Second generation preimplantation genetic testing for aneuploidy in assisted reproduction: A SWOT analysis

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ABSTRACT

Second generation preimplantation genetic testing for aneuploidy (PGT-A 2.0) in patients with unfavourable reproductive and in vitro fertilization (IVF) prognosis is becoming a common practice with the aim of improving reproductive outcomes. However, there is still no clear evidence on the possible advantages and drawbacks with regard to this procedure. In this discussion paper, based on a SWOT (strengths, weaknesses, opportunities, threats) analysis, the different aspects of this strategy are evaluated.

Current evidence suggests that PGT-A 2.0 should not have an indiscriminate application at present, but might be indicated in cases in which the risk of aneuploidy is increased.

Keywords: Preimplantation genetic testing, assisted reproduction, infertility, implantation, live birth, blastocyst biopsy.

Key message

Clinical studies suggest that second generation preimplantation genetic testingfor aneuploidy (PGT-A 2.0) helps to optimize some IVF outcomes; however, it has a number of limitations. PGT-A 2.0 should not be used indiscriminately at present but may be indicated in cases with an increased risk of aneuploidy.

INTRODUCTION

Several techniques for embryo selection have been developed in *in vitro* fertilization (IVF), including screening procedures for numeric or structural chromosome abnormalities which are collectively known as preimplantation genetic testing for aneuploidy (PGT-A) (**Rubio et al., 2017**). In the 1990s, a first version of PGT-A (PGT-A 1.0), based on a day 3 biopsy and the genetic analysis by fluorescence in situ hybridization (FISH), became widespread. However, results from several randomized

controlled trials (RCTs) showed that this approach did not clearly increase live birth rates and in some cases may have even reduced them (**Mastenbroek et al., 2011**).

During the last few years a new generation of preimplantation genetic testing (PGT-A 2.0) has been introduced. PGT-A 2.0, as contrasted to PGT-A 1.0, is characterized by trophectoderm biopsy and aneuploidy assessments of all chromosome pairs instead of FISH of a limited set of chromosomes **(Gleicher et al., 2014)**. The PGT-A 2.0 has been routinely used since 2008 with the aim of improving IVF outcomes (**MastenbroeK and Repping, 2014**). However, success was defined by various authors in many different ways making it very difficult to compare the outcomes of numerous studies (**Geraedts and Sermon, 2016**). Moreover, in the last decade, PGT-A 2.0 technologies have advanced considerably and there are no updated assessments with regard to their possible role in assisted reproductive technology (ART), as well as the possible impact of the biopsy at the blastocyst stage on reproductive outcomes (**Gleicher et al., 2017**).

For this reason, we designed the following SWOT (strengths, weaknesses, opportunities, threats) analysis to assess the available published evidence on the possible recommendation of PGT-A 2.0 for women of reproductive age. Additionally, this SWOT analysis provides the scientific level of evidence for each of the reviewed papers to avoid subjectivity in their statements.

METHODS

In this study, a SWOT analysis was carried out to understand the perceived strengths and major pitfalls of the PGT-A 2.0, to identify the opportunities that can be taken and the key threats of this technology according to the bibliography analyzed, and to know the experts' point of view. The SWOT method is recently applied in fertility medical research when there is insufficient scientific evidence to assess the applicability or not

of a particular technique, give light on specific issues and evaluate the possible pros and cons. (**Barrow, 2016; Blockeel et al. 2016;Engmann et al., 2016; Esteves et al. 2017**). First, a bibliographic search was carried out aimed at "preimplantation genetic screening" and "PGT-A 2 preimplantation" limited to the last 5 years. The primary review of the literature revealed 170 relevant articles. It is important to note that all published papers in the English literature addressing PGT-A 2.0 during the search years were reviewed. A division of the total number of references among the researchers and an Excel spreadsheet for each of the sections of the SWOT was proposed, which would be available on the **SISGtool.org platform** along with the corresponding references. In each of the tables the ideas/phrases proposed for each section would be noted and each researcher would add to each of them the studies classified by the degree of evidence. To unify the criteria for the evaluation of the evidence, the Oxford Centre for Evidence-based Medicine (CEBM) levels of quality of evidence were followed (Oxford CEBM, 2009) (**Table 1**).

RESULTS

1. STRENGTHS

1.1 PGT-A 2.0 is the strongest and most evaluated technique

The diagnostic platforms used to perform PGT-A 2.0 have improved considerably in recent years (**Scott 2017: evidence 5).** Current data strongly support the utilization of technologies that are capable of simultaneously evaluating the ploidy status of all 23 chromosome pairs (**Brezina et al., 2015; 2016**). Therefore, other more limited technologies, including fluorescence-in-situ hybridization (FISH), are discouraged (**Dahdouh et al., 2015: evidence 3a)** and the use of more comprehensive and reliable analytical platforms such as single nucleotide polymorphism (SNP) array, quantitative polymerase chain reaction, array comparative genomic hybridization (aCGH), and next-

generation sequencing (NGS) have been validated with class I data to provide improved results (increased implantation rates, improved delivery rates, greater proportion of pregnancies continuing beyond 20 weeks' gestation and achieving lower multiple pregnancy rates for the same ongoing pregnancy rate) (**Kane et al., 2016; Scott 2017: evidence 1b).**

