

Substrates and Clearance Products of Fetal Adrenal Glucocorticoid Synthesis in Full-Term Human Umbilical Circulation

Heather E. Edwards¹ and Katherine E. Wynne-Edwards²

¹*Department of Obstetrics and Gynecology, Cumming School of Medicine, University of Calgary, Calgary, Alberta T2N 4N1; and* ²*Department of Comparative Biology and Experimental Medicine, Faculty of Veterinary Medicine & Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta T2N 4N1*

ORCID number: 0000-0002-2944-4516 (K. E. Wynne-Edwards).

In full-term elective caesarian sections, fetal flow of adrenal substrate steroids to products differs by sex, with males (M) in molar equilibrium whereas females (F) add net molarity and synthesize more cortisol. Using the same sampling design, paired, full-term, arterial, and venous umbilical cord samples and intrapartum chart records were obtained at the time of vaginal delivery (N = 167, 85 male) or emergency C-section (N = 38, 22 male). Eight steroids were quantified by liquid chromatography coupled to tandem mass spectrometry (adrenal glucocorticoids [cortisol, corticosterone], sequential cortisol precursor steroids [17-hydroxyprogesterone, 11-deoxycortisol], cortisol and corticosterone metabolites [cortisone and 11-dehydrocorticosterone], and gonadal steroids [androstenedione, testosterone]). Fetal sex was not significant in any analytic models. Going through both phase 1 and phase 2 labor increased fetal adrenal steroidogenesis and decreased male testosterone relative to emergency C-sections that do not reach stage 2 of labor (ie, head compressions) and elective C-sections with no labor. Sum adrenal steroid molarity arriving in venous serum was almost double the equivalent metric for deliveries without labor. No effects of operative vaginal delivery were noted. Maternal regional anesthetic suppressed venous concentrations, and fetal synthesis replaced that steroid. Approximate molar equivalence between substrate pool depletion and net glucocorticoid synthesis was seen. Paired venous and arterial umbilical cord serum has the potential to identify sex differences that underlie antenatal programming of hypothalamic-pituitary-adrenal axis function in later life. However, stage 2 labor before the collection of serum, and regional anesthetic for the mother, mask those sex differences.

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In 2013, we used liquid chromatography coupled to tandem mass spectrometry to compare cortisol and corticosterone concentrations in umbilical cord circulation for vaginal deliveries, emergency caesarian sections, and elective C-sections without antecedent labor [1]. Results identified preferential fetal synthesis of corticosterone, rather than cortisol, in response to head compressions during vaginal delivery and during emergency C-sections whose indication was from cephalopelvic disproportion and failure to progress in stage 2 of labor (FTP2). We also identified a sex difference in the absence of labor. With delivery by elective C-section that was not preceded by labor, females were exposed to higher circulating concentrations of cortisol than males, and it also added more cortisol to arterial circulation than males [2].

Previous studies using radioimmunoassay [3] and a similar mass spectrometry method [4] have expanded the range of cortisol precursors quantified in umbilical arterial serum or newborn infants (day 4 after birth) and identified cortisol suppression from prenatal infection and small-for-gestational age (SGA) growth restriction. Following this general approach, we recently quantified 6 adrenal and 2 gonadal (androstenedione and testosterone) steroids in umbilical cord arterial and venous blood samples from an independent group of elective C-sections [5]. Results confirmed that cortisol concentration in umbilical venous circulation approaching the fetus was higher for females than males, and that corticosterone was preferentially added (greater proportional increase) to umbilical arterial circulation by the fetus [5]. Expansion of the adrenal steroids quantified from 2 to 6 also allowed us to look at molar changes in substrates (depletions expected) and products (net additions expected) across the fetus. Specifically, 2 sequential steroids in the synthesis pathway to cortisol (17-hydroxyprogesterone and 11-deoxycortisol), plus the 2 immediate breakdown products of cortisol and corticosterone produced by the 11 β -hydroxysteroid dehydrogenase (11 β -HSD) enzyme (cortisone and 11-dehydrocorticosterone), plus 2 androgens (testosterone and androstenedione) were simultaneously quantified. When molar concentrations were summed for each individual, the male, full-term, fetus was in molar equilibrium, with substrate pool depletion across the fetus approximately equal to the cortisol molar increase. In contrast, depletion of substrate pools in females accounted for only 20% of net cortisol synthesis, suggesting that the flux from substrates to products in neonatal adrenal steroidogenesis is sex-specific [5].

The present study consists of vaginal deliveries and emergency C-sections collected concurrently with the elective C-sections reported in Wynne-Edwards et al [5]. Hierarchical analyses will use pool size (concentration) for each adrenal pathway steroid in matched umbilical vein and artery to: (1) confirm the absence of a sex difference for umbilical cord serum collected after antecedent labor predicted by Wynne-Edwards et al [1]; (2) compare the sum molarity, steroid composition, and molarity change across the fetus to patterns seen for elective C-sections [5]; (3) confirm the predicted preferential synthesis of corticosterone in response to intrapartum stress [1, 5]; and (4) compare operative interventions, regional anesthesia, antepartum risk factors, and rationale for emergency C-sections [6–9] to identify differences in adrenal responses with potential clinical relevance.

1. Methods

A. Ethics Statement

This research was approved by the University of Calgary Conjoint Health Research Ethics Board (CHREB) as Project E22197R2.

B. Subject Pool

Between March 2014 and January 2015, written consent was obtained from 328 pregnant subjects at the time of admission to labor and delivery at the Rockyview General Hospital or the Foothills Medical Centre in Calgary, Alberta. No attempt was made to assess ethnicity or socioeconomic status. Four births were excluded for known serum sampling errors/missing data or active maternal glucocorticoid treatment. For the purposes of this analysis, 66 preterm deliveries (< 37 weeks) were also excluded. Full-term elective caesarian deliveries without antecedent labor (N = 53) were previously analyzed by sex [5] and were recombined and included in analyses of the impact of labor history. Eight sets of dichorionic, diamniotic twins (3 emergency C-section, 5 vaginal deliveries) were treated as independent for analyses. No births involved recent glucocorticoid treatment to the mother because that intervention for anticipated preterm birth stops at 34 weeks [10], and all births in this study were \geq 37 weeks. The final cohort consisted of 167 vaginal deliveries and 38 emergency C-sections (Table 1). The intrapartum delivery record provided the sex of the baby, birth

weight, gestational age, fetal health (Apgar score at 5 minutes), regional anesthetic, operative vaginal delivery, and intrapartum rationale for the decision to proceed to C-section, as well as antepartum maternal and fetal risk factors.

C. Sample Collection

There was no interference with normal postpartum protocol that routinely samples umbilical arterial and umbilical venous blood for pH and acid-base status [11]. One non-heparinized, “red top,” vial of venous blood and another of arterial whole blood were collected by needle aspiration (16 gauge) for this study. Samples were refrigerated and allowed to stand for at least 1 hour before clot removal and centrifugation (4000 xg for 5 minutes). The serum was separated and stored at -20°C until delivery to the research laboratory.

D. Sample Preparation and Quantitation

Methods for sample preparation and LC-ESI+/MRM quantitation are detailed in Wynne-Edwards et al [5]. Briefly, crystalline steroid reference standards were sourced from Steraloids Inc (Newport, Rhode Island) and deuterium labeled bioidentical internal standards (IS) were obtained from CDN Isotopes Inc (Pointe-Claire, Quebec, Canada). The protein precipitation solution consisted of 60 µl of ZnSO₄·7H₂O solution (9 mg/mL) spiked with the deuterated IS mixture, plus 60 µl of serum, vortexed for 30 seconds, then incubated at 4°C for 20 minutes. All samples were centrifuged at 14 000 rpm for 15 minutes. 90 µL of supernatant was submitted to LC-ESI+/MRM analysis. Equipment was an Agilent 1200 binary liquid chromatography (LC) system coupled to an AB SCIEX QTRAP® 5500 tandem mass-spectrometer equipped with an electrospray ionization (ESI) source in positive mode. Liquid chromatography separation used an Agilent ZORBAX Eclipse plus C18 column (100 × 2.1 mm, 1.8 µm particle size) at 40°C. Mobile phase A was ACN/H₂O (5/95, v/v, 2mM NaF) and mobile phase B was 100% ACN (2mM NaF). The gradient was 12 minutes at a flow rate of 0.6 mL/minute and an injection volume of 15 µL. Gradient details, internal standard specifics, and acquisition parameters are provided in Wynne-Edwards et al [5].

