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REVIEW *Reproductive genetics*

The clinical effectiveness of preimplantation genetic diagnosis for aneuploidy in all 24 chromosomes (PGD-A): systematic review

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STUDY QUESTION: Is preimplantation genetic diagnosis for aneuploidy (PGD-A) with analysis of all chromosomes during assisted reproductive technology (ART) clinically and cost effective?

SUMMARY ANSWER: The majority of published studies comparing a strategy of PGD-A with morphologically assessed embryos have reported a higher implantation rate per embryo using PGD-A, but insufficient data has been presented to evaluate the clinical and cost-effectiveness of PGD-A in the clinical setting.

WHAT IS KNOWN ALREADY: Aneuploidy is a leading cause of implantation failure, miscarriage and congenital abnormalities in humans, and a significant cause of ART failure. Preclinical evidence of PGD-A indicates that the selection and transfer of euploid embryos during ART should improve clinical outcomes.

STUDY DESIGN, SIZE AND DURATION: A systematic review of the literature was performed for full text English language articles using MEDLINE, EMBASE, SCOPUS, Cochrane Library databases, NHS Economic Evaluation Database and EconLit. The Downs and Black scoring checklist was used to assess the quality of studies. Clinical effectiveness was measured in terms of pregnancy, live birth and miscarriage rates.

PARTICIPANTS/MATERIALS, SETTINGS, METHODS: Nineteen articles meeting the inclusion criteria, comprising three RCTs in young and good prognosis patients and 16 observation studies were identified. Five of the observational studies included a control group of patients where embryos were selected based on morphological criteria (matched cohort studies).

MAIN RESULTS AND ROLE OF CHANCE: Of the five studies that included a control group and reported implantation rates, four studies (including two RCTs) demonstrated improved implantation rates in the PGD-A group. Of the eight studies that included a control group, six studies (including two RCTs) reported significantly higher pregnancy rates in the PGD-A group, and in the remaining two studies, equivalent pregnancies rates were reported despite fewer embryos being transferred in the PGD-A group. The three RCTs demonstrated benefit in young and good prognosis patients in terms of clinical pregnancy rates and the use of single embryo transfer. However, studies relating to patients of advanced maternal age, recurrent miscarriage and implantation failure were restricted to matched cohort studies, limiting the ability to draw meaningful conclusions.

LIMITATIONS, REASONS FOR CAUTION: Relevant studies may have been missed and findings from RCTs currently being undertaken could not be included.

WIDER IMPLICATIONS OF THE FINDINGS: Given the uncertain role of PGD-A techniques, high-quality experimental studies using intention-to-treat analysis and cumulative live birth rates including the comparative outcomes from remaining cryopreserved embryos are needed to evaluate the overall role of PGD-A in the clinical setting. It is only in this way that the true contribution of PGD-A to ART can be understood.

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preimplantation genetic services to patients in Australia Dr Leeanda Wilton is an employee of and a shareholder in Virtus Health which is a provider of PGD services in Australia Dr Chambers previously received grant support to her institution from the Australian Government, Australian Research Council (ARC) Linkage Grant No. LP1002165; ARC Linkage Grant Partner Organisations were IVF Australia, Melbourne IVF and Queensland Fertility Group.

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Introduction

Aneuploidy is the most common type of chromosome abnormality and the leading cause of implantation failure, miscarriage and congenital abnormalities in humans (Hassold *et al.*, 1996; Vialard *et al.*, 2011). In the context of assisted reproductive technologies (ART), preimplantation genetic diagnosis (PGD) is the practice of obtaining a cellular biopsy of an embryo to evaluate the genetic composition, allowing the selection of a genetically unaffected embryo for transfer (Brezina *et al.*, 2012). Initially, PGD was used to detect embryos at risk for inheriting sex-linked monogenic diseases (Handyside *et al.*, 1990). More recently, the indications for PGD have expanded to include comprehensive aneuploidy screening of all 24 chromosomes in patients with a presumed normal karyotype (PGD-Aneuploidy, PGD-A). In theory, this approach should enhance ART clinical outcomes by improving implantation rates and reducing miscarriage rates, particularly in those patients at an increased risk of producing aneuploid embryos, such as women of advanced maternal age and those with recurrent implantation failure or recurrent miscarriage (Kahraman *et al.*, 2000; Rubio *et al.*, 2003; Voullaire *et al.*, 2007). PGD-A also encourages ART best clinical practice by supporting the selection of a single euploid embryo for transfer, thus minimizing iatrogenic multiple births and the concurrent risk to mothers and babies (Helmerhorst *et al.*, 2004; Ombelet *et al.*, 2006; Lemos *et al.*, 2013).

One of the first PGD-A techniques introduced into clinical practice used comparative genomic hybridization (CGH), with the first baby born from this technique in 2001 (Wilton *et al.*, 2001). Over the last decade more advanced and automated techniques have become widely available, including array CGH (aCGH), single nucleotide polymorphism (SNP) arrays and real-time quantitative polymerase chain reaction (qPCR). However, as previous experience with aneuploidy screening using fluorescence *in situ* hybridization (FISH) of cleavage stage embryos has shown, caution is needed before the widespread adoption of PGD-A into routine clinical practice. While initially promising, FISH-based PGD-A was limited by its capacity to assess only a subset of chromosomes and suffered from a lack of standardized techniques and interpretation which lead to substantial variation in reproducibility of results between laboratories (Colls *et al.*, 2007; Munné *et al.*, 2007; Hardarson *et al.*, 2008, Northrop *et al.*, 2010). Several prospective studies showed that PGD-A using FISH failed to improve pregnancy rates and in some studies worsened the outcomes in the clinic setting (Staessen, 2004; Blockeel *et al.*, 2008; Staessen *et al.*, 2008; Garrisi *et al.*, 2009; Schoolcraft *et al.*, 2009; Debrock *et al.*, 2010; Ajduk and Zernicka-Goetz, 2012). However, it is worth noting that the most recent prospective RCT demonstrated an increase in the live birth rate in older women after PGD-A using FISH (Rubio *et al.*, 2013). Comprehensive aneuploidy screening of all 24 chromosomes using more automated approaches should overcome the limitations of FISH. However, limited evidence exists on the clinical and cost-effectiveness of this more comprehensive approach in the fertility clinic setting. Most studies to date have sought to evaluate the scientific and preclinical validity of various PGD-A techniques, using different cell types (polar body, blastomere and trophectoderm), rather than directly evaluating its clinical effectiveness in terms of patient outcomes (Fragouli *et al.*, 2011; Mamas *et al.*, 2012).

This paper will review the available evidence regarding the effectiveness of comprehensive PGD-A techniques in different patient groups and using different cells types for biopsy. A number of narrative reviews (Brezina *et al.*, 2012; Fiorentino, 2012; Fragouli and Wells, 2012; Gleicher and Barad, 2012; Gleicher *et al.*, 2014) have attempted to assimilate the clinical outcomes of PGD-A into an overall assessment, but to our knowledge this is the first systematic review to focus on the clinical validity of PGD-A and to objectively assess the quality of published studies. This review is timely given that the scientific principles of comprehensive aneuploidy screening are widely accepted. However, considerable controversy still remains about the clinical and, particularly, economic effectiveness of this approach (Hellani *et al.*, 2008; Treff *et al.*, 2010; Gutiérrez-Mateo *et al.*, 2011; Capalbo *et al.*, 2013a,b).

Materials and Methods

Search strategy and study selection

A computer assisted search to identify relevant articles was performed using the OVID interface to MEDLINE, EMBASE, SCOPUS, Cochrane Library, NHS Economic Evaluation Database and EconLit. The search was limited to studies of humans, English language text, with no date restriction and included the MeSH keywords: embryo transfer, preimplantation genetic diagnosis, PGD, prenatal diagnosis, aneuploidy screening, comprehensive chromosomes, pregnancy, IVF, *in-vitro* fertilisation, polar bod$, cleavage, blastocyst$, comparative genomic hybridisation, arrayCGH, single nucleotide polymorphism arrays, SNP$, quantitative polymerase chain reaction, qPCR. The comprehensive PGD-A techniques included were CGH (array and metaphase), SNP and qPCR. All article abstracts were reviewed for relevance and the reference lists of all identified studies were cross-checked for additional articles. The search covered all eligible articles published up until August 2014. The inclusion and exclusion criteria for retaining the identified studies in this review are shown in Table I. Studies had to report clinical or ongoing pregnancy, live birth or miscarriage rates to be included in the review. The primary review was performed by the first author (E.L.), with the other three authors reviewing selected publications. The first author also contacted individual study authors to clarify study details where required.