PGT-A 2.0 presents a high level of consistency and reproducibility in different centers and with different embryologists (**Capalbo 2015; evidence 2a)**. Error rates with all the methods of 24-chromosome aneuploidy detection are low (1-2%), but clinical error rates with diagnoses of partial aneuploidy, mosaicism, or partial mosaicism are still unknown (**Maxwell and Grifo, 2018**). Predictive positive and negative values have been estimated around 4% **(Scott et al., 2012; Rubino et al. 2016: evidence 1b**). Each platform presents advantages and disadvantages (Table 2), but some such as next-generation sequencing (NGS), are capable of evaluating far more data points than has been previously possible (**Goodrich et al. 2017; evidence 1b; Brezina et al., 2016; evidence 1c)** and with better cost-effective results compared with other platforms **(Sueoka et al. 2015: evidence 4).**

1.2 PGT-A 2.0 Improves embryo selection: improves implantation rates and pregnancy rates

Aneuploidy increases with advanced maternal age (AMA) and is inversely proportional to implantation rates (IR). Transferred aneuploid embryos rarely result in a viable pregnancy (**Spinella et al., 2018**). Normally, to bypass the risk of high embryonic aneuploidy rates in ART, multiple embryos have been transferred with the aim of achieving at least one single live birth. However, this practice has been associated with a high rate of multiple pregnancies, a risky situation to both fetus and mother (**Murray**

et al., 2014). Due to this major drawback, embryo selection techniques such as PGT-A, have been developed to select the best available embryo to transfer into the uterus.

Several studies suggest that PGT-A 2.0 performed at the blastocyst stage with whole-genome screening seems to be a unique procedure; providing an accurate assessment of embryo ploidy, while maintaining high implantation potential (**evidence 1b**) (**Capalbo et al., 2013**; **Forman et al., 2013; Scott et al., 2013; Fragouli et al., 2014; Minasi and Greco, 2014; Minasi et al., 2016; Lee et al., 2015; Brezina et al., 2016; Ubaldi et al., 2017**). Seven cohort studies and four RCTs included in a meta-analysis analyzing reproductive outcomes showed that compared with morphological criteria, euploid embryos identified by comprehensive chromosome screening (CCS)-based PGT-A 2.0 were more likely to be successfully implanted (cohort study RR 1.74, 95% CI 1.35–2.24; RCT RR 1.26, 95% CI 0.83–1.93). CCS-based PGT-A 2.0 was also related to an increased clinical pregnancy rate (cohort study RR 1.48, 95% CI 1.20–1.83; RCT RR 1.26, 95% CI 0.83–1.93) and increased ongoing pregnancy rate (cohort study RR 1.61, 95% CI 1.30–2.00; RCT RR 1.31, 95% CI 0.64–2.66) when considering the cohort studies exclusively, but these results were not confirmed when only the RCTs where analyzed **(Chen et al., 2015: evidence 2a**). With regard to the impact of maternal age, Harton et al. demonstrated that the elective transfer of euploid embryos after array CGH (aCGH) confers equal IRs between reproductively younger and older patients up to 42 years (**Harton et al., 2013: evidence 4**). In patients with normal ovarian reserve, PGT-A 2.0 increases clinical and sustained IRs and is associated with higher ongoing pregnancy rates (**Dahdouh et al., 2015: evidence 1a).** In those women at high risk of producing aneuploid embryos [AMA, repeated implantation failure (RIF), recurrent pregnancy loss (RPL)], a lower level of evidence has been found as the data obtained were only from observational studies (**Dahdouh et al., 2015: evidence 2a; Lee et al.,**

2015: evidence 2a). Some ongoing RCTs are being conducted on different patient populations (e.g., AMA *[NCT028685528]* patients with male factor infertility *[NCT02941965]* to clarify the role of this technology in these populations.

Among the different protocols analyzed, selecting competent blastocysts for transfer by combining time-lapse monitoring and PGT-A 2.0 testing has been shown to significantly increase clinical pregnancy rates (71.1% vs. 45.9%, respectively, p = 0.037) and ongoing pregnancy rates (68.9% vs. 40.5%, p = 0.019) (**Yang et al., 2017**: e**vidence 1b**) compared with the best morphological grade available.

When analyzing different platforms, next generation sequencing (NGS) is considered the most precise technique and reported higher pregnancy rates than the others (**Lai et al., 2017: evidence 3b)**. In a post-hoc analysis of the STAR trial, in women 35 years and older a benefit with NGS in ongoing pregnancy rate (OPR) has been demonstrated (OPR of 50.8% (62/122) PGT-A 2.0 group vs 37.2% (54/145) control group; p<0.0349) (**Munné et al., 2017: evidence 1b**) which is consistent with the 2014 Society for Assisted Reproductive Technology (SART) data (SART, 2014).

Finally, aneuploidy screening with PGT-A 2.0 may help assess the probability of having euploid oocytes/embryos in future ART cycles (**Evidence 2b**) (**Feichtinger et al., 2015**).

