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REVIEW

A review of, and commentary on, the ongoing second clinical introduction of preimplantation genetic screening (PGS) to routine IVF practice

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Abstract
Purpose Current re-introduction of "improved" preimplantation genetic screening (PGS#2) raises the question whether PGS#2 is ready for routine clinical application.
Methods We assessed available evidence via review of published data for years 2005–2012, and review of currently ongoing registered clinical trials, based on searches under appropriate key words in PubMed, MEDLINE, Cochrane Database System Review and Google Scholar and http://www.ClinicalTrials.gov. In absence of prospective clinical trials, and due to limited available data, individual publications/ongoing studies are assessed.
Results PGS#2 offers significant improvements in accuracy of aneuploidy diagnosis over PGS#1. By moving embryo biopsy from day-3 after fertilization (6–8 cell stage) to trophectoderm biopsy at blastocyst stage (day 5–6), PGS#2, however, adds additional co-variables to the analysis of efficacy of the procedure, which have special relevance for women with diminished ovarian reserve (DOR), who usually produce small egg and embryo numbers. Limited published data, claiming efficacy of PGS#2, as well as ongoing clinical trials, do not consider these additional co-variables, do not analyze outcomes by intent to treat and, therefore, have to be considered biased in patient selection.
Conclusions Here reached conclusions are based on absence of adequate data rather than affirmative outcome assessments. They, therefore, are subject to change at any future date with generation of significant new data. Premature introduction of PGS#1 caused significant damage to patients. As currently no reliable PGS#2 data are available to suggest improvements in IVF outcomes, to avoid a repeat of the PGS#1 experience, PGS#2 should be considered experimental until data show otherwise.

Keywords In vitro fertilization (IVF) · Preimplantation genetic screening (PGS) · Aneuploidy · FISH · Array techniques · Embryo biopsy · Experimental procedure

Introduction

Only a few years ago, during the initial introduction of preimplantation genetic screening (PGS#1) to in vitro fertilization (IVF), we attempted to warn about routine utilization of PGS in attempts to improve IVF outcomes. Leading medical journals rejected our submission, which from re-analysis of published Belgian data concluded that PGS, likely, was ineffective, while, possibly, exposing older women, to actually diminished pregnancy chances [1].

Mastenbroek et al. at that point published the clinical trial that finally led to a reassessment of PGS, supporting our manuscript's secondarily obtained conclusions with primary data [2]. Another journal then reconsidered its prior rejection, and accelerated publication of our manuscript [1].

Norbert Gleicher and David H. Barad contributed equally to the work.

Capsule Though the current re-introduction of preimplantation genetic screening (PGS) involves greatly improved aneuploidy testing of embryos, currently available data do not demonstrate outcome improvements for in vitro fertilization (IVF). Until such data become available, PGS, therefore, should be considered an experimental procedure.

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Now, barely 5 years later, our research of published and ongoing research on the re-introduction of PGS to the market place puts us into a similar situation, once again warning about premature introduction of a technically improved form of PGS (PGS#2) by, mostly, the same commercial interests, and for the same clinical indications.

PGS#1 exposed thousands of women, worldwide, to an, in principle, clinically useless procedure. For many, PGS#1, indeed, actually reduced pregnancy chances. Patients underwent an obviously still experimental treatment that was offered as "established." It took above noted Dutch study [2] to stop to a degree this worldwide utilization of PGS. Other studies later reaffirmed the results reported by Mastenbroek et al. [3–6]. Professional organizations followed by unequivocally concluding that PGS, at current practice levels, did not improve clinical IVF outcomes [7–9].

Commercial interests, now, once again, have started promoting an allegedly improved PGS#2. Based on a review of the literature and of ongoing registered clinical trials, we here offer the views of PGS#2 proponents, and report on their recent clinical experiences and ongoing clinical trials. This is then followed by a critical review of available data.

The goal of this communication is not the rejection of PGS as a potentially useful concept for selected patients. Our own recent studies, indeed, support PGS as potentially useful in improving IVF pregnancy chances in some patients [10]. Unfortunately, we still do not know who these patients are.

