Characterizing the Steroidal Milieu in Amniotic Fluid of Mid-Gestation: a GC-MS Study

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Introduction

- Growth and development of an embryo or fetus depend on steroid hormone biosynthesis and metabolism in the feto-placental unit [1]. The human adrenal anlage differentiates into two distinct zones by the eighth week of gestation: the inner large fetal/transient zone and the outer small adult/permanent zone [2].
- The transitional zone of the fetal adrenal is believed to be the site of fetal *de novo* cortisol production from the second half of gestation onwards [3].

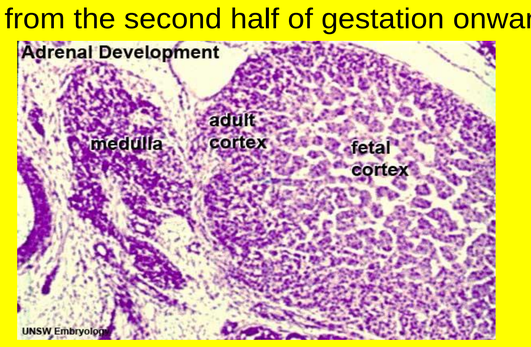

Figure 1. fetal adrenal gland [4]

- Fetal zone steroids refer to those steroids which are only present in fetuses and newborns stemming from the fetal zone of the fetal adrenal, bearing a “3β-hydroxy-5-ene” structure.

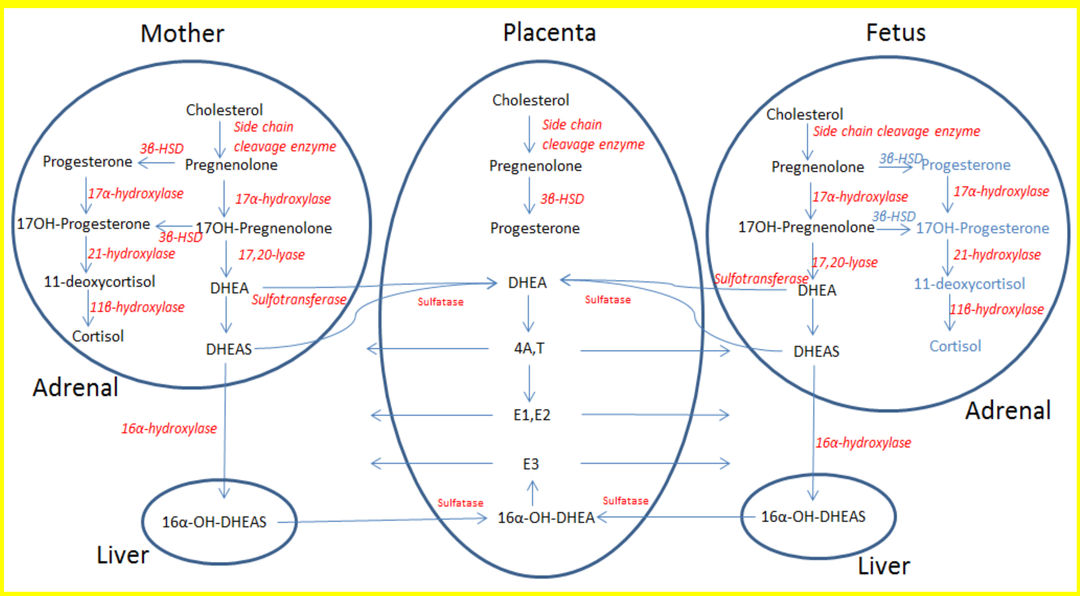

Figure 2. Steroid metabolism and enzyme activities of the feto-placental unit

Aim:
To complement our findings of the LC-MS/MS study [5], we now used GC-MS to delineate further steroid hormone metabolites in AF of mid-gestation by a comprehensive targeted steroid hormone metabolomics analysis approach.

Method

Subjects:
65 pregnant women who underwent amniocentesis agreed to participate in the study. The gestational age of the fetuses was 18.8 ± 1.8 (Mean ± SD) weeks. The mother’s age was 35.6 ± 4.3 (Mean ± SD) years.

Sample analysis:
Free and conjugated urinary steroids were extracted by solid phase extraction, enzymatically hydrolyzed and after recovery of hydrolyzed steroids, methyloxime-trimethylsilyl ethers were formed. GC was performed using an Optima-1 fused silica column (Macherey-Nagel, Düren, Germany) housed in an Agilent Technologies 6890 series GC that was directly interfaced to an Agilent Technologies 5975 inert XL mass selective detector.

References

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[5] R. Wang, D. Tiosano, M.F. Hartmann, S.A. Wudy, Characterizing the steroidal milieu in amniotic fluid of mid-gestation: A LC–MS/MS study, J. Steroid Biochem. Mol. Biol. 185 (2019) 47-56. doi:https://doi.org/10.1016/j.jsbmb.2018.07.007.

Summary

- 52 steroid hormone metabolites in AF of mid-gestation were delineated by a targeted GC-MS approach.
- Pregnenolone and 17-OH-pregnenolone metabolites, DHEA and metabolites, progesterone and 17-OH-progesterone metabolites, androgens, estrogens as well as cortisone and cortisol metabolites were characterized in AF of mid-gestation.
- Reference data of levels of 52 steroids in AF of mid-gestation were provided in the present study. These reflected that all important classes of steroids are present in AF of mid-gestation.
- This set of basic data might lay the foundation for further studies characterizing various diseases affecting steroid metabolism.

Results

Table 1. Concentrations of selected steroids

Steroids	Concentrations (ng/mL) Mean±SD Median (Min-Max)	
Pregnenolone and 17-OH-pregnenolone metabolites (Δ5 unsaturated C_{21} steroids)		
Σ Pregnenolone and 17-OH-pregnenolone metabolites	138.0±59.3 133.4 (40.6-339.7)	
21-OH-P5olon	43.9±24.0 41.9 (0.0-123.5)	
16α-OH-P5olon	17.3±7.1 15.7 (9.1-50.8)	
P5-3β,20α,21-triol	45.3±23.2 41.5 (7.8-123.7)	
15β,17α-OH-P5olon	15.7±10.8 14.0 (3.0-65.7)	
P5-tetrol-15β	13.8±9.9 11.2 (3.3-55.8)	
DHEA and metabolites (Δ5 unsaturated C_{19} steroids)		
Σ DHEA and metabolites	97.1±56.5 83.6 (38.6-361.5)	
DHEA	7.7±6.2 6.1 (3.1-50.6)	
16α-OH-DHEA	42.4±21.8 37.2 (16.3-133.4)	
16β-OH-DHEA	32.9±30.3 24.2 (7.4-185.1)	
Progesterone and 17-OH-progesterone metabolites		
Σ Progesterone and 17-OH-progesterone metabolites	107.3±44.3 101.6 (43.0-299.3)	
Pregnanediol	91.6±41.0 86.8 (33.9-279.9)	
Pregnanetriol	9.0±5.3 7.4 (3.7-33.0)	
Other androgens (saturated C_{19} steroids)		
Androsterone	4.6±2.1 4.4 (0.0-12.1)	
Etiocholanolone	3.6±4.6 2.8 (1.2-38.9)	
Testosterone	Male:1.5±0.4 1.5 (0.9-2.6)	Female: 1.1±0.2 1.1 (0.7-1.5)
Estrogens (C_{18} steroids)		
E3	33.2±26.1 25.5 (10.8-188.0)	
Cortisol (F) and cortisone (E) metabolites (C_{21} steroids)		
(Σ F+Σ E) metabolites	59.6±13.6 60.2 (14.8-103.8)	
Σ F and metabolites	24.1±5.2 24.0 (3.0-35.1)	
Cortisol (F)	8.4±2.3 8.5 (0.0-16.1)	
THF	4.4±1.6 4.0 (0.9-8.2)	
α-THF	4.0±1.9 4.0 (0.0-10.1)	
THE	18.0±4.2 17.0 (10.1-34.1)	

- The concentrations of 52 steroid hormone metabolites which included pregnenolone and 17-OH-pregnenolone metabolites, DHEA and metabolites, progesterone and 17-OH-progesterone metabolites, androgens, estrogens, corticosterone metabolites, 11-deoxycortisol metabolites, as well as cortisol and cortisone metabolites were analyzed. Selected steroids are shown in Table 1.
- The dominating steroids were the group of pregnenolone and 17-OH-pregnenolone metabolites, followed by progesterone and 17-OH-progesterone metabolites, and DHEA and its metabolites.
- Most steroid hormone metabolites did not show any sex dimorphism. Most expressed sex differences were only found for testosterone ($P<0.0001$) with higher values in males, respectively.
- Cortisol metabolites are clearly present in our AF samples of mid-gestation before the start of de novo cortisol synthesis.

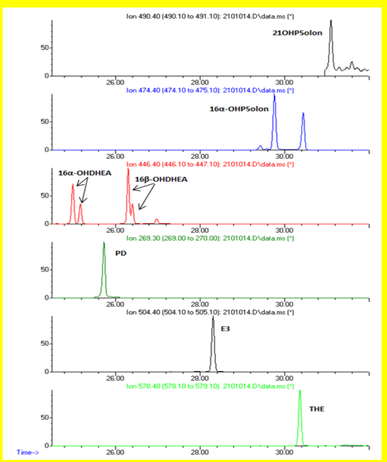

Figure 3. SIM chromatograms of important steroids

Discussion

- The comparable concentrations of 16β-OH-DHEA with 16α-OH-DHEA in AF suggest that 16β-hydroxylase is also active in the feto-placental unit.
- We speculate that 16α-OH-P5olon is the main precursor of 16α-OH-DHEA and 16β-OH-DHEA based on the strong correlation study ($r=0.83, P<0.0001$; $r=0.76, P<0.0001$).
- We found cortisol metabolites to be clearly present in our AF samples of mid-gestation. A possible explanation for a source of cortisol in AF of mid-gestation could be the maternal transfer of cortisol across the placenta. Another reason could be placental progesterone acting as a precursor of cortisol production in the fetus.
- We found the concentrations of cortisone metabolites to be clearly higher than those of cortisol metabolites in our study. This points to a preference of cortisol inactivation by the feto-placental unit.

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