1.3 PGT-A 2.0 decreases miscarriages

Many aneuploid human embryos survive preimplantation development (**Ambartsumyan and Clark, 2008)** and, therefore, the judicious use of embryo screening can minimize the incidence of miscarriages related to chromosomal abnormalities (**Harton et al., 2013; Minasi et al., 2017: evidence 4)**. A meta-analysis of the outcomes showed that compared with morphological criteria, euploid embryos

identified by PGT-A 2.0 decreased miscarriage rates in both cohort study (RR 0.31, 95% CI 0.21-0.46) and RCT-based data (RR 0.53, 95% CI 0.24-1.15) although in RCT-based data it did not reach statistical significance (**Chen et al., 2015: evidence 2a).** However, this finding has not been confirmed in women aged 35-40 (**Munné et al., 2017: evidence 1b**)

1.4 PGS 2.0 increases the chance for a healthy, term, singleton delivery

With PGT-A 2.0, a single euploid blastocyst with high reproductive potential can be selected for transfer. This paradigm increases the chance for a healthy, term, singleton delivery per embryo transfer procedure without requiring patients to undergo an increased number of failed embryo transfers (ETs) (**Chen et al., 2015: evidence 1a).** Particularly, in patients ≤ 42 years with normal ovarian reserve, PGT-A 2.0 resulted in improved obstetrical outcomes shown by higher birthweights, lower rates of preterm delivery, lower rates of neonatal intensive care (NICU) admission, and shorter NICU stays if admission were required (**Forman et al., 2014: evidence 1b**).

Retrospective data also suggest that for patients >37 years, PGT-A 2.0 improved live birth rates for single (aORs, 3.86 [95% CI, 1.25–11.9]; 8.2 [95% CI, 2.28–29.5]) and double ETs (aORs, 9.91 [95% CI, 2.0–49.6]; 8.67 [95% CI, 2.08–36.2]), but no difference was observed among patients <37 years (**Kang et al., 2015: evidence 3b)**. Furthermore, PGT-A 2.0 is not associated with adverse effects on neurological, cognitive and behavioral development, blood pressure, and anthropometrics of 4 year-old (**Seggers et al., 2013: evidence 1b)** and 9-year-old offspring (**Kuiper et al., 2018: evidence 1b)**.

2. WEAKNESS

2.1 Invasiveness and complexity of the technique

An important disadvantage of PGT-A 2.0 is that it requires biopsy of the preimplantation human embryo, which can limit its clinical applicability due to the invasiveness and complexity of the process (**Cimadomo et al., 2016**: **evidence 1a**). To date no sufficiently statistically powered study has clarified the impact of this procedure on embryo reproductive competence, although in young (≤ 35 years) infertile patients, the effectiveness of PGT-A has been suggested to be limited by the effectiveness of TE biopsy **(Ozgur et al., 2019; evidence 1b).** High standards are required for blastocyst culture and cryopreservation, which is an important limiting factor for the widespread implementation of this strategy (**Dahdouh et al., 2015**: **evidence 1a).**

2.2 Non-standardized technique and possibility of errors

One of the biggest limitations found when assessing the effectiveness of PGT-A 2.0 is that it is not a standardized technique which can use different genetic platforms (single nucleotide polymorphism (SNP) microarray, metaphase comparative genomic hybridization (mCGH), array comparative genomic hybridization (aCGH), quantitative polymerase chain reaction (qPCR), and NGS) (**Goodrich et al., 2017: evidence 1b; Yang et al., 2015: evidence 3b; Maxwell et al., 2016: evidence 3b**). Prerequisites for the successful application of PGT-A 2.0 in today's practice should, at least, include experience in extended embryo culture and biopsy and validated and tested CCS platforms (**Dahdouh et al., 2015**: **evidence 1a**). Current laboratory techniques seek to minimize errors as much as possible by utilizing the most sophisticated technology coupled with dozens of systemic quality controls (**Cimadono et al., 2016: evidence 2a).** However, the possibility for error when using PGT-A 2.0 still exists. Examples of technical errors include the following: methodologic misdiagnosis, hybridization errors,

technological misdiagnosis, DNA contamination and human error. Some platforms may be able to limit the impact of some of these problems, such as contamination, through allele mapping. Nevertheless, no diagnostic platform will ever be able to ensure 100% accuracy. This must be acknowledged and discussed with patients during the PGT-A 2.0 counseling process **(Brezina et al., 2016).**

2.3 Laboratory management reliability and poor consistency between centers

Precision in handling, manipulation, biopsy and observation of the gametes and embryos is critical for reducing adverse outcomes and these commonly discussed adverse outcomes are thought to be due to human error. Human and/or laboratory error can occur at every step along the way and laboratory staff have an obligation to follow stringent quality-control mechanisms throughout the process. Furthermore, euploidy rates in donor egg cycles significantly differ between fertility centers (**Munne et al., 2017; evidence 2b**) and training not only in laboratory techniques, but also in interpretation and delivery of reports and results is crucial (**Imudia et al., 2016: evidence 1c)**.

