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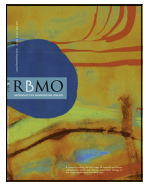

COMMENTARY

# Shooting STAR: reinterpreting the data from the 'Single Embryo TrAnsfeR of Euploid Embryo' randomized clinical trial

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## ABSTRACT

Preimplantation genetic testing for aneuploidy (PGT-A) still remains controversial in clinical practice. Recently, the randomized controlled trial, 'Single Embryo TrAnsfeR of Euploid Embryo' (STAR) by Munné and coworkers showed a similar live birth rate per intention to treat in the two study groups (PGT-A and controls). A wrong diagnosis and/or biopsy-related damage to the embryo might underlie these results. To assess the impact of these factors on the efficiency of PGT-A, the live birth rate of 'euploid' embryos transferred in the PGT-A group was compared with its ideal value, namely the live birth rate of embryos with the potential to implant and to give rise to a baby in the control group. This estimate has been derived using the results of the genetic testing reported in the STAR trial. According to this model, the STAR trial has demonstrated that transferring only blastocysts classified as 'euploid' after PGT-A leads to a reduction from 82.2% to 50.0% of the live birth rate for competent embryos, thus supporting the idea that PGT-A is associated with some embryo wastage.

## INTRODUCTION

This opinion paper aims to provide a different point of view deriving from analysis of the results of the randomized clinical trial (RCT), the 'Single Embryo TrAnsfeR of Euploid Embryo' (STAR) (*Munné et al., 2019*). The study evaluated the pregnancy success rate in women aged 25–40 years randomized for single frozen–thawed embryo transfer with embryo selection based on morphology (control group, *n* = 331) versus preimplantation genetic testing for aneuploidy (PGT-A) euploid status plus morphology (PGT-A group, *n* = 330). The results obtained would indicate that the live birth rate per intention to treat was similar for the two groups (41.8% versus 43.5% for PGT-A and control groups, respectively), suggesting that PGT-A may not improve the success rate of assisted reproductive technology procedures.

Importantly, the outcome of the study by *Munné et al. (2019)* cannot be considered on a par with other RCT, where the lack of differences is only indicative of a lack of efficacy of the technique/treatment tested. Indeed, as previously illustrated by *Paulson (2017)*, if the efficiency of PGT-A was 100%, the pregnancy rate of an unscreened population should be lower than that of the screened patients by the proportion of 'normal' embryos. In contrast, in the study by *Munné et al. (2019)*, there could be three different reasons to explain a similar result in pregnancy rates between the two study groups: (i) the biopsy induces damage to the embryos, causing them to implant less frequently than those not subjected to biopsy; (ii) potential

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normal embryos are classified as 'not euploid' (false positive); (iii) abnormal embryos are classified as 'euploid' (false negative).

### Aim

To assess the impact of these factors on the efficiency of PGT-A, the main objective of this analysis was to compare the live birth rate of 'euploid' embryos (LBR = 50.0%) transferred in the PGT-A group with its ideal value ($LBR_N$), namely the live birth rate of the embryos with the potential to implant and to give rise to a baby in the control group. Because data come from an RCT and the groups were extremely homogeneous, this estimate has been derived using the results of the genetic testing reported in the paper. In the control group, those genetic results were reported for all the embryos except for those that were transferred in the first warming cycle.

### The data analysis

The model is built on the assumption that each of the transferred embryos may or may not have the potential to implant and to evolve into a live birth. The transferred embryos with this potential are referred to as normal 'N'. A hypothetical PGT-A treatment will consequently have the ability to classify as 'euploid' a certain number ($N_{EUP}$) of these 'normal' embryos with pregnancy potential. The number of 'normal' embryos potentially wrongly classified as 'not euploid' by PGT-A analysis is defined as $N_{noEUP}$ (the mosaics are included in this group, according to the definition of mosaics considered in the STAR trial). It is also assumed, to simplify, that there are no false negative results due to the technique.