E. Steroid Quantitation

Linear calibration curves used untransformed analyte concentration (x-axis) versus untransformed analyte/IS peak area ratios (y-axis), and a linear fit with 1/x weighting to improve precision at lower concentrations (all r² > 0.9995). Ten-point calibration curves ranged from 0.1 ng/ml to 100 ng/ml for cortisol, corticosterone, cortisone, and androstenedione. Other steroid calibration curves ranged from 0.05 to 50 ng/ml. The lowest concentration that gave < 20% CV (Coefficient of Variation = standard deviation/mean *100) was deemed the lower limit of quantitation (LLOQ), which was 0.1 ng/ml for all steroids except cortisol, at 0.2 ng/ml. Four quality control (QC) pools in MeOH, matched to ultrahigh (25 or 12.5 ng/ml), high (5 or 2.5 ng/ml), mid (0.5 or 0.25 ng/ml), or low (0.1 or 0.05 ng/ml), plus a triplicate serum pool, were quantified within each of the 4 runs. Details of precision and accuracy are provided in Wynne-Edwards et al [5].

F. Steroid Analytical Parameters

All quantitation results were converted from ng/ml to nmol/l to follow changes in the size of molecular pools of substrate, product, and breakdown product steroids [5]. As established in Wynne-Edwards et al [1], net neonatal steroid synthesis was defined as the concentration difference between the steroid pool in the umbilical artery ([A]; circulation that has passed the neonate and is returning to the placenta) and the umbilical vein ([V]; circulation from the maternal/ placental interface towards the neonate). This is represented in the results as [A-V] in nmol/l. In addition, a parameter representing the percentage of proportional change in steroid

concentration attributable to the neonate ($[A-V]/V$) *100 was calculated, for which negative values represent fetal steroid clearance, zero represents no change, and values of 100 represent a doubling, or 100%, increase in concentration. By definition, steroid clearance across the maternoplacental interface $[V-A]$ is equal in magnitude, but opposite to, fetal synthesis $[A-V]$. However, interpretation of the concentration change across the maternoplacental side of the umbilical circulation is challenging because steroid exchange with maternal circulation was not assessed [1, 2, 5]. Finally, the sum adrenal pathway molarity for each serum sample was calculated as the sum of the 6 adrenal steroidogenesis pathway hormones quantified, excluding the 2 gonadal androgens. In addition, to standardize the visual presentation of hormone changes across different labor histories (no labor, stage 1, and stage 2), all $[V]$ were set to 1.0 and all $[A]$ were expressed relative to that standardized $[V]$ ($[A]/[V]$ parameter).

G. Statistical Analyses

As our explicit intent was to assess molecular pool changes in substrates and clearance products within individual matched pairs of venous and arterial umbilical cord serum, and the LC-MS/MS quantitation method is precise and linear throughout its range (neither the x-axis nor the y-axis is transformed before fitting a high-confidence straight line), untransformed arterial and venous samples were treated as matched pairs for all difference scores. Nonparametric comparisons yielded the same significance patterns as those reported here. In Wynne-Edwards et al [5], an open access supplemental results section detailing the matching of findings between parametric and nonparametric approaches is provided. As the samples were all full-term (≥ 37 weeks), mode of delivery (vaginal versus emergency C-section) and infant sex were the primary class variables.

Subsequently, patterns in adrenal steroidogenesis were further explored through partitioning vaginal deliveries by whether or not they had operative intervention, by the type of operative intervention (forceps or vacuum), by antenatal risk factors (for hypertension, diabetes, and intrauterine growth restriction), and by the presence or absence of regional spinal anesthetic during stage 2 of labor. Emergency C-sections were also compared based on the rationale for the emergency delivery (FTP1: failure to progress in stage 1 of labor = contractions without pushing; FTP2: failure to progress detected after head compressions during stage 2 of labor; and FHR [fetal heart rate abnormalities (decelerations)]). To compare labor histories, the no labor group from Wynne-Edwards et al [5] was combined across sexes and compared by analysis of variance (ANOVA), with post-hoc Tukey HSD against stage 1 and stage 2 labor.

All analyses were conducted using JMP version 13.1.0 (SAS Institute, North Carolina). For two-sample comparisons, such as birthweight, a t-test assuming unequal variances at a critical alpha of < 0.05 was applied. On the other hand, for each steroid hormone, 4 parameters ($[A]$, $[V]$, $[A-V]$, $[A-V]/[V]$) and 2 sum molarity variables were considered in models. The critical alpha threshold applied was therefore modified to reflect those multiple comparisons ($0.5/6 = 0.0083$ critical alpha). Initial models included delivery type, sex, and the interaction between delivery type and sex. This critical alpha threshold was also applied to partitioned vaginal deliveries, sum molarities, and adrenal concentrations standardized to $[V] = 1.0$ ($[A]/[V]$). However, partitioned subsets of the emergency C-section group (ANOVA across 3 levels of rationale for emergency delivery) had a low sample size and therefore less power to detect significant differences. In those cases, comparisons that met an alpha threshold of < 0.05 are reported. They should be interpreted with caution. For associations within individuals for pairs of steroid hormones, values are reported as correlation (r), lower and upper 95% confidence limits on that association, slope, and P value for the model.

2. Results

A. Demographics

Full-term vaginal deliveries and emergency caesarian sections did not differ in maternal age, gestational age, Apgar score 5 minutes after birth, or antenatal risk factor scores (Table 1).

Table 1. Full-Term Cohort Description (N = 205 Births)

	N	Vaginal Delivery	N	Emergency C-section	P
Maternal Demographics	167	30.56 ± 0.40	38	31.79 ± 0.72	0.1424
Maternal age range (yr)	167	17–45	38	20–44	-
Gestational age (wk)	167	39.01 ± 0.10	38	39.27 ± 0.24	0.3304
Regional analgesia (% yes)	135	80.84	38	100.00	
Maternal hypertensive disorders (% yes)	28	16.77	7	18.42	0.8067
Maternal diabetes (% yes)	25	14.97	5	13.16	0.7754
Fetal growth restriction (% yes)	20	11.98	4	10.53	0.8019
Males (% yes)	85	50.9	22	57.9	0.4358
Females (% yes)	82	49.1	16	42.1	
^a Female birthweight (kg)	82	3.18 ± 0.05	16	3.02 ± 0.10	0.1700
^a Male birthweight (kg)	85	3.36 ± 0.06	22	3.47 ± 0.10	0.3382
Apgar (max score of 10)	167	8.90 ± 0.03	37	8.84 ± 0.13	0.6116
Operative vaginal delivery (%)	25	14.97	-	-	-
Forceps (% yes)	16	9.58	-	-	-
Rationale for emergency C-section	-	-	17	44.73	-
Fetal heart rate abnormalities (FHR; % yes)	-	-	13	34.21	-
Failure to progress in stage 1 (FTP1; % yes)	-	-	8	21.05	-
Failure to progress in stage 2 (FTP2; % yes)	-	-	-	-	-

^aMale birthweight is heavier than female birthweight for vaginal deliveries ($P = 0.0028$) and for emergency C-sections ($P = 0.0175$).

Neonatal males were heavier than females in both vaginal deliveries ($P = 0.0028$) and in emergency C-sections ($P = 0.0175$). There was no evidence that males were more likely than females to go to emergency C-section (Pearson's chi-square (2) = 2.757, $P = 0.2519$).

B. Sex Effects on Adrenal Steroid Concentrations

Initial models with delivery type (vaginal delivery or emergency C-section), and sex (male or female neonate), plus their interaction were run for venous [V] and arterial [A] concentration of each adrenal steroid, plus the individual sum of the 6 steroids on the adrenal biosynthesis pathway. In arterial serum, each model was significant (arterial serum all $P < 0.0032$), with effects of delivery type (all $P < 0.0025$), but not sex (all $P > 0.1205$), and no interactions. In venous serum the pattern was the same, with all models significant (all $P < 0.0018$), all effects of delivery mode significant (all $P < 0.0025$), no significant effects of sex (all $P > 0.1701$), and no interactions. Likewise, sum venous, and sum arterial molarity, differed by delivery mode (both $P < 0.0001$), but not sex (both $P > 0.5431$), with no interactions. There was, therefore, no evidence supporting a sex difference in adrenal pathway steroid hormone concentrations. Gonadal androgen models are reported in section K, below.

