Characterizing the Steroidal Milieu in Amniotic Fluid of Mid-Gestation: A LC-MS/MS Study

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Introduction

- Growth and development of an embryo or fetus depend on intact hormone biosynthesis and metabolism in the feto-placental unit [1].
- Most studies on steroids in AF have been carried out by immunoassays for several decades. Due to cross-reactivity of immunoassays, GC-MS and LC-MS nowadays have become the main prevalent tools for the qualitative and quantitative analysis of steroids [2].
- Recently, studies found that with specific uptake carriers(e.g. SOAT), sulfated steroids can also enter cells as unconjugated steroids [3]. As far as we know, except for DHEAS, no other sulfated steroids have yet been quantified in AF by LC-MS/MS [4].

Aim: We therefore aimed at developing a LC-MS/MS method to simultaneously determine unconjugated and sulfated steroids in AF of mid-gestation.

Methodology

- **Instrumental equipment**:
  - SL 1200 HPLC system
  - TSQ Quantum Ultra (Thermo Fisher)
- **Development of the method:**
  - Sample preparation
  - LC conditions
  - MS conditions
- **Validation of the method**:
  - Specificity
  - Linearity
  - Accuracy
  - Precision
  - Recovery
  - Matrix effect

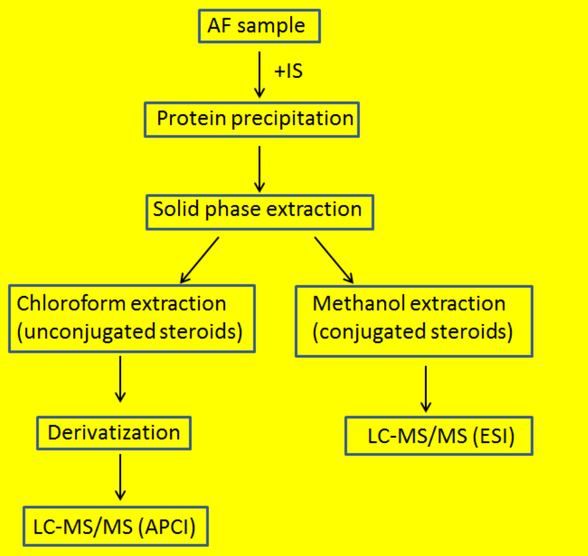

Figure 1. The workflow for sample preparation

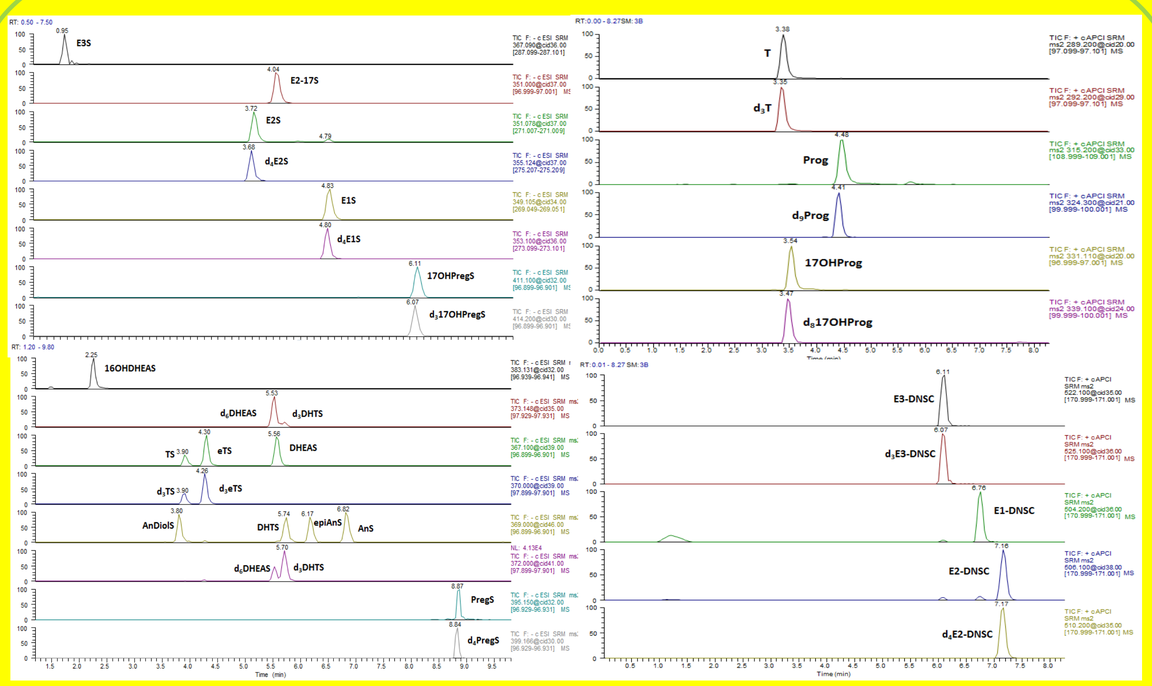

Figure 2. Chromatographic behavior of standards in matrix (10 ng/mL)

Reference

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[6] R. Wang, D. Tiosano, A. Sánchez-Guijo, 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. (2018)

Summary

- A new LC-MS/MS method for the determination of unconjugated and sulfated steroids in AF of mid-gestation was developed and validated.
- LC-MS/MS based reference data of 14 sulfated and 6 unconjugated steroids in AF of mid-gestation was provided. Most steroids have been analyzed for the first time in AF of mid-gestation.
- Correlation study confirmed the classical steroid pathway and a sulfated steroid pathway in the feto - placental unit.

