Analysis of steroid pattern in serum during ovarian stimulation

M C Menet, G Damiens, E Méténier, N Sraouia, V Gayet, L Marcellin, M C Leguy, J Guibourdenche

Hormonology and AMP departments, AP-HP CHU Cochin, Université Paris Descartes, Paris, France

Abstract

Biochemical 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** (Δ4-androstenedione, testosterone), **progestatives** (progesterone, 17 hydroxyprogesterone) 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 $pmol.L^{-1}$), Δ4-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 IIU, 1.8-fold for IVF, 1.9-fold for ICSI). **Δ4-androstènedione, 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 Δ4-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, 17OHP = 17 OH progesterone, A = Δ4 androstenedione, T = Testosterone E1 = Estrone, E2 = Estradiol)

	IVF			
Day	**6**	**8**	**10**	**12**
P	n = 16 900,1225, **2000**, 3575, 6300	n = 20 400, 1125, **1700**, 2375, 4200	n = 14 700, 1300, **1500**, 2750, 6300	n = 10 600, 1300, **1650**, 2125, 4400
17OHP	n = 20 319, 1006, **1755**, 3198, 11049	n = 21 217, 1250, **2017**, 4033, 4909	n = 15 1300, 1800, **2700**, 3600, 8300	n = 11 1480, 2000, **2213**, 3900, 4621
A	n = 19 1012, 3600, **4480**, 5997, 12800	n = 20 2103, 4061, **5446**, 7589, 17100	n = 14 4000, 5038, **6235**, 8447, 33600	n = 10 2524,5020, **6200**, 7375, 9600
T	n = 20 633, 912, **1273**, 1844, 3956	n = 21 630, 901.5, **1283**, 2027, 3175	n = 15 580, 1200, **1700**, 2562, 3993	n = 11 740, 1000, **1400**, 1700, 4084
E1	n = 19 38,173, **351**, 964, 10869	n = 20 137, 318, **594**, 2626, 8467	n = 13 203, 497, **1037**, 4720, 13184	n = 9 250, 708, **1343**, 3068,3956
E2	n = 20 172, 305, **534**, 2012, 7948	n = 21 435, 875, **1194**, 4171, 7991	n = 15 327, 2179, **3550**, 7033, 21869	n = 11 671, 2128, **4040**, 4638, 6365
	ICSI			
Day	**6**	**8**	**10**	**12**
P	n = 16 1000, 1325, **1800**, 2725, 3900	n = 19 700, 1200, **2000**, 2900, 11000	n = 19 <LOQ, 1200, **1600**, 2200, 3700	n = 13 2000, 2450, **2800**, 3450, 3900
17OHP	n = 18 775, 1210, **2400**, 3332, 3737	n = 20 1200, 1483, **2286**, 4338, 6795	n = 19 1600, 1930, **3133**, 3800, 7024	n = 13 2900, 3200, **4265**, 5462, 7700
A	n = 16 1876, 2725, **4500**, 6630, 10700	n = 19 2500, 3222, **5600**, 8800, 12500	n = 18 2177, 4596, **6250**, 10802, 16020	n = 13 4329, 6035, **7800**, 10950, 22990
T	n = 18 444, 677, **1200**, 2285, 3863	n = 20 350, 1002, **2144**, 3157, 3309	n = 19 728, 1300, **1895**, 2731, 5752	n = 13 628, 1200, **1700**, 2517, 6823
E1	n = 18 85, 202, **410**, 568, 1911	n = 19 121, 312, **718**, 1035, 2791	n = 19 111, 517, **833**, 1551, 6206	n = 13 282, 981, **2042**, 2748, 3701
E2	n = 18 217, 527, **640**, 1477, 5649	n = 20 508, 1000, **1562**, 2440, 7774	n = 18 1464, 2230, **3038**, 5028, 12363	n = 13 3313, 3715, **4139**, 5724, 14796
	IUI			
Day	**8**	**9**	**11**	**12**
P	n = 2 1400, 1400, **2950**, 4500, 4500	n = 8 1300, 1375, **1750**, 2575, 3700	n = 8 800, 1425, **1600**, 2150, 2800	n = 5 800, 1100, **1700**, 2350, 2700
17OHP	n = 2 1495, 1495, **2397**, 3300, 3300	n = 8 908, 1250, **1786**, 3224, 4695	n = 8 1100, 1268, **2252**, 2625, 3443	n = 5 1497, 1498, **1900**, 5338, 6277
A	n = 2 5600, 5600, **5618**, 5636, 5636	n = 8 3479, 4731, **5600**, 9855, 12810	n = 8 4200, 4538, **5058**, 8708, 10790	n = 5 4900, 5240, **6100**, 11529, 12040
T	n = 2 1500, 1500, **1606**, 1713, 1713	n = 8 730, 1100, **1475**, 2224, 6236	n = 8 232, 667.5, **1110**, 2513, 3951	n = 5 1000, 1350, **2329**, 2882, 3282
E1	n = 3 164, 164, **165**,166, 166	n = 8 83, 153, **244**, 352, 630	n = 4 97, 122, **219**, 377, 422	n = 5 197, 259, **389**, 697, 706
E2	n = 2 281, 281, **314**, 347, 347	n = 8 287, 370, **431**, 543, 1166	n = 8 303, 407, **704**, 803, 837	n = 5 410, 642.5, **951**, 1293, 1298