Table I Exclusion and inclusion criteria.

Inclusion criteria	Exclusion criteria
A cohort of patients using one of CGH, aCGH, SNP array or qPCR for PGD-A Study with 20 and more patients Papers written in English	Patients who had PGD for single gene disease, translocations or monogenic diseases (To avoid additional selection bias, studies were also excluded if they had recruited predominately patients with a known genetic disorder or who had donated oocytes for analysis)
Experimental and observational studies	Narratives, commentaries, letters to editor or reviews
Inclusion of outcome measures that include either rates of clinical pregnancy, ongoing pregnancy, miscarriage or live births	Studies deficient of an outcome measure
Published in peer reviewed journal	Published in non-peer reviewed journal, abstract, conference proceeding

Assessment of study quality

Critical appraisal of the articles was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) Statement (Moher *et al.*, 2009). Additionally, the methodological quality of the studies that met the inclusion criteria were assessed using a modified version of Downs and Black checklist which is considered valid and reliable for assessing randomized and non-randomized studies of healthcare interventions and covers reporting, external validity, internal validity (bias and confounding) and statistical power (Downs and Black, 1998). Because not all of the items in the Downs and Black checklist are relevant to non-randomized studies, a modified checklist was developed using of 22 of the 27 items. For this review, each item in the checklist was scored either 0 or 1, with a score of <8 considered a poor-quality study, a score of 8–15, a moderate-quality study and a score of more than 15, a high-quality study.

Results

Figure 1 outlines the results of the search strategy which resulted in 19 articles meeting the inclusion criteria for this systematic review. Studies were excluded if they were designed primarily to validate technical aspects of comprehensive PGD-A, used predominately donated oocytes, or lacked clinical outcome measures. Details of each study are included in Supplementary data Table SI.

The three RCTs identified (Yang *et al.*, 2012; Forman *et al.*, 2013b; Scott *et al.*, 2013a) sought to compare the clinical outcomes in young, good prognosis patients assigned to embryo selection based on either PGD-A or morphological assessment alone. The remaining 16 studies used observational designs, with only five studies including a control group for comparison (matched cohort studies) (Wilton *et al.*, 2003; Sher *et al.*, 2007, 2009; Fragouli *et al.*, 2010; Schoolcraft, 2010; Fishel *et al.*, 2011; Geraedts *et al.*, 2011; Schoolcraft *et al.*, 2011; Traversa *et al.*, 2011; Forman *et al.*, 2012, 2013a,b; Hodes-Wertz *et al.*, 2012; Adler *et al.*, 2013; Harton *et al.*, 2013; Keltz *et al.*, 2013; Mir *et al.*, 2013).

Study characteristics

Supplementary data, Table SI summarizes the characteristics and outcomes of the 19 eligible studies. The studies were published between 2003 and 2014, with the majority ($n = 15$) published between 2010 and 2013. There was little geographical spread in the studies, with most ($n = 12$) being performed in the USA.

Significant heterogeneity existed among the studies in terms of study design, size, comparators and patient characteristics. The number of study participants ranged from 20 to 320 patients with results from 2983 patients included in the review. Three studies (Fishel *et al.*, 2011; Hodes-Wertz *et al.*, 2012; Harton *et al.*, 2013) presented the results based on ART cycles and provided little detail on the number of patients in their study. Overall, the mean age of study patients in the review ranged from 31.2 to 40 years. Most studies ($n = 14$) reported outcomes on patients with a poor prognosis, i.e. advanced maternal age, recurrent implantation failure or recurrent pregnancy loss.

Eight studies (Schoolcraft, 2010; Schoolcraft *et al.*, 2011; Traversa *et al.*, 2011; Forman *et al.*, 2012; Yang *et al.*, 2012; Forman *et al.*, 2013a,b; Scott *et al.*, 2013a) assessed trophectoderm biopsy, four blastomere biopsy (Wilton *et al.*, 2003; Sher *et al.*, 2009; Keltz *et al.*, 2013; Mir *et al.*, 2013) and three polar body biopsy (Sher *et al.*, 2007; Fishel *et al.*, 2011; Geraedts *et al.*, 2011). Three studies directly compared trophectoderm with blastomere biopsy (Hodes-Wertz *et al.*, 2012; Adler *et al.*, 2013; Harton *et al.*, 2013) and one study directly compared trophectoderm with polar body biopsy (Fragouli *et al.*, 2010). Seven studies reported outcomes for fresh embryo transfers only (Sher *et al.*, 2007; Fishel *et al.*, 2011; Yang *et al.*, 2012; Forman *et al.*, 2013a; Keltz *et al.*, 2013; Mir *et al.*, 2013; Scott *et al.*, 2013a), six studies reported on fresh and frozen embryo transfers (Geraedts *et al.*, 2011; Forman *et al.*, 2012; Hodes-Wertz *et al.*, 2012; Adler *et al.*, 2013; Forman *et al.*, 2013b; Harton *et al.*, 2013) and the remaining studies reported outcomes based for frozen embryo transfers only (Wilton *et al.*, 2003; Sher *et al.*, 2009; Fragouli *et al.*, 2010; Schoolcraft, 2010; Schoolcraft *et al.*, 2011; Traversa *et al.*, 2011). Twelve studies reported live birth rates, but only six used the number of initiated cycles as the denominator in line with intention-to-treat principles (Sher *et al.*, 2009; Schoolcraft, 2010; Fishel *et al.*, 2011; Schoolcraft *et al.*, 2011; Forman *et al.*, 2013b; Scott *et al.*, 2013a). No cost-effective analysis studies on comprehensive PGD-A techniques were identified.

Quality evaluation

The Downs and Black scores for the non-randomized studies ranged from 6 and 15 (mean: 12.8) indicating studies of poor to moderate quality, while the scores for the three randomized studies ranged from 18 to 20 indicating studies of high quality. The main weaknesses included lack of randomization, lack of patient relevant outcome measures, failure to analyse data according to intention-to-treatment principles, failure to include the outcome of subsequent frozen embryo cycles to calculate cumulative live birth rates, and failure to control for confounding variables either within the study design or analysis (e.g. variable numbers of embryos transferred between case and controls). Only 8 of the 19 studies included a control group. Moreover, while a number of the observational studies were well designed, such studies tend to overestimate treatment effects, and generally can only infer causation (Stroup *et al.*, 2000). Furthermore, a number of studies, including two of the RCTs recruited