2.4 Costs of the technique

Cost has been identified as a significant determinant for the decision on whether or not to use PGT-A 2.0 (**Gebhart et al., 2016: evidence 5)**, since its application can suppose about a 50% increase with respect to the cost of a conventional IVF cycle. One option to improve PGT-A 2.0 efficiency in some patients with poor response would be to accumulate and split a larger number of embryos, but the cost per cycle would increase without the certainty that the healthy embryo comes from the first cycle (**Martínez et al., 2016).**

2.5 Risks of long-term culture to the blastocyst stage and the possibility of loss of embryos during extended culture

There are concerns with regard to epigenetic influence of long-term culture to the blastocyst stage (**Calle et al., 2012**). Blastocyst stage culture has been associated with increased risk of premature delivery in comparison to embryos transferred on day 2 or 3 (**Maheshwari et al., 2013; Dar et al., 2014: evidence 1a)** and the possibility of loss of embryos during extended culture. In fact, a previous Cochrane analysis of blastocyst vs. cleavage stage transfer showed that the cumulative pregnancy and live birth rates were better for cleavage stage transfer (**Glujovsky et al., 2016**).

2.6 Loss of embryos

Higher implantation rates are often used to claim that PGT-A 2.0 increases overall success rates after IVF/ ICSI, but this is somehow incorrect as PGT-A 2.0 is also associated with less embryos being available for transfer and/or cryopreservation (**Gleicher and Barad, 2012; Gleicher et al., 2014**: **evidence 3a**). This could be particularly relevant for young women with premature ovarian aging or older women with age-associated diminished ovarian reserve (DOR) (**Gleicher y Barad, 2012: evidence 3a**). The proportion of embryos that are unsuitable for transfer likely varies among clinical settings, but it has been estimated to be rather relevant (**Paulson 2017**: **evidence 5**). For these reasons, individual programs may need to examine their own embryo implantation rates with and without PGT-A 2.0, calculate their embryo loss rate, and take particular care in drafting and explaining informed consent**.**

2.7 Undiagnosed embryos

There is a small percentage of situations that PGT-A 2.0 is not able to detect (**Tiegs et al., 2016: evidence 4)**. Cases involving a parent with a balanced translocation provide a unique opportunity to characterize the capabilities and limitations of detecting segmental imbalances with a variety of chromosome screening platforms (**Treff et al., 2017: evidence 4**). It is important for clinicians to be aware of the specific PGT-A 2.0 clinical error rate for their respective modality of PGT 2.0 testing in order to best counsel patients when considering this costly, yet rewarding, endeavor.

2.8 Overdiagnosed embryos

Whole-chromosome aneuploidy screening has become a common practice to improve IVF outcomes. As technology has evolved, detection of subchromosomal imbalances and embryonic mosaicism has become possible and these serve as potential explanations for euploid embryo transfer failures (**Treff et al., 2017**). False-positive diagnosis or failure to determine clinical significance in non-selection studies may result in the discarding of reproductively competent embryos and embryos with the ability to self-repair and eliminate aneuploid cells. The source of these errors can either be biological or analytical and thus both must be characterized (**Juneau et al., 2016: evidence 3a**).

2.9 Mosaicisms

One important limitation of PGT-A 2.0 is the presence of chromosomal mosaicism within the developing embryo, a phenomenon in which different chromosomal cell lines reside within a single embryo. The obvious concern for PGT-A 2.0 misdiagnosis associated with embryonic mosaicism is that the cells biopsied may not reflect the chromosomal status of the resulting embryo. Mosaicism is thought to be less common

in blastocyst stage embryos than in previous stages (**Brezina et al., 2015**), so current data strongly support obtaining embryo biopsy at this point in time (**Dahdouh et al., 2015**). However, discordance in the ploidy status between the inner cell mass and the trophectoderm (TE) is still relatively common (**Brezina et al., 2015**). Embryo re-biopsy studies in mosaic embryos show that the reproducibility of TE biopsy demonstrating mosaicism is only 41-58%, and that TE biopsy of five cells may not be representative of the degree of mosaicism of the entire embryo. Therefore, there is still a chance of misdiagnosis based purely on the biology of the developing embryo with PGT-A 2.0 at the biopsy. This represents a biological limitation that is not possible to overcome even with the best diagnostic techniques (**Brezina et al., 2016**).

Nevertheless, validation experiments outlined in a recent retrospective study (**Munné et al., 2017: evidence 2b**) demonstrate that high resolution NGS succeed in detecting mosaicism in the vast majority of trophectoderm biopsies in which it is present and the frequency of false positives and negatives appears to be low. More recently, an overall high diagnostic sensitivity (90%) and relatively low specificity (67%) in the context of PGT-A 2.0 has been demonstrated with NGS (**Popovic et al., 2018**) and this suggests that a considerable proportion of embryos are potentially being classified as clinically unsuitable. Embryos classified as being mosaic by means of NGS miscarry more and implant less, but 40% of them can still result in a viable pregnancy (**Fragouli et al., 2017**: **evidence 2b**). Considering this fact, current recommendations suggest consider for transfer if there are no euploid embryos available and patient is aware of and understands all associated risks (**PGDIS position statement**, **2016; Munné et al., 2017: evidence 2b).** Among the possible options, the most recent data suggest that the majority of embryos with 20%–40% aneuploid cells in their biopsy sample have euploid inner cell mass and could be considered for transfer. Blastocysts with 40%–80%

abnormal cells and those with complex mosaicism should be given the lowest priority for transfer or be excluded **(Munné et al., 2017: evidence 2b**) but will require additional studies to be accurately quantified **(Maxwell et al., 2016: evidence 3b).**