C. Glucocorticoid Pathway Steroid Concentrations

Irrespective of delivery type, cortisone was the most abundant steroid, followed by cortisol and 17-hydroxyprogesterone. Corticosterone, 11-dehydrocorticosterone, and 11-deoxycortisol were the least abundant (Fig. 1). In both venous and arterial samples, all 6 steroids were at higher concentration with vaginal delivery than with emergent C-sections. There was no net gain or loss in sum molarity between artery and vein in individuals born by vaginal delivery or emergency C-section.

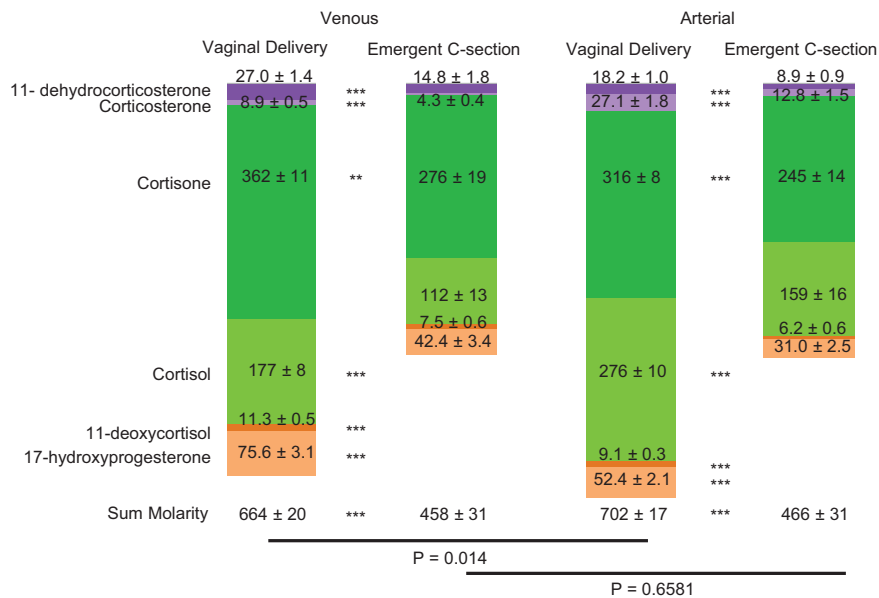


Figure 1. Stacked nmol/l concentrations (mean ± SE) for the 6 adrenal pathway steroids quantified. Results are stratified by venous and arterial serum samples, and by delivery type. Sum molarity (sum of the 6 adrenal steroid pathway molarities) is also shown. Significant differences between vaginal deliveries and emergency C-sections are indicated (** $P < 0.0083$; *** $P < 0.0001$). For sum molarity, lines connecting venous and arterial samples for the same vaginal delivery type show the P value for that paired comparison.

D. Neonatal Steroid Synthesis and Clearance [A-V]

No models including sex were significant for [A-V] (all $P > 0.5135$). In both delivery types, the adrenal stress steroids, cortisol and corticosterone, were added to arterial serum by the neonate at the same time that pools of cortisone, 17-hydroxyprogesterone, 11-dehydrocorticosterone, and 11-deoxycortisol were significantly depleted (Fig. 2). Vaginal deliveries added more cortisol and corticosterone, and depleted more 17-hydroxyprogesterone, than emergency C-sections. In general, cortisol pathway substrate depletion and product additions were approximately in equilibrium. In other words, the sum of the cortisone, 11-deoxycortisol, and 17-hydroxyprogesterone pool depletions were roughly equivalent to the cortisol added for both emergency C-sections (3.50 ± 16.54 nmol/l, $P = 0.8334$) and vaginal deliveries (24.43 ± 12.99 nmol/l, $P = 0.0618$). We did not quantify deoxycorticosterone (a substrate for corticosterone), preventing a parallel analysis of the corticosterone pathway.

E. Preferential Corticosterone Synthesis [A-V]/[V] and Cortisol/Corticosterone Ratio

No models including sex were significant for [A-V]/[V] (all $P \geq 0.4995$). There was also no evidence for impact of delivery type on proportional hormone change (Table 2). As predicted, the proportional increase in corticosterone was larger than in cortisol in vaginal deliveries (274% vs 92%) and in emergency C-sections (223% vs 55%), confirming preferential net corticosterone synthesis. Thus, the ratio of cortisol to corticosterone in venous serum approaching the neonate was higher than in arterial serum, after preferential addition of corticosterone by the neonate (paired t-test, vaginal deliveries: ratio difference = -10.45 ± 0.77 , $P < 0.0001$; emergency C-sections: ratio difference = -13.27 , $P < 0.0001$). Sex did not alter these relationships (both $P > 0.65$). The proportional depletion in 11-dehydrocorticosterone was also larger than in cortisone in vaginal deliveries ($t = 6.52$, $df = 164$, $P < 0.0001$) and in emergency C-sections ($t = 6.74$, $df = 37$, $P < 0.0001$). Preferential corticosterone synthesis by the fetus was therefore confirmed.

F. Adrenal Pathway Steroid Correlations Within Individuals

As the synthesis (11 β -hydroxylase) and back-synthesis (11 β -HSD1) enzymes for cortisol and corticosterone are shared, there was an expected positive association between the 2

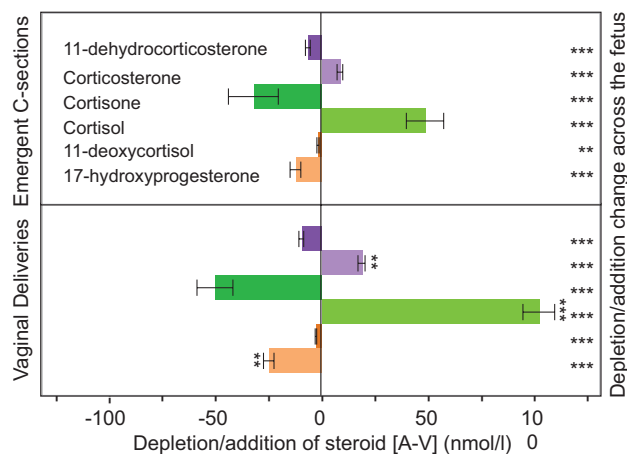


Figure 2. Within-individual change in the concentration (nmol/l) of each adrenal pathway steroid across the neonate [A-V] for emergency C-sections and vaginal deliveries. Significant difference within 1 hormone, between the 2 delivery types, is indicated (** $P < 0.0083$; *** $P < 0.0001$). Significance of the change across the neonate (difference from a distribution with a mean of zero) is shown on the right-hand side of each panel using the same symbols. Color legend applies to all figures.

Table 2. Proportional Steroid Concentration Changes ([A-V]/[V] (nmol/l \pm SE %; 100 = Doubling; -50 = 50% Decrease)

	Vaginal Delivery (N = 165)	Emergency C-section (N = 38)	P
Corticosterone	274.36 \pm 25.02	222.55 \pm 27.49	0.1663
11-dehydrocorticosterone	21.04 \pm 4.08	31.58 \pm 4.45	0.0836
Cortisol	91.90 \pm 14.00	54.50 \pm 9.37	0.0261
Cortisone	4.24 \pm 4.66	6.50 \pm 4.04	0.7146
17-hydroxyprogesterone	22.18 \pm 3.94	21.53 \pm 3.88	0.9070
11-deoxycortisol	9.78 \pm 4.01	13.36 \pm 4.80	0.5683

glucocorticoid concentrations in venous (vaginal deliveries: $r = 0.7841$, 95% limits 0.7177 - 0.8364, slope 0.0490, $P < 0.0001$; emergency C-sections: $r = 0.8343$, 95% limits 0.7017 - 0.9110, slope 0.0290, $P < 0.0001$) and arterial serum (vaginal deliveries: $r = 0.6166$, 95% limits 0.5221 - 0.7032, slope 0.1124, $P < 0.0001$; emergency C-sections: $r = 0.7550$, 95% limits 0.5738 - 0.8657, slope 0.0725, $P < 0.0001$). There were no changes when sexes were assessed separately except that, in the group with the smallest sample size (emergency C-section females, $N = 16$) the P value increased to $P = 0.0015$).

