Cortisol, progesterone, 17α-hydroxyprogesterone, and TSH responses in dogs injected with low-dose lipopolysaccharide

1. BASIC REPORTING

- Everywhere where you say “saline and LPS”, I would replace by “saline or LPS” for improved clarity
- You can’t afford to say “various time points” within the material and method part: this is the only part where everything needs to be detailed: this is the part where the reader goes for clarification or precision in the study design
- List the Siemens kits for each hormone other than 17OHP4

2. EXPERIMENTAL DESIGN

The study design is unclear, the following points need to be specified:

- Emphasize more that there were 5 dogs total and they got both treatments in a row in the same order (rather than twice 5 different dogs, meaning 10 dogs total – I understood it only in the discussion once explaining the limits). This needs to be addressed in details within the material and method.
- Why did the dogs get crystalloids before each treatment? And how long before each treatment? And when compared to the pre-injection blood drawing for hormones measurements?
- If baseline corresponds with 24 hours before treatment injection, then clarify, as the notation “baseline, time -24 hours” seems unclear to me – one wonders if the two propositions separated by a coma are the same or different.
- You also need to specify the assumed ovarian cycle status of the bitches within the material and method, the reader can’t discover this in the discussion. I would add something like: “bitches were selected to be for sure outside of the estrus phase of the ovarian cycle, and hopefully in anestrus.”
- Please add one sentence to explain what a mixed model analysis is. Were the results analyzed by ANOVAs or not?
- It is unclear to me which statistical method you used to come up with the results:
    - You need to specify what was analyzed for normality and equal variance assumption; just saying “analysis” is insufficient to allow the reader to picture what you did. Was it the set of 4 results for each dog for each time point for each hormone? If so, how reliable is a normality test performed on only 4 values, especially with a cutoff for the p value at 0.05?? Probably extremely low.
    - Which normality test did you use?

    - It is because you mentioned checking normality and equality of variances that I deducted that you used ANOVAs, but this is not mentioned anywhere actually; was it actually the case? If yes, then specifies that you used Tuckey to address the result of the ANOVAs. If not, then you really need to re-explain your statistical approach: if the reader can't picture what you did, it's difficult to trust the result.
    - (Did you use Tuckey or another test to address the results that did not verify normality and equality of variances?)
- I would mention the delay of the freezing (2011 – 2017) in the Summary

Are you sure that the Immulite does indeed 10 measurements and provides the mean? I had never heard this and tend to doubt it; I thought it measured the value only once (whereas RIA does indeed 2 measurements and provides a mean with a CV). The Immulite uses an antibody-coated bead for each measurement, and I'm almost sure it needs one bead per measurement. I don't think it uses 10 beads per value to perform 1 measurement: to be double-checked.

3. VALIDITY OF THE FINDINGS

Cortisol results:

- Are you sure the saline was not significantly increased at 3 hours versus baseline ?

TSH:

- Are you sure there was no significant difference between 24 hours and baseline ?? The figure really shows the opposite…

To me, 2 findings need to be added and discussed:

1) Limitations about the normality tests (and thus all the following stats) for tests about 4 values (+the chosen normality test needs to be all mentioned, as all other statistical tests)
2) It seems very important to me that:
    a. All steroid hormones increased clearly after LPS in the 5 dogs
    b. Whereas no steroid hormones increased in 2 out of 5 dogs, meaning almost half the dogs.

The second point was mentioned, but too late in the article in my opinion, and it probably needs more discussion, especially mentioning that then:

- LPS triggers the steroids
- Whereas it's probably not the saline but the process that triggers the steroids in the saline part (otherwise the steroids would increase in the 5 dogs post saline all the same)

4. General comments

Very interesting work, English is good, references are good, and the topic is definitely relevant and useful. You just need to:

- Develop the material and method
- Include a more detailed stat paragraph (mandatory in my opinion)
- And add a couple specifications mentioned above.

5. Confidential notes to the editor

Articles lacking statistical clarity are weak. I truly think this article is good and it would be a pity to weaken it by some missing stats, in my opinion.