A scoring system according to the chromosomes involved in the mosaic has been developed to help the clinician in the counselling of patients, taking into account the risk of miscarriage or having an affected fetus. (Maxwell and Grifo, *Best Practice Research Clinical Obstetrics and Gynaecology*, 2018, in press). However, there are doubts about the real implication of mosaic embryos in women under 35 years regarding successful pregnancies (**Forman et al., 2019**).

2.10 PGT-A 2.0 does not improve pregnancy rate per cycle

PGT-A 2.0 for the diagnosis of aneuploidies are associated with inconsistent results in terms of improving pregnancy rates (**Dahdouh et al., 2015; evidence 1a; Okun et al., 2014: evidence 1c.; Garrido- Gimenez et al., 2015: evidence 2a; Barash 2016: evidence 2a; Kushnir et al., 2016: evidence 2a)**. It is essential to assess pregnancy rates by "intent to treat" and some authors point out that this rate should be calculated with denominator *cycle start* rather than *embryo transfer* **(Gleicher et al. 2014: evidence 3a)**. A retrospective cohort study analyzing this matter found that IVF-PGT-A in women aged >37 improved live birth rates. However, per cycle, the PGT-A 2.0 advantage in this age group did not persist **(Kushnir et al., 2016; Kang et al., 2016: evidence 3a)**.

2.11 Lack of evidence of the benefit of PGT-A 2.0 in certain situations or populations (Recurrent pregnancy loss (RPL), recurrent implantation failure (RIF) and male factor, women of AMA >40, and low response)

Studies relating to patients of AMA, recurrent pregnancy lost (RPL) and recurrent implantation failure (RIF) are restricted to matched cohort studies, limiting the ability to draw meaningful conclusions (**Lee et al., 2015**: **evidence 2a**) and the current evidence examining the use of IVF with PGT-A 2.0 in patients with RPL reveal variable results, probably due to differences in technologies used and variable patient populations **(Shahine et al., 2014: evidence 2a)**.

2.12 Lack of well-designed randomized studies and long –term data

The lack of large well-designed RCTs is one important limitation of PGT-A 2.0. Only three RCTs have been published, all of which have been criticized because of poor study design (Yan et al. 2012, ; Scott et al. 2013; Forman et al., 2013). The pilot RCT by Yang et al. (2012) included a small sample size of 45 young, good prognosis patients. Scott et al. (2013) performed a RCT on 72 good prognosis patients, who were between the ages of 21-42 years and who 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's failure to account for the difference between the unit of randomization (patients) and unit of analysis (individual embryos). The third RCT studied 89 patients aiming to compare PGT-A and single embryo transfer with the transfer of two embryos without genetic diagnosis (**Forman et al., 2013**). The same methodological problem encountered by the Scott trial was introduced and even so, the wide confidence interval for pregnancy did not demonstrate a beneficial effect (**Chen et al., 2015: evidence 1a).** Intention-to-treat studies of deferred ET with and without PGT-A 2.0 and eventually of all transferred embryos will be required to fully assess the impact of contemporary PGT-A 2.0 (**Meldrum et al., 2016: evidence 5)**, and further studies evaluating long-

term paediatric outcomes and the overall cost-efficacy of this approach are necessary (**Forman et al., 2014**).

3. OPPORTUNITIES

3.1 Increasing parental age

The age at which a pregnancy is sought is progressively increasing in western countries, as shown by the increasing average age of the mothers in the first pregnancy. Aneuploidy is associated with maternal and paternal age and is only subtly related to the morphologic appearance of the embryo **(Franasiak et al., 2014: evidence 4**). Also, the fact that IR of euploid embryos are the same at any maternal age up to 42 years has been described (**Harton et al., 2013: evidence 4).** Given the aging of women, PGT-A 2.0 would be a method that would increase the odds and shorten the time to pregnancy in women with "euploid" embryos biopsy to transfer.

3.2 Fewer abnormal frozen embryos

Frozen blastocysts can be warmed and biopsied for PGT-A 2.0 aneuploidy screening. The benefit of frozen embryo transfer (FET) cycles is its ability to increase the number of biopsied embryos for patients utilizing PGT-A 2.0 **(Coates et al., 2017; evidence 1a**) and streamlines the clinical processes by scheduling single embryo transfer in subsequent cycles until a patient achieves a pregnancy or exhausts available euploid embryos **(Huang et al. 2015; evidence 4)**. This avoids the cryopreservation of abnormal embryos with probably lower possibility of achieving a healthy baby (**Rodríguez-Purata et al., 2016; evidence 4),** thereby reducing the logistical problems of storage as well as the ethical dilemmas associated with their disposal.

