Technology versus biology: the limits of pre-implantation genetic screening

Better methods to detect the origin of aneuploidy in pre-implantation embryos could improve the success rate of artificial reproduction

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Assisted reproductive technologies (ART) have become the standard of care for the treatment of infertility. Within this realm, reliable prediction of the developmental potential of the pre-implantation human embryo remains an overriding priority. One such technology, pre-implantation genetic screening (PGS), is being increasingly deployed to select against embryonic aneuploidy [1]. However, a growing number of seemingly contradictory outcome reports are forcing a reevaluation of this approach. Here, we discuss how biological factors, notably mitotic aneuploidy during early embryonic development, limit the very rationale for PGS as a clinical diagnostic method.

“... PGS is an example of technology compromised by biology”

The development of safe and efficient methods to select healthy euploid embryos constitutes a pressing need to improve the success of ART. PGS, as one strategy to achieve this, relies on the ploidy status of the trophectoderm layer of the blastocyst-stage embryo [1] (Table 1). Considerable numbers of patients are undergoing PGS: In the USA alone, more than 5% of 106,902 non-donor assisted reproduction cycles resorted to PGS during 2011 and 2012 [2]. However, the capacity of PGS to select euploid embryos has recently been questioned by the birth of healthy newborns whose originating blastocysts were deemed

mosaic (euploid–aneuploid) [3]. Similar inconsistencies have previously been reported for euploid human embryonic stem cell lines, which have been derived from purportedly aneuploid blastocysts. We suggest herein that the utility of PGS is undermined by innate karyotypic mosaicism, the ontogeny and significance of which in early human development remain uncertain. Viewed in this light, PGS is an example of technology compromised by biology. It follows that the ability of PGS to reliably predict the ploidy status of the human embryo and its developmental potential is limited.

The causes of embryonic aneuploidy

Following fertilization and karyogamy, the human zygote undergoes 8–9 rounds of cell division to create the multicellular blastocyst. During this process, maternal RNA transcripts are degraded, the zygotic epigenome undergoes reprogramming, and the embryonic genome is activated. The blastocyst, the first lineage-specified embryonic structure, comprises an outer cohesive monolayer of polarized epithelium cells known as the trophectoderm (TE), a compact inner cell mass (ICM), and a fluid-filled cavity (blastocoel). The extraembryonic TE develops into the placenta while the ICM-derived epiblast and primitive endoderm (PE) give rise to the developing fetus. Overall, however, and despite significant progress in research, the biology of early human development remains largely unknown. Insights into the fate-specifying molecular programs of individual embryonic

cells in particular are wanting, although recent advances in single-cell RNA-sequencing have led to the identification of several lineage-specific transcription factors [4]. Further progress is anticipated from functional analyses using genome-editing technology.

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Aneuploidy, an abnormal number of chromosomes owing to erroneous segregation during meiosis or mitosis, is innate to early human development. Maternal age-dependent meiotic errors likely arise during the first meiotic division of the oocyte. In contrast, age-independent mitotic errors likely arise through non-disjunction, anaphase lag, endoreplication, or multipolar cell division during post-zygotic development [5]. The incidence of aneuploidy in day-3 cleavage-stage embryos is surprisingly high ($\leq 80\%$). A substantial aneuploidy rate still persists in day-5 blastocyst-stage embryos [5]. The origin of the aneuploidy, the number of extra chromosomes, their distribution in the early embryo, and the identity of the cells affected collectively determine the fitness of the embryo.

The lethal potential of embryonic aneuploidy depends largely on how many embryonic cells are affected, which in turn

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Table 1. Blastocyst developmental potential as inferred from the PGS biopsy ploidy status.

