PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Women's pre-pregnancy lipid levels and number of children: a Norwegian prospective population-based cohort study
AUTHORS	Pirnat, Aleksandra; De Roo, Lisa; Skjaerven, Rolv; Morken, Nils-Halvdan

VERSION 1 – REVIEW

REVIEWER	eric van exel VUmc/GGZinGeest, department of psychiatry, the Netherlands
REVIEW RETURNED	10-Jan-2018

GENERAL COMMENTS	This is an important study, it is one of the largest studies on human fertility, in western society. The issue of lipid metabolism and fertility is certainly overlooked in the literature. Nonetheless, the only minor points of criticism I have is 1) that in 2017 the following paper was also published on this subject. 1 Pugh SJ, et al. Preconception maternal lipoprotein levels in relation to fecundability. Hum Reprod. 2017 1;32:1055-1063. 2) I was wondering if the authors also have data on APOE genotype, a gene strongly related to lipid metabolism. If they do have this data whether they can adjust their analysis for APOE genotype. 3) Similarly low grade inflammation is also associated with fertility and is associated with lipid metabolism, do the authors have data on low grade inflammation, i.e. CRP (Sjaarda LA, et al. Prevalence and Contributors to Low-grade Inflammation in Three U.S. Populations of Reproductive Age Women. Paediatr Perinat Epidemiol. 2017 Sep 15. doi: 10.1111/ppe.12409. If they do have this data whether they can determine if lipid metabolism or low grade inflammation affects fertility more. 4) Most importantly, one could argue that the choice to analyze the TG/HDL ratio is chosen a bit arbitrary. One could also argue that the Total cholesterol/HDL ratio is a far more accepted ratio in the field of cardiovascular disease. Perhaps an additional analysis with Total cholesterol/HDL ratio should be presented and a possible discrepancy or similarity between the TG/HDL ratio and the Total cholesterol/HDL ratio could be discussed.

REVIEWER	Prof. Malgorzata Karbownik-Lewinska Department/Chair of Oncological Endocrinology, Medical University of Lodz, Poland
REVIEW RETURNED	19-Jan-2018

GENERAL COMMENTS	Manuscript ID: bmjopen -2017-021188

	The report is aimed at a possible association between pre-pregnant serum lipid levels and number of children. That was prospective population-based cohort study. The topic of the study is of great importance as currently different factors affecting fertility are vigorously discussed in the literature. The study is properly designed, also with respect to methodology. The manuscript is properly written and the results are properly discussed. However, the study has some small limitations, especially related to how the results are discussed. The authors listed strengths and limitations of the study. However, the lack of thyroid tests/ thyroid antibodies should be added to that list. Whereas these data were probably not available to the authors, this fact should be stressed, as prevalence of thyroid dysfunction, especially subclinical hypothyroidism, in women at reproductive age is very high, with prevalence of positive thyroid antibodies being as high as at least 15% in this population. It should be also stressed that both thyroid dysfunction and positive thyroid antibodies affect strongly fertility rate. The authors have found that women with unfavorable pre-pregnant lipid profile had higher risk of having no or only one child. Taking into account few previously published studies and from logical point of view, these results were expected. When discussing potential mechanisms of the results, the authors underlined the linkage with cardiovascular disease. Whereas this is undoubtedly true, other – more direct mechanisms – should be discussed. The authors mentioned the potential role of oxidative stress (reference 19) and, in my opinion this issue should be discussed more broadly. It has been published recently (TSH ≥2.5 mIU/l is Associated with the Increased Oxidative Damage to Membrane Lipids in Women of Childbearing Age with Normal Thyroid Tests. Horm Metab Res 2017;49:321-326) that in women of childbearing age with normal thyroid tests, TSH in higher normal ranges was associated with higher oxidative damage to membrane lipids and less favorable lipid profile, and the direct linkage between unfavorable lipid profile and increased oxidative damage was confirmed in this study. The authors have found that nulliparous women were older at the time of examination and had higher BMI. Because both are associated with higher oxidative stress, this issue should be discussed more broadly. The finding that a significantly higher number of onechild mothers had in-vitro fertilization (IVF) in their first pregnancy (7.2% versus 2.6% in women with ≥ 2 births, p< 0.001) suggests that without assisted reproductive technologies a number of no-child mothers would be even higher, and this issue should be also discussed more broadly. In summary, the manuscript is worth publishing in a journal such as BMJ Open as a full length paper after adding some information suggested above.

