

## PEER REVIEW HISTORY

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### ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	Pre-treatment maternal lifestyle and outcomes of assisted reproduction: an Italian cohort study.
<b>AUTHORS</b>	Ricci, Elena; Noli, Stefania; Ferrari, Stefania; La Vecchia, Irene; De Cosmi, Valentina; Castiglioni, Marta; Somigliana, Edgardo; Agostoni, Carlo; Cipriani, Sonia; Mauri, Paola; Parazzini, Fabio

### VERSION 1 – REVIEW

<b>REVIEWER</b>	Gian Mario Tiboni Dipartimento di Scienze Mediche, Orali e Biotecnologiche, Università "G. d'Annunzio" Chieti Pescara, Italy
<b>REVIEW RETURNED</b>	02-May-2020

<b>GENERAL COMMENTS</b>	Several studies have shown the negative impact of wrong lifestyle factors on ART outcomes. A simple interview cannot improve knowledge in this area.
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<b>REVIEWER</b>	Heather Hipp Emory University United States
<b>REVIEW RETURNED</b>	18-May-2020

<b>GENERAL COMMENTS</b>	<p>Thank you for the opportunity to review this manuscript, "Pre-treatment maternal lifestyle and outcomes of assisted reproduction: an Italian cohort study." In an effort to determine the impacts of tobacco, alcohol, and physical exercise on IVF success, the authors gathered self-reported surveys on 492 number of women undergoing 492 ART cycles from 2014-2016. They did not find any of these exposures to have a significant impact on ART outcomes. This manuscript was well-written and covers a topic of import, especially given the dearth of well-constructed studies in the field.</p> <p>There are a few limitations, however. The study is presented as a prospective cohort, but the data regarding exposures was actually gathered retrospectively at time of oocyte retrieval. Although the study cannot be re-done with prospective reporting (e.g. with diaries for 3-6 months prior to oocyte retrieval), the retrospective nature of data collection should be explained in the abstract and reported in the discussion as a limitation given risk of recall bias. In addition, there are no data regarding the exposures from a chronologic perspective. Having information about when heavy drinking or strenuous physical may affect outcomes would have been very helpful.</p> <p>Lastly, the authors report clinical pregnancy rate (CPR) will be their primary outcome, with live birth rate (LBR), implantation rate and number of oocytes retrieved as secondary outcomes. In the</p>
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	<p>results, however, they report failure of these outcomes. Recommend consideration to report CPR/ LBR (rather than failure).</p> <p>Abstract:</p> <ul style="list-style-type: none"> <li>- Recommend adding the years in which the study was conducted</li> <li>- Although the conclusion of the abstract seems intuitively reasonable, it is not supported by the results presented.</li> </ul> <p>Introduction:</p> <ul style="list-style-type: none"> <li>- Page 5, Line 42: Recommend re-word “.. largely differ in different populations”</li> </ul> <p>Methods</p> <ul style="list-style-type: none"> <li>- Page 6 Line 51- 17: Many women are likely casual drinks and may start abstaining either a few months prior to retrieval or during stimulation. It would be helpful to report these exposures chronologically, since, presumably, alcohol during the stimulation would have a stronger impact than six months prior.</li> <li>- Page 8, line 5-12: It appears that the authors are reporting cumulative pregnancy rate (or cumulative live birth rate) per retrieval. If this is the case, I would recommend defining it as such (rather than “best outcome”). In addition, the denominators in the results should be clear and the information added to the abstract.</li> </ul> <p>Results</p> <ul style="list-style-type: none"> <li>- Page 10, line 8: Recommend changing terminology of “not achieving embryo transfer” to “cancellation” rate if this is accurate</li> <li>- Page 10, line 10-15: I believe this sentence (“The corresponding figures were 1.16...”) is RR for clinical pregnancy rate and live birth rate failure (not clinical pregnancy rate or live birth).</li> <li>- Line 10, line 38-44 &amp; Table 2: Recommend defining “embryo transfer failure” as either be a cancelled cycle or absence of live birth</li> <li>- It would be interesting to see tobacco use and alcohol intake analyzed as continuous variables</li> </ul> <p>Discussion:</p> <ul style="list-style-type: none"> <li>- Page 11, Line 10: Recommend clarifying “worse outcome of embryo transfer.” I presume this to mean higher rates of cancellation</li> <li>- Limited knowledge regarding type of physical exercise (e.g. since only calculated by total number of hours, rather than exertion/ intensity) should be added as a limitation</li> </ul>
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<b>REVIEWER</b>	Adam Watkins University of Nottingham, United Kingdom
<b>REVIEW RETURNED</b>	08-Sep-2020

