Author’s response to reviews

Title: Pilot randomized trial of short-term changes in inflammation and lipid levels during and after aspirin and pravastatin therapy

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Reviewer 1

1. Comment: Well written manuscript. Data is sparse but useful to others who have similar research interests who are evaluating the dosing of this statin.

Prevalence and Contributors to Low-grade Inflammation in Three U.S. Populations of Reproductive Age Women. Paediatr Perinat Epidemiol. 2018;32(1):55-67) It is also known that higher circulating lipid levels are also associated with a decreased fecundity in women in the EAGeR trial and in prior studies. (Pugh SJ, et al. Preconception maternal lipoprotein
levels in relation to fecundability. Hum Reprod. 2017;32(5):1055-63). Unfortunately, the optimal treatment(s) for improving pregnancy rates among women with low-grade inflammation coupled with greater adiposity is not known. Statins may be one "add on" agent capable of reducing inflammation, as well as lipids. However, there is little published prior knowledge regarding its use in the peri-conceptual period. This study may provide subsequent researchers, institutional review boards and the FDA additional information relative to statin dosages in vivo.

Carbohydrate, lipid, and protein metabolism as well as the cardiovascular system are all responsive to endocrine signals, and especially those that originate in the adipose endocrine organ. While cardiometabolic health is adversely affected by visceral adiposity and its resultant inflammatory adipocytokine production (Amato MC, et al. Visceral Adiposity Index: a reliable indicator of visceral fat function associated with cardiometabolic risk. Diabetes Care. 2010;33(4):920-2), intermediate markers, like infertility occurring earlier in one's life course, may provide novel interventional opportunities. For research to occur in and around the time of conception to test the hypothesis that lipid and inflammation reductions by statins may improve fertility treatment outcomes, preliminary data must be generated and published to guide research protocol dosing and cautions around the critical window of conception. This manuscript provides some of the pieces of that puzzle.

Inflammation is a key player in cardiovascular disease independent of cholesterol levels. Lipid levels have also been routinely reduced with the use of statin therapy.


An evaluation of the effects of statins on total mortality show a significant reduction. (Nunes JP. Statins in primary prevention: impact on mortality. A meta-analysis study. Minerva Cardioangiol. 2017;65(5):531-8) Statins also lower hsCRP and lipids that are associated with inflammation and may be a simple intervention that will improve pregnancy rates in women with excess adipose tissue. (Hoghnestad, et al. Plasma C-reactive protein as a marker of cardiac

As a mechanism of action for the reduction in inflammation, statins have been shown to reduce cholesterol, Amyloid-β and apolipoprotein E (ApoE) levels, decrease reactive oxygen and nitrogen species (ROS and RNS) formation, inhibit excitotoxicity, modulate matrix metalloproteinases (MMPs), and stimulate endothelial nitric oxide synthase (eNOS). (Saeedi Saravi SS, et al. The beneficial effects of HMG-CoA reductase inhibitors in the processes of neurodegeneration. Metab Brain Dis. 2017;32(4):949-65.)


Because of the notable effects of statins on inflammatory pathways associated with lipids and their metabolism, the addition of a statin to low dose aspirin may potentially improve fertility treatment outcomes in a selected group of patients. Preliminary trial data for dose finding and anticipated effects are needed to foster further investigations on this potential pharmacologic combination therapy as it relates to fertility.

While in other situations the sparse data set would preclude publication, the novel utility of statin use in this situation would benefit by the publication of this limited human data set, in my opinion.

Response: Thank you. We agree that these medications have the potential to improve pregnancy rates among women with inflammation, and we appreciate this thorough and detailed review of related literature.

Reviewer 2

1. Comment: Line 34: Why do these conditions impair fertility? (Answered in line 52)

Response: We have added some of the information from line 52 in order to clarify this statement:

Lines 34-35: “Inflammation and lipids may impair fertility because they are related to reproductive disorders.”
2. Comment: Line 35: How do these drugs treat infertility? What is a statin?

