rFSH in AMP and exhaustive characterization of the ovarian steroidogenesis: evidence for an ovarian follicular hyperplasia and potential interest for mass spectrometry to measure 17-hydroxyprogesterone and Δ4-androstenedione.

MC Menet [1,2], MC Leguy [1], L Marcellin [3], V Gayet [3], MLHébert-Schuster [2,4], J Guibourdenche [1,2]
Department of hormonology [1], department of gynecology and AMP[3], department of automated biology [4], Cochin Hospital, AP-HP, ParisDescartes University [2] , Paris, France

Abstract Ovarian monitoring involves serum estradiol and progesterone measurement. We investigate the whole follicular steroïdogenesis under rFSH in AMP (26 IVF, 24 ICSI) compared to 11 controls (IUI). Estradiol and estrone; Δ4-androstenedione and testosterone; progesterone and 17-hydroxyprogesterone were measured using immuno-assay and mass spectrometry. At the beginning of spontaneous or induced cycle (day 6 and 8), steroids levels widely fluctuate within the normal ranges both in AMP and controls. 17-hydroxyprogesterone , Δ 4-androstenedione and estradiol were the predominant serum steroids. Only estrogens (estradiol and estrone) significantly increase during the follicular phase in controls until day 12. In PMA , rFSH injections induced a sharp increase in estrogens associated with an increase in 17-hydroxyprogesterone and Δ4-androstenedione, disrupting estrogens/androgens ratios. rFSH stimulation induces an ovarian hyperplasia affecting the Δ4 pathway which could turn abnormal in recurrent PMA failure. Measurement of 17-hydroxyprogesterone and Δ4-androstenedione using LC-MS/MS could be of interest for the diagnosis and the management of those cases.

Introduction

rFSh injections are widely used in AMP to induce the ovary folliculogenesis (Messinis I et al, 2010). In a daily practice, the biochemical follow up mainly relies on the measurement of estradiol using automated immuno-assay. However, little is known about the effect of this treatment on each step of the ovarian steroidogenesis (Kushnir M et al, 2009; Rothman M et al, 2011). We recently developed sensitive and specific analytical methods using liquid chromatography on line with tandem mass spectrometry (LC MS/MS) to identify and quantify progestatives and androgens (Dufour-Rainfray D et al, 2015). In this study, we aim to analyze the steroid pattern in the serum of women under rFSH.

Material and methods

Patients:

- Controls : IUI (n=11): first line therapy of couple infertility; no treatment
- AMP : IVF(n=26) for usual female infertility;ICSI (n=24) for usual male infertility; same standardised AMP ovarian stimulation protocol (rFSH 150 UI/day since day 8)

Assay:

- P4, E2 (CLIA, Roche); 17OHP, Δ4A, E1, T (RIA IBA; Beckman Coulter).

LC MS/MS: UPLC Acquity: C18 column, MeOH in water in gradient mode, on line with TQ mass spectrometer (Quattro Premier, WatersR)

