Peer Review File

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Reviewer A

It seems that the authors of this paper misunderstand the relationship between FSH and testosterone. FSH does not affect testosterone levels and is not increased with LHRH-agonist therapy but decreased, since the agonists suppress both LH and FSH. The study reports relationships between pre-treatment FSH levels and clinical outcome, which is not very relevant without providing FSH levels during LHRH-agonist treatment.

Reply: We agree that lack of FSH levels while on ADT is a limitation. This has been mentioned in various places including in future directions, but we have explicitly now stated this in the limitations paragraph in the Discussion, "Finally, our analyses only included FSH levels prior to ADT."

That being said, we do believe the current study provides value in showing no relationship between pre-ADT FSH levels and outcomes. Specifically, the pre-ADT FSH levels reflect the hormonal milieu in which the tumor developed. This fact, coupled with the complete lack of associations in our study challenge the FSH-prostate cancer hypothesis. As such, we do believe there is value in presenting the current data, despite the limitation acknowledged by us and the reviewer.

Changes in text: Discussion, "Finally, our analyses only included FSH levels prior to ADT." in (Page 12, line 202-203).

Reviewer B

General comments:

This study examines in prostate cancer patients whether the pretreatment levels of FSH are associated with cardiological or oncological outcomes of the patients. No associations were found, which is not surprising, because it would have been much more important to assess the association of FSH levels during or following the treatment with the outcomes. The main main point in the potential association of FSH with prostate cancer is that there are data on direct FSH action of prostatic cancer growth and the fact that FSH levels rebound during GnRH agonist treatment after an initial suppression. This association remains unfortunately unexplored in this study, and the pretreatment FSH levels sound quite unlikely to predict the cardiovascular or oncological

outcomes of the patients. This was proven by the negative findings. The study is technically sound, well written and the results appear reliable. Unfortunately, the findings are not very interesting.

Reply: As noted above, while we agree that lack of FSH levels while on ADT is a limitation, we do believe the current data contribute meaningfully to the literature.

Some more detailed comments:

Comment 1. L57 and L97: FSH is not increased during GnRH agonist treatment. Instead, it is insufficiently suppressed, or more specifically rebounded after initial suppression.

Reply 1. Thank you for providing this important clarification regarding FSH levels during GnRH agonist treatment in prostate cancer. We appreciate the correction. To accurately represent the information, we modified the text to now state that FSH is not increased during GnRH agonist treatment, but rather insufficiently suppressed or rebounded after initial suppression.

Changes in text: Abstract (Page 3, line 8), introduction (Page 5, line 48)

Comment 2. L59: Please state that these are pretreatment FSH levels.

Reply 2. To accurately convey the information, we specified that this study focus on pretreatment FSH levels in prostate cancer.

Changes in text: Materials and Methods section (Page 6, line 73-74) and in the Limitations section (Page 12, line 202).

Comment 3. The following studies provide additional evidence for the direct FSH effects in prostate cancer, and might be relevant to cite in Introduction: DOI: 10.1530/EC-21-0 https://tau.amegroups.com/reviewer/submission/323559?key=7S7prbaW639, doi: 10.1096/fj.202002168RR, doi: 10.1016/j.urolonc.2018.12.011.

Reply 3. Thank you for sharing these studies! I appreciate the additional evidence you provided regarding the direct effects of FSH in prostate cancer.

Changes in text: We added this study by Dizeyi et al in the Introduction (Page 5, line 45). We couldn't find the other study suggested (DOI: 10.1530/EC-21-0).

Comment 4. Table 1: It appears death rate was higher in the high-FSH group. Was it due to age?

Reply 4. The reviewer is correct. On univariable analysis (Table 5 and figure 3), the high FSH group had worse overall survival. However, no differences were seen on multivariable analysis. Thus, we can conclude that any differences by FSH were due to confounding factors and not FSH itself. In reviewing table 1, the most notable difference is age. As such, we agree with the reviewer that the differences in univariable results are likely driven by age.

Changes in text: No further changes have been made to the manuscript.

Reviewer C

COMMENTS FOR THE AUTHOR:

This article is the first study to analyze a cohort of men all undergoing ADT assessing pre-ADT FSH levels as risk factors for cardiovascular outcomes. As a result, there was no association between pre-ADT FSH levels with cardiovascular events (MACE). Also, authors investigated the association of FSH levels prior to ADT with the development of CRPC, or death via the VA medical records. As a result, there was no association between FSH levels prior to ADT and long-term risk of CRPC, or death.

Specific comments

In material and methods, there were no data regarding comorbidities such as diabetes mellitus, hypertension, hyperlipidemia, hyperuricemia etc. Also, there were no data regarding smoking history and drinking history. These parameters mentioned above have an important influence on the onset of MACE. So authors should reveal the content of patients' comorbidities and smoking or drinking history. The association between pre-ADT FSH level and cardiovascular event should be investigated by using the propensity matching method.

Reply 1: We utilized the Charlson Comorbidity Index (CCI) to assess and account for comorbidities in our study but did not look at each specific comorbidity separately. However, we did not include information on the drinking history of the participants as it was not available in our dataset.

Changes in text: We added this in the limitations section (see Page 12, line 196-197)

Reply 2: Regarding the reviewer's suggestion to use propensity matching, in our study the exposure variable is FSH which is a biological measure. It would not make sense to balance the propensity of being in a high vs. low FSH category since this is a biological measure. If the reviewer is suggesting using propensity score methods to balance which patients received an FSH test vs. not, we only included patients who had an FSH test in our study so this would be answering a different question.

Changes in text: None

Reviewer D

Comment: Overall I feel this is a well written paper with a straightforward hypothesis. The associations of FSH and ADT use in prostate cancer outcomes have previously studied as cited by yourselves. As FSH is a continuous changing parameter subject to normal physiology (endogenous) and (ADT) exogenous treatment, it is important to study the temporal association of FSH with ADT use and outcomes in prostate cancer patients. If FSH is prognostic of outcomes then certain ADT treatments that affect FSH levels could be associated with better outcomes. Even as the study is a "negative" study, hopefully it will point us the right directions to further clarify the association between FSH and ADT use and cancer outcomes. Therefore, this could still be of interest to the medical community researching in this field.

Reply:

Thank you for your thoughtful feedback on our paper. I appreciate your recognition of the paper's strengths and it is indeed crucial to consider the associations of FSH and ADT in relation to prostate cancer outcomes.

As you rightly mentioned, previous studies have explored the relationship between FSH and ADT use in prostate cancer outcomes, which is acknowledged in our paper. Given that FSH is a continuously changing parameter influenced by both normal physiology (endogenous) and exogenous ADT treatment, it becomes imperative to investigate the temporal association of FSH with ADT use.

Even though our study may be considered a "negative" study, we hope that it will provide valuable guidance for further investigations that aim to elucidate the association between FSH, ADT and cancer outcomes. Thus, this research could still hold significance for the medical community engaged in this field of study.