<b>GENERAL COMMENTS</b>	<p>The study by Ricci et al has investigated the effects of maternal lifestyle such as alcohol intake, physical exercise and smoking, on success rates in subsequent rounds of ART. Here, the authors have focused their investigation on a specific group of Italian women who underwent IVF or ICSI after completing the questionnaire. the authors show that excessive drinking was associated with a reduced change of becoming pregnant. However, other lifestyle factors in their study did not display any significant associations with changes of achieving a pregnancy.</p>
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	<p>This is a well conducted, well written and clearly presented study. As such, I only have minor comments to make. My main comments would be that the novelty of this study is made evident early on. As the authors indicate, there have been many studies looking at the effects of these same lifestyle characteristics on pregnancy rates following ART. The authors state later in the Manuscript about them using a very specific demographic of women and in a country where alcohol intake during pregnancy is more acceptable than in other countries. I feel that that some of these aspects should be highlighted earlier in the manuscript to establish how this study differs from those already published.</p> <p>While I appreciate that the focus of the study is on the impacts of maternal lifestyle factors, it would be good to indicate in the Introduction studies that have, or have not, shown similar effects in men. The authors do state that they observe no significant associations with male characteristics, but the role that a father plays in the success of pregnancy is becoming more prominent. A such, some highlighting of how lifestyle can also affect sperm quality would be welcome in the Introduction.</p> <p>Following on from this, on page 10, the authors indicate that male factors did not affect the outcomes of this study. Did they account for male age?</p> <p>Was there any difference in pregnancy rates if the authors separated their data for embryos derived using IVF and embryos derived from ICSI?</p>
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### VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name: Gian Mario Tiboni

Institution and Country: Dipartimento di Scienze Mediche, Orali e Biotecnologiche, Università "G. d'Annunzio" Chieti Pescara, Italy

Competing interests: none declared

Please leave your comments for the authors below

Several studies have shown the negative impact of wrong lifestyle factors on ART outcomes. A simple interview cannot improve knowledge in this area.

A: Thank you for your commitment to evaluate our work. In our opinion, adding further information has some utility even if it regards known factors. Moreover, the effect of different levels of physical activity is still under discussion.

Reviewer: 2

Reviewer Name: Heather Hipp

Institution and Country:

Emory University

United States

Competing interests: None

Please leave your comments for the authors below

Thank you for the opportunity to review this manuscript, "Pre-treatment maternal lifestyle and outcomes of assisted reproduction: an Italian cohort study." In an effort to determine the impacts of tobacco, alcohol, and physical exercise on IVF success, the authors gathered self-reported surveys on 492 number of women undergoing 492 ART cycles from 2014-2016. They did not find any of these exposures to have a significant impact on ART outcomes. This manuscript was well-written and covers a topic of import, especially given the dearth of well-constructed studies in the field. There are a few limitations, however. The study is presented as a prospective cohort, but the data regarding exposures was actually gathered retrospectively at time of oocyte retrieval. Although the study cannot be re-done with prospective reporting (e.g. with diaries for 3-6 months prior to oocyte retrieval), the retrospective nature of data collection should be explained in the abstract and reported in the discussion as a limitation given risk of recall bias. In addition, there are no data regarding the exposures from a chronologic perspective. Having information about when heavy drinking or strenuous physical may affect outcomes would have been very helpful. Lastly, the authors report clinical pregnancy rate (CPR) will be their primary outcome, with live birth rate (LBR), implantation rate and number of oocytes retrieved as secondary outcomes. In the results, however, they report failure of these outcomes. Recommend consideration to report CPR/ LBR (rather than failure).

