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Letter

Response: how PGS/PGT-a laboratories succeeded in losing all credibility

To the Editor

We commend the passion with which Dr Gleicher et al. (2018) defend their business model, denouncing preimplantation genetic screening (PGS) as their marketing differentiator from other centres. It would be idealistic if this was a purely academic discussion, however there are commercial interests on both sides and whilst the authors discuss theories and opinions, this reply reports facts and data supporting PGS.

The scientific evidence that euploid embryos have higher ongoing pregnancy rates per transfer than embryos replaced at random in patients 35 and older is overwhelming (Forman et al., 2013; Munné et al., 2017a; Rubio et al., 2017; Scott et al., 2013a; Yang et al., 2012). In addition, as we reported initially (Harton et al, 2013) and as is now also evident in the latest Society for Reproductive Technology (SART) data on implantation rates, cycles without preimplantation genetic testing (PGT) – the new terminology for PGS – show the typical decrease with age, while fresh transfer cycles with PGT show a lower decrease, and frozen cycles with PGT show no decrease at all (the difference between fresh and frozen being very interesting *per se* but the subject of another commentary). Miscarriages are also significantly reduced and therefore ongoing pregnancy rates per transfer increased. PGT, being a selection tool, does not improve cumulative pregnancy results. However, it does reduce time to pregnancy and pain and suffering resulting from miscarriages or affected babies.

Dr Gleicher and colleagues are both too clever to be confused, and clever enough to cause confusion. Let us clarify (as opposed to confuse) some of their statements:

- The ASRM Practice Committee from 2008 they mention comments on PGT-v1 (day 3 biopsy, FISH), a technique not used nowadays and not related to the issue of mosaicism at hand since mosaicism is not detectable on single blastomere biopsies.
- 'Unacceptably high rates of false-positives': PGT-v1 error rates have been widely published and the same has been done for PGT-v2 (Fiorentino et al., 2014; Kung et al., 2015; Wells et al., 2014; Yang et al., 2015). Unacceptable or acceptable is a personal decision between the patient and the physician. For PGT-v1, the error rate was higher and well known at about 10%, and stated clearly in reports and consents. For PGT-v2 using NGS technology and adding mosaicism screening, the error rate for classified euploid and fully aneuploid embryos is <1%. However, we now classify an intermediate group as mosaic, which has intermediate pregnancy potential (Fragouli et al., 2017; Friedenthal et al., 2018; Greco et al., 2015; Grifo et al., 2015; Maxwell et al., 2016; Munné et al., 2016, 2017b; Spinella et al., 2018).
- Embryos are graded morphologically based on their potential for implantation. What is wrong with NGS results offering a gradient? A gradient is always better than a black or white result with a 10% error rate. Now the black and white (euploid or aneuploid) sections of the gradient have close to 0% error rate.
- There is no evidence that 'large numbers of perfectly normal embryos with normal pregnancy potential' have been discarded, beyond the mentioned error rate. The fact that the error rate for euploid and aneuploid has been now reduced to almost 0% using NGS should be a matter to rejoice, not the contrary. Gleicher and colleagues seem to promote the idea of proactively transferring fully aneuploid embryos. There is limited data available on that approach, but in a small series of 10 fully aneuploid embryo transfers only one implanted and died shortly after birth (Munné, unpublished data). Who is being irresponsible?
- The authors mention increasing numbers of healthy newborns were reported following transfer of so-called 'aneuploid' embryos, but again the authors introduce confusion by mixing reports that used techniques that did not differentiate aneuploid from mosaic embryos with those that did. Mosaic embryos are not fully aneuploid embryos: these two terms should not be used interchangeably.
- The upper and lower limits of the mosaic gradient are not arbitrary. On average, a blastocyst biopsy contains 5 cells; thus the technique can at most recognize 1/5 – 4/5 abnormal cells to classify the sample as mosaic. Obviously, this is an average, as sometimes there are fewer and sometimes more cells, and depending on the sample quality and cells biopsied the mosaicism

https://doi.org/10.1016/j.rbmo.2018.06.021

detection could increase or decrease. The fact is, depending on the abnormal cell load there is evidence that the embryos will have different pregnancy potential (Fragouli et al., 2017; Munné et al., 2017b; Spinella et al., 2018). A 25% mosaic is closer to the potential of a euploid embryo, while a 75% is closer to the potential of an aneuploid embryo.

