Sir,

We read with interest the recent review by Santiago Munné entitled, “Preimplantation Genetic Diagnosis for Aneuploidy and Translocations Using Array Comparative Genomic Hybridization” (1). As part of the review of array comparative genome hybridization (aCGH), the author provides additional information on other 24-chromosome preimplantation genetic diagnosis/screening (PGD/PGS) techniques. As a commercial lab that offers single nucleotide polymorphism (SNP) microarray analysis for PGD/PGS, we would like to comment on a few claims that were made within this article regarding SNP microarrays.

(Table 1) in the article summarizes the differences between 24-chromosome PGD/PGS techniques. This table contains a number of errors regarding SNP microarray detection capabilities as it groups the different approaches of SNP microarray analysis under a single heading of “SNPs”. SNP technologies that employ a combination of qualitative and quantitative data analysis detect far more abnormalities than those that use just one type of analysis. First, it is not accurate to say that “SNPs” cannot detect tetraploidy. SNP microarrays using qualitative/quantitative analysis can detect some forms of tetraploidy; this method will not detect 2:2 tetraploidy, though is indeed capable of detecting 3:1 tetraploidy. Second, SNP microarray technologies that use a qualitative/quantitative approach can detect meiotic and mitotic duplications without recombination (3); the table incorrectly states that SNP microarray approaches cannot detect these abnormalities. Lastly, it is an exaggeration to state that aCGH approaches are able to detect all unbalanced translocations and SNP microarray approaches can only detect some; both approaches are equally limited in their inability to detect very small deletions and duplications (both have a similar threshold in that they typically detect DNA segments greater than 6Mb).

We also question a number of statements the author makes about aCGH and SNP microarray within the body of the paper. The author acknowledges that aCGH cannot detect haploidy or polyploidy but claims that this is a small limitation, as the majority of the haploid or polyploid embryos tested (7.7%) had additional detectable abnormalities; however, these additional abnormalities are not named. It is our experience that other abnormalities are not typically found with 69,XXX. Next, the author credits SNP microarray with the ability to detect uniparental disomy (UPD) but then goes on to use the incidence of UPD of chromosome 15 (UPD-15) to say that UPD in general is a very rare event. Chromosome15 is only one of six imprinted chromosomes (6, 7, 11, 14, 15, and 16), which if UPD is present, could lead to the birth of a baby with a severe genetic syndrome (4). We feel that detection of UPD prior to embryo transfer decisions is highly beneficial to, and desired by, couples undergoing IVF with PGD/PGS. Furthermore, in regards to PGD/PGS for reciprocal and Robertsonian translocations, we would like to clarify that not all SNP microarray approaches can differentiate between normal and balanced (carrier) embryos. Last, the author correctly points out that SNP microarray approaches require parental DNA analysis prior to the embryo sample analysis. However, our lab does not charge a cancellation fee for this parental analysis when IVF cycles are cancelled, thus patients do not pay for unnecessary parental testing.

We appreciate that the author mentions SNP microarray analysis that employs a qualitative/quantitative approach will avoid many of the limitations inherent to the purely qualitative or quantitative approaches. However, the review references our article (Johnson DS et al. [2], reference 77 in the original paper) as an aCGH technology, when in fact we utilize a SNP-based approach with bioinformatics analysis. Natera’s PGD/PGS Parental Support™ method utilizes SNP measurements of parental and embryonic samples, giving us the ability to analyze both qualitative and quantitative data from each chromosome to determine the embryonic chromosomal copy number. In addition, the Parental Support™ method utilizes a much more sophisticated bioinformatics-based analysis (2) than the combined approach described in the article.

New methods should always be validated against more established ones, but given the errors rates reported with FISH PGD/PGS (as stated by the author at the beginning of the paper), we strongly disagree that validation studies using FISH for reanalysis should be considered the gold standard (2, 5). Natera thus supports the creation of an oversight body to administer proficiency testing for laboratories offering 24-chromosome PGD/PGS.

Sincerely,

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REFERENCES

[1] Munné S. Preimplantation genetic diagnosis for aneuploidy and translocations using array comparative genomic hybridization. Current Genomics 2012; 13: 463-470.
[2] Johnson DS, Gemelos G, Baner J, et al. Preclinical validation of a microarray method for full molecular karyotyping of blastomeres in a 24-h protocol. Hum Reprod 2010; 25: 1066-1075.
[3] Rabinowitz M, Ryan A, Gemelos G, et al. Origins and rates of aneuploidy in human blastomeres. Fert Steril 2012; 97: 395-401.
[4] Shaffer LG, Agan N, Goldberg JD, et al. American College of Medical Genetics Statement on Diagnostic Testing for Uniparental Disomy. Genet Med 2001; 3: 206-211.
[5] Staessen C, Platteau P, Van Assche E, et al. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. Hum Reprod 2004; 19: 2849-2858.
LETTER TO THE EDITOR BY SANTIAGO MUNNÉ

Sir,

We stand by our paper and the previous one by Bisignano et al. (2011) but would like to add a few clarifications.