Such procedures may particularly benefit poor responders (**Goldman et al., 2015: evidence 2a)**, patients who have had previous implantation failures, patients who did not have embryo screening before cryopreservation due to lack of embryo screening technology or to other reasons (**Liu et al., 2016**: **evidence 1c; Chamayou, 2017: evidence 2a**).

3.3 Endometrial synchrony

A freeze-all strategy that plans to utilize subsequent FET cycle(s) offers the opportunity to control the window of implantation and possibly improve embryo implantation. A strategy of close monitoring and synchronization of the window of implantation that is based on frozen embryo transfer (FET) combined and strengthened with PGT-A 2.0-based embryo selection gives the opportunity to optimize reproductive potential **(Rodríguez-Purata et al. 2016: evidence 4)**.

3.4 PGT-A 2.0 reduces the multiple pregnancy rates

Elective single frozen-thawed euploid embryo transfer coupled with enhanced embryo selection using PGT-A 2.0 reduced the multiple pregnancy rates, while maintaining the cumulative success rate of the IVF program (**Schoolcraft et al., 2013; Scott et al., 2013; Forman et al., 2013; Yang et al., 2012**; **evidence 1b; Haddad et al., 2015: evidence 3b)**.

Owing to the increased maternal morbidity and perinatal complications related to multiple pregnancies, some authors recommended to extend the uses of PGT-A 2.0.

PGT-A 2.0 might allow a reduction in the number of embryos transferred and the number of transfers to be performed without affecting the total efficacy of the treatment, but reducing the risk of multiple pregnancy and the subsequent complications **(Chen et**

al., 2015; evidence 1a), particularly in the AMA population (**Ubaldi et al., 2015**; **evidence 2b)**. However, Kissin et al. (2015) showed that triplets and twinning are not very common among older patients, even in the presence of transfer of 3 or 4 embryos. So, multiple embryo transfers may be acceptable in some cases and possibly a way to reduce the false positive result of "no euploid embryos" in cases of mosaic embryos with low aneuploidy percentage and with chances of generating healthy live births.

3.5 Shorter time to pregnancy and live birth and fewer dropouts

New data support that PGT-A 2.0 significantly decreased time to live birth by an average of three months in patients with diminished ovarian reserve (**Franasiak et al., 2017; evidence 1b**). As a result, the number of dropouts is reduced and the psychological burden decreases. Time to pregnancy is critical for AMA patients and its reduction through proper embryo selection is highly desirable. Also, further expanded use of PGT-A 2.0 into the domain of gestational surrogacy seems likely (**Sills et al. 2016: evidence 5**).

3.6 Decreased psychological burden, drop outs and the possibility of a shared decision

Reductions in miscarriage and futile transfers markedly reduce stress, which may reduce drop out from further treatment and promote successful outcomes. For patients seeking to conceive, understanding the etiology of reproductive loss can in the same manner be beneficial to their mental health and can assuage feelings of guilt or irresponsibility (**Kong et al. 2016**), and on the other hand it opens the possibility of reaching a shared medical decision **(Su et al. 2016: evidence 1c)** in which providing adequate patient information is important **(Tiegs et al. 2016: evidence 4)**.

3.7 A reduction in the final costs of achieving a live birth and less social costs

With PGT-A 2.0, a single euploid blastocyst with high reproductive potential can be selected for transfer. By being able to better select the embryo to be transferred, the number of failed attempts **(Forman et al., 2014; evidence 1b.)** and required transfers (**Coates et al., 2017; evidence 1c)** is reduced. This paradigm increases the chance for a healthy, term, singleton, delivery, which has been demonstrated to be cost-effective per live birth in women >37 years **(Collins et al. 2017: evidence 1c)** and per embryo (**Sills et al., 2014; evidence 4**). Two papers have been recently published estimating the cost-effectiveness of PGT-A in comparison with conventional IVF without PGT-A, and assuming a 100% of frozen embryo tranfers (ETs) after PGT-A, and 30% or 100% of a first fresh ET, respectively, in the non-PGT-A arm. In the first study (**Neal et al., 2018**), for patients up to 42 years with > 1 embryo, IVF with PGT-A was already cost-effective by reducing total healthcare costs. In the second study (**Somigliana et al., 2019**), cost-effectiveness profile of PGT-A improved with female age and number of available blastocysts, but even in a scenario of cost reduction for the ET, the threshold ages favoring the PGT-A strategy were 42, 40, 39, and 38 years of age for one, two, three, and four blastocysts, respectively. So, these two studies show an economic advantage of PGT-A in women with advanced age even with only a few blastocysts available to screen and considering the additional need of delayed frozen ETs. Nevertheless, a recent opinion paper questions the conclusions of the Somigliana's study since a cost effectiveness analysis based on mathematical models relies on the underlying assumptions, and they are far from agreed upon in the field, not clarifying the question of efficiency and cost-effectiveness of PGT-A (**Paulson, 2019**).

3.8 Future reduction of PGT-A costs

The appearance of new technologies, such as NGS, could help to significantly reduce PGT-A costs while retaining a high level of detection accuracy as compared with the existing techniques. These are essential for PGT-A 2.0 to be widely adopted **(Chen et al. 2015: evidence 1a; Huang et al. 2016: evidence 4)** and represent a cost-effective strategy for the routine detection of aneuploidy in human embryos **(Vendrell et al. 2017)**.