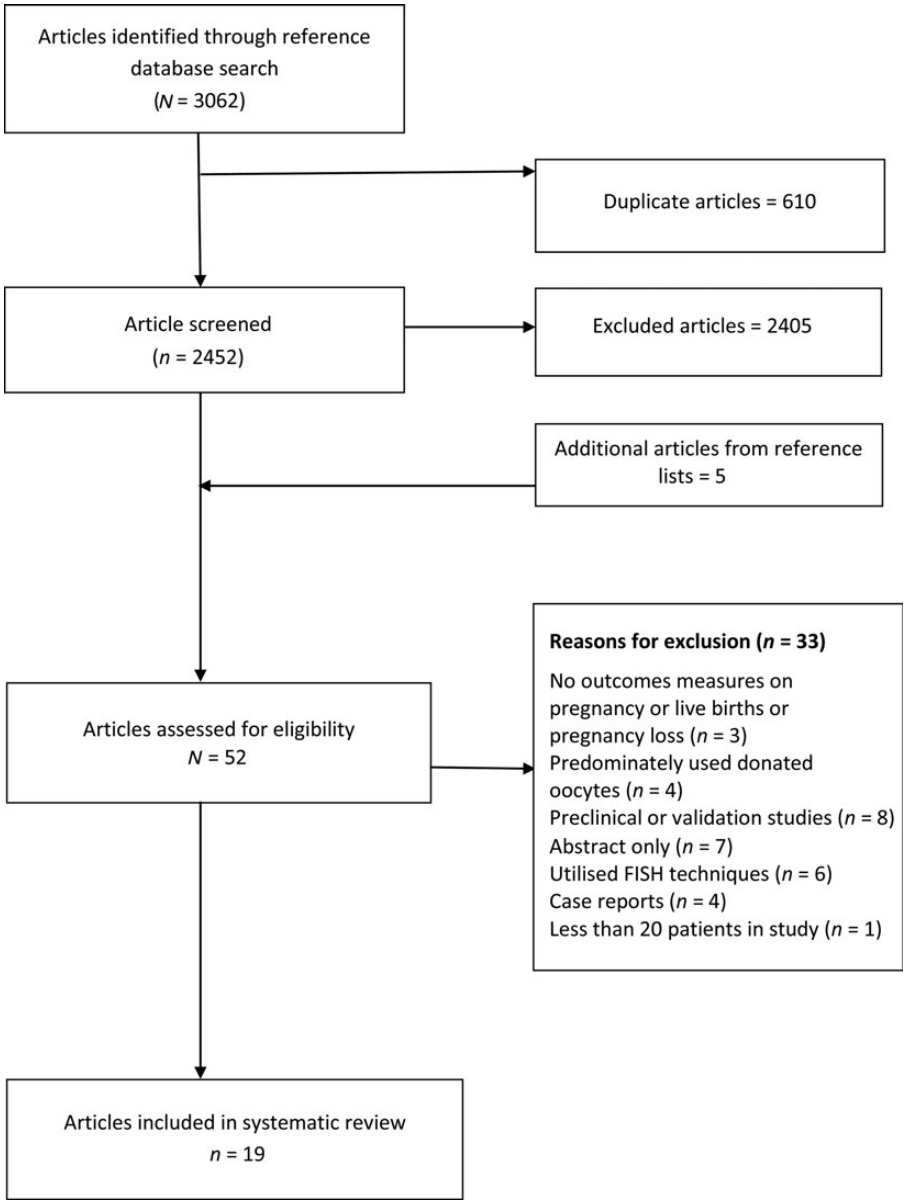

Figure 1 Search flow diagram based on PRISMA.

patients from the same centre (Schoolcraft, 2010; Schoolcraft *et al.*, 2011; Forman *et al.*, 2012, 2013a,b; Scott *et al.*, 2013a).

Clinical outcomes of PGD-A in young patients with good prognosis

Three RCTs (Yang *et al.*, 2012; Forman *et al.*, 2013b; Scott *et al.*, 2013a) investigated the clinical outcomes of using PGD-A on young and good prognosis patients demonstrating a benefit in terms of implantation and pregnancy rates in this group (Fig. 2).

Yang *et al.*'s RCT (2012) evaluated aCGH-based PGD-A to select a single blastocyst for transfer in a fresh cycle (mean age 31.2 years). The ongoing pregnancy rate was significantly higher in the PGD-A group compared to the morphologically assessed embryo group (69.1 and 41.7%, respectively, $P = 0.017$). Although patients in the study were allocated to their intervention using a random number table, a power calculation was not reported and assessors (clinical and laboratory staff) were not blinded. Outcomes from subsequent frozen embryo transfer cycles were not included.

Scott *et al.*'s RCT (2013a) evaluated qPCR-based PGD-A for intended transfer of two Day 6 embryos versus morphologic-based embryo selection for transfer on Day 5. Patients were aged between 21 and 42 years (mean age 32.2 years) with at least two blastocysts available for biopsy. Overall, the results favoured the use of PGD-A producing higher clinical implantation rates (presence of a gestational sac) per transfer (79.8 and 63.2%, $P = 0.002$), and higher delivery rates per cycle (84.7 and 67.5%, $P = 0.01$). Outcomes from subsequent frozen embryo transfer cycles were not included.

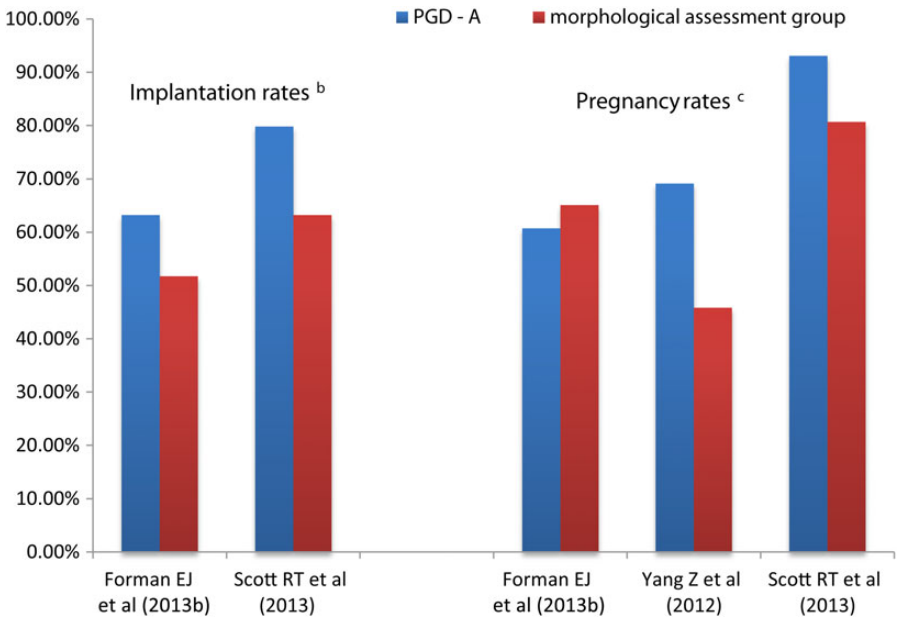

Figure 2 Implantation and pregnancy rates for PGD-A randomized controlled trials of young patients with good prognosis[a]. [a]Data extracted from the text and tables from each publication with results relating to the first cycle. [b]Implantation rates: Forman *et al.* (2013b) reported sustained implantation rates of 63.2% (55/87 embryos transferred) in PGD-A and 51.7% (89/172 embryos transferred) in morphological assessment group. The numerator was gestational sacs with fetal cardiac activity, the denominator was the number of embryos transferred (fresh and frozen). Scott *et al.* (2013a) reported the clinical implantation rate as 79.8% (107/134 embryos transferred) in PGD-A and 63.2% (103/163 embryos transferred) in morphological assessment group. The numerator was gestational sacs, the denominator was number of embryos transferred. Yang *et al.* (2012) did not report implantation rates. [c]Pregnancy rates: Forman *et al.* (2013b). Based on intention to treat analysis, ongoing pregnancy rates (fresh and frozen transfer) were 60.7% (54/89 cycles) in the PGD-A group and 65.1% (56/86 cycles) in morphological assessment group. The numerator was pregnancies of $\geq$24 weeks gestational age, the denominator was the number of initiated fresh and frozen cycles. Single embryo transfer was used for the PGD-A group ($n = 87$). Double embryo transfer was used for the morphological assessment group ($n = 86$). Yang *et al.* (2012) reported ongoing pregnancy rates of for 69.1% (38/55 fresh cycles) in PGD-A group and 41.7% (20/48 fresh cycles) in the morphological assessment group. The numerator was pregnancy at $\geq$20 weeks gestation, the denominator was initiated fresh cycles. All transfers were of a single embryo, PGD-A group ($n = 55$) and morphological assessment group ($n = 48$). Scott *et al.* (2013a) reported clinical pregnancy rates of 93.1% (67/72 cycles) in PGD-A and 80.7% (71/83 cycles) in morphological assessment group. The clinical pregnancy rates were calculated per initiated fresh cycle. Mean number of embryos transferred: PGD-A group = 1.86, Morphological assessment group = 2.

