The Effects of Sedation on Basal and Stimulated Serum Cortisol Concentrations in Healthy Dogs

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1. Basic Reporting

- This manuscript is a concisely, well written original research article. The introduction provides a good summary of background information on the subject and demonstrates the relevance of the research.
- The literature cited is current and relevant to the manuscript.
- The structure of the article mostly conforms to PeerJ standards. There are minor edits needed to fully conform to PeerJ standards. These include:
    - references being italicized within the manuscript
    - providing the full or complete name of journals in the reference list
    - adhering to guidelines for capitalization of article titles in the reference list (e.g. references 1, 5, 10, 15 and 17)
- The two provided tables are clearly labelled and well described. In my opinion, Table 1 is not needed as this information could be included in the text (see specific comment for line 174 in the attached document).
- The raw data is provided for review in the supplemental material.

2. Experimental Design

- The objective and hypothesis of the study are clearly stated.
- The methodology used was able to provide results for the objective and hypothesis and provided further information to what is already reported in the literature on this topic.
- The study design was valid and sound.
- Ethical standards were maintained.
- The methods were described well, with sufficient information to replicate if needed.

3. Validity of The Findings

- The raw data was provided, and it appears to me that appropriate statistical tests were used.
- The results add to the current literature on this topic.
- Overall, the conclusions are quite well stated; however, I did recommend some revisions (see the attached detailed review report).

4. General Comments

- A limitation not mentioned is the low number of dogs used (n = 12). This could be discussed with the other limitations.

- If it is possible based on PeerJ standards, it would be nice to also include cortisol results in standard international units (nmol/L) along with the already provided results in conventional units (µg/dL). This makes it more accessible for a global readership.

5. Confidential Notes to The Editor

- Overall, this manuscript is well written and many of my comments reflect minor edits and/or clarification of the data and its interpretation.

Detailed Review Report to Editor and Authors:

1. Title: Current – "The Effects of Sedation on Basal and Stimulated Serum Cortisol Concentrations in Healthy Dogs"

   This title is very broad with respect to the use of the term sedation. It is the effect of select sedatives (3 in this article) on basal and post-ACTH cortisol concentrations that is being evaluated, not the overall effect of sedation. I would recommend revising the title to reflect this.

2. Lines 41 and 64: Please provide the complete word for ACTH (adrenocorticotropic hormone) prior to using this abbreviation.

3. Line 49: I would recommend adding the word "administration" at the end of the sentence (after 3-5 mg/kg PO).

4. Line 56: If keeping sub-headings within the abstract, I would recommend adding the subheading of "Conclusion" before "In conclusion, butorphanol…".

5. Lines 56-57: "In conclusion, butorphanol should be avoided during HPA interrogation in dogs."

   The use of the term "HPA interrogation" is too broad as a conclusion as low dose dexamethasone suppression testing was not evaluated in the current study. I would recommend changing the wording in this sentence to only reflect ACTH stimulation testing.

6. Line 70: Please specify which endocrinology laboratory. Is it the endocrinology laboratory at your institution?

7. Line 71: Please specify from which author does the (personal experience) originate from.

8. Line 103: In the abstract on line 46, it species the breed as beagles. Please include this information here as well.

9. Lines 117-118: "General anesthesia or euthanasia were not part of the study design; no dogs met the criteria for euthanasia prior to the planned end of the study (i.e. severe illness, cardiopulmonary arrest)." In my opinion this sentence is not necessary for this manuscript.

10. Line 125: "…7-day washout…" On line 47, "1-week washout" is used. For consistency, please use 1-week or 7-day in both locations. Also, what led to the decision for a 7-day washout between treatments versus a different number of washout days? Please include this information in the discussion.

11. Line 126: Trazadone is the only treatment administered PO, while the other treatments are all administered IV. Do you think this could have influenced any of the results? If so, please mention this in the discussion.

12. Line 144: Add a period after "Guzmán et al., 2022)".

13. Lines 159-160: "…minimum of 12 subjects required to detect a difference of 5 µg/dL in post-ACTH stimulation cortisol concentration…" Why was a difference of 5 µg/dL chosen?

14. Line 162: "A 4-treatment, 4-sequence crossover design was used to evaluate changes…" In the methods section (lines 98-99), it is described as "The study was a prospective, controlled, randomized, repeated measure 4-way, 4-period crossover design." For consistency, I would recommend using the same terminology.

15. Line 169: Please provide complete word for QQ.

16. Lines 173-174 and Table 1: "…; the reference ranges are specific to the sex and reproductive status of each dog (see Table 1)."

    The authors have used the term reference interval in the earlier part of this sentence and now have switched to reference range. These terms are not statistically interchangeable. Does the laboratory utilize reference intervals or reference ranges? The term reference range is also used in the raw data title in column H. Please be consistent.

    In Table 1, it mentions that the laboratory provided reference intervals (RIs) are for female spayed and male neutered dogs.

    - Are the laboratory provided RIs based on a diverse population of dog breeds? In this study, only beagles are sampled. How can this influence the comparison of the study results to the laboratory provided RIs? (I do see that using healthy animals of the same breed and age are listed as limitations of the study in lines 300-301).
    - The laboratory provided RIs are for neutered males and spayed females which is fine for the study population as they are also spayed or neutered. Dogs having an ACTH stimulation test performed in a clinical setting may not be neutered or spayed. Does this influence the results? What is reported in the literature about the effect of reproductive hormones on basal cortisol concentrations and cortisol concentrations post-ACTH stimulation?