Sequential precursor steroids for cortisol synthesis, 17-hydroxyprogesterone, and 11-deoxycortisol were also positively associated in venous (vaginal deliveries: $r = 0.7491$, 95% limits 0.6739 - 0.8089, slope 0.1098, $P < 0.0001$; emergency C-sections: $r = 0.6698$, 95% limits 0.4455 - 0.8150, slope 0.1202, $P < 0.0001$) and arterial serum (vaginal deliveries: $r = 0.7190$, 95% limits 0.6361 - 0.7855, slope 0.1173, $P < 0.0001$; emergency C-sections: $r = 0.6630$, 95% limits 0.4357 - 0.8109, slope 0.1548, $P < 0.0001$). All venous and arterial models remained significant at $P < 0.0001$ when partitioned by sex as well as delivery type, with the exception being a loss of correlation in the smallest cohort (emergency C-section females, $P = 0.1488$ venous, $P = 0.1561$ arterial).

Expected positive associations were also present for the 11 β -HSD breakdown products of cortisol and corticosterone, namely cortisone and 11-dehydrocorticosterone, in venous (vaginal deliveries: $r = 0.6536$, 95% limits 0.5571 - 0.7328, slope 0.0849, $P < 0.0001$; emergency C-sections: $r = 0.7311$, 95% limits 0.5369 - 0.8517, slope 0.0701, $P < 0.0001$) and arterial serum (vaginal deliveries: $r = 0.5167$, 95% limits 0.3951 - 0.6205, slope 0.0620, $P < 0.0001$; emergency C-sections: $r = 0.6108$, 95% limits 0.3618 - 0.7785, slope 0.0389, $P < 0.0001$). All venous and arterial models remained significant at $P < 0.0004$ when partitioned by sex as well as delivery type.

G. Antenatal Risk Factors

Births with hypertensive risks, diabetic risks, or intrauterine growth restriction were included in the population sampled (Table 1). A total of 68 vaginal births had antenatal risk factors (5 births [4M] had both hypertensive and diabetic maternal risk factors). Given the overall excellent health of the neonates, it was not surprising that maternal hypertensive risks ($N = 28$; all $P > 0.05$) and maternal diabetic risk factors ($N = 25$; all $P > 0.041$) did not alter any steroid hormone parameters for vaginal deliveries. Intrauterine growth restriction (IUGR) occurred in 20 vaginal births (8M). Both venous ($P = 0.0122$) and arterial ($P = 0.0104$; interpret this critical alpha < 0.05 with caution) androstenedione were at lower concentrations with IUGR, although [A-V] and [A-V]/[V] did not differ ($P = 0.3494$, $P = 0.8247$, respectively). Antenatal risk factors were not included as factors in subsequent analyses. For emergency C-sections, antenatal risk factors affected a large percentage of emergency C-section births (16/38 = 42.1%). However, they were also distributed across 3 rationales with different expected levels of fetal distress (see section J) so statistical comparisons were not attempted for emergency C-sections.

H. Operative Vaginal Deliveries

No models including sex were significant for venous (all $P > 0.2338$) or arterial (all $P > 0.0646$) samples. A total of 41 women had an operative assist from forceps or the vacuum (Fig. 3). There was no evidence of steroid or total molarity parameter changes across operative versus spontaneous vaginal deliveries. There were also no differences between the 2 operative interventions (forceps or vacuum; data not shown).

I. Regional Anesthetic

Within the vaginal deliveries, 135 women received a regional anesthetic and 32 did not. In the presence of maternal pain relief, cortisol and corticosterone concentrations in venous serum were reduced (Fig. 4). In arterial serum, the differences had been eliminated. There were no significant changes across the fetus ([A-V] cortisol $P = 0.0214$; corticosterone $P = 0.0204$). However, proportional increases were larger in the presence of regional anesthetic. There were 2 other responses to maternal pain relief. Cortisone pool size was depleted in venous serum (No: 36.26 ± 3.69 , Yes: 24.74 ± 1.49 , $P < 0.0060$) and 11-dehydrocorticosterone pool size was depleted in arterial serum (No: 23.61 ± 2.19 , Yes: 16.94 ± 1.06 , $P < 0.0086$). All other comparisons yielded $P > 0.05$. The highest sum molarities seen in this study (776.53 ± 53.17 and 757.15 ± 43.29) belonged to venous and arterial umbilical serum from vaginal deliveries in the absence of regional anesthetic, although neither differed from the sum in the presence of regional anesthetic (venous 634.29 ± 20.21 , $P = 0.0166$; arterial 684.27 ± 18.53 , $P = 0.1290$).

Sum venous adrenal steroid pathway steroids did not differ by sex in the absence ($P = 0.4196$) or presence ($P = 0.4766$) of regional anesthetic. In the 135 vaginal deliveries (69M) with regional anesthetic, no sex differences were seen in venous serum (P values ranged from 0.1652 for 17-hydroxyprogesterone to 0.8984 for corticosterone, with $P = 0.7278$ for cortisol). In the absence of regional anesthetic (N = 32, 16M), cortisol also did not show a sex difference ($P = 0.3203$). Although not significant at the reduced critical

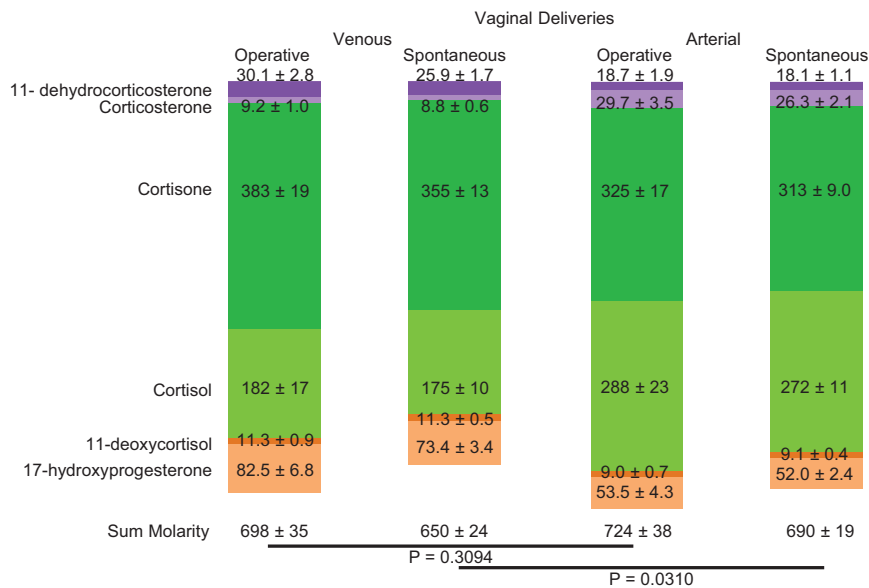


Figure 3. Stacked concentrations (nmol/l ± SE) for 6 adrenal pathway steroids in vaginal deliveries that were operative (forceps or vacuum) versus spontaneous vaginal deliveries. Within venous serum and within arterial serum, and even in sum steroid molarity, there were no significant differences between the 2 vaginal delivery types, so significance is not indicated on the figure. For sum molarity, lines connecting venous and arterial samples for the same vaginal delivery type show the P value for that paired comparison.