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:

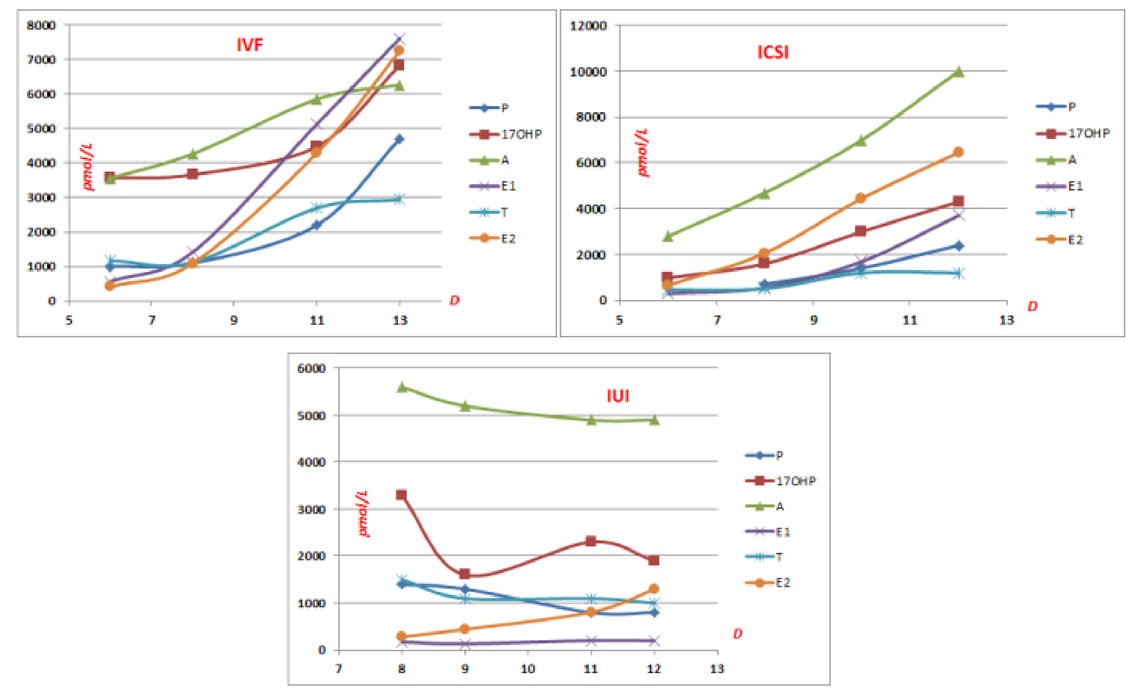
- agonist: rFSH daily injection for 10-14 days with GnRH agonist started 2-8 days before; monitoring each 48 hours
- antagonist: rFSH daily injection for 10-14 days with GnRH antagonist from day 6; monitoring each 48 hours

Assay:

- P, E2 (immunoassays by Cobas^{R}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, Waters^{R}),

* UHPLC Accela: C18 column, ACN in water in gradient mode, on line with TQ mass spectrometer (TSQ Quantum Ultra,ThermoFischer^{R}).

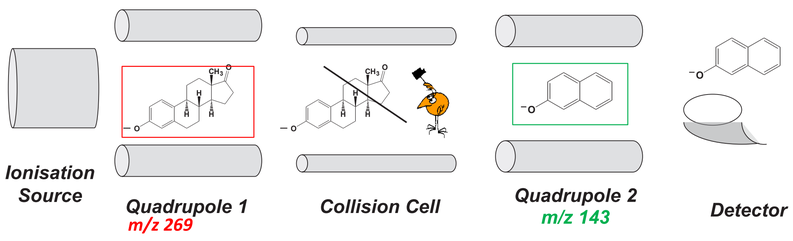


Representative patterns of steroid concentration during IVF, ICSI and IUI

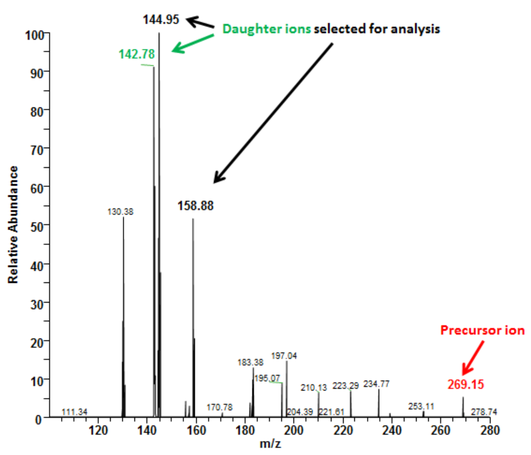
Quantification of steroids by LC MS/MS and SIDA (Stable Isotope Dilution Analysis)

based on determination of « transitions ». The precursor ion (produced in ion source) is selected in the first quadripole 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

