Assessment of preimplantation genetic testing for embryo aneuploidies: a SWOT analysis

A. Alteri[1], L. Corti[1], A. M. Sanchez[2], E. Rabellotti[1], E. Papaleo[1,2], and P. Viganò[2]

[1]Obstetrics and Gynaecology Department, IRCCS San Raffaele Scientific Institute, Milano 20132, Italy.

[2]Reproductive Sciences Laboratory, Division of Genetics and Cell Biology, IRCCS San Raffaele Scientific Institute, Milano 20132, Italy.

Correspondence: Alessandra Alteri, PhD, Obstetrics and Gynaecology Department, IRCCS San Raffaele Scientific Institute, Milano 20132, Italy. E-mail: alteri.alessandra@hsr.it

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Abstract

The recently re-named preimplantation genetic testing for determining embryo aneuploidies (PGT-A) is presently very popular although its acceptance by the scientific community is controversial. This approach still encounters drawbacks. This paper uses a SWOT (strengths, weaknesses, opportunities and threats) analysis to discuss salient points to be considered when examining the PGT-A strategy in order to gather information from a range of perspectives. One of the strength associated with the procedure is represented by an increase in implantation rate although data from the highest level of evidence do not support an increase in cumulative pregnancy rates. The current difficulty in the management of mosaicisms represents a weakness of PGT-A. The application of

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the strategy represents an opportunity to favor the single embryo transfer while other advantages such as reduction of time to pregnancy and emotional distress are controversial. Potential important threats, at present still undefined, are represented by the biopsy-related damage to the blastocyst and the impact on neonatal and long term outcomes.

Key words: IVF/ PGT-A/ aneuploidy/ SWOT analysis.

Introduction

The acceptance by the clinical community of the recently re-named preimplantation genetic testing (PGT) for determining embryo aneuploidies (PGT-A) is still evolving. While PGT for monogenic/single gene defects (PGT-M) and PGT for chromosomal structural rearrangements (PGT-SR) represent a well-established clinical practice, PGT-A is presently under debate. Indeed, its popularity declined following the publication in 2007 of the New England Journal of Medicine paper by Mastenbroek *et al* also called "Mastenbroek controversy" demonstrating that PGT-A of day 3 embryos by fluorescence in situ hybridization (FISH) actually reduced live birth rate in women with advanced maternal age instead of improving it.[1] Other randomized controlled trials

(RCTs) confirmed that there was insufficient data to determine a beneficial effect of PGT-A applied on the live birth rate.[1-2]

On this basis, newer genetic technologies have been developed to achieve the 24-chromosome screening, allowing the assessment the whole chromosome complement. Moreover, the trophectoderm (TE) biopsy of Day 5/6 blastocyst stage embryos has become the common practice based on evidence showing that implantation potential of the biopsied embryos would not be affected if the biopsies are taken at blastocyst stage.[3] Notwithstanding these improvements referred as preimplantation screening version 2.0, whether PGT-A should be offered routinely to in vitro fertilization (IVF) patients is still at the heart of the debate.

This review uses a SWOT (strengths, weaknesses, opportunities and threats) analysis to identify and discuss salient points to be considered when examining the PGT-A strategy. This tool represents a means of gathering information from a range of perspectives. The outcome shows the strengths of PGT-A while also helping to identify the major urgent needs. More specifically, the analysis underlies the dichotomy between the existing gaps in the current literature on PGT-A and the accelerated uptake of the procedure in clinical care.

Strengths

In general, novel strategies procedures aiming at enhancing fertility should be introduced in clinical practice with well-designed and conducted RCTs. Nonrandomized studies tend to show larger treatment effects than RCTs.[4] Improvement in research methodology limits biasing factors that inflate effects as supported by the progressive reduction of effect sizes over time as the quality of studies on gynaecological procedures ameliorated.[5] Producing robust data on assisted reproduction innovation seems feasible.

Prospective randomized controlled trials; implantation and pregnancy rates

Three RCTs have evaluated the performance of the new version of PGT-A with the biopsy at the blastocyst stage associated with comprehensive chromosome screening (CCS) in good prognosis patients (Table 1).[6-8]

In the first RCT, patients with a good prognosis (age < 35, no previous miscarriage, tubal or male factor infertility and no prior IVF cycle) and normal karyotype undergoing a single embryo transfer (SET), were prospectively randomized. In one group, blastocysts were selected on the basis of both morphology and CCS by array comparative genomic hybridization (aCGH) and in the other group, blastocysts were morphologically assessed only. Although no power calculation was provided, a significantly higher implantation rate and significantly increased clinical and ongoing pregnancy rates per randomized patient were found in the PGT-A group.[6]

In a second RCT, good prognosis patients (≤ 42 years, AMH ≥ 1.2 ng/ml, day 3 FSH < 12 IU/L) were randomized when at least two blastocysts were available for biopsy. Patients underwent a single euploid blastocyst transfer after PGT-A using quantitative PCR (qPCR) or a transfer of two untested blastocysts, whether fresh or frozen. A similar ongoing pregnancy rate per randomized patient was observed between the two groups. However, a positive trend toward a higher implantation rate (P=0.08) after a single euploid blastocyst was found.[7] The risk of multiple pregnancy was reduced sharply in the PGT-A group.[7]

In the third RCT, Scott and colleagues[8] randomized infertile women (maternal age 32.2±0.5 in PGT-A group vs 32.4±0.5 in control group) with no more than one previous failed IVF cycle, with a basal follicle count of eight or more and basal FSH ≤ 15 IU/L. Significantly higher delivery rate per cycle and implantation rate resulted in the PGT-A group using qPCR, compared to the untreated group. The study design considered a maximum of two euploid embryos for transfer but ten patients had only a single euploid blastocyst, resulting in a statistically significant difference in the number of blastocysts transferred in the study group compared to the control group (1.8±0.04 vs 2±0.00; P<0.0001).[8]

Two meta-analyses have analyzed overall data from RCTs. Pooled analysis showed a significant increase in implantation rate while no difference was observed for clinical, ongoing pregnancy and miscarriage rates.[9] Dahdouh and coworkers[10] confirmed that PGT-A was associated with an increase of clinical and sustained implantation rates. Analysis of other outcomes has not been performed[10] (Figure 1).