Forman *et al.*'s RCT (2013a) used similar inclusion criteria as Scott *et al.*, (2013a) and Yang *et al.* (2012) but the comparison was between elective single embryo transfer of an embryo that had been screened by PGD-A against two morphologically assessed embryos. Similar pregnancy rates were observed between the two groups (fresh transfer 63.9 and 70.5%, $P = 0.4$, frozen transfer 53.6 and 52.0%, $P = 0.9$). Follow-up results from this RCT that take into account up to one subsequent frozen transfer cycle reported cumulative delivery rates of 69% after euploid single embryo transfer and 72% after untested double embryo transfer (non-significant *P*-value; Forman *et al.*, 2014). It was concluded that the use of PGD-A could be an effective tool in facilitating single embryo transfer, thus reducing the considerable morbidity associated with multiple pregnancy.

Clinical outcomes for PGD-A on women of advanced maternal age

Thirteen observational studies (Sher *et al.*, 2007, 2009; Fragouli *et al.*, 2010; Schoolcraft *et al.*, 2010; Fishel *et al.*, 2011; Geraedts *et al.*, 2011; Schoolcraft *et al.*, 2011; Traversa *et al.*, 2011; Forman *et al.*, 2012; Hodes-Wertz *et al.*, 2012; Adler *et al.*, 2013; Keltz *et al.*, 2013; Mir *et al.*, 2013) examined the use of PGD-A on women of advanced maternal age (mean age >35 years old). Among the studies which included a control group where embryos were selected based on morphology, all demonstrated consistently improved implantation rates in the PGD-A group (Schoolcraft *et al.*, 2010; Fishel *et al.*, 2011; Keltz *et al.*, 2013; Fig. 3). For example, a small matched-cohort study of women with advanced maternal age by Schoolcraft *et al.*, (2010) reported implantation rates after PGD-A of 68.9% compared with 44.8% in the control group. Four of the five studies reported significantly improved pregnancy rates, with the remaining study achieving equivalent pregnancies rates despite fewer embryos being transferred in the PGD-A group (Schoolcraft *et al.*, 2010). However, due to limitations with observational studies, the validity and generalizability of the findings makes it difficult to conclude whether PGD-A is clinically effective in this group of patients.

Effect of cell biopsy stage

Seven observational studies (Wilton *et al.*, 2003; Sher *et al.*, 2009; Hodes-Wertz *et al.*, 2012; Adler *et al.*, 2013; Harton *et al.*, 2013; Keltz *et al.*, 2013; Mir *et al.*, 2013) investigated the clinical outcomes of blastomere biopsy of cleavage stage embryos. Of these, only two studies had a

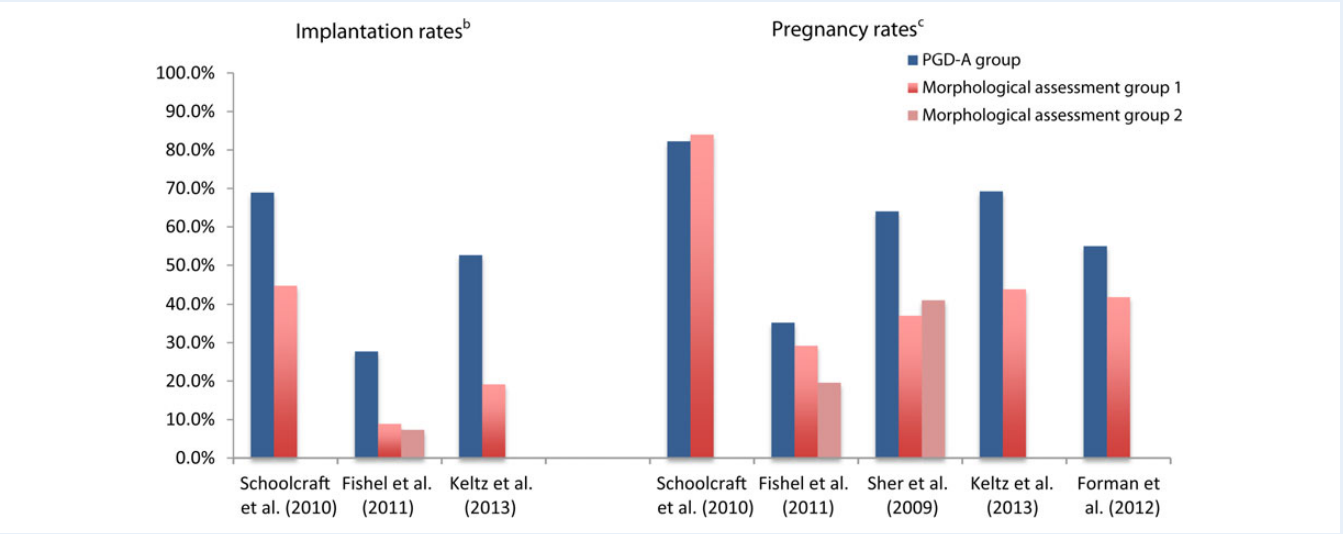

Figure 3 Implantation and pregnancy rates for PGD-A on women of advanced maternal age[a]. [a]Data were extracted from text and tables of each of the included studies. [b]Implantation rates: Schoolcraft *et al.* (2010). The implantation rates were 68.9% (62/90 embryos transferred) in the PGD-A group and 44.8% (134/299 embryos transferred) in morphological assessment group. The numerator was number of fetal hearts, the denominator was the number of embryos transferred. Fishel *et al.* (2011) included two control groups of patients with embryo selection based on morphological assessment. Morphological assessment Group 1 included patients with at least two miscarriages and morphological assessment Group 2 included patients with no previous successful pregnancy and at least two failed implantations after previous IVF attempts. The implantation rates were 27.7% (31 fetal hearts/112 embryos transferred) in the PGD-A group and 8.9% (7/78 embryos transferred) and 7.3% (8/109 embryos transferred) in the two morphological assessment groups, respectively. The numerator was number of fetal hearts, the denominator was the number of embryos transferred. Keltz *et al.* (2013): the implantation rates were 52.6% (30/57 embryos transferred) in PGD-A and 19.2% (253/1321 embryos transferred) in the morphological assessment group. The numerator was number of gestational sacs, the denominator was the number of embryos transferred. Forman *et al.* (2012) did not report implantation rate. Sher *et al.* (2009) did not report implantation rate. [c]Pregnancy rates: Schoolcraft *et al.* (2010) reported biochemical pregnancy per cycle of 82.2% (37/45 cycles) in the PGD-A group and 84.0% (95/113 cycles) in the morphological assessment group. The numerator was positive pregnancy tests, the denominator was initiated cycles. Mean number of embryos transferred; PGD-A group = 2, Morphological assessment group = 2.7. Fishel *et al.* (2011) reported biochemical pregnancy per embryo transfer as 35.2% (38/108 embryo transfers) in the PGD-A group and 29.2% (14/48 embryo transfers) and 19.6% (11/56 embryo transfers) in the morphological assessment Group 1 and 2, respectively. Mean number of embryos transferred: PGD-A group = 1.04, Morphological assessment groups 1 and 2 = 1.63 and 1.94, respectively. Sher *et al.* (2009) included two morphological assessment groups of patients with embryo selection based on morphological assessment. Morphological assessment Group 1 included patients who had frozen–warmed transfer cycles and morphological assessment Group 2 included patients who had fresh blastocyst transfers. The clinical pregnancy rate per transfer was 64% (36/57 embryo transfers) in the PGD-A group, 37% (43/117 embryo transfers) and 41% (132/329 embryo transfers) in morphological assessment Groups 1 and 2, respectively. The numerator was the presence of a gestational sac on ultrasound, the denominator was number of embryo transfers. Mean number of embryos transferred: PGD-A group = 1.7, Morphological assessment groups one and two = 2.2 and 1.9, respectively. Keltz *et al.* (2013) reported clinical pregnancy per fresh cycle as 69.2% (27/39 cycles) in the PGD-A group and 43.9% (173/394 cycles) in the morphological assessment group. The numerator was sonographically confirmed gestational sac, the denominator was number of fresh cycles. Mean number of embryos transferred: PGD-A group = 1.46, Morphological assessment group = 3.35. Forman *et al.* (2012) reported the ongoing pregnancy rate per embryo transfer (fresh and frozen transfer) as 55.0% (77/140 embryo transfers) in the PGD-A group and 41.8% (76/182 embryo transfers) in the morphological assessment group. The ongoing pregnancy rate was defined by live births and sustained pregnancies (beyond the first trimester) per embryo transfer. Single embryo transfer was used for all embryo transfer cycles, PGD-A ($n = 140$) and morphological assessment group ($n = 182$).

control group consisting of embryos assessed morphologically (Sher *et al.*, 2009; Keltz *et al.*, 2013). The results favoured using cleavage stage biopsy compared with morphology-based criteria. For example, a matched cohort study by Sher *et al.* (2009), in poor prognosis patients reported birth rates per transfer of 48% in the PGD-A group compared with 15–19% in control groups.