Response: We have clarified the sentence to more explicitly define statins and the mechanism by which the study drugs could treat infertility:

Lines 35-37: “When these women undergo infertility treatment, taking aspirin and cholesterol-lowering statin drugs could improve their outcomes by reducing inflammation and cholesterol.”

3. Comment: If aspirin is safe to take during pregnancy what is the significance of including aspirin in the study? (perhaps eluded to in line 60 and beyond)

Response: In order to clarify the purpose of including aspirin in the study, we have edited the summary to explicitly discuss dual therapy with aspirin and statins as the potential intervention of interest for infertility:

Lines 40-42: “This would indicate that short-term statin therapy stopped before pregnancy, along with aspirin, a common anti-inflammatory medication, could still support the events of early pregnancy.”

4. Comment: How was the sample size determined? Was there a limit on size for example?

Response: We have clarified this in the methods:

Lines 86-88: “To ensure enrollment of enough women with hsCRP between 2 and 10 mg/L, indicating low-grade inflammation, the target sample size was 39; however, enrollment was stopped at 27 due to practical considerations related to the larger trial.”

5. Comment: Line 86: how were the doses of each medication determined?

Response: We have added an explanation of how the doses were determined to the methods section:

Lines 93-95: “The aspirin dosage is based on previous data showing lowered inflammatory markers at this dose (18), and the dosage of pravastatin was chosen because it is used in normal therapy.”

6. Comment: Line 87: how was the time period of medication intake and washout determined? Where there specifications on the time of day that the medication was to be taken orally?
Response: We have added explanation of both of these points in the methods section:

Lines 97-98: “The study medication was taken daily by mouth for 2 weeks at no specific time of day.”

Lines 99-103: “The treatment period was designed to mimic a realistic course of treatment in our future trial, where study medications would be taken from the beginning of the menstrual cycle until ovulation 2 weeks later. The washout period was designed to determine whether effects of the medications would last throughout events of pregnancy establishment during the 2 weeks following ovulation and treatment cessation.”

7. Comment: Line 108: What method was used to randomize the women into treatment groups?
Response: We have clarified the details of the randomization method:

Lines 95-97: “A block-randomized list of treatment assignments was generated and written in sealed envelopes prior to study initiation, and the next envelope in order was chosen as each participant was enrolled.”

8. Comment: Was there any blinding of the participants?
Response: We have included this information in the methods:

Line 78: “The study design was an unblinded, randomized trial.”

9. Comment: Do precision values need to be included for median and ranges?
Response: Given the small sample sizes in our dataset, we chose not to include measures such as 95% confidence intervals around medians. We prefer to include simply the raw medians and ranges from our data, which we feel give a better sense of precision in this case.

10. Comment: Any potential harm or adverse effects of the medication?
Response: We have added discussion of known potential adverse effects of the medications, as well as adverse events in our study:

Lines 177-181: “None of the participants in this study reported adverse effects of the study medications such as bleeding or muscle pain, suggesting that these treatments can be well-tolerated. Since statins are contraindicated in pregnancy because of possible teratogenicity (17),
the timing of inflammation dynamics during and after statin therapy have potential implications for its use in this context.”

11. Comment: Conclusion seemed a little short. Data presented supports authors conclusions. In the future larger randomized trial, will the population be the same? How can this trial be included into public health and clinical work?

Response: The conclusion now includes an expanded explanation of the intended study population for the future trial, and the broader clinical utility of this work:

Lines 205-210: “We are currently in the process of designing a larger trial of medication use to improve fertility treatment outcomes in a similar population of women with chronic, low-grade inflammation, including those with and without obesity. In the long-term, if statins and aspirin prove beneficial for women with chronic inflammation who wish to become pregnant, these drugs could be added to clinical practice as part of first-line fertility treatments such as OI/IUI, in order to improve the success rates of these procedures.”