A: thank you for your thoughtful considerations. We agree on most and tried to address them in the revised version of our paper. However, as regards the reporting, we found that expressing RR as risk of failure was more readily interpretable than the opposite (alcohol or smoking use associated with RR of failure > 1 are easily interpreted as risk factors, as compared to RR of success < 1, in our opinion).

Abstract:

- Recommend adding the years in which the study was conducted

A: we added the study years

- Although the conclusion of the abstract seems intuitively reasonable, it is not supported by the results presented.

A: we tried to interpret our results in the most conservative way, therefore a relative risk > 1, albeit not statistically significant, was evaluated in the light of previous literature results and it led us to conclude that alcohol and smoking should be avoided. We modified the conclusions to highlight the difference between indication from literature and from our results: "Conservatively, all women seeking pregnancy should be advised to limit alcohol drinking and smoking. Moreover, our study suggested that maintaining a moderate level of physical activity could be beneficial."

Introduction:

- Page 5, Line 42: Recommend re-word "...largely differ in different populations"

A: thank you for this indication. Reword: "...largely vary in different populations"

Methods

- Page 6 Line 51- 17: Many women are likely casual drinks and may start abstaining either a few months prior to retrieval or during stimulation. It would be helpful to report these exposures chronologically, since, presumably, alcohol during the stimulation would have a stronger impact than six months prior.

A: In our study we basically investigated the effect of recent exposures (occurred within the last year). In fact, women are advised to stop smoking and alcohol consumption with the onset of ovarian stimulation, so no exposition should occur during ART cycle. We added this detail in the text: "To evaluate the effect of recent exposure, patients were asked to report about their usual weekly food consumption in the last year, using a reproducible and valid food frequency questionnaire. (...) ...and

a non-smoker if she had never smoked  $\geq$  one cigarette/day. Before starting ovarian stimulation, women were advised to abstain from alcohol and smoking, thus no such exposure should occur during ART cycle.”

- Page 8, line 5-12: It appears that the authors are reporting cumulative pregnancy rate per retrieval (or cumulative live birth rate) per retrieval. If this is the case, I would recommend defining it as such (rather than “best outcome”). In addition, the denominators in the results should be clear and the information added to the abstract.

A: We rephrased this explanation in the Abstract:

“The primary outcome measure was clinical pregnancy. Secondary measures were number of retrieved oocytes, embryo transfer, and live birth.

Methods: “In this analysis, we considered as outcome the cumulative pregnancy rate per retrieval in the cycle immediately following the interview.”

Results. In 492 women undergoing an ART cycle, 427 (86.8%) underwent embryo transfer, 157 (31.9%) had clinical pregnancy, 121 (24.6%) had live birth.”

#### Results

- Page 10, line 8: Recommend changing terminology of “not achieving embryo transfer” to “cancellation” rate if this is accurate

A: We would prefer to replace “not achieving embryo transfer” with “missed embryo transfer”, referring to those couples for whom no vital embryo was available after the oocyte retrieval for any reason.

- Page 10, line 10-15: I believe this sentence (“The corresponding figures were 1.16...”) is RR for clinical pregnancy rate and live birth rate failure (not clinical pregnancy rate or live birth).

A: thank you, we corrected the sentence.

- Line 10, line 38-44 & Table 2: Recommend defining “embryo transfer failure” as either be a cancelled cycle or absence of live birth

A: As above, we would prefer the term “missed embryo transfer”

- It would be interesting to see tobacco use and alcohol intake analyzed as continuous variables

A. we added the results of these analyses in the Results.

“Analysing alcohol intake (g) and number of cigarettes as continuous variables, we did not find any significant correlation with number of high-quality oocytes. Since most women did not smoke, median intakes were 0 (IQR 0-0) in all categories and no differences could be observed. As regards alcohol, women who underwent embryo transfer had median intakes lower than those who did not (1.9 (IQR 0-5.3) vs. 2.7 (IQR 0-7.5 g/day),  $p=0.16$ ); those who achieved clinical pregnancy consumed 1.8 (IQR 0-4.7) g/day and those who did not achieve it consumed 2.4 (IQR 0-5.6) g/day ( $p=0.11$ ). Women with livebirth had lower alcohol intake than those without livebirth (1.8 (IQR 0-5.3) vs 2.3 (IQR 0-5.6) g/day,  $p=0.20$ ). None of these differences was statistically significant.”

