Abstract

Biophysical monitoring of the ovarian function after ovarian stimulation is restricted to the measurement of serum estradiol and progesterone. We aim to investigate the entire ovarian steroidogenesis after ovarian stimulation. 50 women (26 IVF, 24 ICSI) who underwent AMP induction were retrospectively involved and compared to 11 IUI (control). Estrogens (estrone, estradiol, estriol), androgens (4α-androstenedione, testosterone, progesterone), androstenedione (17α-androstenedione, testosterone, progesterone) were measured each 48 hours. Steroid profiles were characterized using mass spectrometry. Results are expressed as median values and a p <0.05 was considered significant.

Steroids basal secretion was within the normal range (IVF, ICSI and IUI respectively): estradiol (223, 317.5, 314 pmol.L⁻¹), testosterone (1124.5, 903.5, 1606.5 pmol.L⁻¹), 4α-androstenedione (3902, 3100, 5618 pmol.L⁻¹), 17 hydroxyprogesterone (1186, 1350, 2397.5 pmol.L⁻¹), progesterone (1150, 1150, 2950 pmol.L⁻¹). We observed a significant increase in estradiol as expected but also in estrone. The 48 hours increase for estradiol was significantly different between control and AMP (1.5-fold for IUI, 1.8-fold for IVF, 1.9-fold for ICSI). 4α-androstenedione, 17 hydroxyprogesterone and to a smaller extent testosterone increase significantly in IVF and ICSI under rFSH. There was no difference in the 48 hours increase of those steroids between ICSI and FIV except for 4α-androstenedione which increased (1.15-fold for FIV; 1.31-fold for ICSI).

rFSH treatment in AMP is likely to induce an ovarian hyperplasia, however we have highlighted individual variations, which we are exploring by mass spectrometry.

Introduction

rFSH is widely used in AMP for the ovary inducing. It is combined to the measurement of estradiol in a daily practice of the folliculogenesis monitoring. However, little is known about the effect of this treatment on each step of the ovarian steroidogenesis during this strong induced folliculogenesis. We recently developed sensitive and specific analytical methods using liquid chromatography on line with tandem mass spectrometry to identify and quantify steroids. In this study, we aim to validate these methods and analyze the steroid pattern in serum of women under rFSH in AMP.

Results

Concentration pmol.L⁻¹, n, minimum, first quartile, median, third quartile, maximum (P = Progesterone, 17OHPP = 17 OH-progesterone, A = 4α-androstenedione, T = Testosterone E1 = Estrone, E2 = Estradiol)

Materials and methods

Patients:
- IUI (n=11): first line therapy of couple infertility; no treatment (= control).
- IVF (n = 26) for usual female infertility and ICSI (n = 20); for usual male infertility.

With AMP ovarian stimulation protocols:
- agonist: rFSH daily injection for 10-14 days with GnRH agonist started 2-8 days before; monitoring each 48 hours
- antagonist: rFSH daily injection for 10-14 days with GnRH antagonist from day 6; monitoring each 48 hours

Assay:
- P, E2 (immunoassays by Cobas®Roche), 17OHP, A, E1, T (RIA (Cisbio Bio and Beckman Coulter).
- LC MS/MS:
  - UPLC Acquity: C18 column, MeOH in water in gradient mode, on line with TQ mass spectrometer (Quattro Premier, Water®).
  - UPLC Acclera: C18 column, ACN in water in gradient mode, on line with TQ mass spectrometer (TSQ Quantum UltraThermoFischer®).

Quantification of steroids by LC MS/MS and SIDA (Stable Isotope Dilution Analysis) based on determination of transitions. The precursor ion (produced in ion source) is selected in the first quadrupole of mass spectrometer, dissociated in collision cell, the produced daughter ions are selected in quadrupole 3 and detected.

Representative patterns of LC MS/MS results for estrogens, androgens and progestatives

Chromatographic analysis of 17 hydroxyprogesterone

Selective detection (by MS/MS) of progestagens and their deuterated derivatives for the quantitative analysis

Chromatographic analysis of Δ₄- androstenedione

Selective detection (by MS/MS) of androgens and their deuterated derivatives for the quantitative analysis

Representative patterns of steroid concentration during IVF, ICSI and IUI

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Analysis of steroid pattern in serum during ovarian stimulation

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Reproduction, endocrine dysfunctions and signaling

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