

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Effect of cigarette smoking on serum anti-Mullerian hormone and antral follicle count in women seeking fertility treatment – a prospective cross-sectional study
AUTHORS	Bhide, Priya; Timlick, Elizabeth; Kulkarni, Abhijit; Gudi, Anil; Shah, Amit; Homburg, Roy; Acharya, Ganesh

VERSION 1 – REVIEW

REVIEWER	Yong, Eu-Leong NUS Yong Loo Lin School of Medicine
REVIEW RETURNED	16-Mar-2021

GENERAL COMMENTS	<p>This study addresses an important issue- the effect of cigarette smoking on ovarian reserve. The findings were negative: no effect of smoking on serum anti-Mullerian hormone and antral follicle count. Such a null finding will add to the literature on smoking and fertility.</p> <p>A strength of the study is that it is clearly written. Another plus is the use of objective measures to document smoking: breath CO and urine nicotine products.</p> <p>A major weakness is the small numbers of smokers/ex-smokers in the study. There were only 12 smokers and 25 ex-smokers. Smoking would need to have a catastrophic effect on fertility, akin to ovariotoxic chemotherapy, for these differences to be evident with these small numbers. For example, differences in AMH found in the Dolleman study involved 760 current smokers and 678 ex-smokers. These considerations need to be further clarified in Abstract, Methods and Discussion to help the reader put this null data into context.</p> <p>Abstract: Setting: mention Fertility Center. Results: number of subjects and median age (IQR) for each of the three categories should be stated.</p> <p>Discussion: Lines 295, 297: Please number references Kline and Dollemen.</p>
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REVIEWER	Tulandi, T McGill University, Obstetrics and Gynecology
REVIEW RETURNED	06-May-2021

GENERAL COMMENTS	A good and interesting manuscript. The authors studied women younger than 35 years (good ovarian reserve). Whether the findings could be applied to those with reduced ovarian reserve remains to be seen.
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REVIEWER	Jamil, Zehra The Aga Khan University
REVIEW RETURNED	30-Aug-2021

GENERAL COMMENTS	In this paper, the authors have "assessed the effect of cigarette smoking on the quantitative ovarian reserve parameters, serum AMH and AFC in women seeking fertility treatment. However, exploring such effect in infertile population adds to a lot of biasness. For example, women with PCOS have a higher level of AMH as compared to others. Similarly there is a border line significant difference in the ages of the two groups where is AMH is age-sensitive as well as varies in different ethnic populations. Therefore, in order to study the effect on smoking on AMH and AFC the population should have been healthy fertile females in narrow age groups in order to minimize the age-related AMH variation. In the presence of diverse causes of infertility, one cannot say with confidence that no significant association between smoking status and AMH/AFC is masked due to infertility. In order to publish this data, the authors should support it with replicated findings in fertile population supported by normal AMH values in the same population.
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VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Dr. Eu-Leong Yong, NUS Yong Loo Lin School of Medicine

Comments to the Author:

This study addresses an important issue- the effect of cigarette smoking on ovarian reserve. The findings were negative: no effect of smoking on serum anti-Mullerian hormone and antral follicle count. Such a null finding will add to the literature on smoking and fertility.

A strength of the study is that it is clearly written. Another plus is the use of objective measures to document smoking: breath CO and urine nicotine products.

A major weakness is the small numbers of smokers/ex-smokers in the study. There were only 12 smokers and 25 ex-smokers. Smoking would need to have a catastrophic effect on fertility, akin to ovariotoxic chemotherapy, for these differences to be evident with these small numbers. For example, differences in AMH found in the Dolleman study involved 760 current smokers and 678 ex-smokers. These considerations need to be further clarified in Abstract, Methods and Discussion to help the reader put this null data into context.

We thank the reviewers for their comments.

Our study assessed the effect of smoking on ovarian reserve markers in young women seeking fertility treatment. It was designed and appropriately powered to detect a difference in ovarian reserve

markers of a magnitude which would be meaningful to clinical practice – i.e. a change in ovarian reserve that would reduce pregnancy rate – which remains the most relevant marker for women seeking fertility treatment.

We agree that large sample sizes would detect statistically significant differences between the groups. However the effect size in these studies would be small and hence not clinically significant. The study by Dolleman detects only a 3.6% decrease in age specific AMH, which we believe is clinically insignificant. This difference may be relevant in non-fertility settings and to answer other research questions.

We have already discussed this in the 'sample size calculations' in the materials and methods section and the 'strengths and limitations' section of the discussion. We have however added further to the abstract and discussion sections to highlight this issue.

Abstract:

Setting: mention Fertility Center.

This has been updated in the manuscript

Results: number of subjects and median age (IQR) for each of the three categories should be stated.

This is detailed in table 1 but now also added to the text in the results section.

Discussion:

Lines 295, 297: Please number references Kline and Dollemen.

This has been updated in the manuscript

Reviewer: 2

Dr. T Tulandi, McGill University

Comments to the Author:

A good and interesting manuscript. The authors studied women younger than 35 years (good ovarian reserve). Whether the findings could be applied to those with reduced ovarian reserve remains to be seen.

We thank the reviewers for their comments. We have already discussed this in the 'strengths and limitations' section of the discussion.

Reviewer: 3

Dr. Zehra Jamil, The Aga Khan University

Comments to the Author:

In this paper, the authors have "assessed the effect of cigarette smoking on the quantitative ovarian reserve parameters, serum AMH and AFC in women seeking fertility treatment. However, exploring such effect in infertile population adds to a lot of biasness. For example, women with PCOS have a higher level of AMH as compared to others. Similarly there is a border line significant difference in the ages of the two groups where is AMH is age-sensitive as well as varies in different ethnic populations. Therefore, in order to study the effect on smoking on AMH and AFC the population should have been healthy fertile females in narrow age groups in order to minimize the age-related AMH variation. In the presence of diverse causes of infertility, one cannot say with confidence that no significant association between smoking status and AMH/AFC is masked due to infertility.

In order to publish this data, the authors should support it with replicated findings in fertile population supported by normal AMH values in the same population.

We thank the reviewers for their comments.

We agree that ovarian reserve markers are influenced by several variables such as age, ethnicity and the presence of PCOS. We have hence collected data on all these confounding variables and compared them between the study groups. None of these confounding variables were significantly different between groups (table 1). In response to the reviewer's concern about borderline significance in age amongst the comparison groups, we have performed a ANCOVA to assess the differences in serum AMH and AFC, with age as a co-variate. No significant differences were observed between the groups.

The study population was limited to subfertile women seeking fertility treatment and the aim to assess the effect of smoking in this population. We did not include fertile women nor aim to assess the effect of smoking in these women. Determining the differences in the effect of smoking between these two populations and hence the effect of infertility as a confounding variable remains outside the remit of this study. However the results of our study are in agreement with population based studies such as those by Bressler et al (referenced in the manuscript) which included fertile women of a similar age range.

Reviewer: 1

Competing interests of Reviewer: None declared

Reviewer: 2

Competing interests of Reviewer: None

Reviewer: 3

Competing interests of Reviewer: I have read the terms and condition and agree to be named for this review.

VERSION 2 – REVIEW

REVIEWER	Yong, Eu-Leong NUS Yong Loo Lin School of Medicine
REVIEW RETURNED	24-Nov-2021
GENERAL COMMENTS	Questions answered.