Search strategy

We searched PubMed, MEDLINE, Cochrane Database System Review, and Google Scholar for publications between 2005 and 2012 under key words preimplantation genetic screening (PGS), preimplantation genetic diagnosis (PGD), embryo biopsy, trophectoderm biopsy, polar body biopsy, blastocyst-stage biopsy, embryo aneuploidy screening, embryo mosaicism and under the names of selected leading investigators in the field. The reference lists of so identified publications were then further reviewed, and additional publications from these reference lists, even preceding the year 2005, were reviewed if relevant.

To determine ongoing clinical trials, we searched ClinicaTrials.gov (http://www.ClinicalTrials.gov), a service of the U.S. National Institutes of Health (accessed May 28, 2012). Reference lists of submitted trial registrations were then also further reviewed.

We reviewed six studies and eight clinical trials in detail, presented in Tables 1 and 2. In absence of data from prospectively randomized studies, every published study and every clinical trial was individually reviewed, and assessed for quality and content of data and conclusions (if any).

The argument in favor of PGS

Already almost 10 years ago, Verlinsky and Kuliev predicted that PGS would become integral to every IVF cycle [20]. Considering the theoretical attractiveness of the concept of PGS, their initial enthusiasm was understandable. The failure of PGS#1, however, refuted their prediction in its universality. As noted before, studies demonstrated not only lack of efficacy in improving IVF outcomes but, actually, decreased pregnancy chances as consequence of PGS in older women [1–3]. The theoretical concept that pretransfer elimination of aneuploid embryos should improve IVF outcomes has, however, nevertheless survived, though remains in search of a suitable patient population where PGS can, indeed, be unequivocally demonstrated to be effective in improving IVF outcomes.

The concept is based on the undisputed fact that human reproduction is highly inefficient, producing high percentages of aneuploid embryos even at young ages [21]. PGS is meant to identify aneuploid embryos prior to embryo transfer, thereby increasing pregnancy chances and reducing miscarriage rates [22].

Since the prevalence of embryo aneuploidy increases with advancing female age [21], older women were initially considered the most promising beneficiaries of PGS. They, therefore, often became primary targets of PGS investigations [21–23].

Following publication of the study by Mastenbroek et al. [2], PGS#1 was, however, widely reassessed, resulting in authoritative statements that PGS#1 neither improved IVF pregnancy rates nor reduced miscarriages [7–9]. Astutely, those, however, were not blanket condemnations of the concept of PGS but left open the possibility that PGS might be improved, leading to better outcomes and, therefore, different conclusions about the utility of PGS.

Most criticism of the procedure had been directed at technical limitations of PGS#1 and different technical performance levels at different laboratories [24]: Single (and at times 2-cell) blastomere embryo biopsy on day-3 after fertilization (6- to 8-cell stage embryos) was criticized since embryos at this developmental stage often are mosaic, resulting in false-positive and false-negative results, depending on which blastomeres of an embryos are assessed [25] and which embryos "self-correct" by segregating abnormal cell lines [24, 25].

PGS#1 also routinely utilized fluorescence in-situ hybridization (FISH) to assess embryo ploidy; — a technique subject to considerable interpretation errors [24]. Verlinsky

Table 1 Reported validation and clinical experiences with PGS#2

Authors	IVF cycles	Indication(s)	Study format	Authors' conclusions
Fragouli et al. [11]	32	Implantation failure	Uncontrolled	PGS#2 may assist patients with implantation failure, capable of producing blastocyst stage embryos.
Schoolcraft et al. [12]	45	>1 prior IVF failure	Uncontrolled	PGS#2 overcomes many problems of PGS#1 and, may allow PGS achieve benefits predicted by theory.
Rius et al. [13]	Single case	Unknown	Case report	Twin pregnancy established
Brezina et al. [14]	Single case	PGS + PGD for single-gene disease	Case report	Destined for increased use to optimize IVF pregnancy outcomes.
Scott et al. [15]	146	113 cleavage stage blastomeres and 142 trophectoderm biopsies	Cohort study	96 % of aneuploid embryos failed, and 41 % of euploid embryos maintained implantation
Yang et al. [16]	103	55 trophoectoderm CGH cycles versus 48 blastocyst-stage transfers after only visual inspection in young, good prognosis patients.	Randomized study	70.9 % clinical pregnancy rate after CGH and 45.8 % after inspection only. Additional studies required for verification

