Human Reproduction, Vol.33, No.9 pp. 1767–1776, 2018
Advanced Access publication on August 6, 2018 doi:10.1093/humrep/dey262

human reproduction

ORIGINAL ARTICLE ***Reproductive genetics***

Preimplantation genetic testing for aneuploidy by microarray analysis of polar bodies in advanced maternal age: a randomized clinical trial

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Submitted on January 3, 2018; resubmitted on June 28, 2018; accepted on July 12, 2018

STUDY QUESTION: Does preimplantation genetic testing for aneuploidy (PGT-A) by comprehensive chromosome screening (CCS) of the first and second polar body to select embryos for transfer increase the likelihood of a live birth within 1 year in advanced maternal age women aged 36–40 years planning an ICSI cycle, compared to ICSI without chromosome analysis?

SUMMARY ANSWER: PGT-A by CCS in the first and second polar body to select euploid embryos for transfer does not substantially increase the live birth rate in women aged 36–40 years.

WHAT IS KNOWN ALREADY: PGT-A has been used widely to select embryos for transfer in ICSI treatment, with the aim of improving treatment effectiveness. Whether PGT-A improves ICSI outcomes and is beneficial to the patients has remained controversial.

STUDY DESIGN, SIZE, DURATION: This is a multinational, multicentre, pragmatic, randomized clinical trial with intention-to-treat analysis. Of 396 women enroled between June 2012 and December 2016, 205 were allocated to CCS of the first and second polar body (study group) as part of their ICSI treatment cycle and 191 were allocated to ICSI treatment without chromosome screening (control group). Block randomization was performed stratified for centre and age group. Participants and clinicians were blinded at the time of enrolment until the day after intervention.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Infertile couples in which the female partner was 36–40 years old and who were scheduled to undergo ICSI treatment were eligible. In those assigned to PGT-A, array comparative genomic hybridization (aCGH) analysis of the first and second polar bodies of the fertilized oocytes was performed using the 24sure array of Illumina. If in the first treatment cycle all oocytes were aneuploid, a second treatment with PB array CGH was offered. Participants in the control arm were planned for ICSI without PGT-A. Main exclusion criteria were three or more previous unsuccessful IVF or ICSI cycles, three or more clinical miscarriages, poor response or low ovarian reserve. The primary outcome was the cumulative live birth rate after fresh or frozen embryo transfer recorded over 1 year after the start of the intervention.

MAIN RESULTS AND THE ROLE OF CHANCE: Of the 205 participants in the chromosome screening group, 50 (24%) had a live birth with intervention within 1 year, compared to 45 of the 191 in the group without intervention (24%), a difference of 0.83% (95% CI: −7.60 to 9.18%). There were significantly fewer participants in the chromosome screening group with a transfer (relative risk (RR) = 0.81; 95% CI: 0.74–0.89) and fewer with a miscarriage (RR = 0.48; 95% CI: 0.26–0.90).

LIMITATIONS, REASONS FOR CAUTION: The targeted sample size was not reached because of suboptimal recruitment; however, the included sample allowed a 90% power to detect the targeted increase. Cumulative outcome data were limited to 1 year. Only 11 patients out of 32 with exclusively aneuploid results underwent a second treatment cycle in the chromosome screening group.

WIDER IMPLICATIONS OF THE FINDINGS: The observation that the similarity in birth rates was achieved with fewer transfers, less cryopreservation and fewer miscarriages points to a clinical benefit of PGT-A, and this form of embryo selection may, therefore, be considered to minimize the number of interventions while producing comparable outcomes. Whether these benefits outweigh drawbacks such as the cost for the patient, the higher workload for the IVF lab and the potential effect on the children born after prolonged culture and/or cryopreservation remains to be shown.

STUDY FUNDING/COMPETING INTEREST(S): This study was funded by the European Society of Human Reproduction and Embryology. Illumina provided microarrays and other consumables necessary for aCGH testing of polar bodies. M.B.'s institution (UZBrussel) has received educational grants from IBSA, Ferring, Organon, Schering-Plough, Merck and Merck Belgium. M.B. has received consultancy and speakers' fees from Organon, Serono Symposia and Merck. G.G. has received personal fees and non-financial support from MSD, Ferring, Merck-Serono, Finox, TEVA, IBSA, Glycotope, Abbott and Gedeon-Richter as well as personal fees from VitroLife, NMC Healthcare, ReprodWissen, BioSilu and ZIVA. W.V., C.S., P.M.B., V.G., G.A., M.D., T.E.G., L.G., G.Ka., G.Ko., J.L., M.C.M., M.P., A.S., M.T., K.V., J.G. and K.S. declare no conflict of interest.

TRIAL REGISTRATION NUMBER: NCT01532284.

TRIAL REGISTRATION DATE: 7 February 2012.

DATE OF FIRST PATIENT'S ENROLMENT: 25 June 2012.

