Dear Authors,

Many thanks for your revised preprint entitled “Sex differences in the relationship between maternal and neonate cortisol in a free-ranging large mammal”. It has now been reviewed by one of the previous reviewers and their comments are appended below. These comments will need to be adressed before your preprint can be recommended.

Regarding the statistical procedure, to be clear,I was not asking for a change of procedure (and I do not think it should be changed) but simply asking for justification and explanation for the readers with references. The justification and references in the response are great to me but I don’t see any associated changes for the reader in the main text (justification of such choice, e.g. statistical power required for testing interactions) and the associated references, so please update those aspects as well. I do appreciate that the description of the procedure itself is now more detailed.

1) We agree and have added the explanation along with a proper reference (lines 169-174).

Similarly, the results regarding the new repeatability analysis seem worth mentioning in the main text, together with the references that make the authors think that such low repeatability was “expected”, and a short statement regarding the implications of such low (and statistically “non-significant”) intra individual repeatability for the investigation of the relationship between maternal and neonate cortisol.

2) We agree that it would be useful to have this in the main text. Therefore we have added the part on repeatability (lines 165-166; 199-200) along with an addition in the discussion regarding its implications (lines 263-266).

I look forward to reading the revised version of this preprint.

Best wishes,

Matthieu

Review by anonymous reviewer 2, 13 Oct 2023 20:27

I was pleased to review the revised version of the manuscript entitled “Sex-specific differences in the relationship between maternal and neonate foetal cortisol glucocorticoids in a free-ranging large mammal”.

First, I have to highlight the great work conducted by the authors to revise their manuscript according to the feedback of the Editor and the 2 Reviewers.

Modifications clarify the fact that this article is a research article rather than a methodological one. However, according to me, it requires to go a bit further to anchor the issue in its research theme. In the introduction notably (~ lines 70-73), I would describe in more details the foetal programming concept, and the associated maternal GC effects on offspring phenotype, as it is the key concept below your research issue. Concretely, I would give some striking examples, indicating exactly how maternal GC levels influence cited offspring features (is it a positive or a negative relationship? Is it species-specific? Is it age-dependent?).

3) We read the whole manuscript again, and noticed that, after the definition of foetal programming (line 70-71), we also provided many examples in the introduction (lines 67-80) and discussion (lines 230-238). After careful evaluation, we decided that adding additional examples would have been redundant and brake the ideas flow.

To note, I didn’t perfectly understand the answers provided by the authors to justify why they couldn’t switch the statistical analyses order and the associated presentation of results (i.e., from the most completed model with both sexes in a unique model and then the two distinct sex-specific models to characterize in more details the relationship between maternal GC levels and foetal ones).

4) We agree that there was detail missing here. We have added additional explanation regarding our choice for the order in which we did the test (lines 169-174). We hope that the rationale is now clearer for the reader.

Finally, the discussion would be more coherent with the introduction by adding a part dealing with the highlighted relationship in the stress context (line 69-70).

5) We agree that the stress part (previously line 69-70) was a bit out of place. It also is not touched upon in the discussion, which creates a feeling of incoherence. That is why we removed the sentence and rephrased the start of that paragraph in the introduction.

Here are line-to-line corrections:

Line 47: have shown

6) Corrected.

Line 48: I would rephrase: “However, how maternal GC levels precisely relate to foetal levels is still not completely understood.”

7) We agree and have taken the suggestion over.

Line 50: I would add one or two sentences describing the methods as it is an innovative and original one, as well as the sample size.

8) This is now added to the abstract.

Lines 51-52: Your relationship of interest should be described in the same order all along the manuscript, i.e. maternal GC levels with foetal GC levels (or the contrary) but being consistent.

9) Corrected.

Line 78: in utero should be written in italics.

10) Corrected.

Line 77-81: it is unclear here why you are dealing with steroid hormones in these two examples while you started this part with stress. This information would be important to add here.

11) We agree that the stress part is a bit incoherent here. We have removed this part and have elaborated it in one of the replies above (reply #5).

Line 100-101: (Regarding the cited examples) some predictions could be emitted. At least, all possible relationship would be described.

12) Since this was an explorative study without clear predictions on a topic rarely studied in a context such as ours, it was not possible for us to dive too much into our expectations (because these were lacking).

Line 111: Fallow deer is a hider species.

13) Corrected.

Line 111: per should be written in italics (to correct all along the manuscript).

14) We don't understand why that should be the case, as 'per' (in the context of the sentence here, i.e. 'per year') is used commonly in the English language and we want to avoid overusing italics throughout the manuscript.

Line 137: How old are the fawns at capture? Do you measure cortisol on the entire hair or do you exclude a part of it to not measure the GC levels accumulated after birth?

15) We typically capture fawns during their first week of life. We agree that this is important information and have added it to the manuscript (line 135). As for the hair, we use a trimmer to shave the belly of the fawn. We also have only used the hair taken at the first capture (fawn age typically 1-3 days). Due to this way of sampling, and since the fawns are still within their first days of life, we do not expect that the values will be strongly affected by new growth of hair.

Line 170-175: This adding increases the complexity of comprehension according to me and it is quite repetitive (with line 190-191 notably). I would place such sentences at the beginning of each part describing in details the statistical analyses achieved, as an "introduction" sentence.

16) We aimed at giving a initial, general, overview of the rationale at the start of the statistical analysis so that the reader knows what to expect. We agree that it was a bit repetitive previously. Now we have also added more rationale about the order of our testing (see reply #1) and also details regarding the repeatability test (see reply #2). We hope that this has helped with the issue of repetitiveness with the later section.

Line 185: mid-May

17) Corrected.

Line 207: Homogenize the format (italics or not) of the word “post-hoc”.

18) Corrected, now italics throughout the manuscript.

Line 210: Be clearer about which outlier you are considered here (the male fawn GCS vs the FCMs value for one mother).

19) Agreed and adjusted in the figure caption.

Between the previous and the current Figure 1B (males), one point has changed (the one ~600 maternal FCMs in the previous Figure 1B), why such change occurred between the two versions of the manuscript.

20) This followed from the reviewer comment in the previous version and is described in more detail in our response in the previous round. In short, they indicated that we also had an outlier in our maternal FCM levels. We agreed and, after investigation, found that it was caused by one sample that we now also omitted from the FCM data. Since this was an individual with multiple sample, we decided to just omit the outlier from this individual, while keeping her other data and thus, she remained in our final figure and results, but with a lower value. So the change is that her data point is now a lower value due to the omission of the one outlier.

Line 235-239: This part should be placed in the introduction.

21) We have added some of the information of this paragraph to the introduction (lines 77-80), namely the part that is about sex differences, since that is the main topic of this manuscript. We hope that there is now more coherence between the introduction and the discussion.

Line 245-251: Such part could support one prediction for your study. If such part would be keep in the Discussion part, transition with the previous/next argument should be rewritten to be easier to read.

22) This paragraph is the start of the discussion regarding the functional mechanism underlying the sex differences. We have rephrased the start of this paragraph to emphasize this transition.

Line 252: what is this hormone? A steroid produced by the HPA axis? Is it only produced by placenta? Transitions with previous part should be rewritten to be easier to read.

23) This is an enzyme that is active specifically to inactivate cortisol. The transition with the previous paragraph follows from the fact that this enzyme is also highly active in the placenta. We have emphasized its activity in the placenta in this sentence now and hope that this has improved the clarity.

Line 274: hypotheses which should be tested.

24) Corrected.