REVIEWER	Giovanna Muscogiuri Endocrinology, University Federico II, Naples
REVIEW RETURNED	21-Jan-2018

GENERAL COMMENTS	The manuscrit aimed to investigate the association between pre-pregnant lipid profile and number of children. The experimental design of the study is adequate as well as the power of the sample size. Policistyc Ovarian Syndrome could be a cause of both lipid derangements profile and infertilityy. Did the authors take into

	account this issue? Further, I suggest to provide an overview of this association in the discussion quoting and providing a comment on the following manuscripts: 1: Kim JY, Tfayli H, Michaliszyn SF, Arslanian S. Impaired Lipolysis, Diminished Fat Oxidation and Metabolic Inflexibility in Obese Girls with Polycystic Ovary Syndrome. J Clin Endocrinol Metab. 2017 Dec 6. doi: 10.1210/jc.2017-01958. [Epub ahead of print] PubMed PMID: 29220530. 2: Kiranmayee D, Kavya K, Himabindu Y, Sriharibabu M, Madhuri GLJ, Venu S. Correlations Between Anthropometry and Lipid Profile in Women With PCOS. J Hum Reprod Sci. 2017 Jul-Sep;10(3):167-172. doi: 10.4103/jhrs.JHRS_108_16. PubMed PMID: 29142444; PubMed Central PMCID: PMC5672721. 3: Moran LJ, Mundra PA, Teede HJ, Meikle PJ. The association of the lipidomic profile with features of polycystic ovary syndrome. J Mol Endocrinol. 2017 Jul;59(1):93-104. doi: 10.1530/JME-17-0023. Epub 2017 May 12. PubMed PMID: 28500248. 4: Palomba S, Falbo A, Chiossi G, Muscogiuri G, Fornaciari E, Orio F, Tolino A, Colao A, La Sala GB, Zullo F. Lipid profile in nonobese pregnant women with polycystic ovary syndrome: a prospective controlled clinical study. Steroids. 2014 Oct;88:36-43. doi: 10.1016/j.steroids.2014.06.005. Epub 2014 Jun 16. PubMed PMID: 24945113.

VERSION 1 – AUTHOR RESPONSE

Reviewer #1
This is an important study, it is one of the largest studies on human fertility, in western society. The issue of lipid metabolism and fertility is certainly overlooked in the literature. Nonetheless, the only minor points of criticism I have is :

1) that in 2017 the following paper was also published on this subject.(1 Pugh SJ, et al. Preconception maternal lipoprotein levels in relation to fecundability. Hum Reprod. 2017 1;32:1055-1063).
Response: Thank you. We have added it to our manuscript as reference number 28.

2) I was wondering if the authors also have data on APOE genotype, a gene strongly related to lipid metabolism. If they do have this data whether they can adjust their analysis for APOE genotype.
3) Similarly low grade inflammation is also associated with fertility and is associated with lipid metabolism, do the authors have data on low grade inflammation, i.e. CRP (Sjaarda LA, et al. Prevalence and Contributors to

Low-grade Inflammation in Three U.S. Populations of Reproductive Age Women.
Paediatr Perinat Epidemiol. 2017 Sep 15. doi: 10.1111/ppe.12409. If they do have this data whether they can determine if lipid metabolism or low grade inflammation affects fertility more.
Response: Unfortunately, data on APOE genotype and CRP was not registered in CONOR or the MBRN. We realise that this could be seen as a limitation, and have added a comment in the strengths and limitations section (P.17, Lines 7-10).