$LBR_N$ is defined as the probability for 'normal' embryos with pregnancy potential to implant and evolve into a live birth in the control group, and based on previous assumptions it is expressed here as:

$$LBR_N = \frac{\text{No. of babies}}{N_{EUP} + N_{noEUP}}$$

If it is assumed that the PGT-A makes no mistakes (in other words, supposing an efficiency of 100%, which is the same assumption as in the STAR trial), then the only embryos with potential for pregnancy would be those classified as 'euploid' by a hypothetical PGT-A analysis. In this case, the $N_{noEUP}$ term should be removed from the formula, giving the following:

$$LBR_N = \frac{\text{No. of babies}}{N_{EUP}}$$

where $N_{EUP}$ is the estimate of the potentially 'euploid' blastocysts in the control group obtained assuming the same distribution of results from genetic testing observed in the PGT-A group. The calculation of this value is described in the Methods section and in TABLE 1 and resulted in a total of 173.9 potentially 'euploid' blastocysts (95% confidence interval [CI] 131.1–217.1) transferred in the control group. Thus, given the number of babies obtained in the PGT-A group ($n$ = 137) and in the control group ($n$ = 143) and considering that 274 'euploid' blastocysts have been transferred in the PGT-A group, the percentage of live births according to the number of 'euploid' blastocysts transferred can be calculated. The live birth rate was 50.0% (137/274, 95% CI 44.1–55.9%) for the PGT-A group, which is much lower than that deriving from the potentially 'euploid' blastocysts in the control group (82.2%; 143/173.9, 95% CI 65.9–100).

To evaluate whether the inclusion of mosaic embryos in the control group could explain this difference, a second estimate was conducted. This time the $N_{noEUP}$ term, containing the estimate for the number of transferred blastocysts that would have been potentially classified as 'mosaic', was included in the analysis. Even assuming that every 'mosaic' embryo can implant with the same probability as a 'euploid' embryo, the $LB_N$ for the control group would be equal to 60.4% (95% CI 51.2–73.7%, calculated using the previous approach).

## DISCUSSION

The efficiency of PGT-A is the subject of fierce debate and mathematical models have already demonstrated a risk of embryo loss (*Alteri et al., 2019*; *Paulson, 2017*; *Somigliana et al., 2019*). According to the model used here, the STAR trial has demonstrated that transferring only blastocysts classified as 'euploid' after PGT-A leads to a reduction from 82.2% to 50.0% of the LBR for 'normal' embryos. This is equivalent to an embryo loss rate of 39.2%, expressed according to Paulson's view (see formula for embryo loss rate by *Paulson, 2017*). In other words, the possibility of a wrong diagnosis or embryo damage induced by the biopsy would represent an important limitation of the technique.

It could be postulated that this embryo loss is associated with the presence of mosaic embryos in the control group. For this reason, in a subsequent analysis, the potential impact on live birth rate of the embryos classified as 'mosaic' according to the frequency observed in the PGT-A group were included. It is important to underline that the conditions used for this second estimate are intentionally unrealistic and exaggerated, in order to guarantee a very conservative evaluation. Despite the use of assumptions in favour of the PGT-A group, the result still shows a non-negligible loss of embryos. This result is equivalent to an embryo loss rate of 17.2%.

The use of a mathematical model like the one presented here certainly has some limits. First of all, the use of an inference for the number of potentially 'euploid' and 'mosaic' embryos in the control group based on the real frequency of the PGT-A group. However, it must be acknowledged that the whole clinical study (RCT) was planned so that the two groups were comparable, and it is therefore legitimate to expect the same frequencies of 'euploid' and 'mosaic' embryos in the two groups. It should also be emphasized that the model did not just use the estimates of 'euploids' and 'mosaics' of the PGT-A group. Indeed, data derived from PGT-A performed on the supernumerary embryos in the control group were also employed to estimate the number of 'euploid' and 'mosaic' embryos transferred in the control group. This approach has allowed inclusion in the model of the enrichment in 'euploid' and 'mosaic' embryos associated with the embryologist's selection of the embryos with the best morphology to be transferred in the control group. On the other hand, due to the lack in the STAR trial of detailed data for the genetic testing in the control group, the use of this approach does not allow separate analysis of the two main age groups (25–34 years and 35–40 years) presented. However, it is possible to perform a simplified calculation not taking into account the enrichment in 'euploid' and 'mosaic' embryos associated with the embryologist's selection of the embryos. The results, calculated assuming the same implantation rate for 'euploid'