Five observational studies (Schoolcraft *et al.*, 2010; Schoolcraft *et al.*, 2011; Traversa *et al.*, 2011; Forman *et al.*, 2012; Forman *et al.*, 2013a), and the three RCTs (Yang *et al.*, 2012; Forman *et al.*, 2013b; Scott *et al.*, 2013a) assessed trophectoderm biopsy. The studies varied in the type of PGD-A technique, age of patients, methodological quality and reported outcome measures. Only the three RCTs and two observational studies (Schoolcraft *et al.*, 2010; Forman *et al.*, 2012) included a control group of patients who received morphologically assessed embryos; all reporting improved implantation rates after PGD-A but not necessarily improved clinical pregnancy rates.

Three studies (Sher *et al.*, 2007; Fishel *et al.*, 2011; Geraedts *et al.*, 2011) evaluated polar body (PB) 1 or both polar body (PB) 1 and 2 for PGD-A. The numbers of participants included in these studies were modest, with two studies (Sher *et al.*, 2007; Geraedts *et al.*, 2011) consisting of <50 patients. Only the study by Fishel *et al.* (2011) included controls (two poor prognosis groups with embryo selection based on morphology assessment), and despite the patients in the PGD-A group being older (median 41 years), they had higher delivery rates

per embryo transfer than those who had morphological assessment alone (24.1 versus 16.7 and 12.5%, $P < 0.0001$).

Three observational studies (Hodes-Wertz *et al.*, 2012; Adler *et al.*, 2013; Harton *et al.*, 2013) directly compared the clinical outcomes of blastomere versus trophectoderm biopsy-based PGD-A. All studies reported an increase in the ongoing/clinical pregnancy rates with trophectoderm biopsy although there was no adjustment for significant differences between patient characteristics and clinical practice, such as the quality of embryos or the number of embryos transferred. Furthermore, the voluntary reporting of outcomes in two of the studies could have contributed to substantial selection bias (Hodes-Wertz *et al.*, 2012; Harton *et al.*, 2013). A study by Fragouli *et al.* (2010) which compared the clinical outcomes of CGH on polar body to trophectoderm biopsy in 32 poor prognosis women of advanced maternal age (mean age 38.4–39.8 years), also reported improved pregnancy rates among patients who had trophectoderm biopsy-based PGD-A. However, the lack of prospective randomization and differences in patient and practice characteristics between groups (e.g. number of embryos transferred, variation in biopsy methodology) also limit the validity of these findings.

Implantation rate

The implantation rate is a measure of the capacity of an individual embryo to implant and is generally expressed as the number of fetal hearts/100 embryos transferred. Five studies, two RCTs (Forman *et al.*, 2013b; Scott *et al.*, 2013a) and three matched cohort studies (Schoolcraft *et al.*, 2010; Fishel *et al.*, 2011; Keltz *et al.*, 2013), presented sufficient data to enable the implantation rate to be compared with a control group and these studies are presented in Figs 2 and 3. In four of the five studies, there was a significant increase in the implantation rate per embryo when the embryo had been screened with PGD-A. The remaining study reported a non-statistically significant improvement ($P = 0.08$) in the implantation rate in the PGD-A group (Forman *et al.*, 2013b). Despite the heterogeneity of the studies, it is reasonable to conclude from this that an embryo that has been screened with PGD-A has a higher implantation rate than an unscreened embryo.

Pregnancy loss

Fourteen studies reported pregnancy loss (variously defined as miscarriage, biochemical loss, missed abortion) as an outcome measure (Sher *et al.*, 2007; Sher *et al.*, 2009; Fishel *et al.*, 2011; Geraedts *et al.*, 2011; Schoolcraft *et al.*, 2011; Hodes-Wertz *et al.*, 2012; Forman *et al.*, 2012; Yang *et al.*, 2012; Adler *et al.*, 2013; Forman *et al.*, 2013a,b; Harton *et al.*, 2013; Keltz *et al.*, 2013; Mir *et al.*, 2013). Pregnancy loss ranged from 2.6% (Yang *et al.*, 2012) to 31.6% of pregnancies (Fishel *et al.*, 2011) in the PGD-A group. Two of the three RCTs (Yang *et al.*, 2012; Forman *et al.*, 2013a,b) reported miscarriage rates and found no significant difference between patients who had PGD-A and those who had embryos selected by morphology alone. Harton *et al.* (2013) undertook a retrospective review of the miscarriage rates between biopsy at cleavage and blastocyst stage and by age group, and found no significant difference between blastocysts and cleavage stage euploid embryos transferred in women up to 42 years, averaging 9.9% for Day 3 and 7.9% for blastocyst stage. However, two observational studies with control groups of patients who received morphologically selected embryos reported significantly lower miscarriage rates after PGD-A (Sher *et al.*, 2009; Forman *et al.*, 2012).

Discussion

The purpose of this review was to evaluate the best available evidence on the effectiveness of PGD-A to improve clinical outcomes. Nineteen studies comprising three RCTs and 16 observational studies were identified.

The main limitation of the synthesis of the evidence was the paucity of published high-quality studies. The 19 studies identified exhibited significant variation in study design and quality. According to the Downs and Black scoring system, only the three RCTs were scored as high quality, with 14 of the remaining studies scored as moderate quality and two as poor quality.

The first criteria for assessing embryo selection techniques is whether the implantation rate of the selected embryo is higher than the implantation rate when conventional morphological assessment of the embryo is used. Despite the limitations of the studies presented in this review, the data presented in Figs 2 and 3 demonstrate, a consistent effect of PGD-A in improving implantation rates.

The evidence from two of the three RCTs also indicates that PGD-A results in an improved clinical pregnancy rates in the index cycle. However, there is an argument that outcomes from subsequent cycles should be included in any analysis of PGD-A because embryo selection may not improve cumulative live birth rates given recent advances in cryopreservation which allow almost all embryos to be cryopreserved and transferred in subsequent cycles (Mastenbroek *et al.*, 2011), and that a proportion of euploid embryos are likely to be excluded as a result of the PGD-A process (Scott *et al.*, 2012). There is also evidence to suggest that all embryos should be cryopreserved and transferred in an unstimulated cycle to improve perinatal outcomes of ART conceived babies (Pelkonen *et al.*, 2010; Wennerholm *et al.*, 2013).

Despite the promise of PGD-A being able to improve live birth rates in women of advanced maternal age and those suffering from repeated implantation failure and recurrent miscarriage, only observational studies were identified in these patient groups (Wilton *et al.*, 2003; Sher *et al.*, 2009; Fragouli *et al.*, 2010; Schoolcraft *et al.*, 2010; Schoolcraft *et al.*, 2011; Adler *et al.*, 2013). While findings from these studies tend to favour PGD-A, observational studies cannot infer causality.