    In my opinion, Table 1 is not needed in this manuscript. This information could be made available in Table 2 with a column for laboratory provided RIs. This information from Table 1 can also be included in the title of column H of the raw data, which I think would be good to include as another supplementary table. Some modification to the title of the last column (current Column H title: Cortisol Ref Range (normal = 0, high = 1) female (5.9 pre, 17.5 post); male (5.6 pre, 15.1 post)) is recommended as it is not accurate. It is a scoring of 0 or 1 based on comparison of participant results to the laboratory provided RIs.

17. Line 184: "…ptyalism was noted in 5 dogs and 3 dogs were administered maropitant." I would recommend adding "after all sampling was completed" (or something to that effect) to clearly indicate that no additional medications were administered during the sampling time period.

18. Line 198: “After saline adm5nistration, no…” Correct typo in the word administration.

19. Lines 201, 202 and 205: Please be consistent with spacing by hyphens or dashes. (1.4- 4 times > RI) (1.1- 1.7 times > RI) (1.03-1.08 times > RI)

20. Lines 210, 218 and 220:

    “Butorphanol causes clinically and statistically significant increases in basal and post-ACTH cortisol concentrations in healthy dogs.”

    “Butorphanol had the greatest effects on the HPA of tested sedatives.”

    “Additionally, butorphanol significantly increased post-ACTH cortisol concentrations.”

    Please reword these sentences to reflect that administration of butorphanol to dogs resulted in significantly increased cortisol concentrations. As we do not know exactly how butorphanol is causing this effect, it is not accurate to state that the butorphanol is directly causing the effect (i.e. the effect on cortisol may be a secondary effect related to changes in the dog due to butorphanol administration). I would suggest reviewing the entire manuscript for this type of wording (e.g. abstract, conclusion (lines 339-340), etc.) as it pertains to butorphanol.

21. Lines 221-222: “Possible mechanisms for this change might include increased hormone release, transcellular movement, or decreased hormone clearance.” Is this a hypothesis from the authors? If so, please indicate this. If not, please cite the source of this information.

22. Lines 244-246: “In contrast, 2/12 (16.6%) healthy dogs had post-ACTH cortisol concentrations > 20 µg/dL and 7/12 (58%) fell into an equivocal range (16-20 µg/dL) after butorphanol administration (Gilor & Graves, 2011).”

    Where did the “equivocal range” determination come from? Unless I missed it, I looked at the Gilor & Graves, 2011 article and did not appreciate this information in that article. Or is this information based on interpretations with the diagnostic laboratory used in this current manuscript? What specific information from the Gilor & Graves, 2011 reference is being referred to in this sentence?

23. Lines 246-247: “These differences could have resulted in misdiagnosis of hyperadrenocorticism.” This sentence applies to the results from this study when compared to the laboratory provided RIs, which I think should be specified.

24. Lines 249-252: “Given significant increases in basal cortisol in healthy dogs after sedation in this study, use of sedation prior to collection of pre-trilostane or 3 hours-post trilostane unstimulated cortisol concentration measurements could result in inappropriate dosage escalation.” For this sentence, please refer specifically to use of butorphanol as this sentence does not apply to the results for trazodone or dexmedetomidine in this study.

25. Lines 257-259: “Of dogs receiving saline, dexmedetomidine, and trazodone in this study, 11/12 (92%), 10/12 (83%) and 12/12 (100%), respectively, had baseline cortisol concentrations < 2 µg/dL.” Please modify to < or = 2 µg/dL or change 10/12 (83%) for dexmedetomidine to 9/12 as sample ID 7 had a cortisol concentration of 2 µg/dL.

26. Lines 260-262: ‘‘Butorphanol should not similarly elevate cortisol concentrations in dogs with hypoadrenocorticism given lack of adrenal reserve and, thus, failure to respond to both endogenous and exogenous ACTH (Guzmán et al., 2022).”

    I understand the validity of this statement. However, in lines 221-222 you mention “Possible mechanisms for this change might include increased hormone release, transcellular movement, or decreased hormone clearance”. If there is an effect of decreased hormone clearance from butorphanol use, although the cortisol concentration may not be significantly increased in a dog with hypoadrenocorticism, the cortisol concentration may still be impacted (and potentially still increase a small amount) if there is decreased hormone clearance. Please comment on this.

27. Lines 262-264: “As such, butorphanol administration could increase the specificity of baseline cortisol concentrations for hypoadrenocorticism.” In my opinion, this statement cannot be made based on the results from this study as it in particular mentions specificity (too much extrapolation). Either delete or if wanting to still portray this message, use a different word other than specificity.

28. Lines 279-280: “Dexmedetomidine at a dose of 4 µg/kg did not significantly increase basal cortisol concentrations in the present study.” That is true, however, it was hypothesized in lines 93-95 that dexmedetomidine would decrease basal and post-ACTH cortisol concentrations. Please discuss the outcome of this hypothesis in the discussion.

29. Lines 341-344: “However, caution should still be used when interpreting ACTH stimulation results after dexmedetomidine or trazodone is administered because this was a study of healthy dogs, with a single selected dose of these drugs given.” I would recommend adding that a single breed of dog was also used for the study in this sentence.

30. Table 2 legend: *“*Significance was defined as $P < .05$. Values within a row that do not share a superscript letter differed significant based on post-hoc analysis.” I think this sentence reads better if significant is changed to significantly.