Table 2 Registered clinical PGS trials, separated into PGS#1and PGS#2

Title of trial (Year)	Sponsor	Clinical trials.gov. identifier	Comments
Still registered PGS#1 studies:			
Establishment of Comprehensive Genetic Analysis From a Single Cell (2005)	National Taiwan Hospital	NCT00173732	Unknown recruitment status; no publication;
Preimplantation Genetic Screening in Women Over 35 Years (2008)	Katholieke Universteit Leuven	NCT00593671	Completed, Debrock et al. [5], No outcome improvement in older women;
Preimplantation Genetic Diagnosis for the Indication of Advanced Reproductive Age (2008)	Reprogenetics	NCT00646893	Suspended for lack of funding;
Preimplantation Genetic Screening in Women of Advanced Maternal Age (2008)	Instituto Valenciano de Infertilidad	NCT00795795	Completed; Milán et al. [17]. Only PGS study in literature, suggesting benefits>age 40 and none <40.
Registered PGS#2-relates studies			
Concurrent Single Gene and 24 Chromosome Aneuploidy Preimplantation Genetic Diagnosis (PGD) (IVF008) (2009)	Natera, Inc.	NCT01023048	Ongoing, not recruiting; Results reported by Rabinowitz et al. [18]. No significant relevance;
Study of Efficacy of 24 Chromosomes Preimplantation Genetic Diagnosis (PGS) (2010)	Reproductive Medicine Associates of New Jersey	NCT01219283	Ongoing, not recruiting; Partially reported as Scott et al. [15]. and Treff et al. [19].
Preimplantation Genetic Diagnosis (PGD) by Array Comparative Genome Hybridization (CGH) and Blastocycst Biopsy (2011)	Reprogenetics, NJ Latinoamerica S. A. C., Lima Yale University McGill University	NCT01332643	Recruiting; All embryos undergo blastocyst-stage biopsy.
Single Embryo Transfer Of a Euploid Embryo Versus Double Embryo Transfer (2011)	Reproductive Medicine Associates of New Jersey Ferring Pharmaceuticals	NCT01408433	Ongoing, not recruiting; Comparison of single embryo transfer after PGS#2 with 2-embryos without.
Preimplantation Genetic Diagnosis Using Blastocyst Biopsy and Array CGH (2012)	Reprogenetics, NJ & multiple IVF centers	NCT01546350	Recruiting; Comparison of blastocyst stage transfer after CGH with transfer without CGH
Polar Body Biopsy for Preimplantation Genetic Screening (2012)	Weill Medical College of Cornell University	NCT01574404	Ongoing comparison of polar body biopsy with FISH and blastocyst biopsy & array analysis
Preimplantation Genetic Screening (PGS) in Advanced Female Age and Male Severe factor (2012)	Instituto Valenciano de Infertilidad	NCT01571076	Not yet open for recruitment; Will involve all blastocyst-stage embryos & CGH.

et al. reported that only approximately 81 % of oocytes were interpretable by FISH [23]. Single cell (or 2-cell) analyses with day-3 embryo biopsies further aggravates shortcomings of FISH since availability of so few cells for analysis does not allow for proper controls [24].

FISH also permitted only analyses of limited chromosome numbers, in most published studies between seven and nine. Utilized probe combinations usually included chromosomes most frequently identified as abnormal in first trimester spontaneous abortions. Aneuploid embryos often demonstrate multiple chromosomal defects. Linkages between different chromosomes, therefore, were believed to detect most chromosomally abnormal embryos, even when assessing only limited chromosome numbers [25, 26].

Complete chromosome complement analyses by comparative genomic hybridization (CGH) recently, however, demonstrated that FISH fails to detect even more chromosomal abnormalities than has been suspected. While Munné et al. suggested that by selecting new probe combinations for 10 to 12 chromosomes, FISH could be improved to an accuracy of 89 to 91 % of aneuploidies [26], assessments of a full chromosome complement have to be assumed more accurate.

Because the failure of PGS#1 by many experts was primarily attributed to above noted technical difficulties and varying levels of laboratory expertise [24], attempts at improvement almost exclusively only concentrated on these technical and procedural aspects of PGS. Australian investigators were the first to suggest trophectoderm biopsy at blastocyst stage in place of day-3 biopsies [27], arguing that it offered distinct technical and clinical advantages by allowing switching from single (or 2-cell) to multiple cell analysis and, thus, reducing technical error opportunities. Trophectoderm biopsy could also be expected to improve false positive and false-negative results due to mosaicism. It on technical grounds, therefore, can be expected to increase accuracy of chromosomal analyses.

