

Dear Professor Lian-Sheng Ma,

We received the comments regarding the Manuscript "*The role of Urocortin in obstetrics and gynaecology: updates and future perspectives*" (ESPS Manuscript NO: 24874), and we are grateful for your useful suggestions and the opportunity to clarify a number of elements from our work.

According to Reviewer 1 (Reviewer's code: 01427317) suggestions:

- *Since the expression and localization of CRF, UCN, CRF-R1 & R2 are dramatically changed during pregnancy and labor, the authors are recommended to add summary schema showing these changes, in order to make readers easily understood the dynamics of CRF/UCN pathways during pregnancy.*

We fully agree with the Reviewer: we have added a Table to provide a brief summary of Corticotropin-Releasing Factor (CRF), Urocortin (UCN), Corticotropin-Releasing Factor Receptor 1 (CRF-R1) and 2 (CRF-R2)'s actions during pregnancy and labor.

According to Reviewer 2 (Reviewer's code: 00733642) suggestions:

- *Please clarify the effect of UCN on myometrium; how it act as stimulator and inhibitor at the same time?*

We have evidenced how the UCN plays a strictly contractile stimulation role on the myometrium and how, through a feedback mechanism, the inflammatory factors induce its production.

- *In page 6, ..."CRF increases vasodilation placental" is an editing mistake, the correct is: placental vasodilation. "As is known". Better to be "As previously known" -Page 7, line 9.....atresic follicles" should be corrected to atretic follicles. Page 8. delivered at term (37 weeks of gestation)". At term is (37 weeks or more). "Disease should be diseases".*

We have corrected the editing mistakes.

- *Looking at obstetrics and gynecology, the administration of UCN and/or AM may reduce the inflammatory phenomena typical of several obstetrics as well as gynecological disease". The paragraph is inaccurate: Is UCN is commercially available for administration?*

UCN is not currently available for administration. There are only some papers concerning its endogenous role and some in vitro studies. In order to introduce UCN commercially, it is necessary to begin a series of appropriate trials in order to assess its safety together with the pharmacokinetic and pharmacodynamic characteristics of this drug.

According to Reviewer's 3 (Reviewer's code: 00038621) suggestions:

1. *Often, the authors cite other review articles for original findings. Instead, they should cite original research articles. E.g. the citations in the final two sentences in the section entitled "BIOCHEMICS AND GENETICS ASPECTS". Also some important papers were missed in later sections, such as "Hypoxia and preeclampsia: increased expression of urocortin 2 and urocortin 3" by Imperatore A. et al. 2010 should have been cited.*

We fully agree with the Reviewer and as suggested, we have considered the appropriate items such as "Imperatore A. et al. 2010".

2. *Urocortins comprise UCN1, UCN2, and UCN3. This should be mentioned in the “BIOCHEMICS AND GENETICS ASPECTS” section, where they introduce urocortin. Throughout the manuscript, the authors mostly refer to “UCN” and it is not clear which of the urocortins they mean. On page 7, use of terminology UCN1 and UCN2 appears and later they also mention UCN3. It is therefore confusing what they mean with UCN. Similarly, the title refers to urocortin, but which one? And if they mean all of them, it should be clarified.*

We fully agree with the Reviewer and we have specified the three different family members together with their characteristics in the “BIOCHEMICS AND GENETICS ASPECTS” section. In this review we have tried to describe the role of urocortin with greater emphasis on its possible implications in obstetrics and gynecology. As requested, we have specified which of these peptides we are referring to.

3. *Abstract, first sentence- the authors write coupling “to the G protein”. There are several G proteins to which a particular CRF receptor can couple to and therefore, it would be more precise to say coupling “to G proteins” or mention the specific one they mean and cite appropriate reference.*

We have specified which G protein we considered and we have included the appropriate citation.

4. *In the abstract the authors write “In term pregnancies, maternal plasma levels of CRF and UCN are lower and correlated to labor onset. Conversely, they do not decrease in post-term pregnancies (when the labor did not physiologically occur).” It is unclear what the comparison is being made to. Levels are lower compared to what? Also the comment in the brackets needs to be corrected for grammar and needs to be more specific, e.g. induced labor? It’s not clear what the authors are referring*

We specified that the levels of CRF and UCN seem to be correlated directly with the onset of spontaneous labor. In fact, they have a tendency to decrease in women who deliver without inductions. This does not happen in women who deliver with induced labour.

5. *Section entitled “Core tip”, the authors wrote “Urocortin is present at higher concentrations in women with endometriomas, and its measurement may be useful for the differential diagnosis of endometriosis compared with other benign ovarian cysts.” It is not clear where the measurement is taken from, which specific tissue, or do they mean plasma?*

Urocortin is a neuropeptide belonging to the corticotrophin- releasing hormone family, known to be expressed in the endometrium. On this basis, serum urocortin has been investigated as a possible marker for endometriosis. Serum levels of urocortin were found to be significantly higher in women with endometriomas than in women with other benign ovarian cysts, giving a sensitivity of 88% and specificity of 90%, while CA-125 detected only 65% of the cases with the same specificity.

6. *The sentence “There are some differences between human and rat amino-acidic UCN.” in the BIOCHEMICS AND GENETICS ASPECTS section is vague and somewhat out of place. It’s not clear what significant information this sentence contributes. The authors should either elaborate on the differences, or leave this sentence out. Also, it the word amino- acidic needs to be revised.*

Following the suggestion, we have decided to remove the phrase as it is not essential for the review.