On the other hand, the main cause of infertility is advanced maternal age (AMA) which is associated with a decrease in ovarian reserve, impairment of oocyte quality and increased embryo aneuploidy, resulting in implantation failure and miscarriage.[11] The incidence of chromosome abnormalities varies from about 40% in fertile egg donors to 80% in patients 41 to 42 years old.[12] The literature presents only one RCT in women aged 38-40 using CCS applied on day 3 embryos.[13] This study confirmed the same results obtained in the good prognosis population also in AMA patients showing an increase in implantation rate using PGT-A. Moreover, the delivery rate after the first transfer attempt was significantly increased in the PGT-A group both per transfer and per cycle.[13]

Miscarriage rate

To transfer a single euploid blastocyst might represent an optimal strategy to reduce pregnancy loss and potential live births with chromosomal abnormalities. In young and good prognosis patients, a decreased but not significant miscarriage rate was observed in the PGT-A group compared to controls, a result also confirmed by a meta-analysis.[6-7,9] Only the RCT on AMA patients showed that PGT-A was associated with a significantly lower miscarriage rate (2.7% vs 39.0%, P=0.0007) (Figure 1).[13] Therefore, only in older patients, PGT-A might have the advantage of reducing dramatically the miscarriage rate, encouraging the use of PGT-A in this population.

Weaknesses

Prospective randomized controlled trials on PGT-A version 2.0

With the advent of new technologies allowing CCS of blastocysts, PGT-A has been actively marketed as increasing implantation rates, and therefore decreasing time to pregnancy, recurrent miscarriages and repeated implantation failure.[14] Despite the initial enthusiastic attitude toward successful selection of euploid embryos, it is necessary to highlight limits and shortcomings of PGT-A. The only three RCTs published have been criticized because of poor study design, restriction to good prognosis patients and great heterogeneity in terms of techniques and conditions applied. Yang et al.[6] included a small sample size of 55 young, good prognosis patients. The 72 good prognosis patients recruited by Scott et al.[7] were randomized quite late, i.e. if they had at least two blastocysts available for analysis. Although the authors claimed that PGT-A increased implantation and delivery rates, there was a fundamental methodological flaw in the study that failed to account for the difference between the unit of randomization (patients) and the unit of analysis (individual embryos). Forman and coworkers[8] included 89 good prognosis patients and the RCT suffered of the same methodological problem encountered in the trial by Scott et al.[7]

Concerning CCS technology, the aCGH was used in one study[6] while qPCR was applied in the other two.[7-8] Next-generation sequencing (NGS) technology is presently subverting these techniques.[15]

Moreover, in two RCTs, the interpretation of the implantation rate was difficult as in one, a single euploid blastocyst versus two untested blastocysts were transferred[8] while in the other, two fresh blastocysts were transferred in both arms, except for 10 patients in the PGT-A group with only one euploid embryo to transfer.[7] Finally, the implantation rate should be abandoned as main outcome for clinical trials essentially for two reasons. First, women are interested in having babies and not implantations; the implantation rate represents a surrogate outcome to demonstrate the effectiveness

of a treatment for our patients. Second, in randomized studies involving PGT-A, based on the embryo selection so that fewer embryos were classified as transferable after the intervention, an unequal number of embryos have been transferred in the two groups with fewer embryos frequency transferred in the study group. Consequently, the implantation rate resulted significantly higher in the study group only as a consequence of the higher number of embryos transferred in the control group (Figure 1).[16]

Cumulative IVF outcomes

With advances in embryo cryopreservation, the real success of an IVF cycle is represented by the cumulative live birth rate that incorporates fresh and thawed frozen embryo transfer.[17-18] For every 'add-on' intervention introduced in the IVF clinical practice, an improving of the cumulative chance of a live birth would be advisable.[17]

According to the single RCT study that has addressed this outcome, the cumulative IVF success was not improved. However, this study was performed using day 3 embryos (Figure 1).[13] Other RCTs are urgently needed. Currently, two larger RCTs are ongoing and the results are expected soon. Both the CESE-PGS (Cumulative Live Birth Rate with elective single embryo transfer (eSET) after Preimplantation Genetic Screening Versus Conventional In-vitro Fertilization) (ClinicalTrials.gov Identifier: NCT03118141) study and the Single Embryo TrAnsfeR of Euploid Embryo (STAR) study (ClinicalTrials.gov Identifier: NCT02268786) entail the NGS analysis on blastocysts biopsy. A notable difference is that, for the CESE-PGS study the primary outcome is represented by the cumulative live birth rate, while for the STAR study it is the ongoing pregnancy rate after one transfer, an outcome measure that has been strongly criticized. Furthermore, of the two studies, only the CESE-PGS study includes an intention-to-treat analysis. To avoid overestimated results, the definition of statistically correct clinical outcomes for PGT-A should imply the calculation of pregnancy rates with number of started cycles rather than embryo transfer as the denominator.[19]

The spectrum of genetic techniques

Some methods are commonly employed to test CCS for PGT-A, including aCGH, single-nucleotide polymorphism array (SNP array), qPCR and NGS. These screening tests differ in terms of genomic coverage, ability to detect unbalanced translocations, partial aneuploidies, polyploidy and mosaicisms. Each of them shows advantages and drawbacks. aCGH, SNP array and NGS require the whole genome amplification (WGA) of genomic DNA, potentially leading to artifacts. aCGH can detect aneuploidies but also unbalanced translocations, partial aneuploidies and mosaicisms but cannot identify uniparental disomies and polyploidies.[20-22] SNP array can detect unbalanced translocations, partial aneuploidies, uniparental disomies but can identify mosaicisms only if an adequate number of TE cells are analyzed.[21] On the other hand, qPCR does not use WGA, can identify aneuploidies in a rapid fashion but has lower genomic coverage, is not able to distinguish small deletions and duplications and cannot detect structural chromosome aberrations or mosaicisms.[23] NGS is the newest platform for PGT-A that permits to reduce DNA sequencing cost, increases number of samples that can be simultaneously sequenced, detects unbalanced translocations, partial aneuploidies and enhances the detection of mosaicisms.[24-25] The higher rate of mosaicism detected by NGS is likely explained by a superior sensitivity of this method for detecting minor lines in mixed cell populations compared with aCGH.[26] Even so, depending on the depth of sequencing and the specific NGS platform used, the sensitivity for detecting cytogenetically distinct subpopulations of cells varies as NGS strategies change. Unfortunately, NGS cannot directly detect balanced chromosomal rearrangements, because there is no imbalance in the total DNA content.[24]

Due to differences in protocols and methodologies, current data do not exist to irrefutably determine the superiority of any platform to the others (Figure 1).

