation, Writing – review & editing. M.R.: Data curation, Investigation, Resources, Validation, Writing – review & editing. M.O.: Investigation, Resources, Validation, Writing – review & editing. E.I.: Investigation, Resources, Validation. S.F.: Investigation, Methodology, Resources, Validation. E.P.: Investigation, Methodology, Resources, Validation. P.Ve.: Conceptualization, Supervision, Validation, Writing – review & editing. P.Vi.: Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing. L.B.: Funding acquisition, Investigation, Supervision, Validation. E.S.: Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Validation, Writing – review & editing.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethical Committee of the Institution. All participants provided informed consent to participate in the study.

Consent for publication

All participants provided informed consent for publication of the study's findings in anonymized form.

Competing interests

P.Ve. is a member of the Editorial Board of Human Reproduction Open and the Journal of Obstetrics and Gynaecology Canada and the International Editorial Board of Acta Obstetricia et Gynecologica Scandinavica; has received royalties from Wolters Kluwer for chapters on endometriosis management in the clinical decision support resource UpToDate; and maintains both a public and private gynecologic practice. P.Vi. has received honoraria as Co-Editor in Chief of Journal of Endometriosis and Uterine Disorders. E.S. reports payments from Ferring, Theramex and IBSA for research grants and honoraria from IBSA, Gedeon-Richter and Sandoz for lectures. The remaining authors have no conflicts of interest to disclose.

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