7. *The sentence “The inactivation of CRF-BP induces an increase of CRF and free UCN.” in the BIOCHEMICS AND GENETICS ASPECTS section needs to be clearer. Where is the increase, in the brain or plasma? Why do the authors refer to “free UCN” but not free CRF? Is the increase in CRF newly synthesized CRF or is it CRF detached from CRF-BP?*

Corticotropin releasing factor-binding protein (CRF-BP) binds CRF and urocortin 1 with high affinity, thus preventing CRF receptor (CRFR) activation. urocortin 3 mRNA expression is found in areas of the brain including the hypothalamus, amygdala, and brainstem, but is not evident in the cerebellum, pituitary, or cerebral cortex. The increase has been seen in a number of animal models in the plasma.

8. *In the IMPLICATIONS IN OBSTETRICS AND GYNECOLOGY section the first sentence should be more specific. The authors wrote about “the concentration of free CRF increases during pregnancy”. They need to at least state whether they mean plasma CRF concentrations? Or placental derived CRF? Placental CRF concentrations? How much increase? And give some citations.*

In a recent paper, LE Borges shows that Ucn1 mRNA expression in viable intrauterine pregnancy is 16.63 ± 0.16 . No difference in decidual Ucn1 mRNA expression was observed between non-viable compared to viable intrauterine and ectopic pregnancy.

9. *The authors wrote a section on HYPOTHESIS OF THERAPEUTIC APPLICATIONS. While they whole review was on several different obstetric and gynecological pat*

We have specified that the assumptions regard obstetrics and gynecology.

We were not able to read the end of point 9 suggestion, which appeared to be incomplete.

According to Reviewer 4 (Reviewer’s code: 00742121) suggestions:

1. *The content of the Abstract and the main text are substantially discordant as compared with that of the title and the “Core tip”. According to the title, this article presents the role of Urocortin in Obstetrics and Gynaecology. However, the Abstract and most of the main text present only the possible role of Urocortin during pregnancy. Hence, the authors should either change the title to “The role of Urocortin in pregnancy: an update and future perspectives” or add more information about gynaecologic disease and add the words “gynaecologic disease” in the title.*

We fully agree with the Reviewer and we have changed the title.

2. *The authors should use for the main text either the structure of a systematic review (i.e. Introduction, Methods, Results and Discussion) or the structure of a narrative review (without Methods, Results and Discussion). In any case, an “Introduction” should be added.*

We have modified the structure of the paper as a narrative review (without Methods, Results and Discussion), and have added an “Introduction”.

3. *Comments regarding the second section of the main text entitled “Biochemics and genetics aspects”: a) the title of this section should be changed to “Biochemical and genetic aspects”; b) this section should be divided into at least three paragraphs; c) the authors should make clear in each and every case throughout this section if the information provided refers to humans or other species; d) in the last five lines of this*

section the authors should be more precise on how UCN correlates with congestive heart failure, gastritis, irritable bowel syndrome, rheumatoid arthritis, major depression, atopic disorders, the onset of labor, spontaneous abortion and preeclampsia.

We have changed the title of the section “Biochemics and genetics aspects” in “Biochemical and genetic aspects”. We have divided this section into three paragraphs. We have specified whether the reported information refers to humans or other species. We have carefully evidenced how UCN correlates with several diseases (both in obstetrics, gynecology and other specialties).

4. Comments regarding the third section of the main text entitled “Implications in Obstetrics and Gynecology”: a) As mentioned in comment 1, this section mainly presents the possible role of Urocortin during pregnancy; hence the title of this section should be changed accordingly. b) This section should be better subdivided in subsections, and long paragraphs should be divided in shorter paragraphs. c) At the end of this section the authors mention that “UCN is increased in women with endometriomas, and its measurement may be useful for differential diagnosis etc.”. More information is needed on this issue: How was UCN measured? Is UCN increased in the serum of patients with endometriomas or in endometrioma tissue? Is there any evidence on UCN levels in other ovarian cysts or other disease states?

We have made the requested changes. Regarding endometriosis, in the reported study UCN was measured in endometrioma tissue. Furthermore, we would like to clarify that there is no robust evidence about UCN levels in other ovarian cysts.

5. Comments regarding the fourth section of the main text entitled “Hypothesis of therapeutic applications”: a) This section contains data from animal studies, and this should be mentioned in the section’s title; the title should be changed to e.g. “Evidence from animal studies suggesting possible therapeutic applications”. b) How safe would be the use of UCN/AM during pregnancy? Is there any evidence available regarding this issue? The authors should discuss these issues at the end of this section.

We have mentioned in the section “Hypothesis of therapeutic applications” that the reported data came from animal studies, changing the subtitle to “Evidence from animal studies suggesting possible therapeutic applications”. We discuss the hypothesis of UCN/AM treatment during pregnancy.

6. The authors should either use British or American spelling, not both.

A native English speaker and biologist performed an accurate and complete language revision of the manuscript.

Once again, we thank the Reviewers for their precious suggestions and the Editor for giving us the opportunity to clarify a number of issues. We hope that you will appreciate our work. We remain at your disposal for any further details you might want to discuss.

On behalf of the co-Authors,

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