Births of 3 normal neonates after transfer of "aneuploid" embryos: Evidence against use of PGS, in poor prognosis patients

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ABSTRACT

BACKGROUND

In poor prognosis patients, preimplantation genetic screening (PGS) has been suggested to reduce pregnancy chances in association with IVF. Proponents of PGS have, however, disputed this finding. A better understanding of how PGS may reduce pregnancy chances in poor prognosis patients is, therefore, of importance.

METHODS

Human embryos are frequently mosaic and can self-correct. False positive PGS results, therefore, can potentially lead to discarding of normal embryos with normal pregnancy potential. This will impact outcomes especially in poor prognosis patients who usually produce low oocyte and embryo numbers with high aneuploidy rates and, therefore, frequently do not reach embryo transfer. We, therefore, developed a formal center policy, which with appropriate informed consent allows transfer of embryos, reported to carry supposedly lethal monosomies if no euploid embryos are available for embryo transfer after PGS. Embryo biopsies were performed at cleavage stage, and PGS was performed by one of the country's most prominent preimplantation genetic diagnosis laboratories. We here report on the births of three normal children after transfer of blastocyst-stage embryos, previously reported as aneuploid.

RESULTS

Eight couples were identified in the three participating centers to qualify for potential embryo transfer of monosomic embryos since initiation of above noted policy. Among those, 5 chose to undergo embryo transfers with monosomic embryos. Among these 5 patients, 3 conceived and delivered healthy, chromosomally normal offspring. The other 2 patients failed to conceive.

CONCLUSIONS

These outcomes confirm that, especially in poor prognosis patients with small embryo numbers, PGS may actually reduce pregnancy chances by preventing embryo transfer of viable embryos. The procedure should, therefore, be avoided in such patients. Though trophectoderm embryo biopsies at blastocycst stage offer technically more reliable chromosomal analyses than cleavage stage biopsies, abnormal cell lines in embryos are usually segregated into the trophectoderm. Risks of false positive diagnoses in poor prognosis patients, therefore, likely exist whether embryos are biopsied at cleavage or blastocyst stage.

Especially in women with abnormally low ovarian reserve, embryos are characterized by high degrees of aneuploidy, which further increases with advancing female age.[1] Investigators have, therefore, since the 1990s pursued the concept of preimplantation genetic screening (PGS), which involves testing of embryos for chromosomal abnormalities prior to their transfer into a uterus.[2] Since aneuploid embryos relatively rarely implant, the hypothesis driving PGS has been from the beginning that the elimination of aneuploidy embryos prior to embryo transfer should lead to higher implantation and pregnancy and lower miscarriage rates.[3]

Unfortunately, when PGS was first introduced over a decade ago (PGS#1), these expectations were not met,[4,5] and professional organizations quite uniformly declared the technique ineffective in improving IVF outcomes and reducing miscarriage rates.[5-7]

Supporters of PGS attributed its failure to inadequate techniques and technologies, which in those days involved embryo biopsy at cleavage stage (day-3 after fertilization) and fluorescence in-situ hybridization (FISH) of a restricted number of chromosomes.[8] Because cleavage stage embryos often are mosaic and can self correct,[9] they further argued that later embryo biopsy at blastocyst stage (days 5/6 after fertilization) should be more accurate.[10]

From these criticisms evolved a new generation of PGS procedures (PGS#2), based on 24-chromosome assessments, utilizing a variety of different next-generation platforms in place of FISH, and of trophectoderm biopsies on days 5/6 in place of cleavage-stage embryo biopsies on day-3 after fertilization.[6,7] Since trophectoderm biopsies allow for removal of more cells than cleavage stage biopsies and since the new diagnostic platforms, unquestionably, are more accurate in determining ploidy than FISH, PGS#2 found even more rapid acceptance than the earlier PGS.