New array techniques in replacement of FISH took time to develop. A first report on the utilization of CGH to detect chromosomal abnormalities in first polar bodies of metaphase II oocytes was reported in 2004. It suggested superior accuracy of this technique over FISH in detecting aneuploidies [28]. CGH, of course, allowed the analysis of all chromosomes at once. The European Society for Human Reproduction and Embryology (ESHRE) a few years later in 2010 decided to start a proof of principle study for CGH, utilizing polar body biopsy [29].

Other investigators began reporting promising CGH results, utilizing polar body as well as trophectoderm biopsies: Fragouli et al., utilizing CGH, reported implantation and pregnancy rates of 11.5 and 21.4 %, respectively, after polar body biopsy but of 58.3 and 69.2 %, respectively, after trophectoderm biopsy at blastocyst stage [11]. At approximately the same time Harper and Harton concluded in a review of the subject that, if array-based testing is to be proven useful, the array platform has to be validated on appropriate tissue, including on single cells; the best embryo stage for biopsy has to be determined, polar body, cleavage (day-3) or blastocyst stage; and, finally, improvements in delivery rates after IVF have to be demonstrated by appropriately designed clinical trials [30]. Their words have been proven prophetic!

While trophectoderm biopsy at blastocyst stage offers obvious advantages over day-3 embryo biopsy, this new approach also adds significant complexity: Embryos have to be cultured for two more days. CGH results after day-5/6 biopsy, at least initially, took too long to allow for embryo transfers in the same IVF cycle. In PGS#2 embryos initially, therefore, underwent trophectoderm biopsy at blastocyst stage (day 5 or 6), then were cryopreserved and, if at least one embryo was euploid, transfer took place in a subsequent thaw cycle.

Table 1 summarizes the very limited number of reports, attempting to validate CGH in association with trophectoderm biopsy or using this clinical approach towards PGS, here given the acronym PGS#2. Despite, obviously, limited data in support, proponents of such an approach have voiced strong expectations that such an approach would, ultimately, benefit IVF outcomes [11, 12, 14–16, 31].

Recently, the time needed for analysis of ploidy has been speeded up: Treff et al. reported a speeded up quantitative real-time polymerase chain reaction-based assay for comprehensive chromosomal aneuploidy screening of human blastocysts, which permits accurate chromosomal analysis of a complete chromosome complement within 4 hours, and, therefore, in time for in cycle embryo transfer. Their technique, therefore, potentially eliminates any need for cryopreservation [19]. Using whole genome amplification, Yang et al. performed analysis in time for embryo transfer on day-6, using a proprietary so-called SurePlex DNA amplification system (BlueGnome Ltd; Cambridge, UK) [16].

Replacing day-3 embryo biopsy with trophectoderm biopsy at blastocyst stage, and replacing FISH with comprehensive chromosome analyses, indisputably, improves the technical accuracy of aneuploidy testing. Convinced that these technical improvements will beneficially impact on clinical efficacy, PGS #2 is, therefore, widely viewed as a significant improvement over PGS#1, which, according to opinion leaders, will finally produce the clinical benefits predicted by the “theory” of PGS [12].

Such a conclusion is, however, predicated on the unproven assumption that PGS#1 in principle failed because of technical shortcomings, now remedied by above described procedural and technical modifications. Evidence for such a conclusion, however, is lacking. Indeed, as we will discuss below, data generated at our center suggest that technical and procedural shortcomings of PGS#1, likely, played only

a marginal role in the earlier failure of PGS#1. If correct, such a conclusion, however, raises the specter of a similar failure with PGS#2, if again utilized indiscriminately without prior definition of suitable patient populations.

Arguments against current clinical use of PGS#2

Even proponents of PGS#2 have published evidence that other than technical and procedural issues led to the failure of PGS#1. For example, Schoolcraft et al. reported that PGS#1 was ineffective in older women [32], first, of course, demonstrated by Mastenbroek et al. [2]. Except for a small study, performed at our center [10], no attempts have been made, however, to determine why PGS#1 really failed.

