PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Relating the number of human leukocytes antigen mismatches to pregnancy complications in oocyte donation pregnancies: study protocol for a prospective multicentre cohort study (DONOR study)
AUTHORS	van Bentem, Kim; Lashley, Eileen; Bos, Manon; Eikmans, Michael; Heidt, Sebastiaan; Claas, Frans; le Cessie, S; van der Hoorn, Marie-Louise

VERSION 1 - REVIEW

REVIEWER	Dina El Demellawy University of Ottawa, Faculty of Medicine, Ottawa, Ontario, Canada
REVIEW RETURNED	05-Dec-2018

GENERAL COMMENTS	Thank you for giving me the opportunity to review this well written and valuable manuscript. The study parameters are clear and certainly fills a deficit in the literature on the parthenogenesis and mechanism of increased feto-maternal complications with oocyte donation. i have no reservation or suggestion on this manuscript.

REVIEWER	Rita Vassena Clinica Eugin, Spain
REVIEW RETURNED	11-Dec-2018

GENERAL COMMENTS	I would like to thank the authors for planning this study, which should shed light on the possible immunological connection between OD pregnancies and hypertensive pregnancy disorders. I would like the authors to consider the following points: In the abstract, the authors mention that their objective is to study the number and nature of HLA mismatches, however the study is powered to only study the association between said disorders and a high number of HLA mismatches. This could be made clearer in the abstract. In the abstract the authors describe the study in the future tense, but they also report in the main text that the coordinating center has started recruiting in 2016. It is unclear if there is still room to improve the study design, and to what extent. About the study design, the authors indicate that the maternal age will be taken into account by frequency match women in 5 years categories. However, in the general population, the average age for OD is significantly higher than IVF, which itself is significantly higher

	than natural conception. It is expected that the treatment group will cluster at the high end of the 5 year category, while the controls will cluster on the low end of it. Will this affect the results and their interpretation? Again about the design: there is no mention of the source of semen, however the use of donor semen has been shown in the literature to have a facilitating effect on hypertensive disorders, and that it adds to the chances of developing PE when OD is also used. The authors should take this into account by either excluding "embryo donation" as well as IVF with donated semen from their recruiting strategy (and possibly also gestational surrogacy). Along the same lines, it would be better to only include primiparous women in the study (a previous non PE pregnancy decreases the risk of a second PE pregnancy, but OD patients are typically primiparous). About the ethics, I suggest that the authors include in the report the information and consent forms, and describe in more details the procedures to obtain such consent. Also, it seems that the consent to participate is only requested from the patient included in the study (the pregnant woman). However, it seems that, at least for the request to collect cord blood after birth, which could be banked for the child's own future use or donated to a cord blood bank, the partner could also be informed (even if their consent might not be strictly needed). Finally, it seems from the "ethics and dissemination paragraph, that both partner and oocyte donor (and sperm donor one assumes) will be asked to fill a webform questionnaire. When will the donor and partner be asked for participation, and how? Will their consent be recored in writing? What happens when the donor is from abroad (the protocol includes OD carrier out abroad)?

REVIEWER	Juan A Garcia-Velasco IVI RMA Madrid, Spain Rey Juan Carlos University, Madrid, Spain
REVIEW RETURNED	28-Jan-2019

GENERAL COMMENTS	Much needed study, looking forward to their results

VERSION 1 – AUTHOR RESPONSE

Comments reviewer #1

Thank you for giving me the opportunity to review this well written and valuable manuscript. The study parameters are clear and certainly fills a deficit in the literature on the parthenogene-sis and mechanism of increased feto-maternal complications with oocyte donation. i have no reservation or suggestion on this manuscript

Thank you for your response and positive feedback on our manuscript. Indeed, we aim to clari-fy the association between hypertensive complications and oocyte donation pregnancy and hopefully, this may facilitates future strategies for immune tolerization and reduction of compli-cations.

Comments reviewer #2

In the abstract, the authors mention that their objective is to study the number and nature of HLA mismatches, however the study is powered to only study the association between said dis-orders and a high number of HLA mismatches. This could be made clearer in the abstract.

We adjusted the sentence on objective in the abstract in: ‘The association of high number of HLA mismatches (>5) between mother and fetus will be determined and related to clinical out-come and pregnancy complication’

In the abstract the authors describe the study in the future tense, but they also report in the main text that the coordinating centre has started recruiting in 2016. It is unclear if there is still room to improve the study design, and to what extent.