Management of mosaicisms

Management of mosaicisms represents a critical issue for PGT-A. The primary origins of embryonic mosaicisms are the post-zygotic chromosome segregation errors due to mitotic non-disjunction, anaphase lag, chromosome deletion or duplication.[27] An embryonic mosaicism might also be induced by IVF treatments such as the ovarian stimulation and/or in vitro culture of human embryos. Indeed, the type of ovarian stimulation may influence the rate of chromosomal mosaicisms.[28] In mouse models, changes in oxygen tension during embryo culture have been shown to affect chromosomal mosaicism rates.[29] Recently, the incidence of chromosome abnormalities in human embryos has been demonstrated to be fertility center-dependent, indicating that mosaicism rate could very likely be influenced by culture conditions as temperature, pH and media composition.[30]

Although embryonic mosaicism was initially observed 25 years ago[31], the emerging attribution of embryos as mosaics are due to two phenomena. Firstly, genetic technologies for detecting chromosomal copy number variations in embryos have evolved from FISH to CCS platforms that represent a superior method for the assessment of mosaicisms. If, on one hand, NGS is more accurate to detect a low level of mosaicism in an embryo biopsy[26], on the other, some studies consider the impact of technical artifacts introduced by WGA and the lack of robustness of testing methods for the diagnosis as one possible cause contributing to the overestimation of the embryo mosaicisms.[32] Secondly, the advancement from cleavage stage to TE biopsy of blastocysts has allowed the analysis of multiple biopsied cells. A single TE biopsy usually includes approximately 5-10 cells that do not always show the same chromosomal content. Moreover, Gleicher and coworkers[33] have observed that multiple biopsies of the same blastocyst show different genetic results. Although the sample size of this paper is very small, the intra-embryo variability suggested that TE mosaicism is more frequent than previously reported. High divergence in results among biopsies of the same blastocyst was reported also by other authors.[34-35] This represents a biological limitation, difficult to overcome with the most effective diagnostic techniques, and an inaccurate prediction of mosaicism could lead to a false positive diagnosis. Therefore, the actual rate of

mosaicism is not known and varies based on the stage of the embryo, the sensitivity of PGT-A techniques used and the percentage of aneuploid cells within the TE specimen.[20,36-43] Limitations and details of the different genetic techniques and platforms are described in the specific dedicated paragraph.

The possibility to detect chromosomal mosaicisms in embryos has given rise to novel clinical challenges in PGT-A result interpretation and patients' counseling. At the introduction of PGT-A, only euploid embryos had been considered for transfer while mosaic embryos were not transferred being considered as abnormal. Recent studies revealed that mosaic blastocysts miscarry more often and implant less frequently than an euploid blastocyst but a proportion of mosaic embryos can implant and result in healthy babies.[41,44-45] In a chimeric murine model, mosaic embryos have been shown to undergo a "self-correcting" mechanism if they contain sufficient euploid cells.[46]

Taking into account these new findings, mosaic embryos can be considered a distinct category in terms of viability between euploid and fully aneuploid embryos. Recently, the Preimplantation Genetic Diagnosis International Society (PGDIS) recommended that these embryos should not be discarded but transferred when there is no alternative after appropriate genetic patients' counseling. According to this position statement, a cut-off point for the definition of mosaicism would be > 20% abnormal cells from a TE biopsy. Lower levels should be treated as euploid, more than 80% as aneuploid and between 20 and 80% abnormal cells as mosaic embryos. Mosaic monosomies, generally not viable, should be favored over mosaic trisomies, that can result in live births with associated physical and cognitive deficits.[47] Moreover, the same guidelines recommended to PGT-A laboratories the use of NGS, which is capable of measuring chromosomal copy numbers, as the only diagnostic platform adequate in assessing TE mosaicism.[47] In support to the PGDIS Position Statement, at the 2016 World Congress on Controversies in Preconception, Preimplantation, Prenatal Genetic Diagnosis (CoGEN) Meeting, a group of investigators reached similar conclusions, advising to prioritize transfer of mosaic embryos with lower levels (20–40%) of

aneuploidy over those with higher levels (40–70%), and defining any embryo TE biopsy with more than 70% aneuploidy as aneuploid and, consequently, not to be transferred.[48] In contrast to PGSIS position statement, GoGEN Consensus statement considered embryos mosaics for monosomies with similar implantation rate to mosaic trisomies because mosaic monosomies might contain trisomic cell lines, enabling them to implant.[48]

The chance of a healthy live birth seems to vary depending on the rate of mosaicism and on the type of aneuploidy. In fact, according to the group of Fiorentino and coworkers[49], embryos with a high chromosomal mosaicism (≥ 50%) showed a significant reduction in terms of implantation and live birth rates, compared with mosaic embryos with a lower aneuploidy percentage (<50%). Furthermore, blastocysts with lower levels of mosaicism (<50%) were associated with outcomes similar to euploid embryos. Miscarriage rates did not appear significantly different.[49] These findings are in contrast with those from Kushnir and coworkers[50] that, reanalyzing the raw data reported from Munnè *et al.*[45] to assess accuracy of the mosaicism percentage in predicting ongoing pregnancy and miscarriage rates, found that the degree of TE mosaicism, at any threshold of aneuploidy, was a poor predictor for both IVF outcomes. Given the financial and emotional impact of failed IVF cycles on patients, research to delineate the characteristics of mosaicisms potentially compatible with healthy live births is urgently needed (Figure 1).

Opportunities

Single embryo transfer policy

Multiple pregnancies carry adverse outcomes such as an increased risk of premature birth and perinatal death.[51] To reduce the rate of multiple births, a limitation on the number of transferred embryos has been recommended, especially, in good prognosis patients.[52] The eSET is the most obvious way of avoiding the risk of twins following IVF cycles. The uptake of this strategy has

been variable, and it is mandatory only in some countries.[53] In this context, the application of the PGT-A strategy represents an opportunity to select embryos to transfer (Figure 1). Importantly, the eSET does not impact negatively on the cumulative live birth rates. A Cochrane study showed no evidence for a significant difference in the cumulative live birth rate when a single cycle of double embryo transfer was compared with repeated eSETs.[54] According to Ubaldi and coworkers, the application of eSET in combination with the appropriate selection of blastocysts by PGT-A could be considered an efficient approach also in AMA patient. Indeed, after the introduction of an eSET policy coupled with PGT-A, multiple pregnancy rate decreased from 21.0 % to 6.8%, maintaining constant the cumulative success rate of the IVF programme.[55]