3.9 Differentiating between paternal and maternal aneuploidy origins

The possibility of differentiating between aneuploidies of paternal or maternal origin, would help to establish the indication of the gamete to be substituted when it was necessary to resort to a treatment with donation of one or both gametes. In addition, this technique could allow additional information to be obtained with regard to the health of the embryo **(Sills et al. 2016: evidence 4)**.

4. THREATS

4.1 Ethics and eugenics

With the generalized use of PGT-A 2.0, one possible threat that must be considered is that the results are not always interpretable and the risk of eugenics may arise as well as some ethical considerations with regard to embryo selection and transfer and the disposal of abnormal embryos or embryos not transferred (**Bolton et al., 2015; evidence 1c; Hens et al. 2013: evidence 5)**. Conflicts on embryo selection may also arise between the couple and the doctor, it is therefore imperative for healthcare

providers and patients using IVF and PGT-A 2.0 to collaboratively make informed decisions **(Gebhart et al. 2016; evidence 5**).

4.2 Different legal issues of each country

Some of the threats derived from the new PGT-A 2.0 technology, where there is continuously more information with access to more complete tests, are the different legal landscapes of each country, different levels of access in different populations, and the complexities that come with making both clinical and ethical decisions (**Harper 2014: evidence 5**). In fact, nowadays there are countries in which mosaic embryos are transferred, while in others they are not (**McCoy RC 2017)**. In the United States and many other countries, reproductive decisions taken regarding PGT-A 2.0 are ultimately left to patients and their physicians, but there are some countries where strict legislation and restrictions on PGT-A and IVF limit access to these treatments, which may contribute to the phenomenon of reproductive tourism **(Imudia et al. 2016; evidence 1c**). The European Society of Human Genetics and European Society of Human Reproduction and Embryology 2013 (**ESHRE 2013**) has published a document comparing the different working methods in Europe and the legal limitations between countries that can be useful.

4.3 Emergence of studies on the adverse effects PGT-A 2.0 for live birth

Because PGT-A 2.0 is a relatively new and emerging technology, deleterious effects of embryo biopsy, vitrification, or manipulation may not be evident until the children are born or become older. The most recent data suggest that PGT-A 2.0 in cleavage stage embryos are not associated with serious adverse effects on neurological, cognitive and behavioral development, blood pressure and anthropometrics of offspring at 4 (**Seggers**

et al. 2013; evidence 1b) and 9 years (**Kuiper et al., 2018; evidence 1b)**. Other studies suggest that vitrification can be associated with an increased incidence of pregnancy hypertension (**Jing et al. 2015; evidence 3b)** and extended blastocyst cultures could induce epigenetic changes **(Calle et al., 2012; Evidence 3b**) and premature death **(Dar et al.,** 2014**; Evidence 3b)**. These findings underline the importance of long-term follow-up of the health and development of children born to couples after PGT-A 2.0.

4.4 Development of a non-invasive and less damaging genetic diagnosis method

The invasive nature of PGT-A 2.0 includes a series of limitations that can be resolved with the development of non-invasive (**Cimadomo et al., 2016)** and personalized (**Takyi et al., 2017**) methods. For instance, a combined evaluation of morphology and developmental kinetics using time-lapse imaging (**Montag et al. 2013; evidence 2a**), the development of an aneuploidy risk model derived from time-lapse imaging without PGT-A 2.0 (**Campbell et al. 2013; evidence 1c**), and non-invasive genetic/proteomic/metabolomic screening of spent IVF culture medium (**Feichtinger et al., 2017; evidence 4; Xu et al. 2016; evidence 4)** have all been proposed as promising tools. A combination of all these advances may in the future allow fertility specialists to predict which embryo will have the highest probability of implantation or even predict with absolute accuracy whether or not an embryo is implanted (**Sermon et al., 2016: evidence 5)**.

4.5 Indiscriminate application of the technique and errors

PGT-A 2.0 is unable to study every chromosome completely, and it therefore cannot guarantee a healthy baby. This may impede some patients from opting for this technique, especially those with implantation failure (**Yang et al. 2015: evidence 4)**.

The main mistake with PGT-A 1.0 was an indiscriminate use before demonstrating its risks, so it is very important that we avoid this situation with the PGT-A 2.0. The risk of the indiscriminate application of PGT-A 2.0 exists in certain situations, such as in oocyte donation cycles without a demonstrated benefit in terms of live births (**Sills et al., 2016: evidence 4**) or in other cases where fertility clinics offer PGT-A 2.0 for non-medical purposes. Before PGT-A 2.0 is performed, thorough education and counseling must be provided by a genetic specialist to ensure that patients fully understand the limitations of the technique, the risk of error, and the ongoing debate on whether PGT-A is necessary to improve live birth rates (**Dadouh et al. 2015; Evidence 3a).**

Finally, a prospective, web-based questionnaire directed to users and non-users of PGT-A 2.0 that included a total of 386 IVF units from 70 countries reports that most respondents (84%) believe that more RCTs are needed to support the extensive use of PGT-A 2.0 (**Weissman et al., 2017; evidence 4**)

CONCLUSIONS

In theory, discriminating between an euploid and an aneuploid embryo before proceeding to its transfer, is undoubtedly a positive fact that helps to optimize the result of the IVF cycle. This way, the transfer of embryos with a practically zero probability of implantation, which would result in a gestational loss or in the worst case an evolutionary gestation of a chromosomally abnormal embryo, is avoided.