Since 2016, the department of gynaecology and obstetrics started with the inclusion of patients as described in section ‘Study population and recruitment’. To increase the number of patient inclusions however, we decided to expand the number of centres including patients. The de-sign of the study, being a prospective cohort study, did not change by the expansion of partici-pating centres. The original protocol of the study was approved by LUMC Medical Research Ethics Committee protocol in 2016 (study number P16.048) and only adjustments in participating centres have been made thereafter. As the multicentre study had yet to start, this manuscript is written in the future tense.

About the study design, the authors indicate that the maternal age will be taken into account by frequency match women in 5 years categories. However, in the general population, the aver-age age for OD is significantly higher than IVF, which itself is significantly higher than natural conception. It is expected that the treatment group will cluster at the high end of the 5 year cat-egory, while the controls will cluster on the low end of it. Will this affect the results and their interpretation?

According to the DAG, adjustment for age is necessary to minimize the effect of confounding. This adjustment is performed in the data collection stage by frequency matching in 5 years cat-egories. In addition, multivariable analyses is performed at the stage of data analysis. We hope that with these two methods confounding by age is limited. We clarified the two methods of limiting for confounding in the ‘control of bias’ section.

Again about the design: there is no mention of the source of semen, however the use of donor semen has been shown in the literature to have a facilitating effect on hypertensive disorders, and that it adds to the chances of developing PE when OD is also used. The authors should take this into account by either excluding "embryo donation" as well as IVF with donated semen from their recruiting strategy (and possibly also gestational surrogacy).

Along the same lines, it would be better to only include primiparous women in the study (a pre-vious non PE pregnancy decreases the risk of a second PE pregnancy, but OD patients are typi-cally primiparous).

Indeed, epidemiological studies show that limited seminal exposure or barrier methods as con-traception are associated with increased risk hypertensive complications [1, 2]. We are however restricted by low numbers of patients that are suitable for inclusion. In the Netherlands com-mercial and anonymous donation is forbidden by law [3], which affects the number of women that apply for oocyte donation. Furthermore, many patients wish to conceal the fact that their pregnancy was

conceived artificially (elsewhere) and will not mention the oocyte donation to medical personnel. To select only women that apply to oocyte donation with sperm of their own partner will further reduce the number of suitable patients. Therefore, to adjust for the confounding effect of ‘source of semen’ this factor will be included in our multivariate analysis.

Primigravidity is a risk factor for the development of hypertensive complications and OD wom-en are indeed typically primigravid women. However, according to our DAG, gravidity (or parity) is not a confounding factor for the relation between HLA mismatches and the development of hypertensive complications and therefore, it is not necessary to correct for this factor.

About the ethics, I suggest that the authors include in the report the information and consent forms, and describe in more details the procedures to obtain such consent. Also, it seems that the consent to participate is only requested from the patient included in the study (the pregnant woman).

However, it seems that, at least for the request to collect cord blood after birth, which could be banked for the child's own future use or donated to a cord blood bank, the partner could also be informed (even if their consent might not be strictly needed).

We have uploaded our patient information form and the Informed consent form. It should be noted that the original study protocol as approved by our Medical Research Ethics Committee protocol in 2016, is a more extensive protocol, with more research objectives than on immuno-logical aspects in oocyte donation than only the association between HLA mismatching and hy-pertensive complications. The procedure to obtain consent is described both in section ‘Study population and recruitment’ as in section ‘Ethics and dissemination’.

The consent to participate is indeed only requested from the patient, the pregnant woman. In the Netherlands, the collection of cord blood for commercial cord blood banks is not recom-mended nor facilitated [4]. We therefore do not find it necessary to request consent from the partner.

Finally, it seems from the "ethics and dissemination paragraph, that both partner and oocyte donor (and sperm donor one assumes) will be asked to fill a webform questionnaire. When will the donor and partner be asked for participation, and how? Will their consent be recored in writing? What happens when the donor is from abroad (the protocol includes OD carrier out abroad)?

As stated, this study is performed within our DONOR project; a more extensive project on im-mulogical aspects with oocyte donation pregnancy. To clarify this, we changed the first sen-tence in section ‘study design’ in ‘This study is performed within the DONOR project; a project on the, the DONation of Oocytes in Reproduction’. For the research objective in current manu-script however, information on the partner and donor is not necessary. We therefore removed this from the ‘ethics and dissemination’ section.

Comments reviewer #3

Much needed study, looking forward to their results

We thank the reviewer for the positive feedback.