Time to pregnancy

Couples undergoing several IVF failures or miscarriages may undergo economic and psychological burdens. Moreover, pregnancy represents a race against time for women older than 35 years. If PGT-A is performed, the couple may conceive a healthy baby in a shorter time transferring only euploid embryos, and this might be an advantage mainly for AMA patients. According to the RCT by Rubio *et al.*, the time for a successful ongoing pregnancy was reduced, but not significantly, in patients undergoing PGT-A.[13] Recently, a retrospective cohort study involving AMA women showed that the PGT-A group achieved a clinical pregnancy leading to a live birth in a shorter time, compared to controls (104.8 days vs 140.6 days, $P<0.05$).[56] Nowadays, this is the only study that supports PGT-A procedure as a good strategy to obtain a clinical pregnancy in a shorter time. Nevertheless, the value of this data was strongly limited by the retrospective nature of the study (Figure 1). A shorter time to obtain a pregnancy entails a reduction in the number of embryo transfers with a consequent reduction in costs.

Psychological aspect of health care

An indication to PGT-A treatment might be represented by repeated miscarriages and recurrent implantation failures on the basis of the idea that the consequent psychological sequelae often involve grief, guilt, loss and, in some cases, psychiatric disturbances including depression, anxiety and posttraumatic stress disorder.[57] In addition, unresolved grief, interpreted as parents who continue to grieve the loss of the previous baby rather than happily anticipating a baby in a new current pregnancy, can impact on attachment to subsequent pregnancies.[58] Therefore, on the basis of the supposed opportunity to reduce the time to pregnancy, PGT-A is often offered in order to reduce the emotional distress of multiple embryo transfers with negative outcomes. Moreover, the cryopreservation of embryos with a real potential to implant because of euploid chromosome status could reassure women about their family planning. In these cases, PGT-A might have a positive impact on psychological wellbeing (Figure 1). No study has evaluated the psychological implications and the consequences of a PGT-A procedure resulting in only aneuploid embryos available for transfer. In this case, PGT-A might represent a psychological turning point.

Threats

Cost analysis

PGT-A is a costly procedure, but the real costs are difficult to quantify, as they include costs for the IVF cycle, molecular techniques, genetic and psychological counseling but also the management of miscarriages and of multiple pregnancies should not be disregarded (Figure 1).

For PGT-A version 1, a single study using an analytic decision model for cost-effectiveness analysis showed that IVF procedure alone was less costly per healthy infant compared to IVF/PGT-A in AMA women.[59] Three studies have evaluated the cost-effectiveness of PGT-A version 2. Murugappan and coworkers, applying PGT-A to patients with unexplained recurrent miscarriages compared to expectant management, showed that PGT-A could decrease abortion rate while live

birth rate was not improved. Results of the analysis of the costs per live birth showed that IVF/PGT-A was 100-fold more expensive and consequently was not cost-effective in increasing live birth.[60] However, this study used the current literature to evaluate the cost effectiveness of IVF cycles and PGT-A compared with expectant management and not the effectiveness of PGT-A in terms of real costs compared with only IVF treatment. Also, Scriven and coworkers[61] demonstrated that adding PGT-A to IVF treatment for women under the age of 40 years has no beneficial effect in terms of costs. Conversely, based on data from published literature, a decision analytic model for an hypothetical fresh IVF cycle in women older than 37 with at least one blastocyst in assessing the expected cost of achieving one live birth, was in favor of the addition of PGT-A as a cost-effective approach.[62] Since these studies were based on hypothetical estimates, it is of critical importance to provide a cost analysis of PGT-A based on real costs. More recently, the cost to attain a 50%, 75% or 90% likelihood of a euploid blastocyst was evaluated, considering patient's age and AMH values. Results would indicate that cost increases in poor prognosis patients.[63] More research on this aspect is needed, considering the potential advantage of NGS in reducing costs by high throughput sequencing technologies and the increasing number of samples that can be simultaneously sequenced during a single experiment.[15]

Invasive and no standardized procedure

To collect cells needed for a genetic analysis, PGT-A requires a biopsy procedure that represents an invasive manipulation of embryos and blastocysts and a time-consuming approach. An overall concern persists over a possible damage deriving from this procedure. Embryo biopsies require well-trained and highly experienced embryologists and consequently the allocation of novel human resources in PGT-A laboratories, with an increase in costs.[64] A prospective paired RCT has demonstrated that cleavage stage biopsy is detrimental while TE biopsy is safe for the implantation process.[3] Nowadays, it is generally accepted that TE biopsy has less impact on embryo viability

than cleavage stage biopsy because, even though more cells are removed during blastocyst biopsy, they represent a smaller percentage of embryo mass. Moreover, only TE cells and no fetal cells are collected. Nevertheless, the impact of a TE biopsy is not well defined and, given the importance of the TE for implantation, a perturbation of this critical event cannot be excluded. In the meantime, high standards are required for blastocyst culture and cryopreservation, which represent important limiting factors for the widespread implementation of this strategy.

Different methods have been described[65] for the blastocyst stage biopsy that may involve a heterogeneous cohort of embryos in terms of both morphology and developmental rate. A hole in the zona pellucida at day 3 can be performed with the aim to favor a blastocyst hatching process and to allow an easier collection of the cells extruded. Alternatively, the zona opening and the TE biopsy may be simultaneously performed, leaving the embryo undisturbed up to day 5-7. In the latter case, the blastocyst is biopsied exclusively after reaching the full expansion. Finally, a hole in zona pellucida can be performed later, at the blastocyst stage, and the hatching may be appreciated after few hours. No RCT has been performed on the efficacy and safety of these different approaches (Figure 1).

Alternatively to blastocyst biopsy, less-invasive techniques are being developed. Many studies focused on cell-free DNA recovered from blastocoel fluid with controversial data regarding the concordance rate with TE cells.[66-69] Recently, cell-free DNA has also been detected in blastocyst-spent culture media but no agreement on concordance rate with the genetic status of embryos has been found.[70-74] Although both strategies appear to be attractive methods, they are characterized by some limitations including the incomplete representation of the whole embryonic genome, the potential maternal DNA contamination, the poor nucleic acid integrity and the unknown sampling time points to obtain acceptable amplification rates.[75] Well-designed studies are needed in order to evaluate the efficacy of cell-free DNA employment on clinical outcomes.