Depending on the density of the array used, aCGH has a resolution between 6Mb and 1Mb. The paper of Fiorentino et al. (2011) in Fig. (3) illustrates an abnormal embryo from a 46,XY,t(3;18)(p26.3;q12) translocation with a 2.5M deletion in chromosome 3 (3p26.3-pter). Furthermore, any unbalanced translocation can be detected if three of the four chromosome arms are detected, therefore for a translocation to be missed with the high-resolution aCGH chip, it will require two fragments to be below 1Mb. Indeed of 926 procedures of PGD for translocations previously analyzed by FISH, none had two such small fragments (Colls et al., 2012), although a few have been described recently.

About polyploidy, it is well known from the times of FISH (Munne et al. 1994) that the majority of monospermic polyploid embryos are chaotic mosaics. For the present paper, we retrospectively reviewed the data on 91,073 embryos analyzed by FISH. Of those 7.7% were polyploid or haploid but the vast majority were again complex chaotic abnormalities, for example 2X2Y, 6[13], 4[14], 2[15], 2[16], 4[17], 6[18], 4[19], 4[20], 4[21], 4[22]. Others were a combination of polyploidy and aneuploidy, and the vast majority of haploid embryos were haplohaploid i.e. XO, 0[13], 1[14], 2[15], 0[16], 0[17], 1[18], 1[19], 0[20], 0[21], 1[22]. The theory advanced already in the 1994 paper is that monospermic polyploid embryos are usually slowly developing ones with some cytoplasmic but not karyoplasmic arrest, with those cells arrested still continuing to duplicate or divide a single chromosome set at random.

We do not argue that 69XXX embryos may be sometimes purely triploid, but there is a huge caveat. There are three or more potential sources of triploid embryos, one are polyspermic embryos, which are invariably complex chromosomally abnormal and selected against by embryologist because they have three pronuclei. The other source is second polar body retention after ICSI. Those could be perfectly polyplolid, but again embryologists usually select them against since they present a three pronuclei and a single PB. The third are giant eggs (Munné 2007), which are diploid instead of haploid, and thus are also selected against by embryologist. The few that escape embryologist detection, might indeed by purely triploid. In our database that is 0.2% of embryos (n= 91,000).

We refer the authors to our previous paper by Bisignano et al. (2011) in which we argued that the finding of UPD is being exaggerated by the fact that most embryos reported with UPD had other chromosome abnormalities, indicative of complex abnormalities. The finding of UPD is relevant in the absence of another deadly chromosome abnormality; otherwise the classification of the embryo is of complex abnormal, mosaic, or other, not UPD.

Error rates by FISH can be high or low depending on the lab. In our hands was 7% (Colls et al., 2007), all explained by mosaicism. The authors group has yet to present data on cell by cell reanalysis of non-replaced embryos avoiding the question of mosaicism. Even the best diagnosis cannot get around day-3 mosaicism and achieve an error rate below 2-3%, measured by full reanalysis of not replaced embryos. Using FISH to analyze all the other cells of previously analyzed abnormal embryos has a very low error rate since many cells are analyzed and compared to each other, and is a cheap way to validate a technique, but any other technique can be used to validate another one, provided that all the cells of the non-replaced embryo are analyzed to differentiate mosaicism from technical error, something not published yet by the corresponding authors of the letter.

Sincerely,
Santiago Munné, PhD
Reprogenetics

REFERENCES

[1] Bisignano A, Wells D, Harton G, Munné S. Preimplantation Ge- netic Diagnosis and Aneuploidy Screening for 24 chromosomes: advantages and disadvantages of competing platforms. Reprod Biomed Online. 2011, 23, 677-85.
[2] Colls P, Escudero T, Zheng X, Lenzi M, Cinnioglu C, Cohen J, Munné S. Increased efficiency of preimplantation genetic diagnosis for infertility through reanalysis of dubious signals. Fertil Steril 2007, 88, 53-61.
[3] Colls P, Escudero T, Fischer J, Cekleniak N, Ben-Ozer S, Meyer B, Damien M, Grifo J, Hershlag A, Munné S. Validation of array comparative genome hybridization for diagnosis of translocations in preimplantation human embryos. Reprod Biomed online, 24: 621-629
[4] Fiorentino, F., Kokkal, G., Biricik, A., Stavrour, D., Ismailoglu, B., De Palma, R., Arizzi, L., Harton, G., Sessa, M., Pantos, K., 2010. Polymerase chain reaction-based detection of chromosomal imbalances on embryos: the evolution of preimplantation genetic diagnosis for chromosomal transloca-
tions. Fertil. Steril. 94, 2001–2011.
[5] Munné S, Alikani M, Grifo J, Cohen J Monospermic polyploidy and atypical embryo morphology. Human Reprod. 1994, 9: 506-510.
[6] Munné S (2007) Chromosomal status of human embryos. In: Human Preimplantation Embryo Selection, chapter 18. Edited by J. Cohen and K. Elder. Taylor & Francis. Boca Raton, FL. Pp. 209-234.