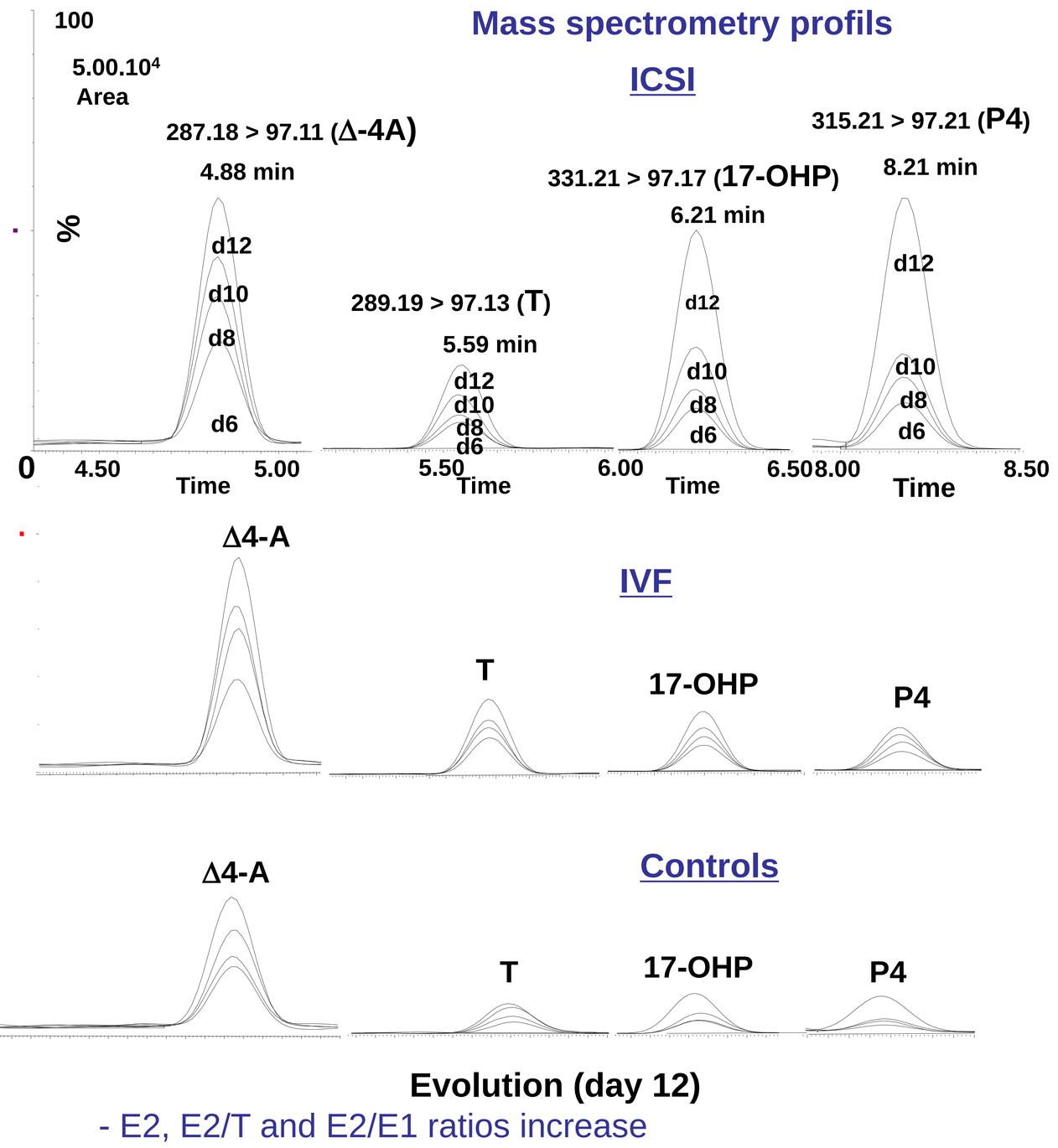
Results

Median (minimum, maximum) serum concentrations

(P4: Progesterone, 17-OHP: 17 OH progesterone, Δ4-A: Δ4-androstenedione, T: Testosterone; E1; Estrone; E2: Estradiol)