The optimal cells to biopsy for PGD-A is a source of considerable debate. The majority of studies identified in this review used trophectoderm biopsy, which is in-line with a recent report by the European Society of Human Reproduction and Embryology (ESHRE) which reported a shift away from blastomere biopsy towards polar body and trophectoderm biopsy (Harton *et al.*, 2011). This has been fuelled by a report that cleavage stage biopsy damages embryos as evidenced by lower implantation rates (Scott *et al.*, 2013b). Blastocysts are known to have higher implantation rates than cleavage stage embryos so it is of some concern that the implantation rates of control cleavage and blastocyst embryos were the same in this study. This is possibly a vagary of the small number of implantations analysed, particularly in the cleavage embryo group, and raises some questions about the conclusions of this study. Furthermore, while a randomized trial by the same group (Scott *et al.*, 2012) found significantly improved predictive values for implantation for trophectoderm biopsy compared with blastomere biopsy, a RCT is needed to confirm the clinical relevance of these findings.

Furthermore, it is acknowledged that many patients who are referred for PGD-A treatment have a poor prognosis and may not be able to

produce sufficient embryos for blastocyst biopsy (Gleicher *et al.*, 2014) and it is incumbent on ART clinics to provide both types of biopsy and on PGD laboratories to have the expertise to test both trophectoderm and single cell biopsies. There also exists much debate around the effectiveness of polar body biopsy due to the incidence of post-zygotic aneuploidy (Capalbo *et al.*, 2013a; Christopikou and Handyside, 2013; Fragouli and Wells, 2013) which occurs after the diagnostic testing is performed.

High-quality studies of embryo selection techniques

Good study design coupled with appropriate statistical analysis is the cornerstone of evidence-based clinical decisions in medicine. However, particular challenges exist in designing and analysing efficacy and effectiveness trials of embryo selection techniques, such as PGD-A, metabolomics, time-lapse imaging (Daya, 2006; van Gelder and Nijs, 2011). The RCT remains the best study design to establish the efficacy of an intervention and is the best method for assessing emerging technologies, such as embryo selection techniques, before more generalizable effectiveness trials are undertaken. Key principles underlying the 'gold-standard' label given to RCTs include defining a clear *a priori* test hypothesis based on clinically meaningful questions, obtaining an appropriate sample size to detect a difference in outcomes, and using blind randomization to ensure similarity among intervention groups. In addition, in embryo selection trials it is important to minimize bias by defining a cycle as the being from the beginning of stimulation (not at oocyte retrieval or embryo transfer), to report success as birth of a live born singleton child, and to control for confounders such as number of embryos transferred and previous experience of ART in either the study design or analysis. Intermediate end-points such as implantation rates are informative but the cumulative rate of live born singleton children from fresh and subsequent frozen embryo cycles provides the most meaningful outcome measures from a clinical and cost-effectiveness perspective. The time required to achieve a live born child is also of importance to patients. Many of these principles were not observed in the three RCTs reviewed or in previous FISH RCTs. Furthermore, ensuring that the clinical and laboratory protocols are highly standardized among centres and quality assurance programs are incorporated into all trial aspects is needed to minimize bias associated varying expertise among centres and single centre trials. Such measures also provide confidence in the reproducibility of results, a problem with previous RCTs using FISH for PGD-A.

Data analysis using intention-to-treat principles is also critical in preserving the unbiased composition of the randomized groups, such that all patients randomized to a group are analysed together. In intention-to-treat analysis, any non-compliance with the original assignment due, for example, to withdrawing from treatment or crossing over to the alternate intervention, are reflected in outcomes measures assigned to the original group. This analytical approach reflects the clinical effectiveness of the interventions and tends to provide a more conservative and cautious estimation of the treatment effect.

Cost-effectiveness studies of embryo selection techniques

No cost-effectiveness analyses of PGD-A using comprehensive techniques were identified. This is an important consideration as PGD-A technologies are currently expensive, and have potentially significant economic implications for funders and patients. Theoretically PGD-A has the potential to be cost-effective if it reduces the number of ART cycles needed by a couple to achieve a live birth. This is particularly true if it supports single embryo transfer (Polinder *et al.*, 2008; Chambers *et al.*, 2014) in fresh and subsequent less expensive thaw cycles with lower miscarriage rates. PGD-A, therefore has the potential to maximize both the clinical and economic benefits of fewer ART cycles resulting in a singleton baby in a shorter period of time.

PGD-A has been criticized on the grounds that it is an expensive intervention, however, it may also limit costs and emotional burden by preventing the storage and later transfer of aneuploid, non-viable embryos and theoretically reduce the time to pregnancy. Therefore, assessing the cost-effectiveness of an embryo selection technique requires not only consideration of the cost of the PGD-A technique and success rates following an the first embryo transfer cycle, but must also include the likelihood of any transfer occurring in the stimulation cycle, the number of remaining embryos, the costs and effectiveness of the cryostorage programme and impact of time to pregnancy. In other words any cost-effectiveness analysis should consider the incremental differences in societal costs and singleton live births between a strategy of repeated cycles transferring untested embryos and a strategy of transferring euploid only embryos.

The available comparative data on PGD-A are very limited in these respects. The three studies from the New Jersey group (Forman *et al.*, 2012, 2013b; Scott *et al.*, 2013a) report remarkably small changes in the number of available vitrified blastocysts after embryo selection with PGD-A. In contrast, the data of Schoolcraft *et al.* (2010) suggest that the number of remaining embryos after the initial transfer could change from 4.4 embryos to 1.1 embryos through the use of PGD-A. Of the other five studies with control groups, none report comparative data on the number of vitrified embryos with and without PGD-A. None of the studies, so far published, in the clinical evaluation of PGD-A provide sufficient data to fully understand the clinical and economic effectiveness of this technology.

An important aspect of cost-effectiveness of PGD-A that needs to be considered is the potential for promoting single embryo transfer. The study by Forman *et al.* (2013b) demonstrated similar pregnancy rates when transfer of a single, PGD-A screened embryo was compared with transfer of two morphologically screened embryos. This raises the possibility that PGD-A may be useful in promoting single embryo transfer in parts of the world where this is not yet established practice, thus reducing the considerable morbidity and economic burden of multiple pregnancy (Chambers and Ledger, 2014; Chambers *et al.*, 2014).

Conclusion

This review did reveal potential benefits of using PGD-A techniques over morphology-based selection of embryos, in particular, demonstrating that a PGD-A screened embryo has a higher implantation rate than a morphologically screened embryo. Three RCTs in young good prognosis patients demonstrated clinical benefit in terms of clinical pregnancy rates and the use of single embryo transfer, however, studies in other patient groups were limited to observational studies.

The previous enthusiasm for adopting FISH based on observational studies which was later dampened by several RCTs (Mastenbroek *et al.*, 2007; Blockeel *et al.*, 2008; Staessen *et al.*, 2008; Schoolcraft

et al., 2009; Debrock et al., 2010) should serve as a cautionary tale against using observational data to guide clinical practice. Well-designed studies that take into account all the elements necessary to evaluate the clinical and cost-effectiveness of PGD-A techniques in the clinic setting are overdue.

Authors' roles

E.L. was involved in the design and acquisition of data, analysis and interpretation of data, construction of tables and figures, and drafting and critical revisions of the manuscript. G.M.C. led the conception and design of the study, analysis and interpretation of data, drafting and critical revisions of the manuscript. P.I. and L.W. were involved in the study concept, analysis of data and critical revisions of the manuscript. All authors approved the final manuscript.

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Conflict of interest

P.I. is the Medical Director of the IVF Clinic, IVF Australia and has a financial interest in the parent group, Virtus Health. L.W. is an employee of and a shareholder in Virtus Health which is a provider of PGD services in Australia. G.M.C previously received grant support to her institution from the Australian Government, Australian Research Council (ARC) Linkage Grant No. LP 1002165; ARC Linkage Grant Partner Organisations were IVF Australia, Melbourne IVF and Queensland Fertility Group. E.L does not report any conflict of interest.

References

Adler A, Lee H-L, McCulloh DH, Ampeloquio E, Clarke-Williams M, Wertz BH, Grifo J. Blastocyst culture selects for euploid embryos: comparison of blastomere and trophectoderm biopsies. *Reprod Biomed Online* 2014;**28**: 485–491.