Obstetrical and perinatal outcomes

Reports on neonatal outcomes and obstetrical complications of PGT pregnancies are limited.[76-82] Some studies have evaluated the effects of embryo biopsy without distinction between PGT-M and PGT-A.[77,80] It is necessary to consider that the technical procedures for PGT-M and PGT-A are similar, while the indication for these techniques is not. Indeed, PGT-M is applied in fertile couples, whereas PGT-A is used in infertile couples undergoing IVF. As infertility condition is associated with obstetrical complications, perinatal adversities and a less optimal neurological development, PGT-A children could be at higher risk for adverse outcomes compared to PGD offspring.[83]

Only a RCT and a retrospective questionnaire analysis have analyzed obstetrical and neonatal outcomes in patients who underwent PGT-A only (Figure 1).[82,84] Forman and colleagues[84] showed that single euploid blastocyst transfer had significantly better obstetrical outcomes than double untested embryo transfer. A lower birthweight and a longer period spent in the neonatal Intensive Care Unit have been reported for untested newborns but the consequences of multiple pregnancies in this group represent an obvious explanation. Jing and coworkers[82] found a higher incidence of gestational hypertension in singleton pregnancies after blastocyst stage biopsy and frozen embryo transfer than after cleavage stage biopsy and fresh embryo transfer. In this study, vitrification may be an important factor for the high incidence of gestational hypertension.

Long-term outcomes

The relative invasiveness of the embryo biopsy inherent to PGT-A raised issues on its safety on children development. Currently, data on developmental status and health of PGT-A offspring is scarce (Figure 1). From the paper by Mastenbroek and coworkers,[1] six prospective, assessor-blinded follow-up studies were derived on children born to women undergone PGT-A or not, in order to evaluate neurodevelopmental outcomes at 18 months, 2-, 4- and 9- years.[85-90] In these studies, no statistically significant differences in mental, psychomotor, neurological and behavioral outcomes were reported between children born after PGT-A and those born without PGT-A. Unfortunately, the power calculation of the original RCT was based on the number of women

needed to detect an increase in ongoing pregnancy rates and not on the number of children to be followed-up. Nevertheless, in two of these studies, according to the post-hoc power analysis, the sample size seemed to be able to detect clinically relevant differences.[88,90] None of the studies has addressed the long-term follow-up of children born after PGT-A at the blastocyst stage.

Conclusions

Various SWOT analyses have been delineated in the last years for novel strategies that are entering dramatically in the clinical practice in the IVF world.[91-93] Although the major advantage of the PGT-A procedure is the selection of the embryo to transfer in order to favor the eSET and reduce the number of transfers, several other factors such as the potential reduction in miscarriage rate, also support a move toward this approach in assisted reproduction technology (ART). The psychological burden and the reduction in costs in a wider perspective than so far investigated are elements that should be better investigated. Taken together, these developments may lead to a new era in modern ART. Nevertheless, confirmation that risks and threats associated with this strategy do not overcome benefits and opportunities is mandatory prior to shifting our current practice toward the routine use of blastocyst biopsy with genetic testing in all infertile patients.

References

1. Mastenbroek S, Twisk M, van Echten-Arends J, et al. In vitro fertilization with preimplantation genetic screening. N Engl J Med. 2007;357(1):9-17.

2. Twisk M, Mastenbroek S, van Wely M, Heineman MJ, Van der Veen F, Repping S. Preimplantation genetic screening for abnormal number of chromosomes (aneuploidies) in in vitro fertilisation or intracytoplasmic sperm injection. Cochrane Database Syst Rev. 2006; (1):CD005291.

3. Scott RT Jr, Upham KM, Forman EJ, Zhao T, Treff NR. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. Fertil Steril. 2013;100(3):624-630.

4. Ioannidis JP, Haidich AB, Pappa M, et al. Comparison of evidence of treatment effects in randomized and nonrandomized studies. JAMA. 2001;286(7):821-830.

5. Selman TJ, Johnson NP, Zamora J, Khan KS. Gynaecologic surgery from uncertainty to science: evolution of randomized control trials. Hum Reprod. 2008;23(4):827-831.

6. Yang Z, Liu J, Collins GS, et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. Mol Cytogenet. 2012;5(1):24.

7. Scott RT Jr, Upham KM, Forman EJ, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. Fertil Steril. 2013 ;100(3):697-703.

8. Forman EJ, Hong KH, Ferry KM, et al. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. Fertil Steril. 2013;100(1):100-107.

9. Chen M, Wei S, Hu J, Quan S. Can Comprehensive Chromosome Screening Technology Improve IVF/ICSI Outcomes? A Meta-Analysis. PLoS One. 2015;10(10):e0140779.

10. Dahdouh EM, Balayla J, García-Velasco JA. Comprehensive chromosome screening improves embryo selection: a meta-analysis. Fertil Steril. 2015;104(6):1503-1512.

11. Hassold T, Hunt P. Maternal age and chromosomally abnormal pregnancies: what we know and what we wish we knew. Curr Opin Pediatr 2009;21:703-708.

12. Ata B, Kaplan B, Danzer H, et al. Array CGH analysis shows that aneuploidy is not related with the number of embryos generated. Reprod Biomed Online 2012;24:614–620.

13. Rubio C, Bellver J, Rodrigo L, et al. In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study. Fertil Steril. 2017;107(5):1122-1129.

14. Sermon K, Capalbo A, Cohen J, et al. The why, the how and the when of PGS 2.0: current practices and expert opinions of fertility specialists, molecular biologists, and embryologists. Mol Hum Reprod. 2016;22(8):845-857.

15. Fiorentino F, Bono S, Biricik A, et al. Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. Hum Reprod. 2014;29(12):2802-2813.

16. Griesinger G. Beware of the 'implantation rate'! Why the outcome parameter 'implantation rate' should be abandoned from infertility research. Hum Reprod. 2016;31(2):249-251.

17. Maheshwari A, McLernon D, Bhattacharya S. Cumulative live birth rate: time for a consensus? Hum Reprod. 2015;30(12):2703-2707.

18. Malizia BA, Hacker MR, Penzias AS. Cumulative live-birth rates after in vitro fertilization. N Engl J Med. 2009;360(3):236-243.

19. Gleicher N, Kushnir VA, Barad DH. Preimplantation genetic screening (PGS) still in search of a clinical application: a systematic review. Reprod Biol Endocrinol. 2014;12:22.