4) Most importantly, one could argue that the choice to analyze the TG/HDL ratio is chosen a bit arbitrary. One could also argue that the Total cholesterol/HDL ratio is a far more accepted ratio in the field of cardiovascular disease. Perhaps an additional analysis with Total cholesterol/HDL ratio should be presented and a possible discrepancy or similarity between the TG/HDL ratio and the Total cholesterol/HDL ratio could be discussed.
Response: We can see the reviewers concern. Given the focus of our study on metabolic aspects of CVD risk, TG/HDL ratio was chosen due to its stronger correlation with insulin resistance compared with other traditional lipids or lipid ratios (Tingting D. Clinical usefulness of lipid ratios, visceral adiposity indicators, and the triglycerides and glucose index as risk markers of insulin resistance. Cardiovascular Diabetology 2014. 13:146). TG/HDL ratio also has a high sensitivity in early detection of individuals with cardiometabolic risk (please see reference numbers 24 and 25 in the current version). For these reasons, and given the extensive literature on direct comparison between traditional and novel lipid ratios in a clinical setting, our belief is that assessment of TG/HDL ratio is more appropriate for the purposes of our paper.

Reviewer #2
The report is aimed at a possible association between pre-pregnant serum lipid levels and number of children. That was prospective population-based cohort study.
The topic of the study is of great importance as currently different factors affecting fertility are vigorously discussed in the literature.
The study is properly designed, also with respect to methodology. The manuscript is properly written and the results are properly discussed.
Response: Thank you.

However, the study has some small limitations, especially related to how the results are discussed. The authors listed strengths and limitations of the study. However, the lack of thyroid tests/ thyroid antibodies should be added to that list. Whereas these data were probably not available to the authors, this fact should be stressed, as prevalence of thyroid dysfunction, especially subclinical hypothyroidism, in women at reproductive age is very high, with prevalence of positive thyroid antibodies being as high as at least 15% in this population. It should be also stressed that both thyroid dysfunction and positive thyroid antibodies affect strongly fertility rate.
Response: We agree with the reviewer that lack of thyroid tests/thyroid antibodies should be acknowledged limitation and we have added this to the limitation section. (P.17, Lines 7-10)

The authors have found that women with unfavorable pre-pregnant lipid profile had higher risk of having no or only one child. Taking into account few previously published studies and from logical point of view, these results were expected. When discussing potential mechanisms of the results, the authors underlined the linkage with cardiovascular disease. Whereas this is undoubtedly true, other – more direct mechanisms – should be discussed. The authors mentioned the potential role of oxidative stress (reference 19) and, in my opinion this issue should be discussed more broadly. It has been published recently (TSH ≥2.5 mIU/l is Associated with the Increased Oxidative Damage to Membrane Lipids in Women of Childbearing Age with Normal Thyroid Tests. Horm Metab Res 2017;49:321-326) that in women of childbearing age with normal thyroid tests, TSH in higher normal ranges was associated with higher oxidative damage to membrane lipids and less favorable lipid profile, and the

direct linkage between unfavorable lipid profile and increased oxidative damage was confirmed in this study.
Response: We agree with the reviewer that the role of oxidative stress should be elaborated more broadly, taking into account more direct mechanisms that link oxidative stress, CVD and fertility issues. This section now reads: “One possible molecular mechanism could be through a mediating role of HDL on Paraoxonase 1 (PON1) activity. Paraoxonase (PON) is an HDL-associated enzyme that inhibits LDL oxidation, and thus protects cells from oxidative stress.(20) Its stability and binding affinity is strongly influenced by changes in shape and size of HDL particles.(21) These changes may lead to decreased antioxidative capacity and consecutively – oxidative stress. Oxidative stress is associated with adverse cardiovascular and fertility outcomes, including atherosclerosis, PCOS, preeclampsia, endometriosis and infertility.(19, 22) A recent study in women of reproductive age with upper normal ranges of thyroid-stimulating hormone has suggested a direct link between unfavorable lipid profile and increased oxidative membrane damage.(23)"

The authors have found that nulliparous women were older at the time of examination and had higher BMI. Because both are associated with higher oxidative stress, this issue should be discussed more broadly.
Response: We have added a comment on this aspect in the Discussion section (please see P. 15, Lines 26-35).

