Author's response to reviews

Title: High resolution array comparative genomic hybridization and flow cytometry analysis of spontaneous abortions and mors in utero samples

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Version: 2 Date: 30 June 2009

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

Dear Iratxe Puebla,

Thank you very much for considering our manuscript entitled: “Array comparative genomic hybridization and flow cytometry analysis of spontaneous abortions and mors in utero samples” for publication in BMC Medical Genetics.

Below we have answered all questions by the reviewers point-by-point, and we have changed the manuscript where necessary.

Best regards,

Björn Menten

Reviewer: Howard R Slater
Reviewer’s report:

This manuscript describes use of array CGH for improved analysis of spontaneous miscarriage samples in combination with flow cytometry (FCM) to detect triploidy. FCM is a novel approach in this setting.

The major problem with testing this type of sample is culture failure and the authors describe how array CGH virtually overcomes this. This has been described previously.

Major Compulsory Revisions
1. The Abstract doesn’t explain why FCM is used; it needs a brief sentence.

This was added in the abstract.

2. It is not clear in the Methods Section whether uncultured cells were used for the FCM although I think they must have been. Details of how single cell preparations were made are absolutely necessary. Similarly, it should indicate that DNA was extracted from biopsy material not from cultures (I assume it had to have been). This is very important in terms of the efficiency argument.

This was adjusted in the Methods section: DNA was isolated from tissue samples.

3. In the Abstract Results and the main Results sections, it isn’t made clear that the higher detection rate is due to the increased test success rate rather than improved sensitivity, ie 23% of successful karyotypes were abnormal versus 24% for successful array CGH tests.

This was adjusted in the both sections
4. A key question is whether array CGH detects subtle, clinically significant abnormalities in this type of sample. None were found perhaps because the arrays used are of relatively low resolution. Comment is needed.

**A comment was added in the discussion section.**

5. In Results, the proportion (33%) of karyotyped samples affected by maternal contamination is staggering. I doubt that reference #4 is valid. I have seen data from external quality assessment programmes over many years containing data from approximately 30 laboratories where the XX/XY ration for this type of testing is essentially 1. Surely there must be a dissection problem and I wouldn’t have thought that the authors would want to publicise this quality of work. It shouldn’t be stated (2nd last para of Discussion) that maternal contamination is a ‘major obstacle’. Avoiding cultural artefact would be a different thing.

*We have indeed a very high proportion of maternal contamination in our samples, this is however probably due to the unbiased collection of samples. Many samples we receive for chromosome analysis are macerated or are from early curettages. In the first instance, the fetal material is very unlikely to grow, in the second instance it is very hard to do a good dissection of fetal material. If we would exclude all these cases for chromosome analysis, we would have much better results for our sex-ratio but we would not have a result in many cases.*

*The data in reference #4 are in keeping with similar data from other Belgian centres.*

*In the discussion it is adjusted that indeed avoiding cultural artefact is the major obstacle.*

6. The lst comment in Discussion raises the issue of whether 1Mb BAC arrays are ‘high resolution’. They are relative to karyotyping but are not relative to dense oligonucleotide arrays. This is relevant in the title.

**High resolution is removed from the title. Indeed, technologies are going very fast and nowadays 1Mb arrays are not the high resolution anymore.**

7. The range of abnormalities found is narrow considering 50% were 1st trimester. A comment would be appropriate. None of the aneuploidies detected would have been missed by MLPA subtelomere testing so what is the advantage of the more expensive array CGH if sensitivity is not higher? With a larger sample set, I'm sure that a few subtle abnormalities would be found, eg STS deletions or CMT1A/HNPP duplications/deletions.

*Although array CGH used to be very expensive, the advent of commercially available microarray platforms, make array CGH a price competitive alternative for molecular techniques such as MLPA subtelomere testing.*
In this study we have not detected any interstitial deletions or duplications, larger follow-up studies making use of high resolution array CGH might indeed reveal interstitial deletions and/or duplications.

8. A comparison with other ways of detecting polyploidy would be appropriate, especially in terms of cost.

In the discussion there is a short paragraph on other molecular techniques. Although these techniques such as subtelomere-MLPA can pick-up unbalanced translocations, array CGH is able to identify interstitial deletions. Moreover, array CGH also immediately shows the size of the rearrangement. In the last sentence of the discussion, it is mentioned that nowadays, commercial microarray platforms are very price-competitive to other molecular techniques. For polyploidy, FCM is by far the cheapest method (~10€) as compared to eg. QF-PCR (~50€).