20. Fragouli E, Alfarawati S, Daphnis DD, et al. Cytogenetic analysis of human blastocysts with the use of FISH, CGH and aCGH: scientific data and technical evaluation. Hum Reprod. 2011;26(2):480-490.

21. Brezina PR, Anchan R, Kearns WG. Preimplantation genetic testing for aneuploidy: what technology should you use and what are the differences? J Assist Reprod Genet. 2016;33(7):823-832.

22. Mamas T, Gordon A, Brown A, Harper J, Sengupta S. Detection of aneuploidy by array comparative genomic hybridization using cell lines to mimic a mosaic trophectoderm biopsy. Fertil Steril. 2012;97(4):943-947.

23. Treff NR, Tao X, Ferry KM, Su J, Taylor D, Scott RT Jr. Development and validation of an accurate quantitative real-time polymerase chain reaction-based assay for human blastocyst comprehensive chromosomal aneuploidy screening. Fertil Steril. 2012;97(4):819-824.

24. Fiorentino F, Biricik A, Bono S, et al. Development and validation of a next-generation sequencing-based protocol for 24-chromosome aneuploidy screening of embryos. Fertil Steril. 2014;101(5):1375-1382.

25. Zheng H, Jin H, Liu L, Liu J, Wang WH. Application of next-generation sequencing for 24-chromosome aneuploidy screening of human preimplantation embryos. Mol Cytogenet. 2015;8:38.

26. Munné S, Wells D. Detection of mosaicism at blastocyst stage with the use of high-resolution next-generation sequencing. Fertil Steril. 2017;107(5):1085-1091.

27. Taylor TH, Gitlin SA, Patrick JL, Crain JL, Wilson JM, Griffin DK. The origin, mechanisms, incidence and clinical consequences of chromosomal mosaicism in humans. Hum Reprod Update. 2014;20(4):571-581.

28. Baart EB, Martini E, Eijkemans MJ, et al. Milder ovarian stimulation for in-vitro fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial. Hum Reprod. 2007;22(4):980-9828.

29. Bean CJ, Hassold TJ, Judis L, Hunt PA. Fertilization in vitro increases non-disjunction during early cleavage divisions in a mouse model system. Hum Reprod. 2002;17(9):2362-2367.

30. Munné S, Alikani M, Ribustello L, Colls P, Martínez-Ortiz PA, McCulloh DH. Referring Physician Group. Euploidy rates in donor egg cycles significantly differ between fertility centers. Hum Reprod. 2017;32(4):743-749.

31. Delhanty JD, Griffin DK, Handyside AH, et al. Detection of aneuploidy and chromosomal mosaicism in human embryos during preimplantation sex determination by fluorescent in situ hybridisation, (FISH). Hum Mol Genet. 1993;2(8):1183-1185.

32. Capalbo A, Ubaldi FM, Rienzi L, Scott R, Treff N. Detecting mosaicism in trophectoderm biopsies: current challenges and future possibilities. Hum Reprod. 2017;32(3):492-498.

33. Gleicher N, Vidali A, Braverman J, et al. Accuracy of preimplantation genetic screening (PGS) is compromised by degree of mosaicism of human embryos. *Reprod Biol Endocrinol* 2016;14:54.

34. Tortoriello DV, Dayal M, Beyhan Z, Yakut T, Keskintepe L. Reanalysis of human blastocysts with different molecular genetic screening platforms reveals significant discordance in ploidy status. J Assist Reprod Genet. 2016;33(11):1467-1471.

35. Orvieto R, Shuly Y, Brengauz M, Feldman B. Should pre-implantation genetic screening be implemented to routine clinical practice? Gynecol Endocrinol. 2016;32(6):506-508.

36. Liu J, Wang W, Sun X, et al. DNA microarray reveals that high proportions of human blastocysts from women of advanced maternal age are aneuploid and mosaic. Biol Reprod. 2012;87(6):148.

37. Johnson DS, Cinnioglu C, Ross R, et al. Comprehensive analysis of karyotypic mosaicism between trophectoderm and inner cell mass. Mol Hum Reprod. 2010;16(12):944-949.

38. Northrop LE, Treff NR, Levy B, Scott RT Jr. SNP microarray-based 24 chromosome aneuploidy screening demonstrates that cleavage-stage FISH poorly predicts aneuploidy in embryos that develop to morphologically normal blastocysts. Mol Hum Reprod 2010;16:590-600.

39. Capalbo A, Wright G, Elliott T, Ubaldi FM, Rienzi L, Nagy ZP. FISH reanalysis of inner cell mass and trophectoderm samples of previously array-CGH screened blastocysts shows high

accuracy of diagnosis and no major diagnostic impact of mosaicism at the blastocyst stage. Hum Reprod 2013;28:2298-2307.

40. Novik V, Moulton EB, Sisson ME, et al. The accuracy of chromosomal microarray testing for identification of embryonic mosaicism in human blastocysts. Mol Cytogenet. 2014;7(1):18.

41. Greco E, Minasi MG, Fiorentino F. Healthy babies after intrauterine transfer of mosaic aneuploid blastocysts. N Engl J Med 2015;373:2089-2090.

42. Ruttanajit T, Chanchamroen S, Cram DS, et al. Detection and quantitation of chromosomal mosaicism in human blastocysts using copy number variation sequencing. Prenat Diagn. 2016;36(2):154-162.

43. Popovic M, Dheedene A, Christodoulou C, et al. Chromosomal mosaicism in human blastocysts: the ultimate challenge of preimplantation genetic testing? Hum Reprod. 2018;33(7):1342-1354.

44. Fragouli E, Alfarawati S, Spath K, et al. Analysis of implantation and ongoing pregnancy rates following the transfer of mosaic diploid-aneuploid blastocysts. Hum Genet. 2017;136(7):805-819.

45. Munné S, Blazek J, Large M, et al. Detailed investigation into the cytogenetic constitution and pregnancy outcome of replacing mosaic blastocysts detected with the use of high-resolution next-generation sequencing. Fertil Steril. 2017;108(1):62-71.

46. Bolton H, Graham SJ, Van der Aa N, et al. Mouse model of chromosome mosaicism reveals lineage-specific depletion of aneuploid cells and normal developmental potential. Nat Commun. 2016;7:11165.

47. PGDIS position statement on chromosome mosaicism and preimplantation aneuploidy testing at the blastocyst stage. [http://www.pgdis.org/docs/newsletter_071816.html].

