**Reviewer's report**

**Title:** The human fetal adrenal produces cortisol but no detectable aldosterone throughout the second trimester.

**Version:** 0  **Date:** 11 Nov 2017

**Reviewer:** Yves Morel

**Reviewer's report:**

This manuscript reports the human fetal adrenal steroidogenesis during the second trimester. This paper is the first to explore all compounds of steroidogenesis: 1) plasma measurements of steroid, 2) intra-adrenal steroid quantification, 3) steroidogenic enzyme expression (mRNA and protein, immunohistochemistry). A true biosynthesis of cortisol is the mean contribution of this paper. The absence of aldosterone synthesis is interesting but could be due to the difficulty to have efficient methods as the physiological level of aldosterone in human is low (pmol/L). The effect of maternal smoking remains weak. It is difficult to deduce a direct effect of smoking and eliminated an associated event due the stressed profile of these mothers.

This paper follows the Goto et al's paper showing an early cortisol biosynthesis (8-12WG) during the first trimester of pregnancy. Nevertheless it shows that cortisol biosynthesis is maintained during the second trimester although previous papers have postulated the absence of cortisol between 12 and 22 WG because of the absence of detection of HSD3B2 expression. Therefore the paper modulates the concept deduced from Goto's data and proposed by Hanley and Arlt that the dexamethasone treatment of woman having a female foetus at risk for 21-hydroxylase deficiency should only necessary for the first half of pregnancy. They are agreed with the clinical observations of elevated 17OH progesterone and androgens in amniotic fluid of fetus affected by severe 21-hydroxylase deficiency during the second trimester.

The reviewer would like to discuss about the increase of corticosterone and the comment of the authors. Although several times reproduced in textbook even recently by Auchus, the pathway of the biosynthesis of corticosterone is incomplete and should lead to a misunderstanding of levels of corticosterone (see the pathway below). Most plasma corticosterone in human is due to the action of CYP11B1 and not CYP11B2 (see Portrat Portrat-Doyen, S., et al, JCEM,1998, 83, 4156). Thus the concentration of aldosterone was the order of pmol/L and corticosterone of nmol/L. Therefore this difference was due to two distinct pathway, the one due to CYP11B2 leading to aldosterone (pmol/L) located in glomerulae zone, the other due to CYP11B1 leading to corticosterone (nmol/L) located in fasciculae zone. A part of the discrepancy between the biosynthesis of aldosterone and corticosterone in the paper should be due to this concept and against the simplified pathway of the figure 2. The best proof was the deficiency of the CYP11B1 with high 11-deoxycorticosterone and low aldosterone biosynthesis due to the inhibition of renin.


The authors explain that the high concentration of cortisol (add corticosterone) produces by the fetal adrenal come from placental progesterone. Nevertheless, although weak, HSD3B2 was detected by western blotting suggesting a complete pathway is the fasciculae zona from cholesterol. Moreover from fetuses of 17-19 WG, Pezzi et al (J Steroid Biochem 2003) have reported the expression of HSD3B2 mRNA in adrenal and testis.

Finally, how the authors explain the large expression of CYP21A2 in fetal zone?

Specific comments:

1- In summary and introduction, the authors have well mentioned the essential role of adrenal for survival early in post-natal life through the secretion of aldosterone but have forgotten the role of cortisol (hypoglycemia, ...).

2- page 2, line 49 The authors have right to mentioned that few studies have been done on human fetuses and their adrenals. Although some outstanding studies have been cited, the review of Jaffe cited later in the paper (ref 36) could be added here. Moreover, another study "Folligan, K., et al. (2005). "[Histological and molecular study of fetal human adrenal cortex (12-36 wk)]." Ann Endocrinol (Paris) 66(6): 519-526" in 119 normal fetus has reported the expression of P450c21 in all adrenal zone and HSD3B2 in definitive and intermediate zone. Although written in French, summary and legends of figures were in English.

3- page 4, lines 43 : although well detailed in the ref 14, the authors should add that the abortions have been done by mifepristone, an anti-progestin. What influence on ACTH and steroids?

4- page 6, lines 14-15 : Why cortisol has been measured by immunoassay kit and not with LC/MS ?

5- page 8, lines 80-81 : The paragraphs of intra-adrenal steroid quantification with LC/MS is well documented and many parameters have been validated. Nevertheless, as the LC/MSMS method should be more known by the reader, the authors should mention if levels of performance for detection of steroids with a Q-Extractive Orbitrap mass spectrometer were equivalent with LC/MSMS. The LOQ of some steroids were higher than these detected by
LC/MSMS. For instance, the absence of detection of androstenedione (page 14 line 82) should be due to high LOQ (10 ng/ml).

6- page 8, line 30: MS run instead of LC run.

7- page 17, lines 160-163 This sentence shows that the authors are not familiar with prenatal diagnosis of 21-hydroxylase deficiency. The diagnosis of 21-hydroxylase in the second trimester by measuring of 17OH progesterone alone and more recently associated with 21-desoxycortisol in amniotic fluid were used in routine since more of three decades since the paper (Forest, M. G., et al. (1981). "Prenatal diagnosis of congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency by steroid analysis in the amniotic fluid of mid-pregnancy: comparison with HLA typing in 17 pregnancies at risk for CAH." Prenat Diagn 1: 197-207). Although to-day the diagnosis in female is done early by molecular method, the steroid analysis remains and is improved by LC/MSMS method (Tardy et al, J Clin Endocrinol Metab 99: 1180-1188).

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable
Declaration of competing interests

Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests' below

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal