Reviewer’s report

Title: Longitudinal reference ranges for maternal plasma laeverin, and its role as a potential biomarker of preeclampsia

Version: 0 Date: 01 Aug 2016

Reviewer: Lesia Kurlak

Reviewer's report:

This manuscript describes plasma laeverin concentrations through the second half of healthy pregnancy and investigates possible differences in pre-eclampsia (PE); this study also aims to explore the possibility of using laeverin as a biomarker for PE by determining whether differences could be detected early in pregnancy, in women who went on to develop PE.

The study is useful in establishing normal ranges in pregnancy and could prove to be an essential reference in investigations of pathological pregnancies.

Nonetheless, the fact that measurements only started at 22 weeks, is clearly a limitation, particularly in light of the authors wanting to determine differences in women who later develop PE. To use laeverin as a predictive biomarker of PE would require detection much earlier than 22 weeks gestation but also would need to look at early-onset and late-onset PE as would expect differences if laeverin is indeed involved in placentation. Although the authors have acknowledged this in the discussion, this would strengthen this study.

Other comments:

Study population: the incidence of PE in this study is towards the higher end of the range for developed countries- is this typical for this population?

There are many subgroups within this cohort with differing numbers of samples: need a much bigger cohort to actually be able to use this study as a definitive reference range.

Methods:

Plasma and serum are used interchangeably - have the authors confirmed that the measurement of laeverin is not affected by preparation of blood samples?

The methods are generally described clearly. I am somewhat cautious that only one method has been used to verify presence and abundance of laevarin protein. The authors state that the lowest detectable laeverin protein in the kit was 0.312ng/ml but report levels of 0.18 ± 0.31 ng/ml in
men, therefore is this actually laeverin? The concentrations are low in men, non-pregnant and postmenopausal women as expected if placentally-derived however, they are detectable with reported means and SD- is this actually laeverin and where is it coming from in these cases?

The authors state the kit was previously validated: could they confirm by other techniques, that this is indeed laevarin: Does protein quantification by Western blotting in the placental samples show the same relative pattern using the ELISA method?

The authors indicate that freeze-thawing had minimal effect on laeverin but do they know what effect of long-term storage is on laeverin, irrespective of freeze-thawing?

The authors speculate that the lower plasma laeverin in PE may be due to it being 'trapped' in the placenta. Is it possible that it may be due to increased degradation in PE? Has this been assessed? The levels postpartum appear to decrease faster in those women who had had PE than in normal, where laeverin was still detectable 6 days after delivery: do the authors have any comments?

Is there any effect of mode of delivery on plasma laeverin? 40% of the women with PE had a Caesarean section compared to only 5.7% of the healthy controls.

Not sure whether Figure 3 is helpful as a separate graph, can these data be superimposed onto Figure 1 with different symbols?

**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.

Yes

**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 recommend additional statistical review

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

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