Results

- The levels of 14 sulfated and 6 unconjugated steroids in 65 AF samples of mid-gestation were measured, see Table 1.
- Only T exhibited a significant sex difference (P<0.0001).
- Strong positive correlations were found between 16OH-DHEAS and DHEAS, 16OH-DHEAS and E3S, 17OHPregS and PregS as well as E3 and E3S.

Table 1. Levels of steroids in AF of mid-gestation

C21 steroids	Concentrations (ng/mL) Mean±SD, median(min-max)
Prog	37.4±12.8, 33.9(16.4-78.6)
17OHProg	1.0±0.3, 1.0(0.4-2.0)
PregS	8.6±3.7, 8.2(2.6-20.3)
17OHPregS	4.9±2.0, 4.5(2.1-13.2)
C19 steroids	
T	<LOQ -0.6
TS	<LOQ -3.9
eTS	7.3±3.6, 5.9(2.9-17.8)
DHEAS	4.6±2.4, 3.8(1.5-12.3)
16OH-DHEAS	21.5±10.7, 19.3(6.9-62.9)
DHTS	<LOQ-7.0
AnS	9.2±7.4, 7.4(0.9-39.4)
epiAnS	<LOQ -2.7
AnDiolS	<LOQ -1.9
C18 steroids	
E1	<LOQ -1.3
E1S	<LOQ-25.3
E2	<LOQ-0.5
E2S	<LOQ -2.3
E2-17S	<LOQ -3.7
E3	1.2±0.4, 1.1(0.6-2.6)
E3S	8.1±4.0, 6.5(2.2-21.0)

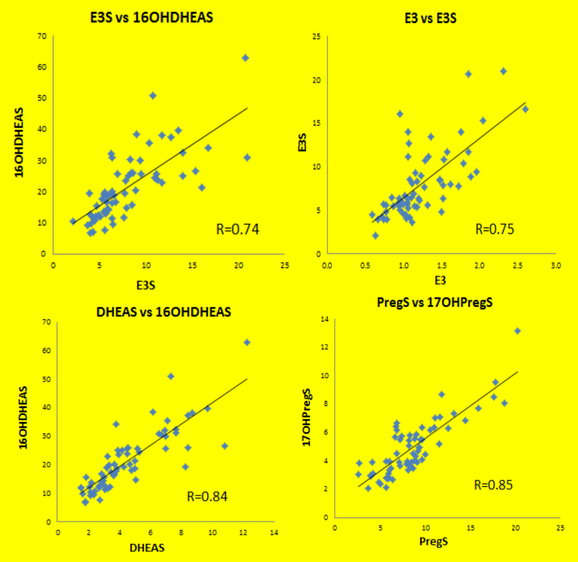

Figure 3. Pearson's correlation between steroid concentrations in AF, X axis represents the concentration for one steroid (ng/mL) and Y axis means concentration for the other steroid (ng/mL).

Discussion

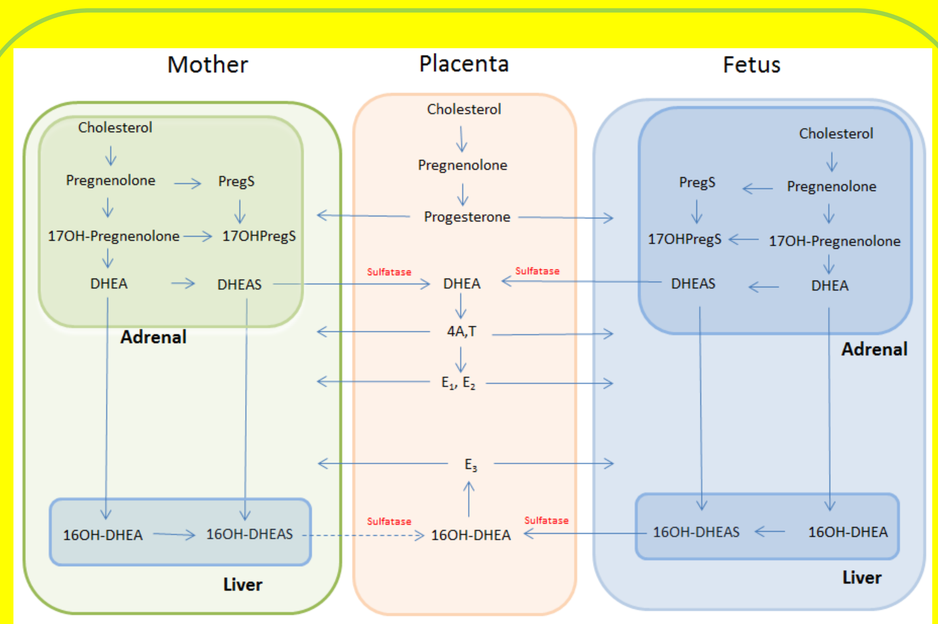

Figure 4. Steroid pathway in the feto-placental unit

- The fetal adrenal produces large amounts of DHEAS. Then, DHEAS is 16α-hydroxylated in fetal liver to produce 16α-OH-DHEAS.
- 16α-OH-DHEAS is the principal precursor of E3 in the placenta. In the fetal compartment, E3 is sulfated at position 3 by sulfotransferase to produce E3S.
- PregS can serve as a substrate for biosynthesis of 17OHPregS in vitro and in human males [5]. This steroidogenic pathway for sulfated steroids seems also to be present in the feto-placental unit [6].