**TABLE 1 DATA FOR THE GENETIC TESTING RESULTS FROM *MUNNÉ ET AL. (2019)* AND RELATIVE CALCULATED ESTIMATES FOR THE CONTROL GROUP**

| | PGT-A group | 95% CI lower limit | 95% CI upper limit | Control group | 95% CI lower limit | 95% CI upper limit | Estimated |
|---|---|---|---|---|---|---|---|
| No. of embryos | 2178 | | | 2071 | | | |
| 'Euploid' frequency | 43.1 | 41.1 | 45.2 | 43.1 | 41.1 | 45.2 | √ |
| No. of 'euploid' | 939 | – | – | 892.9 | 850.1 | 936.1 | √ |
| No. of 'euploid' in supernumerary embryos | 665 | – | – | 719 | – | – | |
| No. of 'euploid' in transferred embryos | 274 | – | – | 173.9 | 131.1 | 217.1 | √ |
| Babies | 137 | – | – | 143 | – | – | |
| Live birth rate | 50.0 | 44.1 | 55.9 | 82.2 | 65.9 | 100 | √ |
| 'Euploid + mosaics' frequency | 59.9 | 57.8 | 62.0 | 59.9 | 57.8 | 62.0 | √ |
| No. of 'euploid + mosaics' | 1305 | – | – | 1240.9 | 1197.9 | 1283.1 | √ |
| No. of 'euploid + mosaics' in supernumerary embryos | 1031 | – | – | 1004 | – | – | |
| No. of 'euploid + mosaics' in transferred embryos | 274 | – | – | 236.9 | 193.9 | 279.1 | √ |
| Babies | 137 | – | – | 143 | – | – | |
| Live birth rate | 50.0 | 44.1 | 55.9 | 60.4 | 51.2 | 73.7 | √ |

and 'mosaic' embryos, showed an embryo loss rate of 39.2% and 29.9%, respectively, for the women aged 25–34 years and 35–40 years. This means that an embryo wastage associated with PGT-A could be demonstrated in both age groups. One therefore may ask why the clinical outcomes were better for the 35–40 years group when PGT-A was performed. In this context, it is important to underline that only the results of the first transfer were presented, but no data were provided for the residual blastocysts.

In conclusion, results from the STAR trial showed, rather than a lack of improvement in live birth rate, that the indiscriminate use of PGT-A implies a loss of potentially competent embryos due to the wrong diagnosis and/or the biopsy-related embryo damage. Based on the results of the study by *Munné et al. (2019)*, the safety of the biopsy should be carefully reconsidered.

## METHODS

The data used to estimate the number of 'euploid' ($N_{EUP}$) and 'euploid + mosaic' ($N_{EUP} + N_{noEUP}$) embryos transferred in the control group are shown in TABLE 1. Considering the total number of embryos obtained in the control group ($n$ = 2071, of which 1758 supernumerary and 313 transferred), it was assumed that the frequency of 'euploids' and 'euploids + mosaics' for the control group was the same as the PGT-A group. Multiplying these frequencies for the number of embryos in the control group, the total number of 'euploid' ($2071 \times 0.431 = 892.9$) and 'euploid + mosaic' ($2071 \times 0.599 = 1240.9$) embryos expected in the control group was then calculated. Because the real number of 'euploid' ($n$ = 719) and 'euploid + mosaic' ($n$ = 1004) embryos, as reported in the paper, is known for the supernumerary biopsied blastocysts of the control group, those numbers were subtracted from the previous estimates to obtain the number of transferred 'euploid' ($892.9 - 719 = 173.9$) and 'euploid + mosaic' ($1240.9 - 1004 = 236.9$) embryos.

Notably, this approach allowed us to also include in the model the enrichment in 'euploid' and 'euploid + mosaic' embryos linked to the selection of the embryos with the best morphology to be transferred in the control group. Because the estimated frequency of 'euploid' embryos transferred in the control group was 55.6% (173.9 'euploid' over 313 transferred embryos), while the reported frequency for 'euploids' in supernumerary embryos was indeed equal to 40.9% (719 'euploid' over 1758 embryos), the estimated enrichment of the 'euploid' embryos thanks to the embryologist's selection corresponded to an odds ratio of 1.81. In the same way, an odds ratio equal to 2.34 has been estimated for the enrichment in the frequency of transferred 'euploid + mosaic' embryos for the control group when compared with the observed frequency in supernumerary embryos.

The 95% CI for the known proportions was calculated using the Wilson score method without continuity correction. The CI for all the estimates were obtained applying the same calculation previously described and using the lower and upper limits of 95% CI for the 'euploid' and 'euploid + mosaic' embryo frequencies.

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