Biopsy ploidy status	Blastocyst ploidy status (potential cell line representation)			Blastocyst developmental potential (Inferred)
	Euploid	Aneuploid (Meiotic)	Aneuploid (Mitotic)	
Euploid	Yes	No	Yes	Uncertain due to potential concurrent aneuploidy of mitotic origin
Aneuploid	Yes	Yes	Yes	Inviabile if aneuploidy is of meiotic origin Uncertain if aneuploidy is of mitotic origin
Euploid–aneuploid mosaic	Yes	No	Yes	Uncertain
Aneuploid–aneuploid mosaic	Yes	Yes	Yes	Inviabile if aneuploidy is of meiotic origin Uncertain if aneuploidy is of mitotic origin

depends on whether the chromosomal aberration is of meiotic or mitotic origin. Meiotic aneuploidy, which occurs mostly during oocyte development, affects all embryonic cells. In contrast, the dispersion of mitotic aneuploidy is defined by the timing of its onset [5]: Errors arising during fertilization or soon thereafter are more broadly distributed than errors that materialize later. Mitotic errors invariably yield a mosaic embryo with two or more chromosomally distinct cell lines, the spatial dissemination of which is ill understood. The outcome of embryonic aneuploidy also depends on the identity of the cells affected. Aneuploidy of the ICM, a certainty in the case of meiotic errors, is often lethal, while aneuploidy of TE cells, owing to mitotic errors, may still lead to a viable fetus.

As the most common human genetic disorder, aneuploidy is a major cause of early pregnancy loss and congenital birth defects [5]. In 2013 alone, the last year for which reliable US data are available, 15.8% of pregnancies resulting from ART ended in a miscarriage [6]. A more nuanced analysis reveals this outcome to be maternal age dependent. The incidence of fetal loss among women aged 36 or younger was < 15% but rapidly increased to 29 and > 50% in women aged 40 and 44 and older, respectively. Autosomal aneuploidy of maternal origin likely plays an important role as maternal meiotic errors constitute the dominant pathology in miscarried conceptions. Fewer clinical miscarriages are caused by aneuploidy of mitotic origin though some cases of mosaicism may go undetected. These observations reinforce the role of meiotic errors in early fetal loss and the importance of their detection in ploidy screening paradigms.

Pre-implantation genetic screening for aneuploidy

Screening against aneuploidy in pre-implantation human embryos has been performed for the better part of the past two decades [1]. Screening of day-3 cleavage-stage embryos using fluorescence *in situ* hybridization (FISH) did not improve live birth rates in women of advanced age [1]. More recent methods have relied on day-5 multicell biopsies of the TE layer of the blastocyst using newer analytic technologies including array comparative genomic hybridization, single nucleotide polymorphism microarrays, quantitative real-time PCR, and whole-genome next-generation sequencing. Nonetheless, most current PGS protocols select against aneuploidy without specifying whether it is of meiotic or mitotic origin. Embryo biopsies are therefore classified as either euploid or aneuploid. In some cases, the diagnosis of aneuploidy of mitotic origin may be deduced by the detection of cellular mosaicism [7]. However, PGS protocols which routinely characterize aneuploidy as meiotic or mitotic in origin are not being broadly applied at this time [5]. Such protocols require that both parents be concurrently genotyped to assign segregation errors to individual parental homologs [5].

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The impact of PGS on the outcome of assisted reproduction remains uncertain. Prospective randomized clinical trials

involving young women with good prognosis revealed that PGS cycles outperform non-PGS counterparts [or “non-PGS cycles”] as assessed by pregnancy and delivery rates [8]. In contrast, use of PGS did not alter the outcome of patients with recurrent pregnancy loss. What is more, a retrospective analysis of US data for the 2011–2012 reporting period revealed reduced delivery rates for PGS cycles as compared with non-PGS controls [9]. Systematic reviews and a meta-analysis of the clinical effectiveness of PGS remain guarded in their assessment of PGS for aneuploidy screening [8]. Taken together, these observations on the utility of PGS suggest the need for larger, high-quality trials that focus on intention-to-treat analysis and on cumulative live birth rates in diverse patient populations [8]. It is likely that the reported variable impact of PGS on ART success is attributable to the limitations of the technology, sample size variance, patient population characteristics, and design considerations to name a few possibilities.