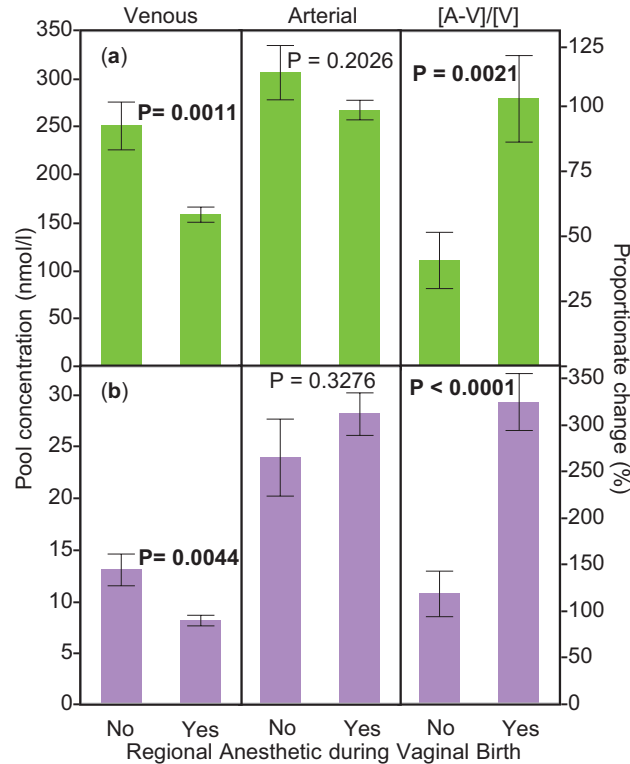


Figure 4. The effect of regional anesthetic during vaginal delivery on concentrations (nmol/l \pm SE) of (A) cortisol, and (B) corticosterone in venous [V] and arterial [A] serum, plus the proportional change across the fetus [A-V]/[V] (%). Emergency C-section deliveries are not shown because all caesarian births occurred under regional anesthetic. P values indicated are for a t-test. Bold values indicate that $P < 0.0083$. Colors match the legend for Fig. 2.

alpha of 0.0083 applied to all multiple comparisons for steroid hormone parameters, venous male adrenal steroid pools trended towards being larger than venous female pools for all 3 cortisol substrates. Specifically, cortisone (male 460.71 ± 35.54 vs female 336.82 ± 41.01 , $P = 0.0298$), 17-hydroxyprogesterone (male 81.39 ± 9.31 vs female 50.27 ± 7.25 , $P = 0.0134$), and 11-deoxycortisol (male 13.45 ± 1.49 vs female 8.54 ± 1.04 , $P = 0.0122$) all differed in the same direction even though cortisol concentration was similar. A larger sample than the current one might therefore identify a sex-specific pattern in substrate pool size in venous serum arriving at the fetus that is masked by regional spinal anesthetic.

Arterial serum did not reveal any sex differences in the presence of regional anesthetic (P values ranged from 0.1288 for 17-hydroxyprogesterone to 0.9501 for corticosterone, with $P = 0.4434$ for cortisol). In the absence of regional anesthetic, the only sex difference was that male arterial cortisone (394.14 ± 22.66 nmol/l) was at higher concentrations than female arterial cortisone (289.92 ± 16.83 ; $P = 0.0010$). Cortisol ($P = 0.7370$), corticosterone ($P = 0.2145$), 11-dehydrocorticosterone ($P = 0.2453$), 17-hydroxyprogesterone ($P = 0.1471$), and 11-deoxycortisol ($P = 0.1640$) did not differ by sex. However, there were no significant sex differences in [A-V] change across the fetus in the presence or absence of maternal anesthetic. Thus, vaginal deliveries without regional anesthetic might help to identify sex differences in fetal adrenal steroidogenesis at full-term, but the effect size is likely to be small.

J. Rationale for Emergency Caesarian Section

There were 3 rationales for choosing emergency delivery. The FHR (N = 17) and FTP1 (N = 13) deliveries both experience some labor. The FTP2 (N = 8) group is the only one with

head compressions from stage 2 labor. Given the sample sizes, the critical alpha for each ANOVA was cautiously left at 0.05 for these comparisons. Within the venous umbilical serum samples, neither cortisol ($P = 0.0543$) nor 11-dehydrocorticosterone ($P = 0.3285$) differed by rationale (Fig. 5). Corticosterone, cortisone, 17-hydroxyprogesterone, and the sum of the 6 steroids each followed the same pattern, with FTP2 concentrations being the highest, FTP1 concentrations significantly lower, and FHR intermediate. For 11-deoxycortisol, FTP2 was at higher concentration than both FTP1 and FHR, which did not differ. Differences were largely eliminated in arterial samples. Only an arterial cortisol pattern with FTP2 concentrations the highest, FTP1 lowest, and FHR intermediate emerged. All other hormones did not differ in arterial serum. Sum molarity followed the pattern suggested by the individual steroids with $\text{FTP2} > \text{FHR} > \text{FTP1}$ in venous and eliminated in arterial samples.

Net addition of cortisol and corticosterone, plus the depletion of 11-dehydrocorticosterone were not different in the 3 rationales (Fig. 6). In contrast, depletions of all 3 substrates for cortisol synthesis differed, with FTP2 showing the largest depletions and FTP1 showing the smallest pool depletions. The proportional change across the neonate $[\text{A-V}]/[\text{V}]$ showed the same pattern as the absolute difference. Proportional addition of cortisol and corticosterone, plus the proportional depletion of 11-dehydrocorticosterone were not different in the 3 rationales ($P = 0.9544$, $P = 0.6098$, $P = 0.0837$, respectively). In contrast, depletions of all 3 substrates for cortisol synthesis, cortisone ($F(2, 35) = 3.48$, $P = 0.0417$), 11-deoxycortisol ($F(2, 35) = 4.71$, $P = 0.0155$), and 17-hydroxyprogesterone ($F(2, 35) = 9.13$, $P = 0.0006$) differed, with FTP2 showing the largest depletions and FTP1 showing the smallest pool depletions.

K. Androgens

Female testosterone concentration was below the limit of detection (≤ 0.10 ng/ml = 0.3467 nmol/l), or nonquantifiable because of matrix interference, in 45/98 venous

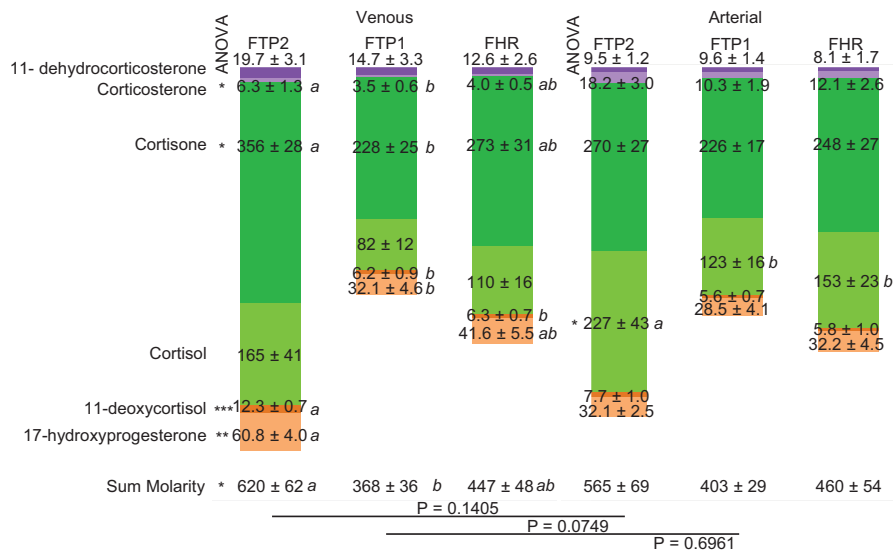


Figure 5. Stacked adrenal pathway steroid concentrations (nmol/l ± SE) separated by rationale for the emergency C-section venous and arterial samples. FTP2 = failure to progress at stage 2 of labor, N = 8. FTP1 = failure to progress at stage 1 of labor, N = 13. FHR = fetal heart rate abnormalities, N = 17. In the columns labelled ANOVA, * indicates $P < 0.05$ (and should be viewed with caution), ** indicates $P < 0.0083$, and *** indicates $P < 0.0001$. Where the ANOVA was significant, lower case letters that differ are used to indicate, within each steroid hormone and serum type, significant Tukey post hoc differences ($P < 0.05$) in the pool size for that hormone. The same indications are used for sum molarity within venous and within arterial. Paired sum molarity comparisons between venous and arterial samples are indicated by lines and P values beneath the frame.