The finding that a significantly higher number of onechild mothers had in-vitro fertilization (IVF) in their first pregnancy (7.2% versus 2.6% in women with ≥ 2 births, $p< 0.001$) suggests that without assisted reproductive technologies a number of no-child mothers would be even higher, and this issue should be also discussed more broadly.
Response: We see the reviewers point. However, in our judgment a broader discussion of this issue will have limited value, as there are very few cases in both groups (35 cases among one-child mothers, 57 cases among women with two or more children).

Reviewer # 3
The manuscript aimed to investigate the association between pre-pregnant lipid profile and number of children. The experimental design of the study is adequate as well as the power of the sample size. Polycystic Ovarian Syndrome could be a cause of both lipid derangements profile and infertility. Did the authors take into account this issue?
Response: Yes we did, but we had only 3 cases in our sample (please see previous version P.9, lines 48 - 50).

Further, I suggest to provide an overview of this association in the discussion quoting and providing a comment on the following manuscripts:
1: Kim JY, Tfayli H, Michaliszyn SF, Arslanian S. Impaired Lipolysis, Diminished Fat Oxidation and Metabolic Inflexibility in Obese Girls with Polycystic Ovary Syndrome. J Clin Endocrinol Metab. 2017 Dec 6. doi: 10.1210/jc.2017-01958. [Epub ahead of print] PubMed PMID: 29220530.

2: Kiranmayee D, Kavya K, Himabindu Y, Sriharibabu M, Madhuri GLJ, Venu S. Correlations Between Anthropometry and Lipid Profile in Women With PCOS. J Hum Reprod Sci. 2017 Jul-Sep;10(3):167-172. doi: 10.4103/jhrs.JHRS_108_16. PubMed PMID: 29142444; PubMed Central PMCID: PMC5672721.

3: Moran LJ, Mundra PA, Teede HJ, Meikle PJ. The association of the lipidomic profile with features of polycystic ovary syndrome. J Mol Endocrinol. 2017 Jul;59(1):93-104. doi: 10.1530/JME-17-0023. Epub 2017 May 12. PubMed PMID: 28500248.

4: Palomba S, Falbo A, Chiossi G, Muscogiuri G, Fornaciari E, Orio F, Tolino A, Colao A, La Sala GB, Zullo F. Lipid profile in nonobese pregnant women with polycystic ovary syndrome: a prospective controlled clinical study. Steroids. 2014 Oct;88:36-43. doi: 10.1016/j.steroids.2014.06.005. Epub 2014 Jun 16. PubMed PMID: 24945113.
Response: We realize that subclinical forms of PCOS or underreporting of cases could be present. We have in the attached version added a comment in the Discussion section, including nr. 3 and 4 of your suggested references (please see P.14, Lines 36-41, included as current references: 29, 30). However, we find that a too extensive elaboration in the light of PCOS would be of limited value as we only had 3 diagnosed cases of PCOS in our study sample. (Please see previous version P.9, lines 48 - 50).

VERSION 2 – REVIEW

REVIEWER	Eric van Exel GGZinGeest/VUmc, department of psychiatry, Amsterdam the Netherlands
REVIEW RETURNED	21-Mar-2018

GENERAL COMMENTS	The authors have all issues adressed earlier by me.

REVIEWER	Karbownik-Lewinska Malgorzata Medical University of Lodz
REVIEW RETURNED	30-Mar-2018

GENERAL COMMENTS	The revised manuscript is acceptable for publication.