The case for screening against aneuploidy of meiotic origin

Implicit in the predictive utility of PGS is the premise that the ploidy status of the TE biopsy faithfully represents the entirety of the blastocyst. However, this presumption only holds true for aneuploidy of meiotic origin, but not for mitotic aneuploidy (Table 1). Moreover, some mosaic embryos—which are usually not implanted—can still grow into healthy newborns [3]. Indeed, recent murine studies suggest that mosaic embryos with a significant complement of euploid cells retain full developmental potential [10]. Mosaicism can go undiagnosed because a single TE biopsy is not representative of the karyotypically heterogeneous monolayer or

of the ICM. Additional biopsies of the same TE monolayer could yield altogether different results. Less well studied, though just as important, is the prospect of non-concordance between the TE and the ICM. Here again, absent detection of meiotic aneuploidy, the ploidy status of the TE does not necessarily represent the ICM [10]. In practice, this means that mosaicism may go undetected simply owing to sampling and/or technological limitations. It follows that embryos may thus be misclassified as uniformly euploid or aneuploid. A recent reanalysis of 46 blastocyst biopsies revealed that a plurality of those deemed to be euploid were indeed mosaic [7].

“...better diagnosis of the causes of aneuploidy and its dissemination [...] can improve the selection of viable embryos and may increase the live birth rates for ART.”

Limiting the multicell TE biopsy to screen for aneuploidy absent, an investigation of whether it is of meiotic or mitotic origin fails to maximize the utility of PGS. First, meiotic errors are the leading genetic cause of pregnancy loss. Second, meiotic errors affect all of the embryonic cells and are therefore almost always lethal. Third, meiotic errors appear in a quarter or more of all blastocysts. Fourth, the incidence of meiotic errors is maternal age dependent. A recent study of more than 18,000 multicell TE biopsies revealed that the rate of meiotic and mitotic errors in women aged 35 or younger is about the same [5]. In contrast, meiotic errors greatly outnumbered mitotic errors as the cause of aneuploidy in women aged 35 and older [5]. As aneuploidy of meiotic origin is highly predictive of an adverse outcome, affected blastocysts should not be used for intrauterine transfer. In contrast, mitotic errors that give rise to aneuploidy in the TE or even the ICM can still lead to normal pregnancy and healthy children. This suggests that better diagnosis of the causes of aneuploidy and its dissemination—whether it affects the TE and/or the ICM—can improve the selection of viable embryos and may increase the live birth rates for ART.

The case for intensifying human embryo research

Concurrent with improved screening methods to determine the origin of aneuploidy in embryos, more research on human embryonic development is needed to better understand how the cells of the ICM form the fetus. In particular, research is needed to address the lineage-specifying programs in the early blastocyst, the causes and consequences of chromosomal abnormalities, the normative topography of a mosaic constitution, and the identity, number, and location of embryo-specifying epiblasts. Such insights may improve the diagnosis and selection of healthy embryos through PGS and hopefully lead to the development of new technologies. Beyond these considerations, relatively little is known about the survivability of mosaic euploid–aneuploid blastocysts and the determinants thereof [5,10]. The finding that a mosaic embryo can survive to the blastocyst stage may be attributable to the proportion of aneuploid cells, their location, and the particulars of their karyotype [5,10]. Absent additional knowledge, however, predicting the viability of mosaic blastocysts is not feasible.

The major problem, however, is not lack of interest by the research community but lack of public funding of human embryo research. In the USA and other scientifically advanced nations, human embryo research is not eligible for public funding or is legally prohibited. Future research and therefore future advances in ART diagnostics are thus relegated to the private sector. This state of affairs hampers the acquisition of new insights into the intricate process of early human development. More importantly, translational breakthroughs intent on improving infertility care are being delayed. Patients afflicted with infertility deserve better.

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Conflict of interest

EYA declares that he has no conflict of interest. RCM is a co-inventor on patent application USSN

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