48. Controversies in Preconception, Preimplantation, and Prenatal Genetic Diagnosis. CoGen position statement on chromosomal mosaicism detected in preimplantation blastocyst biopsies. [https://www.ivfworldwide.com/index.php?option=com_content&view=article&id=733&Itemid=464].

49. Spinella F, Fiorentino F, Biricik A, et al. Extent of chromosomal mosaicism influences the clinical outcome of in vitro fertilization treatments. Fertil Steril. 2018;109(1):77-83.

50. Kushnir VA, Darmon SK, Barad DH, Gleicher N. Degree of mosaicism in trophectoderm does not predict pregnancy potential: a corrected analysis of pregnancy outcomes following transfer of mosaic embryos. Reprod Biol Endocrinol. 2018;16(1):6.

51. Pinborg A. IVF/ICSI twin pregnancies: risks and prevention. Hum Reprod Update. 2005;11(6):575-593.

52. Thurin A, Hausken J, Hillensjö T, et al. Elective single-embryo transfer versus double-embryo transfer in in vitro fertilization. N Engl J Med. 2004;351(23):2392-2402.

53. Maheshwari A, Griffiths S, Bhattacharya S. Global variations in the uptake of single embryo transfer. Hum Reprod Update. 2011;17(1):107-120.

54. Pandian Z, Marjoribanks J, Ozturk O, Serour G, Bhattacharya S. Number of embryos for transfer following in vitro fertilisation or intra-cytoplasmic sperm injection. Cochrane Database Syst Rev. 2013;(7):CD003416.

55. Ubaldi FM, Capalbo A, Colamaria S, et al.. Reduction of multiple pregnancies in the advanced maternal age population after implementation of an elective single embryo transfer policy coupled with enhanced embryo selection: pre- and post-intervention study. Hum Reprod. 2015;30(9):2097-2106.

56. Lee E, Chambers GM, Hale L, Illingworth P, Wilton L. Assisted reproductive technology (ART) cumulative live birth rates following preimplantation genetic diagnosis for aneuploidy (PGD-A) or morphological assessment of embryos: A cohort analysis. Aust N Z J Obstet Gynaecol. 2017 Dec 27.

57. Frost M, Condon JT. The psychological sequelae of miscarriage: a critical review of the literature. Aust N Z J Psychiatry. 1996;30(1):54-62.

58. O'Leary J. Grief and its impact on prenatal attachment in the subsequent pregnancy. Arch Womens Ment Health. 2004;7(1):7-18.

59. Mersereau JE, Plunkett BA, Cedars MI. Preimplantation genetic screening in older women: a cost-effectiveness analysis. Fertil Steril. 2008;90(3):592-598.

60. Murugappan G, Ohno MS, Lathi RB. Cost-effectiveness analysis of preimplantation genetic screening and in vitro fertilization versus expectant management in patients with unexplained recurrent pregnancy loss. Fertil Steril. 2015;103(5):1215-1220.

61. Scriven PN. Towards a better understanding of preimplantation genetic screening for aneuploidy: insights from a virtual trial for women under the age of 40 when transferring embryos one at a time. Reprod Biol Endocrinol. 2017;15(1):49.

62. Collins SC, Xu X, Mak W. Cost-effectiveness of preimplantation genetic screening for women older than 37 undergoing in vitro fertilization. J Assist Reprod Genet. 2017;34(11):1515-1522.

63. Goldman RH, Racowsky C, Farland LV, et al. The cost of a euploid embryo identified from preimplantation genetic testing for aneuploidy (PGT-A): a counseling tool. J Assist Reprod Genet. 2018;35:1641-1650.

64. Alikani M, Go KJ, McCaffrey C, McCulloh DH. Comprehensive evaluation of contemporary assisted reproduction technology laboratory operations to determine staffing levels that promote patient safety and quality care. Fertil Steril. 2014;102(5):1350-1356.

65. Capalbo A, Romanelli V, Cimadomo D, et al. Implementing PGD/PGD-A in IVF clinics: considerations for the best laboratory approach and management. J Assist Reprod Genet. 2016;33(10):1279-1286.

66. Palini S, Galluzzi L, De Stefani S, et al. Genomic DNA in human blastocoele fluid. Reprod Biomed Online 2013;26:603-610.

67. Tobler K, Zhao Y, Ross R, et al. Blastocoel fluid from differentiated blastocysts harbors embryonic genomic material capable of a whole-genome deoxyribonucleic acid amplification and comprehensive chromosome microarray analysis. Fertil Steril 2015;104:418-425.

68. Gianaroli L, Magli MC, Pomante A, et al. Blastocentesis: a source of DNA for preimplantation genetic testing. Results from a pilot study. Fertil Steril 2014;102:1692-1699.

69. Magli MC, Pomante A, Cafueri G, et al. Preimplantation genetic testing: polar bodies, blastomeres, trophectoderm cells, or blastocoelic fluid? Fertil Steril 2016;105:676-683.

70. Galluzzi L, Palini S, Stefani S, et al. Extracellular embryo genomic DNA and its potential for genotyping applications. Future Sci OA 2015;1:FSO62.

71. Xu J, Fang R, Chen L, et al. Noninvasive chromosome screening of human embryos by genome sequencing of embryo culture medium for in vitro fertilization. Proc Natl Acad Sci U S A 2016;113:11907-11912.

72. Vera-Rodriguez M, Diez-Juan A, Jimenez-Almazan J, et al. Origin and composition of cell-free DNA in spent medium from human embryo culture during preimplantation development. Hum Reprod 2018;33:745-756.

73. Li P, Song Z, Yao Y, et al. Preimplantation genetic screening with spent culture medium/Blastocoel fluid for in vitro fertilization. Sci Rep 2018;8:9275.

74. Kuznyetsov V, Madjunkova S, Antes R, Abramov R, Motamedi G, Ibarrientos Z, Librach C. Evaluation of a novel non-invasive preimplantation genetic screening approach. PLoS One 2018;13:e0197262.

75. Farra C, Choucair F, Awwad J. Non-invasive pre-implantation genetic testing of human embryos: an emerging concept. Hum Reprod. 2018;33(12):2162-2167.

76. Bay B, Ingerslev HJ, Lemmen JG, Degn B, Rasmussen IA, Kesmodel US. Preimplantation genetic diagnosis: a national multicenter obstetric and neonatal follow-up study. Fertil Steril. 2016;106(6):1363-1369.

77. Desmyttere S, De Rycke M, Staessen C, et al. Neonatal follow-up of 995 consecutively born children after embryo biopsy for PGD. Hum Reprod. 2012;27(1):288-293.