Ajduk A, Zernicka-Goetz M. Advances in embryo selection methods. *F1000 Biol Rep* 2012;**4**: 11.

Blockeel C, Schutyser V, De Vos A, Verpoest W, De Vos M, Staessen C, Haentjens P, Van der Elst J, Devroey P. Prospectively randomized controlled trial of PGS in IVF/ICSI patients with poor implantation. *Reprod Biomed Online* 2008;**17**: 848–854.

Brezina PR, Brezina DS, Kearns WG. Preimplantation genetic testing. *BMJ* 2012;**345**:e5908.

Capalbo A, Bono S, Spizzichino L, Biricik A, Baldi M, Colamaria S, Ubaldi FM, Rienzi L, Fiorentino F. Sequential comprehensive chromosome analysis on polar bodies, blastomeres and trophoblast: insights into female meiotic errors and chromosomal segregation in the preimplantation window of embryo development. *Hum Reprod* 2013a;**28**: 509–518.

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* 2013b;**28**: 2298–2307.

Chambers GM, Lee E, Hansen M, Sullivan EA, Bower C, Chapman M. Hospital costs of multiple-birth and singleton-birth children during the first 5 years of life and the role of assisted reproductive technology. *JAMA pediatrics* 2014;**168**: 1045–1053.

Chambers GM, Sullivan EA, Ishihara O, Chapman MG, Adamson GD. The economic impact of assisted reproductive technology: a review of selected developed countries. *Fertil steril* 2009;**91**: 2281–2294.

Christopikou D, Handyside AH. Questions about the accuracy of polar body analysis for preimplantation genetic screening. *Hum Reprod* 2013;**28**: 1732–1733.

Colls P, Escudero T, Cekleniak N, Sadowy S, Cohen J, Munné S. Increased efficiency of preimplantation genetic diagnosis for infertility using "no result rescue". *Fertil steril* 2007;**88**: 53–61.

Daya S. Methodological issues in infertility research. *Best Pract Res Clin Obstet Gynaecol* 2006;**20**: 779–797.

Debrock S, Melotte C, Spiessens C, Peeraer K, Vanneste E, Meeuwis L, Meuleman C, Frijns J-P, Vermeesch JR, D'Hooghe TM. Preimplantation genetic screening for aneuploidy of embryos after in vitro fertilization in women aged at least 35 years: a prospective randomized trial. *Fertil Steril* 2010;**93**: 364–373.

Downs SH, Black N. The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. *J Epidemiol Commun Health* 1998;**52**: 377–384.

Fiorentino F. Array comparative genomic hybridization: its role in preimplantation genetic diagnosis. *Curr Opin Obstet Gynecol* 2012;**24**: 203–209.

Fishel SC, Craig A, Lynch C, Dowell K, Ndukwe G, Jenneer L, Cater E, Brown A, Thornton S, Campbell A *et al.* Assessment of 19,803 paired chromosomes and clinical outcome from first 150 cycles using array CGH of the first polar body for embryo selection and transfer. *J Fertiliz In vitro* 2011;1–8

Forman E, Tao X, Ferry K, Taylor D, Treff N, Scott R. Single embryo transfer with comprehensive chromosome screening results in improved ongoing pregnancy rates and decreased miscarriage rates. *Hum Reprod* 2012;**27**: 1217–1222.

Forman EJ, Hong KH, Ferry KM, Tao X, Taylor D, Levy B, Treff NR, Scott RT Jr. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. *Fertil Steril* 2013b;**100**: 100–107. e101.

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**: 157. e151–157. e156.

Forman EJ, Upham KM, Cheng M, Zhao T, Hong KH, Treff NR, Scott RT Jr. Comprehensive chromosome screening alters traditional morphology-based embryo selection: a prospective study of 100 consecutive cycles of planned fresh euploid blastocyst transfer. *Fertil Steril* 2013a; **100**: 718–724.

Fragouli E, Alfarawati S, Daphnis DD, Goodall N-n, Mania A, Griffiths T, Gordon A, Wells D. Cytogenetic analysis of human blastocysts with the use of FISH, CGH and aCGH: scientific data and technical evaluation. *Hum Reprod* 2011;**26**: 480–490.

Fragouli E, Katz-Jaffe M, Alfarawati S, Stevens J, Colls P, Goodall N-n, Tormasi S, Gutierrez-Mateo C, Prates R, Schoolcraft WB. Comprehensive chromosome screening of polar bodies and blastocysts from couples experiencing repeated implantation failure. *Fertil Steril* 2010;**94**: 875–887.

Fragouli E, Wells D. Aneuploidy screening for embryo selection *Seminars in reproductive medicine*. Thieme Medical Publishers, 2012, pp. 289–301.

Fragouli E, Wells D. Questions about the accuracy of polar body analysis for preimplantation genetic screening. *Human Reproduction* 2013;**28**: 1731–1732.

Garrisi JG, Colls P, Ferry KM, Zheng X, Garrisi MG, Munné S. Effect of infertility, maternal age, and number of previous miscarriages on the

outcome of preimplantation genetic diagnosis for idiopathic recurrent pregnancy loss. *Fertil steril* 2009;**92**: 288–295.

Geraedts J, Montag M, Magli MC, Repping S, Handyside A, Staessen C, Harper J, Schmutzler A, Collins J, Goossens V. Polar body array CGH for prediction of the status of the corresponding oocyte. Part I: clinical results. *Hum Reprod* 2011;**26**: 3173–3180.

Gleicher N, Barad DH. A review of, and commentary on, the ongoing second clinical introduction of preimplantation genetic screening (PGS) to routine IVF practice. *J Assist Reprod Genet* 2012;**29**: 1159–1166.

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.

Gutiérrez-Mateo C, Colls P, Sánchez-García J, Escudero T, Prates R, Ketterson K, Wells D, Munné S. Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos. *Fertil sterily* 2011;**95**: 953–958.

Handyside AH, Kontogianni EH, Hardy K, Winston R. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature* 1990;**344**: 768–770.

Hardarson T, Hanson C, Lundin K, Hillensjö T, Nilsson L, Stevic J, Reismer E, Borg K, Wikland M, Bergh C. Preimplantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: a randomized controlled trial. *Hum Reprod* 2008;**23**: 2806–2812.

Harton G, Magli M, Lundin K, Montag M, Lemmen J, Harper J. ESHRE PGD Consortium/Embryology Special Interest Group—best practice guidelines for polar body and embryo biopsy for preimplantation genetic diagnosis/screening (PGD/PGS). *Hum Reprod* 2011;**26**: 41–46.

Harton GL, Munné S, Surrey M, Grifo J, Kaplan B, McCulloh DH, Griffin DK, Wells D. Diminished effect of maternal age on implantation after preimplantation genetic diagnosis with array comparative genomic hybridization. *Fertil Steril* 2013;**100**: 1695–1703.

Hassold T, Abruzzo M, Adkins K, Griffin D, Merrill M, Millie E, Saker D, Shen J, Zaragoza M. Human aneuploidy: incidence, origin, and etiology. *Environ Mol Mutagen* 1996;**28**: 167–175.

Hellani A, Abu-Amero K, Azouri J, El-Akoum S. Successful pregnancies after application of array-comparative genomic hybridization in PGS-aneuploidy screening. *Reprod Biomed Online* 2008;**17**: 841–847.

Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *BMJ* 2004;**328**: 261.

Hodes-Wertz B, Grifo J, Ghadir S, Kaplan B, Laskin CA, Glassner M, Munné S. Idiopathic recurrent miscarriage is caused mostly by aneuploid embryos. *Fertil Steril* 2012;**98**: 675–680.

Kahraman S, Bahce M, Şamlı H, İmirzalıoğlu N, Yakısn K, Cengiz G, Dönmez E. Healthy births and ongoing pregnancies obtained by preimplantation genetic diagnosis in patients with advanced maternal age and recurrent implantation failure. *Hum Reprod* 2000;**15**: 2003–2007.