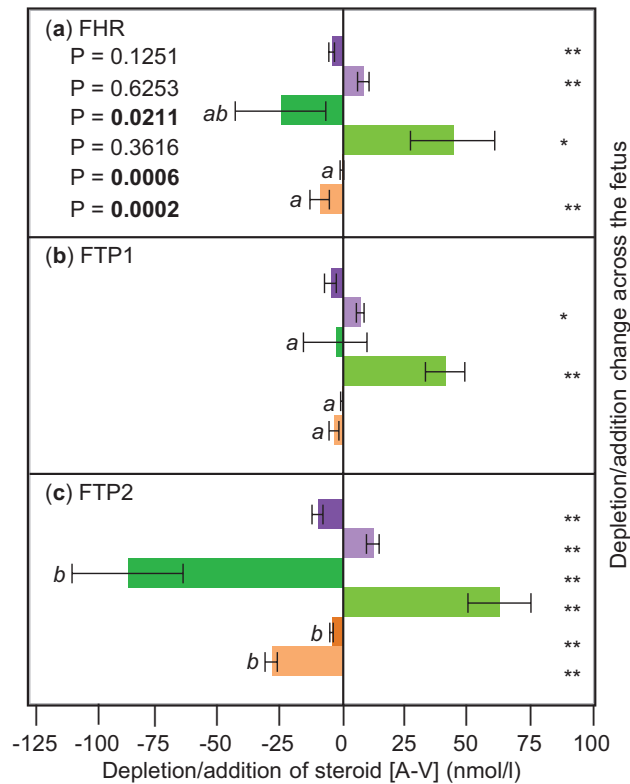


Figure 6. Depletion or addition ([A-V], nmol/l \pm SE) of each adrenal pathway steroid hormone (color legend provided in Fig. 2) across the neonate, separated by rationale for the emergency C-section (see legend for Fig. 5 for definitions). The P values reported in the top panel refer to the ANOVA. Lower case letters that differ indicate, for each steroid hormone, significant Tukey post hoc differences at $P < 0.05$. Significant changes in the pool size across the neonate (comparisons against a mean of zero) are shown on the right side of each panel (* indicates $P < 0.05$ [and should be viewed with caution]; ** indicates $P < 0.0083$).

samples and 21/98 arterial samples involving 47/98 female births. Within the emergency C-section subset, 10/16 females had either or both venous and arterial testosterone pools not quantifiable. There was no effect of delivery type on the probability that testosterone concentration would exceed the lower limit of detection (venous: Pearson's chi-square, $P = 0.1797$; arterial: Pearson's chi-square, $P = 0.4981$). Thus, testosterone in female umbilical circulation was not further compared across the 2 types of delivery.

For males, 5 venous testosterone samples could not be quantified because of matrix interference and were treated as missing data. Four serum testosterone concentrations that fell below the limit of quantitation but above the limit of detection were assigned their imputed value and remained in analyses with the larger dataset. There was no difference in the testosterone concentration approaching the neonate [V], but after passage through the neonate, in arterial serum [A], testosterone was higher in males delivered by emergency C-section than males delivered vaginally (Table 3). Testosterone [A-V] and proportional increase [A-V]/[V] did not differ.

The immediate precursor steroid for testosterone synthesis, androstenedione, was quantifiable in serum from both males and females (Table 3). There was no evidence of a sex effect on any of the 4 variables ([A], [V], [A-V], and [A-V/V], all $P > 0.07$). Delivery type, however, had a significant effect in the opposite direction to testosterone. Venous concentrations of androstenedione were lower in emergency C-sections than vaginal deliveries, but no net addition/subtraction of androstenedione was seen across the neonate [A-V], nor was there any difference for proportional change [A-V/V].

Table 3. Gonadal Sex Steroid Concentrations (nmol/l) and Changes by Delivery Type

Fetal Sex	Steroid	Delivery Type	N	Venous	P	N	Arterial	P	[A-V]	P	[A-V]/[V] (%)	P
A. Males	Testosterone	Vaginal delivery	81	0.41 ± 0.02	-	85	1.34 ± 0.13	-	0.99 ± 0.12	-	271 ± 30	-
		Emergency C-section	21	0.67 ± 0.13	0.0634	22	2.47 ± 0.37	0.0078	1.87 ± 0.42	0.0559	425 ± 101	0.1565
B. Females ^a	Androstenedione	Vaginal delivery	85	1.98 ± 0.10	-	85	2.75 ± 0.14	-	0.76 ± 0.12	-	49 ± 7	-
		Emergency C-section	22	1.44 ± 0.15	0.0041	22	2.12 ± 0.23	<i>0.0228</i>	0.68 ± 0.20	0.7111	71 ± 19	0.2888
	Androstenedione	Vaginal delivery	82	1.71 ± 0.10	-	82	2.23 ± 0.11	-	0.54 ± 0.10	-	48 ± 8	-
		Emergency C-section	16	1.32 ± 0.11	0.0114	16	1.89 ± 0.27	0.2490	0.57 ± 0.23	0.9105	44 ± 17	0.8571

^aFemale testosterone was frequently near or below the limit of detection, preventing statistical comparison.

Stratification of the male emergency C-sections by their rationale (FHR, FTP1, FTP2; N = 8, 7, 6) did not reveal any differences for testosterone (all $P \geq 0.4276$). Similarly, among male vaginal deliveries, there were no differences in testosterone in operative versus spontaneous vaginal deliveries ($P \geq 0.1727$) or regional anesthetic for the mother ($P \geq 0.3418$ for all parameters). For androstenedione, there were no sex effects and no effects of emergency C-section rationale (FHR, FTP1, FTP2; N = 17, 13, 8; all $P \geq 0.0489$). Similarly, there were no differences between operative versus spontaneous vaginal deliveries (all $P \geq 0.4179$) or regional anesthetic for the mother (all $P \geq 0.6671$).

L. Impact of Labor History

Data for elective C-sections from the same sample collection and analysis batches as the current data set was previously analyzed by sex [5]. For labor history comparisons, those deliveries were recombined across sexes as no labor and compared to stage 1 labor (FTP1) and stage 2 labor (vaginal deliveries with head compressions and maternal regional anesthetic). Every ANOVA except androstenedione [A] was significant, with no labor different from stage 2 labor, and FTP1 similar to no labor (Table 4). The same pattern persisted in sum adrenal molarity with vaginal deliveries (sum [V] = 634.29 ± 20.21 , sum [A] = 684.27 ± 18.53 , $P < 0.0001$) higher than both no labor (sum [V] = 323.30 ± 15.75 , sum [A] = 346.89 ± 16.67 , $P < 0.0001$) and FTP1 (sum [V] = 373.52 ± 38.91 , sum [A] = 412.62 ± 30.25 , $P < 0.0001$). However, there was no evidence that the fetus was responding differently depending on labor history. When [V] was set to 1.0, arterial change was never significant (ANOVA for adrenal pathway steroids $0.3550 \leq P \leq 0.9292$; for androgens $P \geq 0.1845$). Thus, although concentration ranges were different, the patterns of steroid substrate depletion and product addition patterns were shared across different labor histories (Fig. 7).

3. Discussion

As expected, this independent data set confirmed the findings of Wynne-Edwards et al [1]. Specifically, no statistical models in the current study identified fetal sex as a significant factor in adrenal steroidogenesis pathway concentrations or parameters. This contrasts with the sex difference in elective C-sections without antecedent labor [2] that was recently replicated [5]. Thus, labor obscures or eliminates the statistical detection of sex differences at full-term in reasonably-sized cohorts. Second, results confirmed that stage 2 labor (vaginal deliveries vs elective C-sections) approximately tripled cortisol and corticosterone concentrations in both arterial and venous serum [1]. In the current study, cortisol after stage 2 labor doubled relative to no labor (cortisol [V] = 1.98-fold, [A] = 2.01-fold) and corticosterone more than doubled ([V] = 2.72-fold, [A] = 2.47-fold). The overall cortisol increase was predicted by the elevation of maternal serum cortisol during labor and delivery [12, 13]. When very high maternal serum cortisol exchanges with the placenta, the cortisol overwhelms the 11β -hydroxysteroid dehydrogenase (11β -HSD2) enzyme activity that converts cortisol to cortisone, allowing more cortisol to reach venous umbilical circulation. Thus, maternal cortisol elevation also explains the venous cortisone increase. Maternal corticosterone has not been studied in human labor and delivery, although the overall increase suggests that it will be elevated and crossing the placenta.