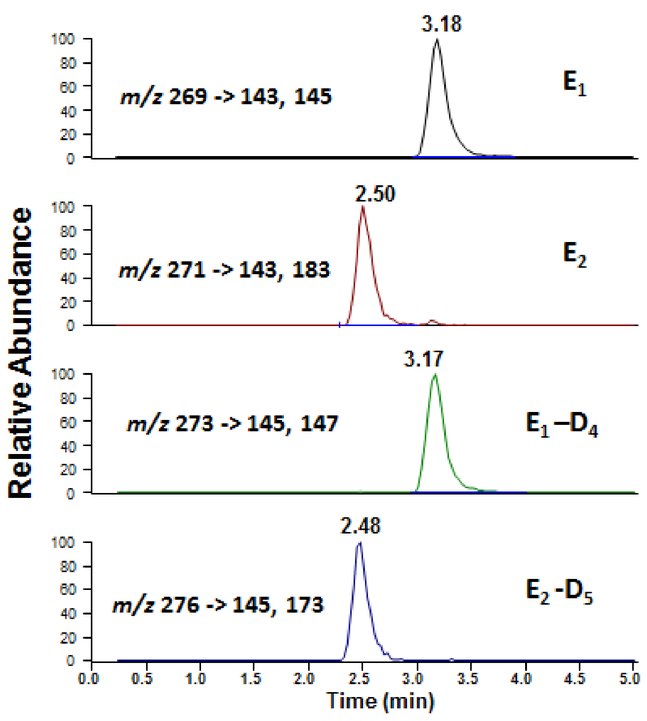


Triple quadrupole



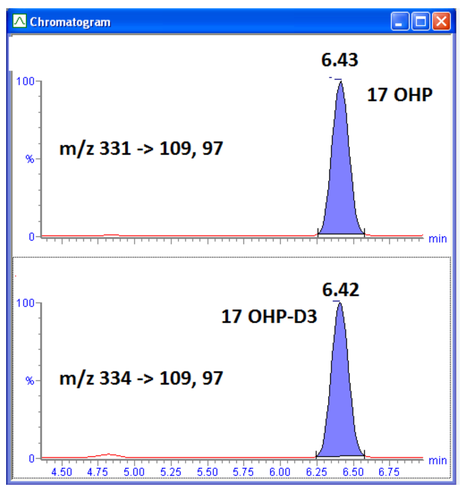
E1 MS² Spectrum

obtained after fragmentation of precursor ion ***m/z* 269** in collision cell and detection of all the daughter ions (***m/z* 143**, 145 , 159)



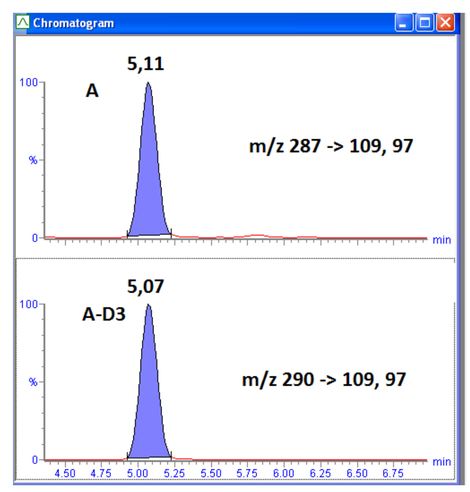
Chromatographic analysis of E1 and E2

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



Chromatographic analysis of 17 hydroxyprogesterone

Selective detection (by MS/MS) of progestatives 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

Conclusions :

- In IUI, a situation closed to the physiological ovulation with wide between variations, only E1 increases with E2
- When the ovary is strongly stimulated (IVF, ICSI) , Δ4-androstenedione and 17 OH progesterone increase with E1 and E2
- rFSH treatment is likely to induce an ovarian hyperplasia which can lead to a pathological increase in specific steroids. We are investigating each step of the induced ovarian steroidogenesis using mass spectometry.

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References: Naessenn T et al, Fertil Steril 2010; Binder H et al, Fertil Steril 2008; Kushnir MM et al, Clinical Chem 2006

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Reproduction, endocrine disruptors and signalling

jean guibourdenche

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