Mosaicism: throwing the baby out with the bath water?

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With the advent of next-generation sequencing (NGS), cytogenetic laboratories now have the ability to detect low-level mosaicism in the trophectoderm of blastocysts [1]. An abstract presented by Dagan Wells, PhD, and his collaborators at the European Society for Human Reproduction and Embryology (ESHRE) Meeting titled: “Evidence that differences between embryology laboratories can influence the rate of mitotic errors, leading to increased chromosomal mosaicism, with significant implications for IVF success rates,” was well-received. In this study, the authors retrospectively analyzed 623 blastocysts from seven fertility clinics and suggested that under different laboratory conditions, the same embryo can have different mosaicism rates ranging from 32 to 60 % [2]. The abstract focuses on mosaicism-introduced post-fertilization (mitotic non-disjunction of the embryo) and therefore during laboratory culture, rather than due to meiotic non-disjunction of the oocyte.

The concept that laboratory conditions may cause post-fertilization aneuploidy or mosaicism in embryos is provocative. This could explain the large dissimilarities seen in aneuploidy rates among different clinical trials and why some

embryology laboratories see a decrease in their live birth rates per cycle start when performing preimplantation genetic screening (PGS) due to no euploid embryo being available for transfer [3–5]. Munne et al. first described a possible increase in post-fertilization mosaicism when comparing different embryology laboratories [6]. More recently, it has been shown that embryos with better morphological scores have fewer chromosomal errors [7, 8]. What specific embryo culture conditions can impact embryo morphology and possibly ploidy status? According to a recent systematic review and meta-analysis of randomized controlled trials (RCTs), culture at 5 % vs. 20 % oxygen concentration increased the number of high/top morphology embryos at the cleavage stage (RR = 1.2, 95 % CI 1.1–1.3) [9]. Different culture media with different recommended pH ranges support blastocyst development in culture, but the optimal pH level has not been established [10, 11]; animal models suggest that strict control of pH at different developmental stages improves embryo quality [12]. A recent RCT showed that embryos cultured at 37 °C have higher blastulation rates (60.1 vs. 51.6 %, p 0.03) and form more usable blastocysts (48.1 vs. 41.2 %, p 0.03) when compared to embryos cultured at 36 °C [13], indicating the need for strictly controlled temperature of incubators and heated surfaces in the laboratory. If we follow this same principle, variations in the biopsy technique such as location of biopsy in the trophectoderm, due to segregation of abnormal cell lines, and/or number of cells removed, may impact the level of mosaicism reported [14]. We know that controlling for these variables by strict adherence to protocols and training of staff lead to comparable and reproducible outcomes with no difference in aneuploidy rates [15].

If indeed there is variation among embryology laboratories of embryos being reported as mosaic, we need to ask ourselves at what stage is the insult occurring and what is its clinical significance? There is evidence that aneuploid cells proliferate more slowly than euploid cells, and if we diagnose an embryo

Capsule Causes and clinical implications of mosaicism detected by preimplantation genetic screening (PGS) have yet to be fully established. This commentary suggests possible evidence-based approaches to diagnosing and reporting mosaicism.

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as mosaic or aneuploid, it can still result in a healthy pregnancy [16, 17]. More recently, Tortoriello et al. performed a second trophectoderm biopsy in embryos initially deemed by cytogenetics to be aneuploid and found that 9/27 (33 %) were euploid when using a different technology and a different genetic laboratory [18]. Gleicher et al. found a similar false positive rate (36.4 %) when re-testing dissected embryos initially reported as aneuploid [19]. To date, studies have reported similar miscarriage rates in patients undergoing in vitro fertilization when compared to a sub-fertile population; thus, even if some laboratory conditions are correlated with increases in post-fertilization mosaicism, that does not automatically correlate to an increase in miscarriages [20]. It is impossible to know which cell line will propagate and so consequently the clinical significance of mosaicism is still not well-defined.

With new genetic platforms, ploidy status of blastocysts may no longer be limited to euploid or aneuploid, but categories in between that quantify the amount of mosaicism present [21]. Recently, the Preimplantation Genetic International Society (PGIS) consortium issued a statement in hopes of adding guidance while evaluating PGS results. They call for embryos with <20 % mosaicism to be treated as euploid, 20–80 % as mosaic, and >80 % as aneuploid [22]. The PGIS statement is followed by guidelines on how to prioritize the transfer of mosaic embryos. This is an important step to standardize reporting of PGS. A survey by Sermon et al. conducted among 12 well-established molecular biologists found that only two participants report the mosaicism level back to practitioners [23]. It will also be important for practitioners to understand the implications of having only mosaic embryos available for transfer.

In summary, while there is much speculation about the reasons for increased aneuploidies and mosaicism rates across laboratories, the clinical significance remains unknown. Laboratories not routinely performing PGS will likely see no change in their protocols, while laboratories routinely performing PGS may see a decrease in embryos available for transfer. PGS has now been widely adopted at IVF programs throughout the world without having been shown to increase live birth rates per cycle start. Recently, a major newspaper ran a story titled: “Questions About ‘Mosaic’ Embryos” [24]. We believe that this study and other recent research reports regarding mosaicism should open a broader dialog among clinicians and embryologists/ART practitioners who are strongly encouraged to practice evidence-based medicine with a clear understanding of the clinical implications of new technologies already in use today.

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