Repetition of the doubling/ tripling pattern in cortisone and 11-dehydrocorticosterone suggests that changes in maternal serum, rather than preferential affinity of the placental (11β -HSD2) enzyme for corticosterone over cortisol, might be responsible for this pattern, allowing the enzyme to convert each in proportion to the substrate pools available. However, the immediate precursor of corticosterone, 11-deoxycorticosterone, was not quantified and maternal serum was not collected. Free cortisol and corticosterone concentrations are also affected by local corticosteroid binding globulin concentrations, which decline in maternal serum at full-term and is higher in the placental intervillous space than in umbilical circulation [14].

Table 4. Impact of Labor History on Adrenal Steroid Hormone Concentrations (nmol/l \pm SE).

Steroid	^a Sample	^b No Labor	Stage 1	Stage 2	^c ANOVA
17-hydroxyprogesterone	[V]	42.58 \pm 3.14	33.88 \pm 4.64	77.93 \pm 3.45	<i>b</i>
	[A]	28.95 \pm 3.25	30.05 \pm 4.10	52.71 \pm 2.29	<i>***b</i>
11-deoxycortisol	[V]	8.76 \pm 0.72	6.30 \pm 1.03	11.39 \pm 0.50	<i>b</i>
	[A]	7.00 \pm 0.73	5.89 \pm 0.79	8.99 \pm 0.35	<i>***b</i>
Cortisol	[V]	80.43 \pm 7.14	86.49 \pm 12.19	159.47 \pm 7.93	<i>b</i>
	[A]	133.52 \pm 11.83	129.26 \pm 16.61	268.37 \pm 10.21	<i>***b</i>
Cortisone	[V]	180.30 \pm 8.88	229.62 \pm 26.92	352.78 \pm 11.78	<i>b</i>
	[A]	160.87 \pm 5.84	227.10 \pm 17.91	309.32 \pm 9.16	<i>***b</i>
Corticosterone	[V]	2.94 \pm 0.27	3.39 \pm 2.38	7.99 \pm 0.51	<i>b</i>
	[A]	11.31 \pm 1.31	10.67 \pm 2.00	27.94 \pm 2.06	<i>***b</i>
11-dehydrocorticosterone	[V]	8.28 \pm 0.98	13.84 \pm 3.44	24.74 \pm 1.49	<i>b</i>
	[A]	5.26 \pm 0.41	9.65 \pm 1.50	16.94 \pm 1.06	<i>***b</i>
Androstenedione	[V]	1.36 \pm 0.10	1.37 \pm 0.18	1.85 \pm 0.08	<i>b</i>
	[A]	2.10 \pm 0.14	2.16 \pm 0.29	2.47 \pm 0.09	<i>***</i>
Testosterone (males only)	[V]	0.73 \pm 0.07	0.47 \pm 0.09	0.38 \pm 0.03	<i>a</i>
	[A]	2.80 \pm 0.24	1.67 \pm 0.43	1.32 \pm 0.15	<i>***a</i>

^aNo Labor = elective C-sections (N = 49; 31M), Stage 1 = FTP1 (N = 12; 7M), Stage 2 = vaginal deliveries (N = 135; 69M). All had maternal regional analgesia.

^bRecalculated from [5] without considering fetal sex.

^cOne-way ANOVA (critical alpha = 0.0083) followed by Tukey HSD. Labor histories that do not share a letter (italics) significantly different. Matched pairs t-test between [V] and [A] for each hormone and labor history (* $P < 0.05$ (Stage 1 only); ** $P < 0.0083$; *** $P < 0.0001$).

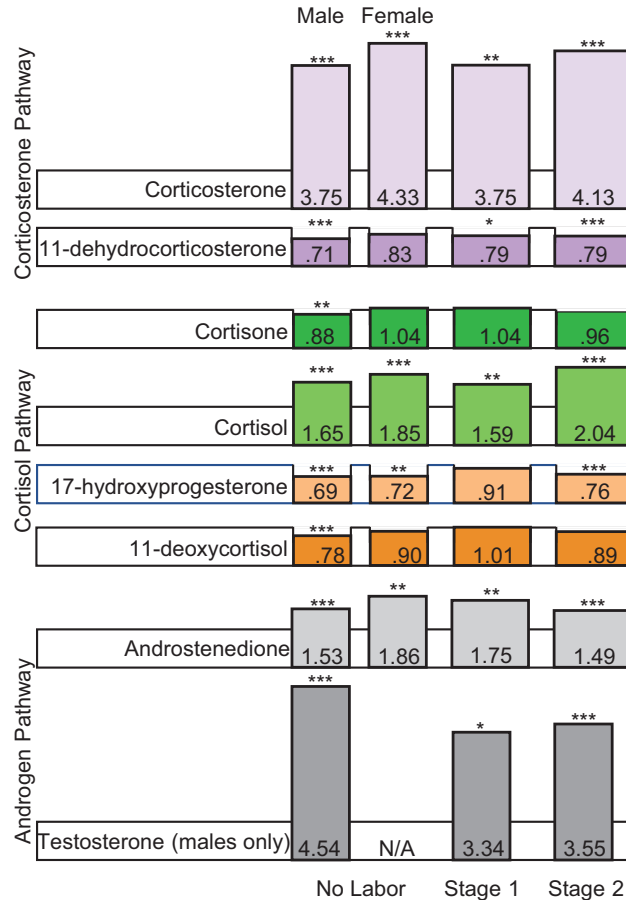


Figure 7. Fetal modification of venous serum concentrations across labor history groups. To align groups with different steroid pool concentrations, [V] for each individual and hormone was arbitrarily set to 1.0 and [A] was then expressed relative to that standardized concentration (eg, if [V] = 200 nmol and [A] = 50 nmol, then the fetal modification will be 0.25 [unitless] and if [V] = 0.5 and [A] = 2.0, fetal modification will be 4.0). The no labor group (N = 49; 31M, 18F) includes deliveries by elective C-section without antecedent labor from Wynne-Edwards et al [5] that were collected and quantified simultaneously with the current study. As the earlier paper identified sex differences, the no labor group is partitioned by sex. Stage 1 (N = 12; 7M for testosterone) consists of the FTP1 emergency C-section cohort. Stage 2 (N = 135; 69M for testosterone) consists of vaginal deliveries with regional anesthetic. All deliveries involved regional spinal anesthetic to the mother. Hormones are batched into the corticosterone pathway, the cortisol pathway, and the androgen pathway. The rectangle containing the steroid name has a height of 1.0 to represent the standardized venous concentration arriving at the fetus. Color matches the legends for other figures and has a height equivalent to the arterial score. Asterisks indicate the probability that the score distribution has a mean of 1.0 where * $P < 0.05$ is only reported for stage 1 with small sample size; ** indicates $P < 0.0083$; and *** indicates $P < 0.0001$. One-way ANOVA across the 3 stages (combining males and females in the no labor group) did not reveal any evidence of differences depending on the labor history.

Results also confirm preferential fetal synthesis of corticosterone [1, 5]. Although corticosterone concentrations are much lower than cortisol concentrations, the [A] corticosterone is approximately 4-fold higher than [V], whereas [A] cortisol only reaches a doubling. These corticosterone increases are in the same range as testosterone increases in males. The difference is likely to reflect immediate-substrate pool sizes competing for the same 11 β -hydroxylase enzyme, but the current data cannot address that hypothesis because 11-deoxycorticosterone was not quantified. Likewise, in the absence of maternal serum samples, this study cannot address the impact of maternal serum corticosterone, and the

published literature does not provide serum measures of maternal corticosterone immediately postpartum. Nevertheless, a 4-fold increase in any steroid should be readily detectable as a biological signal within the fetus and at the placenta. Finally, concentrations of adrenal pathway steroids in arterial and venous samples followed the same pattern in all groups, with cortisone highest, cortisol second, 17-hydroxyprogesterone third, and 11-dehydrocorticosterone, corticosterone, and 11-deoxycortisol at the lowest concentrations.