78. Eldar-Geva T, Srebnik N, Altarescu G, et al. Neonatal outcome after preimplantation genetic diagnosis. Fertil Steril. 2014;102(4):1016-1021.

79. Zakharova EE, Zaletova VV, Krivokharchenko AS. Biopsy of human morula-stage embryos: outcome of 215 IVF/ICSI cycles with PGS. PLoS One. 2014;9(9):e1064.

80. Liebaers I, Desmyttere S, Verpoest W, et al. Report on a consecutive series of 581 children born after blastomere biopsy for preimplantation genetic diagnosis. Hum. Reprod. 2010;25(1):275–282.

81. Strom CM, Levin R, Strom S, Masciangelo C, Kuliev A, Verlinsky Y. Neonatal outcome of preimplantation genetic diagnosis by polar body removal: the first 109 infants. Pediatrics 2000;106(4):650–653.

82. Jing S, Luo K, He H, et al. Obstetric and neonatal outcomes in blastocyst stage biopsy with frozen embryo transfer and cleavage-stage biopsy with fresh embryo transfer after preimplantation genetic diagnosis/screening. Fertil Steril. 2016;106(1):105-112.

83. Thomson F, Shanbhag S, Templeton A, Bhattacharya S. Obstetric outcome in women with subfertility. Br J Obstet Gynecol 2005;112:632-637.

84. Forman EJ, Hong KH, Franasiak JM, Scott RT Jr. Obstetrical and neonatal outcomes from the BEST Trial: single embryo transfer with aneuploidy screening improves outcomes after in vitro fertilization without compromising delivery rates. Am J Obstet Gynecol. 2014;210(2):157.

85. Middelburg KJ, Heineman MJ, Haadsma ML, Bos AF, Kok JH, Hadders-Algra M. Neurological condition of infants born after in vitro fertilization with preimplantation genetic screening. Pediatr Res. 2010;67(4):430-434.

86. Middelburg KJ, van der Heide M, Houtzager B, et al. Mental, psychomotor, neurologic, and behavioral outcomes of 2-year-old children born after preimplantation genetic screening: follow-up of a randomized controlled trial. Fertil Steril. 2011;96(1):165-169.

87. Beukers F, van der Heide M, Middelburg KJ, et al. Morphologic abnormalities in 2-year-old children born after in vitro fertilization/intracytoplasmic sperm injection with preimplantation genetic screening: follow-up of a randomized controlled trial. Fertil Steril. 2013;99(2):408-413.

88. Schendelaar P, Middelburg KJ, Bos AF, et al. The effect of preimplantation genetic screening on neurological, cognitive and behavioural development in 4-year-old children: follow-up of a RCT. Hum Reprod 2013;28:1508-1518.

89. Seggers J, Haadsma ML, la Bastide-van Gemert S et al. Blood pressure and anthropometrics of 4-y-old children born after preimplantation genetic screening: follow-up of a unique, moderately sized, randomized controlled trial. Pediatr Res 2013;74:606-614.

90. Kuiper D, Bennema A, la Bastide-van Gemert S, et al. Developmental outcome of 9-year-old children born after PGS: follow-up of a randomized trial. Hum Reprod. 2018;33(1):147-155.

91. Blockeel C, Drakopoulos P, Santos-Ribeiro S, Polyzos NP, Tournaye H. A fresh look at the freeze-all protocol: a SWOT analysis. Hum Reprod. 2016;31(3):491-497.

92. Engmann L, Benadiva C, Humaidan P. GnRH agonist trigger for the induction of oocyte maturation in GnRH antagonist IVF cycles: a SWOT analysis. Reprod Biomed Online. 2016;32(3):274-285.

93. Massin N. New stimulation regimens: endogenous and exogenous progesterone use to block the LH surge during ovarian stimulation for IVF. Hum Reprod Update. 2017;23(2):211-220.

Figure legends

Figure 1. SWOT analysis of the PGT-A strategy

Table 1 Characteristics of the four prospective randomized controlled trials on PGT-A using comprehensive chromosome screening (CCS).

	Patients, n (PGT-A/control)	Age (mean ± SD) (PGT-A/ control)	Transfer type (PGT-A/ control)	Biopsy day/ Platform for PGT-A	CPR	OPR	IR	AbR	MPR	LBR
Yang et al., 2012	55/48 good prognosis	31.2 ± 2.5/ 31.5 ± 2.7	SET/SET Fresh	Day 5/ aCGH	improved	improved	improved	unchanged	-	-
Forman et al., 2013	89/86 good prognosis	35.1 ± 3.9/ 34.5 ± 4.7	SET/DET Fresh or frozen	Day 5-6/ qPCR	-	unchanged	unchanged	unchanged	reduced	-
Scott et al., 2013	72/83 good prognosis	32.2 ± 0.5/ 32.4 ± 0.5	SET-DET/ DET Fresh	Day 5-6/ qPCR	improved	-	improved	-	-	improved
Rubio et al., 2017	100/105 (AMA patients)	38≤years≤41	SET-DET/SET-DET Fresh or frozen	Day 3/ aCGH	unchanged	-	improved	reduced	-	improved

PGT-A, preimplantation genetic testing for aneuploidy; SET, single embryo transfer; DET, double embryo transfer; PR, pregnancy rate; CPR, clinical pregnancy rate; OPR, ongoing pregnancy rate; IR, implantation rate; AbR, abortion rate; MPR, multiple pregnancy rate; LBV, live birth rate; AMH, anti-mullerian hormone; aCGH, array comparative genomic hybridization; qPCR, quantitative PCR.

Strengths	Impact/Benefit
increased implantation rate	to be defined
decreased miscarriage rate	reduction of medical treatments reduction of distress

Weaknesses	Impact/Risks
3 RCTs in good prognosis patients 1 RCT in AMA patients	to set up clinical procedures based on poor evidence
cumulative IVF success not improved	overtreatment
spectrum of genetic techniques	misdiagnosis
management of mosaicism	decrease in treatment effectiveness

Opportunities	Impact/Benefit
adoption of eSET policy	reduction of multiple pregnancies
reduced time to pregnancy	cost reduction
psychological aspect of healthy care	improvement of patients' management

Threats	Impact/Risks
high cost	patients' dissatisfaction
invasive procedure and not standardized technique	embryo damage
obstetrical and perinatal outcomes: limited data long-term effect: limited data	adverse outcomes