However, despite the development of advances in the technique in recent years, there are still a number of limitations that make the indication for PGT-A 2.0 controversial, such as the obligation to perform an embryo biopsy, where 4-5 cells of the trophectoderm are extracted. This implies an invasive manipulation of the embryo;

whose consequences cannot be determined or ruled out, and can be highly dependent on the ability of the embryologist who performs it. Secondly, the need to vitrify and later de-vitrify the embryo for its transfer introduces a 2 to 10% variable risk for embryo death; with the loss of a viable embryo as a consequence, which in some cases may be the only one available. In addition to these drawbacks, the possibility of diagnostic errors must be added to the still high cost of the procedure. **Figure 1** summarizes the SWOT analysis of the PGT-A 2.0.

In conclusion, evidence shown dictates that PGT-A 2.0 should not have an indiscriminate application at present, but it could be useful in cases in which the risk of aneuploidy is increased, as may be in patients of AMA >37, or in patients with a history of implantation failure or RPL without any other identified cause. However, in this group it would only be useful if the numbers of oocytes produced were enough to allow selection through PGT-A. In order to reach a recommendation for a broader indication, it is necessary to optimize the technique and carry out cost-benefit studies that objectively determine the situations in which it is justified.

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Table 1. Oxford Centre for Evidence-based Medicine (CEBM)-Levels of Evidence (March 2009). Available at: http://www.cebm.net/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/]

Levels of evidence	Type of study
1a	Systematic reviews (with homogeneity) of randomized controlled trials
1b	Individual randomized controlled trials (with narrow confidence interval)
1c	All or none randomized controlled trials
2a	Systematic reviews (with homogeneicity) of cohort studies
2b	Individual cohort study or low quality randomized controlled trials (e.g. <80% follow-up)
2c	“Outcomes" Research; ecological studies
3a	Systematic review (with homogeneity) of case-control studies
3b	Individual case-control study
4	Case-series (and poor quality cohort and case-control studies)
5	Expert opinion without explicit critical appraisal, or based on physiology, bench research or "first principles"

Note: A minus sign "-" may be added to denote evidence that fails to provide a conclusive answer because it is *either* (a) a single result with a wide Confidence Interval; *OR* (b) a Systematic Review with troublesome heterogeneity.

Table 2. Relative strengths and weaknesses of diagnostic platforms currently used in PGT-A 2.0

	Whole chromosome aneuploidy	**Mosaicism**	**Triploidy**	**Large deletions or duplications (>50 Mb)**	**Clinically significant deletions or duplications (800 kb – 1 Mb)**	**Mitochondrion copy number**	**Uniparental disomy**
SNP array	Yes	Yes	Depends on DNA quality	Yes	Depends on the size and chromosome location	No	Yes
aCGH	Yes	Yes/no	No	No	No	No	No
RT-PCR	Yes	Yes	No	No	Yes	No	
MiSeq NGS	Yes	Yes	Yes	Not designed to detect	No	Yes	Yes
Low-density NGS	Yes	No	No	No	No	No	No
PGM NGS	Yes	Yes	Yes	Yes	Yes	Yes	Yes

TABLE 3 SWOT ANALYSIS OF PGT-A 2.0 IN ASSISTED REPRODUCTION

Strengths (positives)	Weaknesses (negatives)
• Is the strongest and most evaluated technique • Improves embryo selection: improved implantation rates and pregnancy rates • Decreases miscarriages • Increases the chance of a healthy, term, singleton delivery	• Invasiveness and complexity of the technique • Non-standardized technique and possibility of errors • Laboratory management reliability and poor consistency between centres • Costs of the technique • Blastocyst stage • Loss of embryos • Undiagnosed embryos • Overdiagnosed embryos • Mosaicisms • Does not improve pregnancy rate per cycle

	• Lack of evidence of the benefit in recurrent pregnancy loss, repeated implantation failure, male factor infertility and advanced maternal age, and low response • Lack of well-designed randomized studies and long-term data
Opportunities (external factors with possible positive impact)	Threats (external factors with possible negative impact)
• Increasing parental age • Fewer abnormal frozen embryos • Endometrial synchrony • Decrease in multiple pregnancies • Shorter time to pregnancy and live birth, fewer pregnancy losses and fewer drop outs • Decreased psychological burden and allows for a shared decision • Reduction in the final costs of achieving a live birth (fewer transfers) and less social cost (reduction in multiple pregnancies) • Future reduction of PGT costs • Differentiating between paternal and maternal aneuploidy origins	• Ethics and eugenics • Different legal issues of each country • Emergence of studies on the adverse effects of PGT on live birth • Development of a non-invasive and less damaging genetic diagnosis method • Indiscriminate application of the technique and errors