That the principal reasons were technical is, therefore, unconfirmed, and unsupported by data. Utilizing day-3 embryo biopsy and FISH, based on IVF outcomes in elective, not infertility-related cycles, we found no evidence that technical reasons substantially contribute to the failure of PGS#1, as in such a highly selected patient population PGS#1, indeed, improved pregnancy chances in IVF cycles [10]. The relative contributions of technical causes versus patient selection, of course, greatly matter in assessing the prospects of PGS#2 to be successful. PGS#2 will in unselected patient populations only succeed if PGS#1, principally, failed due to technical inaccuracies of day-3 embryo biopsy and chromosomal analyses by FISH.

The current status of PGS

An obvious first argument against current routine clinical utilization of PGS#2 is that authoritative bodies, at present, see no clinical value in PGS [7–9]. ESHRE's previously noted effort to set up a prospectively randomized multicenter study also suggests a healthy level of skepticism, and confirms PGS as an experimental procedure of no proven clinical effectiveness, yet. Published reviews in the literature are confirmatory [6, 33, 34]. PGS utilization to improve pregnancy and miscarriage rates, based on currently available data [1, 2, 7, 9, 29, 30, 35, 36] should, therefore, only occur under study conditions, and with appropriate informed consents.

Considering the clarity of best available evidence, it is disturbing to witness increasing utilization of PGS, with patients, often, completely unaware of undergoing experimental treatments. We have seen patients allegedly advised that the new techniques of PGS#2 offer "their only chance of pregnancy;" and that PGS#2 now represents "routine" state-of-the-art IVF care.

We, however, also have seen patients where the failure of PGS#2 was considered evidence enough to advise that their only remaining treatment chance involved donor oocytes. At least two such patients, who failed PGS#2 because none of their embryos reached blastocyst stage, after cleavage-stage, day-3 embryo transfers, and without utilization of PGS, recently conceived at our center with use of autologous oocytes. Both pregnancies are ongoing (Gleicher and Barad, unpublished data).

Based on a single small study, by the authors, themselves, described as a "pilot study in need of verification" [16], two prominent medical journals in the specialty in their June 2012 issues ran an advertisement by a manufacturer touting the company's DNA amplification system under the headline "24 sure study shows increased pregnancy rates." The ad further claimed a 65 % increase in pregnancy rate "even in younger women who are more likely to have favorable IVF outcomes." Publication of such an advertisements by credible medical journals is, of course, destined to cause confusion.

The misdirection of ongoing studies of PGS#2

Eleven ongoing registered related studies further enhance concerns (Table 2). Six involve total chromosome complement screening and, therefore, directly or indirectly refer to PGS#2. None, unfortunately, considers in study design the additional variables trophectoderm biopsy at blastocyst stage (days 5/6) adds in comparison to cleavage-stage biopsy (day-3).

Blastocyst-stage cultures are meant to select embryos with favorable pregnancy potential. In a Cochrane review Blake et al., however, point out that such an effect is only obtained in so-called good prognosis patients who produce high numbers of 8-cell embryos on day-3. They specifically note that blastocyst culture is not effective in unselected patients or poor prognosis patients [37].

Whether because of advanced age or premature ovarian aging (POA), diminished ovarian reserve (DOR) results in small oocyte and embryo numbers and poor quality. DOR patients, therefore, very clearly do not represent good prognosis patients. They, therefore, cannot be expected to derive outcome benefits from blastocyst-stage in comparison to cleavage-stage embryo transfers. Indeed, the opposite effect can be expected: the poorer a patient's embryo quality, the less likely will any one embryo reach blastocysts stage. Only a comparatively small number of embryos from older women and POA patients will, therefore, reach blastocyst stage.

Women with DOR, therefore, at best, will have only small embryo numbers available on days 5/6 for trophectoderm biopsy. The smaller a patient's embryo cohort at that stage, the higher will be the statistical likelihood that all embryos will be found aneuploid. In other words, two

embryos will less likely yield at least one euploid embryo than eight embryos.

Combining in women with DOR the risk that none of their embryos will reach blastocyst stage with the increased risk for 100 % aneuploidy due to small embryo numbers, demonstrates why only relatively few DOR patients ever will reach embryo transfer with PGS#2. Quoted pregnancy outcome data, therefore, are highly biased in patient selection because reported data do not report outcomes based on intent to treat. Instead, universally, in all published studies, pregnancy rates were calculated in reference to only those patients who reached embryo transfer on days 5/6, not considering who started the process, and, therefore, excluding most women with DOR.