We have argued that a small number of studies, which since have claimed outcome benefits for PGS#2 have been misleading,[6,7] and that the reason why neither PGS#1 nor PGS#2 has been able to demonstrate improvements in IVF outcomes lies in varying statistical efficacy of PGS in different patient populations.[11]

Any method of embryo selection will demonstrate outcome improvements only in so-called good prognosis patients, where selection of “best” embryos from among a large cohort of “excellent” embryos can be expected to result in improved implantation and pregnancy rates. Good prognosis patients represent, however, only a small minority of infertile women undergoing IVF. Average prognosis patients, representing a majority of patients in most IVF centers, either have no “best” embryos to select or limited number of available embryos do not allow for embryo selection at all. They, therefore, are usually clinically unaffected by any form of embryo selection, though obviously are exposed to unnecessary costs. The major risks of embryo selection lie, however, with poor prognosis patients.[11]

In poor prognosis patients, usually women with very low egg and embryo numbers, any form of embryo selection will negatively affect IVF outcomes.[11] In association with PGS, this was first demonstrated by Mastenbroek et al,[4] and since in many other follow up studies.[5-7] Schoolcraft and Katz-Jaffe recently claimed excellent results with PGS#2 in women with advanced female age.[12] A closer review of their data, however, reveals a highly selected patient population of extremely good prognosis patients.

Despite overwhelming evidence to the contrary, poor prognosis patients, and especially older women, are because of their high rate of aneuploidy [1] still widely considered the most appropriate patient population to undergo PGS as recent ESHRE data once again demonstrated.[13] One likely reason is that many colleagues still do not understand how PGS can affect IVF outcomes negatively. In poor prognosis patients, with small embryo numbers, false-positive PGS diagnoses, eliminating potentially viable normal embryos, can, however, have significant effects on potential pregnancy chances.

We here offer additional evidence that false-positive PGS can, indeed, affect pregnancy chances in poor prognosis patients.

METHODS

Here presented data were generated at three independent fertility centers in New York City, The Center for Human Reproduction (CHR), Fertility Specialists in New York and Braverman IVF & Reproductive Immunology. All three centers, independently, reached the conclusion that reported aneuploidy rates, even from reputable national preimplantation genetic diagnosis laboratories, were in some patients statistically improbably high. Concern arose especially in poor prognosis patients, who usually produced only small embryo numbers and, therefore, were at particularly high risk to end up with no euploid embryos for transfer into the uterus.

CHR, therefore, developed and published a policy (https://www.centerforhumanreprod.com/fertility/possibility-selectively-transferring-embryos-preimplantation-genetic-diagnosis-pgdpgs-determined-chromosomally-abnormal/), which, if in a poor prognosis patient no euploid embryos were available for transfer after IVF, with appropriate informed consent allowed the transfer of embryos, reported to carry potentially embryo-lethal monosomies. Non-lethal monosomies were not transferred. The two other New York centers, independently, adopted similar policies.

Every patient undergoing such transfers was individually counseled, was fully advised of risks, had to agree to undergo early prenatal genetic diagnosis and to be willing to undergo termination of pregnancy should a chromosomally abnormal pregnancy be established.

We here report the combined experience of all 3 centers, which all used the same highly reputable national laboratory for PGS. All embryos were transferred at blastocyst stage after embryo biopsy at cleavage stage.

RESULTS

Since above described policy was put in place, the three centers so far encountered 8 women who after IVF and PGS had no transferrable embryos but in their PGS reports had embryos with embryo-lethal monosomies. Among those 8 patients, 5 decided to pursue embryo transfers with embryos, reported to be monosomic. Three among those 5 conceived and delivered healthy male offspring. They are presented in Table 1. The other two patients did not conceive. No miscarriage was, thus, encountered in 5 transfers.

Patients 1 and 2 had two and one monosomic embryos transferred, respectively, with birth of singleton males in both cases. Patient 3, who underwent IVF with a desire for sex selection for male, had two embryos transferred, a normal 46XX female and a monosomic 45XY embryo. A healthy male child was born, obviously a product of the monosomic embryo transferred.