#### Discussion:

- Page 11, Line 10: Recommend clarifying “worse outcome of embryo transfer.” I presume this to mean higher rates of cancellation

A: We modified into “higher rate of missed embryo transfer”

- Limited knowledge regarding type of physical exercise (e.g. since only calculated by total number of hours, rather than exertion/ intensity) should be added as a limitation

A: we added this limitation: "A further limitation was that knowledge regarding type of physical exercise was limited, because we recorded total number of weekly hours spent exercising, but not intensity or type of exercise."

Reviewer: 3

Reviewer Name: Adam Watkins

Institution and Country: University of Nottingham, United Kingdom

Competing interests: None declared

Please leave your comments for the authors below

The study by Ricci et al has investigated the effects of maternal lifestyle such as alcohol intake, physical exercise and smoking, on success rates in subsequent rounds of ART. Here, the authors have focused their investigation on a specific group of Italian women who underwent IVF or ICSI after completing the questionnaire. The authors show that excessive drinking was associated with a reduced chance of becoming pregnant. However, other lifestyle factors in their study did not display any significant associations with changes of achieving a pregnancy.

This is a well conducted, well written and clearly presented study. As such, I only have minor comments to make. My main comments would be that the novelty of this study is made evident early on. As the authors indicate, there have been many studies looking at the effects of these same lifestyle characteristics on pregnancy rates following ART. The authors state later in the Manuscript about them using a very specific demographic of women and in a country where alcohol intake during pregnancy is more acceptable than in other countries. I feel that that some of these aspects should be highlighted earlier in the manuscript to establish how this study differs from those already published.

While I appreciate that the focus of the study is on the impacts of maternal lifestyle factors, it would be good to indicate in the Introduction studies that have, or have not, shown similar effects in men. The authors do state that they observe no significant associations with male characteristics, but the role that a father plays in the success of pregnancy is becoming more prominent. As such, some highlighting of how lifestyle can also affect sperm quality would be welcome in the Introduction.

A: Thank you for your thoughtful considerations. We have added some information about these characteristics in men in the Introduction and we remarked the peculiarity of our sample as regards alcohol use.

Following on from this, on page 10, the authors indicate that male factors did not affect the outcomes of this study. Did they account for male age?

A: male age was accounted for in the complete model, since this variable was available for all couples. However, since men's and women's age are strongly correlated, when including women's age any effect disappeared. We'd prefer not to add further details on men, to avoid this article become too long, but if the Reviewer deems it necessary we are willing to add a further section about the relationship between men's characteristics and ART outcome. However, we modified the last sentence of Results as follows: "Finally, we controlled these results for partner's characteristics and lifestyle, in a subgroup of 324 couples with complete information for both male and female. Men's age was significantly associated with higher rate of negative outcomes in the univariate analysis, but when including women's age in the model, this relationship lost significance. As regards to women lifestyle,

results did not change. Men’s lifestyle (smoking, alcohol drinking and physical activity) did not significantly impact on ART outcomes.”

Was there any difference in pregnancy rates if the authors separated their data for embryos derived using IVF and embryos derived from ICSI?

A: thank you for this suggestion, we added this piece of information in the Results.

### VERSION 2 – REVIEW

<b>REVIEWER</b>	Heather Hipp Emory University USA
<b>REVIEW RETURNED</b>	09-Oct-2020