- The authors complain that 'the sensitivity limitations of a piece of laboratory equipment now officially determine embryo fate' but the same occurs for any diagnostic test in medicine. For example, CAP/NYSDoH/ISO15189 requirements state that any assay needs a limit of detection and sensitivity.
- A mathematical model can provide completely different results depending on the premises upon which it is structured. There are questionable premises in their model, such as the high number of cells in a blastocyst, which may invalidate such analysis. Irrespective of that, real-world data provides us with an error rate of close to 0% for PGT using NGS (Fiorentino et al., 2014; Kung et al., 2015; Wells et al., 2014; Yang et al., 2015), as well as very few false negatives. All of this effectively negates statements such as 'The absurdity of this explanation was demonstrated in mathematical models which established that even 100% aneuploidy in a single 6-cell TEB could mathematically not establish high enough probability that such a finding would suggest aneuploidy for the whole trophectoderm'. From doubting mosaicism to doubting the existence of aneuploidy?
- The paper by Bolton et al. (2016) shows that abnormal cells in mice embryos are not clonally distributed but mostly distributed at random. More studies are needed to confirm that finding, but regardless of clonality or not, the fact is that embryos classified by NGS as purely aneuploid or euploid are more likely to be so than with other techniques. The grey zone of mosaic embryos needs to be further studied and segmented, but incipient evidence suggests that a higher load of abnormal cells results in lower pregnancy rates (Fragouli et al., 2017; Munné et al., 2017b; Spinella et al., 2018).
- We question the 50–80% mosaicism rate quoted by the authors. Using FISH in large studies showed a rate of around 30% mosaicism in day 3 embryos (Colls et al., 2007; Munné et al., 2007), and around 20% in day 5 embryos using NGS (Fragouli et al., 2017; Munné et al., 2017b; Spinella et al., 2018). If an embryo is fully aneuploid for one chromosome (meiotic origin) and mosaic for another (mitotic origin), the embryo should be classified as fully aneuploid. Because of that, with advancing maternal age the rate of mosaicism seems to decrease since more embryos are classified as fully aneuploid, ranging from 23% in young patients to 12% in over 42-year olds.
- By their logic, if mosaicism was in 50–80% of embryos and if that had no effect on embryo viability then it would be seen very widely pre/postnatally. However this is not the case after 40 years of chorionic villus sampling (CVS) and more recent non-invasive prenatal testing (NIPT) results, where mosaicism is seen at no more than 2% incidence. This is even before you look at the PGS data where mosaicism is clearly seen and can be correlated with lower live birth rates and higher miscarriage rates (Fragouli et al., 2017; Munné et al., 2017b; Spinella et al., 2018).
- The authors ask why, if there is self-correction in mosaic mice embryos (Bolton et al. 2016), PGT has any validity? Again they try to confuse the reader by equating mosaicism with aneuploidy (in the Bolton paper fully aneuploid embryos did not implant!). The mechanism of self-correction in the Bolton et al. (2016) paper is that euploid cells in mosaic mouse embryos divide faster than abnormal ones and eventually take over and/or that the abnormal ones, especially those created in the Bolton paper, are non-viable. Then, the more euploid cells an embryo has the higher is its chance to develop. But a fully aneuploid embryo cannot self-correct in this way because it has no euploid cells (as also illustrated by the Bolton paper). Thus, PGT has validity. Besides, this is a mouse model. Mice have 3% aneuploidy rates while humans have 40%. This paper might not be relevant to humans, regardless of whether or not it supports PGT.
- In regard to the statistical analysis in the Munné et al. 2017b paper, ROC curves were not considered by our statistician for several reasons. First, if we group the information according to the type of mosaic, a loss of information is produced mathematically and statistically, which is reflected in the ROC curves. In addition, the fact that the data are measured on a dichotomous scale also entails an unreliable interpretation of these curves. Additionally, the sample size is not suitable for the construction of these curves, and even less so if we group by type of mosaic. This would require estimating a distribution of information that also provides a posterior bias to the ROC curves. In short, and due to these facts, we consider that the contribution of the ROC curves in this study is not at all reliable and, on the contrary, we think that the logistic model proposed is the most suitable data analysis available for the study. In retrospect, this is now a moot point because adding the results of Fragouli et al. (2017), Munné et al. (2017b) and Spinella et al. (2018) clearly show a difference in ongoing pregnancy rates between mosaics with a high or low percentage of abnormal cells.

If Dr Gleicher and colleagues want to take issue with PGT, for reasons of business, pleasure, or science, perhaps they should focus on blastocyst biopsy and not mosaicism. How is it possible that with 20–60% abnormality rates in egg donor blastocysts (Munné et al, 2017c) there is no evidence (SART data, Munné et al., 2017a) of PGT benefiting younger patients? To us it seems that the blastocyst biopsy effect should be re-visited since there is no standardization and great variability between centers, Sub-optimal procedures may be causing damage which is only compensated at older ages when the selection potential of the technique is higher. While Dr Gleicher and colleagues are still shaking a fist at PGS v1, the field is moving towards v3, i.e. non-invasive PGS.

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