Fetal aldosterone synthesis is likely to influence preferential corticosterone synthesis [15]. Proportional depletion of 11-dehydrocorticosterone to corticosterone was approximately 2-fold larger than proportional cortisone depletion. As corticosterone is a direct substrate for aldosterone synthesis, that pathway could be driving the corticosterone pool elevation. Mineralocorticoid actions on fetal electrolytes and water balance, as impacted by 11 β -HSD2, are established in late gestation [16]. Aldosterone concentrations were not quantified. In the absence of *in vitro* studies competing the parallel substrates against the shared enzymes, it is not possible to discriminate between all of these possibilities.

A new finding was the broad effect of maternal regional anesthesia and the role of the fetus in compensating for those changes. Births by C-section involved regional spinal anesthesia. However, within the vaginal deliveries, there was a reasonably sized subgroup that did not receive regional anesthetic. For both cortisol and corticosterone, maternal pain relief was associated with a substantial reduction in the concentrations reaching the fetus through umbilical venous circulation. Spinal anesthetic does not reach the blood circulation of the mother and therefore does not reach the fetus. Thus, the expectation is that maternal serum cortisol and corticosterone fall rapidly after pain relief although other anticipatory adrenocortical responses to new parenthood remain. Consistent with this hypothesis, cortisone and 11-dehydrocorticosterone were also reduced in venous samples after maternal anesthetic.

Second, the fetal experience of labor is expected to be unchanged by maternal anesthetic, as contractions continue towards delivery. Thus, it is also not surprising that the fetus adds both cortisol and corticosterone to the arterial circulation. It is also not surprising that none of the individual steroid hormone analyses of the impact of labor identified differences between delivery histories. What was unexpected was the complete fetal compensation for the effect of maternal regional anesthetic. There was no difference between the 2 groups for cortisol or corticosterone in arterial serum. To achieve this “correction” there was more than a doubling (100% increase) in cortisol when the mother was receiving pain relief and the corticosterone increase was even larger at 300%. Like preferential corticosterone synthesis, this pattern is likely to arise from a combination of high activity of the fetal 11 β -hydroxylase enzyme and high activity of 11 β -HSD1 enzyme catalyzing the back-conversion of cortisone and 11-dehydrocorticosterone to cortisol and corticosterone. In the absence of maternal serum data, the parsimonious explanation for this effect is that maternal cortisol and corticosterone decrease rapidly after pain relief. Supporting this hypothesis is the finding that there were no effects of labor history on the standardized arterial concentration of steroids.

This fetal compensatory synthesis can only be detected in paired arterial and venous samples. For example, with paired arterial and venous concentrations in the absence of labor, there were higher cortisol concentrations in female versus male umbilical circulation, plus male substrate depletion was in equilibrium with cortisol synthesis, whereas females had net cortisol synthesis exceeding substrate depletion [5]. A probable explanation for this difference is that females maintain a high flux of substrates through to cortisol synthesis rather than increased pool sizes [5]. Mixed human umbilical cord samples, for reasons related to umbilical cord vascular anatomy consist predominantly of venous serum. In the human umbilical cord, the vein is a large, thin-walled vessel, whereas the arteries are narrow, thick-walled, less likely to drip by gravity, and have their blood pressure constrained by viscosity (hematocrit) [17]. Thus, needle aspiration is technically easier from the vein, and mixed cord blood contains considerably more venous than arterial sample. In a large majority of published umbilical cord steroid hormone analyses, umbilical cord serum is collected either by aspiration of the umbilical vein (as is routine for cord pH and base

excess measures) or as mixed cord blood (eg, [18]). Thus, umbilical vein sampling after vaginal delivery or stage 2 head compressions will incompletely represent the umbilical steroid environment of full-term pregnancy. Similarly, maternal regional pain relief will increase variability in adrenal hormone measures that might mask important sex differences and remains an important variable in future studies of fetal steroidogenesis.

A third key finding was that emergency C-sections are too heterogeneous to be combined in studies of fetal adrenal steroidogenesis at term. All of the emergency C-sections in this study shared the intervention of regional spinal anesthetic. They also shared positive outcomes in terms of neonatal health status. However, they differed in terms of the maternal and fetal stressors before the birth. With FTP2, cephalopelvic disproportion is not detected until stage 2 of labor (pushing) has been attempted without progress towards vaginal delivery. This represents head compressions for the fetus and prolonged labor for the mother. Both FHR and FTP1, on the other hand, are delivered by emergency C-section before stage 2 of labor and have not experienced stage 2 stressors. However, FHR rationale recognizes a specific biomarker of fetal distress (fetal heart rate decelerations), in contrast with FTP1 rationale that is based on maternal progression.

Venous serum approaching the fetus in the 3 emergency C-section groups differed in total molarity, with a consistent pattern of differences across the 3 groups in corticosterone, cortisol, deoxycortisol, and 17-hydroxyprogesterone, with FTP2 concentrations being the highest, FTP1 concentrations the lowest, and FHR between the other two. Cortisol was a notable exception to the pattern and did not vary with the rationale for the emergency C-section. After the fetus, the arterial blood returning to the placenta had lost the differences in total molarity, gained a difference between groups in cortisol, and lost the other hormone differences. Thus, venous serum, which is directly influenced by maternal inputs and placental steroidogenic enzymes, contains a different quantity and balance between steroids than the matched arterial serum. This conclusion supports the earlier finding, in an emergency C-section cohort of similar size, that the corticosterone increases across the fetus ([A]-[V]) were approximately 3-fold larger with the FTP2 rationale than with FTP1 or FHR [1].

Comparison of the 3 emergency C-section rationales represents a second finding of fetal steroidogenic compensation for different levels of steroids arriving in venous circulation. Total molarities for the 3 rationales “correct” to similar levels in arterial serum, with all steroids reverting to similar concentrations arterially. The exception, again, was cortisol, that increased differentially to result in higher arterial concentrations of cortisol with FTP2 rationale than either of the other 2 groups. As described above for regional anesthetic to the mother, failure to distinguish these different rationales for an emergency C-section will increase the variance in hormone concentrations and thereby potentially obscure underlying patterns in fetal steroidogenesis at term. Future studies will need to partition emergency deliveries and increase sample sizes as necessary for the study, retaining the rationale for the emergency delivery in statistical models. Paired sampling of matched arterial and venous samples is also necessary for the future study of fetal compensatory steroidogenesis.

In terms of clinical practice, the results also confirmed that there was no evidence that the use of a vacuum or forceps altered the umbilical steroid environment relative to spontaneous vaginal delivery [19]. Likewise, this sample population had universally positive outcomes in terms of fetal health status (no admissions to the NICU), so that antenatal maternal and fetal risk factors did not have impacts on steroidogenesis. Of course, sample sizes were low for antenatal risk factors, and other impacts could emerge in cohorts with greater statistical power. In addition, suppression of net testosterone synthesis by labor suggests that routine screening of fetal androgens is unlikely to have clinical value, unless labor history is controlled. In terms of research methods, the current results support a paired arterial and venous sampling approach to umbilical cord serum, awareness of the separate impacts of labor (including the difference between a stage 1 and a stage 1 plus 2 labor history), and controls for the impact of maternal regional anesthetic.

There is a sex difference in fetal adrenal steroidogenesis dynamics at full-term [2, 5]. Any developmental sex difference in fetal adrenal axis function has the potential to play

a role in the early-life programming caused by in utero stressors [20–22]. Sex differences in neurodevelopmental and behavioral outcomes follow intrauterine exposure to stress [23] and may be linked to sex differences in later-life psychiatric disorders [24]. The placental cortisol to cortisone enzyme is differentially affected by antenatal steroids in males and females [25, 26] as is the placental microvasculature [27] and placental glucocorticoid isoforms [28]. Male neonates also suffer more growth restriction from a repeated glucocorticoid (betamethasone) treatment antenatally than females [20, 29], are compromised by genetic corticosteroid binding globulin (CBG) deficiency [30], and are more susceptible to intrauterine growth restriction, preterm delivery, and stillbirth [31]. Thus, umbilical steroid dynamics during early uterine development are a pathway towards understanding antenatal hypothalamic-pituitary-adrenal axis function differences between males and females that alter lifetime health outcomes.

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Additional information

Correspondence: Katherine E. Wynne-Edwards, PhD, Department of Comparative Biology and Experimental Medicine, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada T2N 4N1. E-mail: k.wynne-edwards@ucalgary.ca

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