IVF (n=26)				
	day 6	**day 8**	**day 10**	**day 12**
P4 (nmol/L)	2.0 (1.2-3.6)	1.7 (1.1-2.4)	1.5 (1.3-2.7)	1.6 (1.3300-2.1)
17-OHP (nmol/L)	1.8 (1.0-3.2)	2.0 (1.2-4.0)	2.7 (1.8-3.6)	2.2 (2.0-3.9)
Δ4-A (nmol/L)	4.5 (3.6-6.0)	5.4 (4.0-7.6)	6.2 (5.0-8.5)	6.2 (5.0-7.4)
T (nmol/L)	1.3 (0.9-1.8)	1.3 (0.9-2.0)	1.7 (1.2-2.6)	1.4 (1.0-1.7)
E1 (pmol/L)	351 (173-964)	594 (318-2626)	1037 (497-4720)	1343 (708-3068)
E2 (pmol/L)	534 (305-2012)	1194 (875-4171)	3550 (2179-7033)	4040 (2128-4638)
ICSI (n=24)				
	day 6	**day 8**	**day 10**	**day 12**
P4 (nmol/L)	1.8 (1.3-2.7)	2 (1.2-2.9)	1.6 (1.2-3.7)	2.8 (2.4-3.4)
17-OHP (nmol/L)	2.4 (1.2-3.3)	2.3 (1.5-4.4)	3.1 (1.9-3.8)	4.3 (3.2-5.5)
Δ4-A (nmol/L)	4.5 (2.7-6.6)	5.6 (3.2-8.8)	6.2 (4.6-10.8)	7.8 (6.0-10.9)
T (nmol/L)	1.2 (0.7-2.3)	1.6 (1.0-3.2)	1.9 (1.3-2.7)	1.7 (1.2-2.5)
E1 (pmol/L)	410 (202-568)	718 (312-1035)	833 (517-1551)	2042 (981-2748)
E2 (pmol/L)	640 (527-1477)	1562 (1000-2440)	3038 (2230-5028)	4139 (3715-5724)
IIU (n=11)				
	day 8	**day 9**	**day 11**	**day 12**
P4 (nmol/L)	1.9 (1.4-4.5)	1.8 (1.4-3.7)	1.6 (1.4-2.1)	1.7 (1.1-2.3)
17-OHP (nmol/L)	2.4 (1.5-3.3)	1.8 (1.2-3.2)	2.2 (1.3-2.6)	1.9 (1.5-5.3)
Δ4-A (nmol/L)	5.6 (5.6-5.7)	5.6 (4.7-9.8)	5.1 (4.5-8.7)	6.1 (5.2-11.5)
T (nmol/L)	1.6 (1.5-1.7)	1.5 (1.1-2.2)	1.0 (0.7-2.5)	2.3 (1.3-2.9)
E1 (pmol/L)	165 (164-166)	244 (153-352)	219 (122-377)	289 (259-697)
E2 (pmol/L)	314 (281-347)	431 (370-543)	704 (407-803)	951 (642-1293)

Mass spectrometry profils



Basal levels (day 6 and 8)

- no significant differences in steroids levels between IVF and ICSI
- 17-OHP , Δ4-A and E2 are the predominant progestin, androgen and estrogen in each group
- 17-OHP positively correlates with P 4 ($p<0.0001$), Δ4A ($p=0.0003$) in AMP
- E2/T and E1/Δ4-A ratios increase in AMP but not the E2/E1 ratio

Evolution (day 12)

- E2, E2/T and E2/E1 ratios increase
- In AMP, E1 increases with E2 but to lesser extent.

In ICSI, P4,17-OHP, and Δ4-A increase significantly

Discussion and Conclusion

We confirm using immuno-assays and LC-MS/MS that the ovarian steroidogenesis is a dynamic process which highly fluctuates from a woman to another (Rothman M et al, 2011; Bui H et al, 2015; Kushnir M, 2016). These fluctuations affect also intermediate steroids such as 17-OHP and Δ4-A but are not increased under rFSH. Serum steroids distribution is identical in controls and AMP, the key Δ4 pathway being predominant (Kushnir M et al, 2009). In IIU, the steroids pattern reflects the increased and coordinated actions of LH and FSH on the follicular enzymes, avoiding any pathological increase in 17-OHP and Δ4-A (Messinis I et al, 2010; Even M et a, 2012). In contrast , in AMP especially in ICSI, extended high rFSH stimulation disrupt the ovarian steroids equilibrium, leading to an increase in progestins and androgens, and a discrepancy between estrogens/androgens ratios (Even M et al, 2012). As the measurement of 17-OHP and Δ4-A is delicate using immuno-assay, the use of LC MS/MS provides a good alternative to analyze those steroids in a single shot .This could be of interest in recurrent AMP failure cases, as androgens are likely to stimulate the growth and the survival of small follicles in basal growth (Weil S et al, 1999; Vendola KA et al, 1998; Even M et al, 2012).

Ref: Even M, Med Reprod Gyn Endocrinol 2012; Weil S, J Clin Endocrinol Metab 1999; Vendola K, J Clin Invest 1998; Kushnir M, Clin Chem 2009; Bui H, Clin Chim Act 2015; Rothman M, Steroids 2011; Messinis I, AnnNY Acad Sci 2010; Dufour-Rainfray D, Ann Biol Clin 2015; Kushnir M, J Steroid Biochem Mol Biol 2016

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