DISCUSSION

Here presented case series raises additional doubts about PGS, as currently practiced worldwide. We and others have previously questioned the procedure's alleged ability to improve IVF cycle outcomes.[4-7] Here presented evidence, however, raises even more serious concerns since our findings reemphasize that poor prognosis patients may, indeed, be seriously harmed by the procedure, as previously first reported by Mastenbroek et al,[4] and later confirmed others.[5-7]

Poor prognosis patients can afford such harm least because the fewer embryos patients produce, the higher their risk of *all* being aneuploidy and, therefore, not being eligible for transfer. On the other hand, the larger the available embryo pool, the more likely will it contain euploid embryos and, therefore, allow embryo transfer. As previously noted, characteristically, therefore, only good prognosis patients will benefit from PGS; average prognosis patients will emerge neutral, without clinical advantages or disadvantages (except for financial costs), and poor prognosis patients will be clinically negatively affected,[11] as here again demonstrated.

Since here presented data reemphasize this message, they suggest that, except in good prognosis patients, PGS should have no place in IVF. Whether PGS in good prognosis

patients, who even without utilization of embryo selection methods have excellent pregnancy rates, is really cost effective, remains to be determined. The answer to this question is closely related to the question whether elective single embryo transfer is a desirable IVF practice pattern, in itself a still controversial issue.[14]

Even though here presented cases involved day-3 cleavage stage embryo biopsies (all 3 births were day-3 biopsies, genetically tested in one of the nation's leading commercial PGS laboratories), here-suggested conclusions, likely, also apply to the new PGS#2. The reason is that the widely voiced opinion that benefits of PGS are primarily depending on the accuracy of diagnosing aneuploidy[8] appears mistaken. Much more likely, benefits of PGS are depending, as outlined above, on the patient population undergoing the procedure.[6,7]

Here reporting IVF centers have been skeptical of PGR reports for a number of reasons: As the utilization of PGS increased, we have witnessed growing numbers of patients, many still quite young, who in repeated IVF cycles uniformly only produced aneuploid embryos. Considering their young ages, such a findings is statistically highly implausible. Our skepticism was, however, even further enhanced when we observed that a good number of such patients conceived and delivered healthy pregnancies in subsequent IVF cycles with routine cleavage-stage embryo transfers and without use of PGS.[6,7]

We also, and not only in poor prognosis patients, question the benefits of blastocyst-stage culture and trophectoderm biopsies because claims of diminishing mosaicism and improving diagnostic accuracy also appear questionable. What is known about embryo development may actually suggest the opposite: Embryos, at cleavage stage found to be aneuploidy, indeed, have in a good number of cases (especially monosomies) the ability to segregate abnormal cell lines, and, thereby, to correct themselves.[9]

Segregation, however, primarily directs abnormal cell lines toward the trophectoderm, from which the placenta is formed, and where aneuploidy cell lines are relatively common, as was first reported in the 1980s.[15] Trophectoderm is, however, where PGS#2 gets its biopsies from. False-positive trophectoderm biopsies, therefore, should be expected when so segregated abnormal cell lines are accidentally biopsied.

This suspicion was recently, likely, confirmed in a Canadian study, when results from biopsies at different locations of the same embryos' trophectoderm were sent to three different reputable genetic laboratories, and resulted in highly contradictory results.[16] Rejecting outright laboratory errors in testing procedures, an unlikely possibility considering the highly accurate diagnostic platforms utilized by those laboratories, the only explanation for these findings is that different areas of trophectoderm can reflect different chromosomal findings.

Here reported three cases, however, offer the ultimate evidence of potential consequences of false positive PGS, which, especially in patients with very few embryos, can have devastating consequences.

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Table 1. Characteristics of embryos transferred that led to normal delivery

Patient	n Embryos transferred	Monosomy transferred	Outcome
1	2	13, 15, 18 15, 16, 18	normal birth, 46XY
2	1	21	normal birth, 46XY
3	1*	21	normal birth, 46XY

* This patient, who had undergone PGS for sex selection (desired sex male), had a monosomic 45XY and a normal 46XX female transferred.