<b>GENERAL COMMENTS</b>	<p>Thank you for the opportunity to re-review this manuscript. It is much improved and an interesting topic that warrants publication</p> <p>Abstract:</p> <ul style="list-style-type: none"> <li>-I would still recommend description of this design as a retrospective cohort study.</li> <li>- The authors revised the primary outcome to cumulative pregnancy rate per retrieval (which could include multiple transfers) in the methods, however did not include this information in the abstract</li> <li>- The authors state that primary outcome was clinical pregnancy however report risk of pregnancy failure</li> <li>- Although the authors added a caveat to the conclusion (“Conservatively”), it would be helpful for it to describe the actual results (e.g. There were no significant differences in IVF outcomes among women who used alcohol or tobacco in the year prior to treatment. Conservatively, all women should be advised to limit substance abuse, however.)</li> </ul> <p>Methods</p> <ul style="list-style-type: none"> <li>- Authors report clinical pregnancy to be the main objective of the study, but report clinical pregnancy failure in their main analysis (results text and table)</li> </ul> <p>Results</p> <ul style="list-style-type: none"> <li>- Did some women have multiple transfers? If so, it would be helpful to include this information</li> <li>- Missed embryo transfer is not a common term in the literature. They are typically cancelled cycles.</li> </ul>
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<b>REVIEWER</b>	Adam Watkins University of Nottingham, United Kingdom
<b>REVIEW RETURNED</b>	13-Oct-2020

<b>GENERAL COMMENTS</b>	The authors have addressed my comments appropriately. I have no further comments to make.
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### VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Editor comments:

The reviewer(s) have recommended revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript. Please note that we normally allow a maximum of two manuscript revisions. As such, we urge you to make all the necessary revisions at this stage in an effort to convince the reviewers that your work is suitable for publication in BMJ Open.

Editorial requirements:

- We note that the Strengths and Limitations section still discusses the results of the study. Please revise the 'Strengths and limitations' section of your manuscript (after the abstract). This section should contain five short bullet points, no longer than one sentence each, that relate specifically to the methods. The results of the study should not be summarised here.

Thank you, we missed this request in the first revision. The Strengths and Limitations section has been modified as suggested.

Reviewer(s)' Comments to Author:

Reviewer: 2

Reviewer Name: Heather Hipp

Institution and Country:

Emory University

USA

Competing interests: None

Please leave your comments for the authors below

Thank you for the opportunity to re-review this manuscript. It is much improved and an interesting topic that warrants publication

Abstract:

-I would still recommend description of this design as a retrospective cohort study.



We deleted the term “prospective” from abstract and methods.

- The authors revised the primary outcome to cumulative pregnancy rate per retrieval (which could include multiple transfers) in the methods, however, did not include this information in the abstract

This information was added in the Abstract.

- The authors state that primary outcome was clinical pregnancy however report risk of pregnancy failure

According to this observation, we modified the analysis, to show the risk of clinical pregnancy (as well as embryo transfer and livebirth).

- Although the authors added a caveat to the conclusion (“Conservatively”), it would be helpful for it to describe the actual results (e.g. There were no significant differences in IVF outcomes among women who used alcohol or tobacco in the year prior to treatment. Conservatively, all women should be advised to limit substance abuse, however.)

We modified the conclusions as suggested.

## Methods

- Authors report clinical pregnancy to be the main objective of the study, but report clinical pregnancy failure in their main analysis (results text and table)

We modified the analyses to show the risk of successful outcomes.

## Results

- Did some women have multiple transfers? If so, it would be helpful to include this information

This information has been added in the Results: “Seventy-two (14.6%) women underwent two, 28 (5.7%) three, 4 (0.8%) ≥4 embryo transfers. Out of 157 women with pregnancy, 7 had a miscarriage at the first attempt and livebirth at the second one, for a total of 164 pregnancies in 492 women. The cumulative pregnancy rate per retrieval was 33.3% (95% CI 28.5%-38.7%). Women with a previous

miscarriage and a livebirth at the following pregnancy were considered as having a successful ART in the main analysis.”

- Missed embryo transfer is not a common term in the literature. They are typically cancelled cycles.

Since we changed the analyses as suggested, we simply used the term “embryo transfer”.

Reviewer: 3

Reviewer Name: Adam Watkins

Institution and Country: University of Nottingham, United Kingdom

Competing interests: None

Please leave your comments for the authors below

The authors have addressed my comments appropriately. I have no further comments to make.

Thank you.

### VERSION 3 – REVIEW

<b>REVIEWER</b>	Heather Hipp Emory University, USA
<b>REVIEW RETURNED</b>	24-Oct-2020
<b>GENERAL COMMENTS</b>	The authors have addressed my concerns, and I would recommend publication at this point. There is a small typo in the first sentence of the discussion: "In this sample of women referring to an Italian Fertility centre, did not play a significant role..."