Unless PGS#2 is utilized only in good prognosis patients, so far only reported in one study by Yang et al. [16], one has, therefore, to consider the possibility, maybe even likelihood, that in DOR patients embryo culture to blastocyst stage may, actually, reduce pregnancy chances [37]. In such patients a day-3 embryo transfer may result in a viable pregnancy, while the same marginal embryo, cultured to days-5/6, may not survive prolonged culture. As a consequence, PGS#2, like PGS#1 before [2–6] in DOR patients may, therefore, actually reduce pregnancy chances.

While not formally acknowledged by proponents of PGS#2, their study designs, nevertheless, point towards acknowledgment of such a risk. Scott et al., for example, culture to blastocyst stage only if, by day-3, patients have at least 4 high quality embryos [15]. By excluding from PGS#2 women with fewer good quality embryos, they, obviously, attempt to select favorable patients.

According to Blake et al. [37], four high quality embryos are, however, a low cut-off for good prognosis patients. This makes it very likely that published studies and ongoing clinical trials (Table 2) by Scott's group actually represent selected good prognosis patients intermingled with patients Blake et al. would have classified as unselected, neither representing outright good or bad prognosis patients. This assumption is further supported by inclusion of patients with "normal" basal FSH levels of up to 12.0 IU/L [15] or even 15.0 IU/L (ClinicalTrials.gov Identifier: NCT01219283), both FSH cut-offs by most considered at all ages well within DOR range [38, 39]. Being offered PGS #2 may, therefore, harm such patients.

Published PGS#2 claims and ongoing PGS#2 trials are, therefore, for at least two very distinct, and opposing, reasons, uninterpretable: On the one hand, reported data have excluded some unfavorable patients (<4 good quality embryos on day-3); yet, on the other hand, among those who were cultured to blastocyst stage, an unknown number of patients are actually poor prognosis patients, and may include women with DOR. Because they were taken to blastocyst stage, their pregnancy chances may, actually, have been reduced.

Reported PGS#2 outcomes, therefore, have to be recognized for their limitations. Like early studies of PGS#1, they are uninterpretable, and do not allow determinations which patients would benefit from PGS#2, and who would be harmed. Unfortunately, considering published study designs, currently ongoing clinical trials also will not offer satisfactory answers.

One obvious question to be asked is what happens to all those patients who never make it to embryo transfer with utilization of PGS#2? Would they do equally poorly or better, had they not been cultured to blastocyst stage but undergone a routine day-3 transfer without embryo biopsy?

Conclusions

DOR patients represent a significant percentage of patients in most infertility centers. If not properly diagnosed in advance and excluded from PGS#2, young women with POA and older women with age-associated DOR appear at risk to be negatively affected in their pregnancy chances by PGS#2.

We noted earlier our anecdotal experience with two older women with DOR. Both had failed up to four IVF/PGS#2 cycles since they never reached embryo transfer. Both ended up conceiving after day-3 transfers without embryo biopsy and PGS. Anecdotal experiences are, of course, just that! They, however, reemphasize how little is known about the utilization of PGS#2, and how urgently properly designed studies are needed. Unfortunately, current studies in the pipeline do not appear suited to provide needed answers.

We have become convinced that PGS in properly selected patient, indeed, improves IVF pregnancy and, likely, also reduces miscarriage rates. [10]. We are, however, also, more than ever, convinced that PGS has the potential of reducing pregnancy chances if women are incorrectly selected.

While trophectoderm biopsy and array techniques, unquestionably, represent significant technical progress, the switch from day-3 embryo biopsy to blastocyst-stage biopsy adds significant additional co-variables. Efficacy of PGS#2 is, therefore, even more difficult to assess than efficacy of PGS#1.

To prevent repetition of the harm caused by PGS#1, it is essential that the clinical utilization of PGS#2 in routine IVF cycles be considered unethical until proper studies determine who the patients are who benefit from such an approach. Acceptance of advertisements by reputable medical journals, claiming efficacy for PGS#2 in improving IVF pregnancy rates, therefore, appears inappropriate. Until patient populations have been defined who will benefit from PGS#2, the procedure should be offered to patients only under study conditions and with appropriate informed consent.

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