Author’s response to reviews

Title: Genetic and epigenetic characteristics in ovarian tissues from polycystic ovary syndrome patients with irregular menstruation resemble those of ovarian cancer

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Reviewer reports:

Eleni Papaoiconomou, Ph.D (Reviewer 1):

I consider that the paper merits publication as it attempts to shed light on a recently emerged and still obscure subject. Thus, limited data are existing. Small number of PCOS patients is a pitfall of this study. Also, I believe that a brief literature review regarding PCOS and ovarian cancer would be appropriate.

- Special thanks to you for your helpful comments and your kind consideration. The part regarding PCOS and ovarian cancer has been rewritten. Please see the “Introduction” in the revised manuscript.
Pavlina Andreeva-Gateva (Reviewer 2):

This is a paper describing results of the genetic and epigenetic evaluation of polycystic ovary syndrome (PCOS) ovarian tissues with regular menstruation (n = 10) and irregular menstruation (n = 10). DNA methylation sequencing, real-time PCR array, whole-exome sequencing, and bioinformatics analysis were performed. Authors provided genetic and epigenetic basis for the clinical relationship between irregular menstruation and increased risk for ovarian cancer.

Comments:

1. Description of the study participants is to be placed in the main text rather than in the supplement.
   ● Revised.
   We have moved this part from the supplement to the main text as suggested.

   Yet, looking at the patients characteristics, I could not say that the patient have hyperandrogenia.
   ● Available data suggest that testosterone is positively associated with ovarian cancer risk and is differentially associated with ovarian cancer subtype (Cancer Res 2017;77(14): 3951–3960). Therefore, normo-androgenic PCOS women were recruited for avoiding testosterone interference in this study.

   When the hormonal evaluations were performed? What medications were used? How far from the first diagnostic were taken the tissue samples for evaluation? In general, the patients need to be better characterized.
   ● The patient characteristics have been redescribed in detail, please see the “Methods: Patients and tissue collection” in the revised manuscript.

2. What was the definition of irregular menstruation applied? The criteria "2 to 12 months" is it applicable for all 10 patients in the group of irregular menstruation?
   ● Revised.
   Cycles within the range of 27–35 days were defined as regular menstruation, and cycles > 2 months were defined as irregular menstruation. All 10 patients in the group of irregular menstruation had cycles of 2–12 months.
   These patient characteristics have been redescribed in detail. Please see the “Methods: Patients and tissue collection” in the revised manuscript.

3. Figure 1 A - DNA methylation level is calculated as % of what? And the dotes are presenting what? Please, explain.
   ● DNA methylation level was calculated as average DNA methylation based on all CpG sites in each region, and each region was further divided into 20 bins (20 dots) to evaluate the methylation levels. The calculation was redescribed in detail. Please see “Figure legends-Figure 1” in the revised manuscript.

Patricia Canto (Reviewer 3):

Jiao et al., performed a study which objective was to investigate differences in DNA methylation, mRNA expression and miRNA expression profiles between two groups of women; as well as, the authors searched mutations in a critical oncogenic genes.
This study is interesting; however, I have some comments:

Subject and Method section:

The authors must described more the women who participated in the study, i.e, family history of ovarian cancer or PCOS, what were the criteria of inclusion and exclusion? Etc.
- Revised.
These patient characteristics have been redescribed in detail. Please see the “Methods: Patients and tissue collection” in the revised manuscript.

The authors knows What was the histopathologic characteristics of the ovarian biopsias of their women?
- There were no histopathological characteristics of the ovarian biopsy from these women because the laparoscopic wedge resection of PCOS ovarian tissue does not require intraoperative pathology guidance.

In spite that the authors show the information of 46 normal ovary tissue samples downloaded from GEO database, the authors knows the general characteristic and the menstrual cycles of the normal and ovarian serous cystadenocarcinoma tissue women?
- We would like to thank the reviewer for pointing this out.
We have checked each sample-related publication from PubMed, such as articles 1996270 (PMID) for GSE18521; 21351362 for GSE27651; 23762861 for GSE38666; 23029477 for GSE36668; 23714854 for GSE43346; 15075390 for GSE1133. Regrettably, there was no information about menstrual cycles of normal subjects and patients with ovarian serous cystadenocarcinoma. In this regard, clinical follow-up and retrospective studies that focus on menstrual cycles of women with ovarian cancer will be an effective way to validate this observation in the future.

Discussion section:
The identification of women carriers of BRCA1 mutations is essential for the prevention of ovarian cancer and breast cancer, so it is important that the authors discuss these findings further. In addition, although it is not an objective of their study, but they found it in their results, the discussion should also include comparing the mutations they found with those described in ovarian cancer, in order to determine whether women with PCOS with irregular menstruation should be monitored more often.
- This is a very insightful comment. This part has been rewritten. Please see “Discussion” in the revised manuscript.