Author's response to reviews

Title: Twenty-four hours secretion pattern of serum estradiol in healthy prepubertal and pubertal boys as determined by a validated ultra-sensitive extraction RIA

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Twenty-four hours secretion pattern of serum estradiol in healthy prepubertal and pubertal boys as determined by a validated ultra-sensitive extraction RIA
(MS: 9176180091909251) by Carina Ankarberg-Lindgren and Ensio Norjavaara

Thank you for allowing us to resubmit our review. The present manuscript has now been redrafted in order to meet the comments of the reviewers.

Referee 1:
Major compulsory revisions:
We have now revised the manuscript according to the reviewer’s comments. The changes are made in “estradiol determination”, line 3 on page 6 and in “discussion”, second paragraph, page 10.

The cross-reactivity of the estradiol antiserum had been tested by the manufacture for 30 estradiol-related steroids as stated in a previous paper (reference nr 11). However, that information is now incorporated in the present manuscript as well (page 6, 10).

At time for the preparation of the present manuscript, no estradiol results for boys determined by MS-based methods were available in the literature. However, the last few month two papers have been published. The comparisons of our results to their results are now incorporated in the discussion. To convince the reader and for clarification of the assay’s validity, we have added some sentences to the second paragraph on page 10.

Referee 2:
Minor essential revisions:
We have now revised the manuscript according to the reviewer’s comments.

1. The analytical detection limit was calculated as the apparent concentration 3SD from the counts at maximum binding, multiplied with the concentration factor in the extraction step. The functional sensitivity was defined as the lowest concentration that can be measured with an interassay CV of 20%. This has now
been clarified on pages 5-6.

2. The text in figure 3 is clearer now than in its previous form.

3. The regression lines in figure 5 have now been clarified with the following sentence on page 9, second paragraph: The regression analysis for boys in early and mid-puberty showed similar regression lines and were therefore merged to one group in figure 5.

Discretionary revisions:
1. “analytical” has been added to page 3, second paragraph.

2. According the reviewer’s comment, we have changed the sentence in the introduction to “have been shown to give lower values which more resemble values measured by mass spectrometry (MS)-based methods”

3. Thank you for being observant! Figure 1 is now corrected.