Keltz MD, Vega M, Sirota I, Lederman M, Moshier EL, Gonzales E, Stein D. Preimplantation Genetic Screening (PGS) with Comparative Genomic Hybridization (CGH) following day 3 single cell blastomere biopsy markedly improves IVF outcomes while lowering multiple pregnancies and miscarriages. *J Assist Reprod Genet* 2013;**30**: 1333–1339.

Lemos EV, Zhang D, Van Voorhis BJ, Hu XH. Healthcare expenses associated with multiple vs singleton pregnancies in the United States. *Am J Obstet Gynecol* 2013;**209**: 586. e581–586. e511.

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**: 943–947.

Mastenbroek S, Twisk M, van Echten-Arends J, Sikkema-Raddatz B, Korevaar JC, Verhoeve HR, Vogel NE, Arts EG, De Vries JW, Bossuyt PM. In vitro fertilization with preimplantation genetic screening. *N Engl J Med* 2007;**357**: 9–17.

Mastenbroek S, van der Veen F, Aflatoonian A, Shapiro B, Bossuyt P, Repping S. Embryo selection in IVF. *Hum Reprod* 2011;**26**: 964–966.

Mir P, Rodrigo L, Mercader A, Buendía P, Mateu E, Milán-Sánchez M, Peinado V, Pellicer A, Remohí J, Simón C. False positive rate of an arrayCGH platform for single-cell preimplantation genetic screening and subsequent clinical application on day-3. *J Assist Reprod Genet* 2013;**30**: 143–149.

Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med* 2009;**151**: 264–269.

Munné S, Gianaroli L, Tur-Kaspa I, Magli C, Sandalinas M, Grifo J, Cram D, Kahraman S, Verlinsky Y, Simpson JL. Substandard application of preimplantation genetic screening may interfere with its clinical success. *Fertil Steril* 2007;**88**: 781–784.

Northrop L, Treff N, Levy B, Scott R. 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.

Ombelet W, Martens G, De Sutter P, Gerris J, Bosmans E, Ruyssinck G, Defoort P, Molenberghs G, Gyselaers W. Perinatal outcome of 12 021 singleton and 3108 twin births after non-IVF-assisted reproduction: a cohort study. *Hum Reprod* 2006;**21**: 1025–1032.

Pelkonen S, Koivunen R, Gissler M, Nuojua-Huttunen S, Suikkari A-M, Hyden-Granskog C, Martikainen H, Tiitinen A, Hartikainen A-L. Perinatal outcome of children born after frozen and fresh embryo transfer: the Finnish cohort study 1995–2006. *Hum Reprod* 2010;**25**: 914–923.

Polinder S, Heijnen E, Macklon N, Habbema J, Fauser B, Eijkemans M. Cost-effectiveness of a mild compared with a standard strategy for IVF: a randomized comparison using cumulative term live birth as the primary endpoint. *Hum Reprod* 2008;**23**: 316–323.

Rubio C, Simon C, Vidal F, Rodrigo L, Pehlivan T, Remohi J, Pellicer A. Chromosomal abnormalities and embryo development in recurrent miscarriage couples. *Hum Reprod* 2003;**18**: 182–188.

Rubio C, Bellver J, Rodrigo L, Bosch E, Mercader A, Vidal C, De los santos MJ, Giles J, Labarta E, domingo J *et al*. Preimplantation genetic screening using fluorescence in situ hybridization in patients with repetitive implantation failure and advanced maternal age: two randomized trials. *Fertil Steril* 2013;**99**:1400–1407.

Schoolcraft WB, Fragouli E, Stevens J, Munne S, Katz-Jaffe MG, Wells D. Clinical application of comprehensive chromosomal screening at the blastocyst stage. *Fertil Steril* 2010;**94**: 1700–1706.

Schoolcraft WB, Katz-Jaffe MG, Stevens J, Rawlins M, Munne S. Preimplantation aneuploidy testing for infertile patients of advanced maternal age: a randomized prospective trial. *Fertil Steril* 2009;**92**: 157–162.

Schoolcraft WB, Treff NR, Stevens JM, Ferry K, Katz-Jaffe M, Scott RT Jr. Live birth outcome with trophectoderm biopsy, blastocyst vitrification, and single-nucleotide polymorphism microarray–based comprehensive chromosome screening in infertile patients. *Fertil Steril* 2011;**96**: 638–640.

Scott RT Jr, Ferry K, Su J, Tao X, Scott K, Treff NR. Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. *Fertil Steril* 2012;**97**: 870–875.

Scott RT Jr, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, Tao X, Treff NR. 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* 2013a;**100**: 697–703.

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* 2013b;**100**: 624–630.

Scott KL, Hong KH, Scott RT Jr. Selecting the optimal time to perform biopsy for preimplantation genetic testing. *Fertil Steril* 2013;**100**: 608–614.

Sher G, Keskintepe L, Keskintepe M, Ginsburg M, Maassarani G, Yakut T, Baltaci V, Kotze D, Unsal E. Oocyte karyotyping by comparative genomic hybrydization provides a highly reliable method for selecting "competent" embryos, markedly improving in vitro fertilization outcome: a multiphase study. *Fertil Steril* 2007;**87**: 1033–1040.

Sher G, Keskintepe L, Keskintepe M, Maassarani G, Tortoriello D, Brody S. Genetic analysis of human embryos by metaphase comparative genomic hybridization (mCGH) improves efficiency of IVF by increasing embryo implantation rate and reducing multiple pregnancies and spontaneous miscarriages. *Fertil Steril* 2009;**92**: 1886–1894.

Staessen C, Platteau P, Van Assche E, Michiels A, Tournaye H, Camus M, Devroey P, Liebaers I, Van Steirteghem A. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. *Hum Reprod* 2004;**19**: 2849–2858.

Staessen C, Verpoest W, Donoso P, Haentjens P, Van der Elst J, Liebaers I, Devroey P. Preimplantation genetic screening does not improve delivery rate in women under the age of 36 following single-embryo transfer. *Hum Reprod* 2008;**23**: 2818–2825.

Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. *JAMA* 2000;**283**: 2008–2012.

Traversa MV, Marshall J, McArthur S, Leigh D. The genetic screening of preimplantation embryos by comparative genomic hybridisation. *Reprod Biol* 2011;**11**: 51–60.

Treff NR, Levy B, Su J, Northrop LE, Tao X, Scott RT. SNP microarray-based 24 chromosome aneuploidy screening is significantly more consistent than FISH. *Mol Hum Reprod* 2010;**16**: 583–589.

van Gelder P, Nijs M. Statistical flaws in design and analysis of fertility treatment studies on cryopreservation raise doubts on the conclusions. *Facts Views Vis Obgyn* 2011;**3**: 273.

Vialard F, Boitrelle F, Molina-Gomes D, Selva J. Predisposition to aneuploidy in the oocyte. *Cytogenet Genome Res* 2011;**133**:127–135.

Voullaire L, Collins V, Callaghan T, McBain J, Williamson R, Wilton L. High incidence of complex chromosome abnormality in cleavage embryos from patients with repeated implantation failure. *Fertil Steril* 2007;**87**: 1053–1058.

Wennerholm U-B, Henningsen A-KA, Romundstad LB, Bergh C, Pinborg A, Skjaerven R, Forman J, Gissler M, Nygren KG, Tiitinen A. Perinatal outcomes of children born after frozen-thawed embryo transfer: a Nordic cohort study from the CoNARTaS group. *Hum Reprod* 2013;**28**: 2545–2553.

Wilton L, Voullaire L, Sargeant P, Williamson R, McBain J. Preimplantation aneuploidy screening using comparative genomic hybridization or fluorescence in situ hybridization of embryos from patients with recurrent implantation failure. *Fertil Steril* 2003;**80**: 860–868.

Wilton L, Williamson R, McBain J, Edgar D, Voullaire L. Birth of a healthy infant after preimplantation confirmation of euploidy by comparative genomic hybridization. *N Engl J Med* 2001;**345**: 1537–1541.

Yang Z, Liu J, Collins GS, Salem SA, Liu X, Lyle SS, Peck AC, Sills ES, Salem RD. 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**: 1–8.