In summary, the use of FCM is interesting but the manuscript would benefit from including much more detail on how it’s done and its advantages (if any) over other methods. The content of the manuscript is straightforward and sound but the Discussion could be improved with comparison (including costs) with alternative methods.

**Reviewer:** Moncef Benkhalifa  
**Reviewer’s report:**  
this manuscript is confirming the contribution of chromosomal abnormalities in reproductive pathology and mainly in first trimester this article showed clearly the benefit of array CGH for molecular karyotyping and the feasibility of the method non cultured cells

this manuscript is acceptable for publication with minor revisions

Major compulsory Revisions: Non

Minor Essential Revisions:  
Background  
page 3: add more references about chromosomes abnormalities on gametes, zygote and early embryo blocking (Pellestor et al, Benkhalifa et al, Munne et al, Gianarolli et al, Martin et al)

**As this is not an article about the mechanisms underlying chromosomal abnormalities in miscarriages, the authors only included references from Hassold et al., Jobanputra et al., Goddijn & Leschot.**

Methods  
Flow cytometry
give more detail about the protocol specially for the troubleshooting of the technique and clarify if there is a correction factor compared to internal control.

**The technique of flow cytometry is very straightforward. The protocol is carried out as described in the protocol as indicated in the text, no correction factor was used.**

Array CGH
explain if the clones are printed in duplicate or quatruplicate

**This information is available in reference 17.**

what's the risk rate of SNV in your bank

**The authors do not understand the exact meaning of this question.**

the hybridisation was done on reverse and forward slides or not in which base the hybridisation is accepted or rejected give more detail about the software and the data analysis what's the sensitivity of the system for low mosaic give more explanation about the identification of balanced disorders

**For more information on array CGH and the used protocol, we would like to refer to reference 17, as indicated in the text.**

Results
the history of maternal contamination should clarified more

**some words were added in the result section.**

flow cytometry: one result is missing

**this was added**

give more explanation about the DNA index and the risk of mosaicism

Discussions
can you discuss more the benefit of FCM compared to others techniques and the adverse benefit of the method

**FCM is by far the cheapest and easiest method for ploidy status determination. We do not exactly know what is meant by adverse benefit of the method.**

also discuss the limitations of array CGH in genetic diagnosis more than chromosomes and future development of arrays in general diagnostics
This is more a topic for another paper, and again I would like to refer to reference 17 on this issue, as well as a recently submitted article by Buysse et al.

Reviewer: Carla Rosenberg

Reviewer’s report:
The present paper compares the use of karyotyping to combined array-CGH and flow cytometry analysis to access the cytogenetics of spontaneous abortion material. Hundred samples have been analysed by the three approaches and the results discussed, showing that array-CGH is significantly more efficient in detecting abnormalities in abort material than classical cytogenetics, overall if complemented by ploidy evaluation by flow cytometry. Although some papers in the field have been published before (mentioned among the manuscript's references), results are limited and heterogeneous, and the data of this manuscript is wellcome. The high frequency of maternal contaminant is a bit surprising and the paper could benefit of some more discussion about the subjet.

The paper should be published, but carefully reviewing the manuscript is recomended. Some examples:

1. The conclusion of the abstract does not make sense or seem to be incomplete: "This study convincingly shows that array CGH analysis for detection of numerical and segmental imbalances in combination with flow cytometry."

This sentence was completed

2. The flow cytometry results do not add up to 100 samples: "The results of 3 samples could not be interpreted due to the presence of a high percentage of apoptotic cells. Ninety-three samples showed a diploid DNA content whereas 3 samples were DNA-triploid (DNA-index # 1.45)."

This was a mistake in the text: ninety-four samples showed a diploid DNA content.

3. This reviewer does not see how quantitative PCR can be used to determine ploidy.

With quantitative PCR you cannot determine polyploidy, but you can determine aneuploidy. With QF-PCR (quantitative fluorescent PCR) you can detect polyploidy and aneuploidy.

4. In toto and other Latin expressions should be in italic.

This was changed to italics.