Author's response to reviews

Title: Clinical Outcome of Preimplantation Genetic Diagnosis and Screening with the Use of Next Generation Sequencing

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Author's response to reviews: see over
Dear Editors,

Thank you for giving us the opportunity to revise the manuscript 8785075791195923 again. We have revised the main text according to the comments of the reviewers. A point-by-point response to the concerns has been provided and attached.

Please kindly reconsider the revised manuscript for publication in your Journal. If you have any questions, please feel free to contact us.

Best regards.

Yours sincerely,

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Reviewer's report

Title: Clinical Outcome of Preimplantation Genetic Diagnosis and Screening with the Use of Next Generation Sequencing

Version: 2 Date: 15 June 2014
Reviewer: Joris Vermeesch

Reviewer's report:

The authors went a long way in answering the reviewers comments. However, there remain two major and one extra comment they need to address.

1. In abstract: “Four embryos with false negative signals and 2 embryos with false positive signals in SNP array results were validated”. This statement is wrong as it shows prejudice and should read: Following validation, four embryos were proven false negative and 2 false positive in SNP arrays. However, I still disagree with the answer provided: the qPCR is not the right method. Although the qPCR corroborates the sequencing results, it is no proof. Polymorphic marker analysis would proof that only maternal or only a paternal allele is present, rather than it being a SNP array artefact. Hence, either the authors perform additional work or they can be more modest in their claims: this could be removed from the abstract and the discussion could be more cautious.

Thanks for your suggestion.

We agree with you that the qPCR is no proof. But the qPCR data in this study was proved to be reliable. Polymorphic marker analysis would be better. However, there are not sufficient DNA amount for polymorphic marker analysis recently. The claims and statement in the manuscript were revised according to your comment.

The statement in abstract was revised as “All the signals in NGS results were confirmed to be accurate by qPCR validation”.

The statement in discussion was revised as “There were 6 embryos with signals to be inconsistent with NGS and qPCR results in SNP array data. These signals may due to that only a maternal or a paternal allele is present in SNP array results etc., which can easily lead to false-negatives and false-positives”.

2. Within the literature some confusion exists about the clinical validity of aneuploidy screening. The authors state that several reports exist of clinical practice of aneuploidy screening in AMA and refer to Munnué et al and Liang et al. I agree there are a lot of reports using the method, but the proof that it is clinically valid is lacking. Actually, there is no improved baby take home rate in randomized trials (for a review, see for example Harper et al., 2008, Human Reproduction; Harper and Sengupta, 2012) and randomized trials on blastocysts are lacking. Hence, the benefits of aneuploidy screening remain hypothetical and this should be stated as...
such.

Thanks for your comment.

We agree with you that the proof of clinically valid of aneuploidy screening for AMA and randomized trials on blastocysts are lacking. The statement in manuscript was revised according to your suggestions.

The statement in discussion was revised as “The feasibility of NGS-PGS application for the patients with AMA or RM was evaluated as well, though the benefits of aneuploidy screening for this population remain hypothetical”. And the two references you mentioned were cited in the updated manuscript as well.

Extra comments:

1. Table 3: In 99 couples no embryo transfer was possible because lack of euploid embryo’s. From a clinical perspective it is relevant to add this to table 3: No of couples participating, No of couples with embryos transferred.

Thanks. The table 3 was revised adding the No. of couples participating according to your suggestion.

Note to editor and authors

2. I disagree with reviewer 1 that NGS is the better wording: The sequencing is not next generation anymore but is massive parallel sequencing. The argument is circular. I would leave the choice to the authors.

Thanks. As NGS was used in several references (Ref 38, Treff NR et al, Fertil Steril 2013; Ref 40, Martin J et al, Fertil Steril 2013). We will still choose next generation sequencing (NGS) according to the suggestion of reviewer 1.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

see first review