

in Amniotic Fluid by LC-MS/MS

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Background

Determination of steroids in amniotic fluid (AF)(1) has been essentially used in the 3 past decades for the prenatal diagnosis of 21-OH deficiency. With the recent advances of ultrasound technology (US) and the widespread use of amniocentesis, prenatal diagnosis of DSD appears more common especially if a mismatch between karyotype and external genitalia detected by US occurs. An accurate and specific determination of normal value of steroids in AF appears essential to evaluate DSD during this prenatal period.

Objectives

Determination of the pattern of reference value for 17OHP, Delta4-A and Testosterone(T) by Liquid chromatography tandem mass spectrometry (LC-MS/MS method) according to sex.

Methods

UPLC was performed on an Agilent Technologies 1290 Infinity using a Poroshell C18 4.6x50 2.7µm coupled to an Agilent Technologies triple quadrupole 6460. In the sample preparation, internal standard deuterium are added in calibration curve and AF before extraction (SLE). This method separated the steroids was validated according the Norm (linear response, CV less than 2% for the repeatability, less than 15% for the reproducibility and correlation with RIA $R^2 > 0.93$ but the value was lower than in RIA). The lower limit of quantification is for Delta4A, and 17OHP 0.13 nmol/L and for testosterone 0.05nmol/L.

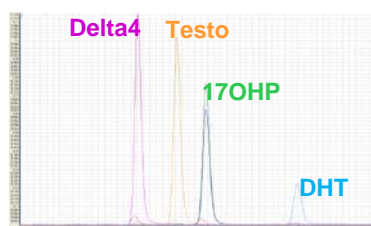
Materials

Amniocentesis, after informed consent, have been performed in 165 women at 14.2 and 23 weeks of amenorrhea for increased maternal serum markers. The 165 fetal karyotype were normal (46, XX or 46, XY) and no morphological abnormality has been observed. We report the quantification of 3 in these AF.

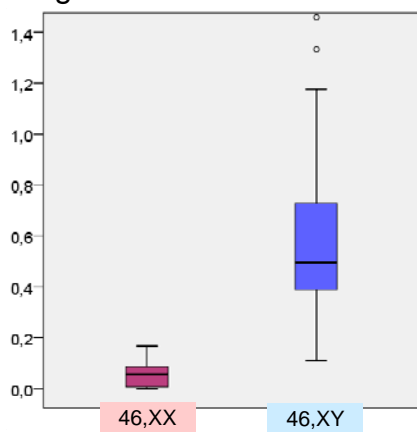
Results

Normal values of 3 steroids

Chromatography method separated the followed steroids:



Testosterone is significantly higher in male foetus.



	17OHP nmol/L Mean (+/-SD)	Delta4A nmol/L mean (+/-SD)	Testostérone nmol/L Mean (+/-SD)
46, XX (n=87)	3.44 (+/-1.18)	0.85 (+/-0.54)	0.08 (+/- 0.08)
46, XY (n=78)	3.24 (+/-1.25)	1.46 (+/-0.78)	0.57 (+/-0.29)

The lower values determined by LC-MS/MS vs chromatography-RIA should be due to a more specific determination.

Conclusions

We report a rapid, sensitive and accurate method for simultaneous measurement of three steroids in AF. Moreover, pathological values for DSD (defect of steroid biosynthesis,...) were in progress.

References

1. Forest et al, JCEM, 1980