Key words: PGT-A / PGS / comprehensive chromosome screening / array comparative genomic hybridization / randomized clinical trial / polar body biopsy

Introduction

Numerical chromosomal abnormalities of either meiotic or mitotic origin in preimplantation embryos have been regarded as the main reason for implantation failure, miscarriage and prolonged time to pregnancy in IVF. It was consequently postulated that selection of euploid embryos for transfer would increase the likelihood of pregnancy and thereby IVF success rates (Sermon, 2017). Although preimplantation genetic testing for aneuploidy (PGT-A), previously known as PGS (Zegers-Hochschild *et al.*, 2017), has been widely applied at the cleavage stage (Goossens *et al.*, 2009), randomized clinical trials (RCTs) clearly showed that PGT-A did not increase pregnancy rates, and in some instances even lowered them (Mastenbroek *et al.*, 2011). This was later explained by the high rate of mosaicism in cleavage stage embryos (Delhanty *et al.*, 1997; Chavez *et al.*, 2012; Mertzanidou *et al.*, 2013a, 2013b), and by the failure of the FISH technology to detect all chromosomal abnormalities present. Moreover, it was later reported that biopsy of one (Scott *et al.*, 2013b) or more blastomeres (De Vos *et al.*, 2009) is potentially harmful to embryo development.

In 2009, the ESHRE PGT-A Task Force undertook a pilot study with the aim to study whether the ploidy of the zygote could be predicted with acceptable accuracy by array comparative genomic hybridization (aCGH) analysis of both polar bodies (PBs) (Geraedts *et al.*, 2010). Array-CGH had not been used for PGT-A at that time, and the use of aCGH needed to be validated. The choice of PB biopsy (PBB) at the onset of the study was guided by the presence of extensive mosaicism at Day 3 precluding biopsy at this developmental stage, while trophectoderm (TE) biopsy was not fully developed (Kokkali *et al.*, 2005; McArthur *et al.*, 2005; Scott *et al.*, 2013a) as there was only one RCT terminated prematurely at that time (Jansen *et al.*, 2008). The advantages of PBB are that it will identify the abnormalities of maternal meiotic origin, which make up for 90% of meiotic errors in embryos, and that at the time of the start of the study, it was the only form of PGT-A allowed in two of the participating countries, Italy and Germany (Geraedts *et al.*, 2010). The disadvantages of PBB are that the paternal contribution is not tested and that mosaicism occurring during later stages is missed (Montag *et al.*, 2013). The most state-of-the-art comprehensive chromosome screening

(CCS) method at that time was selected, i.e. aCGH (24sure® by Illumina).

In 2011, two back-to-back papers were published on the results of this pilot study, a clinical paper (Geraedts *et al.*, 2011) and a technical paper (Magli *et al.*, 2011). The concordance between the chromosome analysis of PBs and those from the resulting fertilized oocytes was 94%. Consequently, an RCT named the ESHRE Study into the Evaluation of oocyte Euploidy by Microarray analysis (ESTEEM) was initiated. The ESTEEM study question was whether the analysis of 23 chromosomes in the PB1 and PB2 to select embryos derived from euploid oocytes for transfer increases the likelihood of a live birth within 1 year amongst women aged 36–40 years, compared to ICSI without chromosome analysis.

Materials and Methods

Experimental design

The ESTEEM trial was designed as a multinational, multicentre, pragmatic, randomized clinical trial (RCT) with intention-to-treat (ITT) analysis, to estimate the effectiveness of PGT-A in broad routine clinical practice (Welsing *et al.*, 2017). Participants were recruited between August 2012 and December 2016. The experimental questions addressed by this trial were: (i) Does PGT-A by analysis of 23 chromosomes in the PB1 and PB2 to select euploid embryos for transfer increase the likelihood of a live birth within 1 year in women aged 36–40 years treated with ICSI compared to ICSI without chromosome analysis and (ii) is PGT-A by analysis of 23 chromosomes in the PB1 and PB2 in women with no euploid embryos in a first cycle indicative of the probability of having no euploid oocytes in a subsequent cycle? Secondary outcomes studied were clinical pathway outcomes, including miscarriage rate, and genetic outcomes, i.e. aneuploidy rate.

Each individual collaborating centre obtained ethical approval by the appropriate review board before study initiation.

The ESTEEM trial was directed by a steering committee independent of the collaborating centres and was registered at Clinicaltrial.gov as NCT01532284. The study was conducted according to the principles of the Declaration of Helsinki and in accordance with ICH-GCP (Good Clinical Practice).

Population

Women aged 36–40 years who were being treated for infertility through ICSI were eligible. Additional inclusion criteria were: (i) BMI between 18 and 30 kg/m^2, (ii) patients prepared to accept the transfer of up to two embryos and (iii) absence of any type of hereditary condition in the patient's or partner's personal and family history. Excluded were couples meeting one or more of the following criteria: (i) the infertility treatment involving the use of donor oocytes; (ii) menstrual cycle irregularity (<24days and >35 days); (iii) three or more previous failed IVF or ICSI cycles with the present partner; (iv) poor ovarian response in previous IVF or ICSI cycles; (v) partner requiring surgical sperm retrieval; (vi) partner with total asthenozoospermia, macrozoospermia and/or globozoospermia; (vii) three or more clinical miscarriages; and (viii) the chronic use of anti-psychotics, anxiolytics or continuous use of non-steroidal anti-inflammatory drugs (NSAID) or any type of medication that may interfere with controlled ovarian hyperstimulation, embryology or early pregnancy.

A failed treatment cycle was defined as the absence of a clinical pregnancy relating to a treatment with embryo transfer (ET) resulting from oocyte retrieval for the current intended pregnancy and with the current partner. A treatment cycle included transfers of fresh or frozen embryos derived from this treatment. Clinical pregnancy was defined as the presence of a gestational sac at the earliest ultrasound and includes those resulting in early clinical miscarriage, late miscarriage or clinically confirmed extrauterine pregnancy. Poor ovarian response was identified if meeting one of the following criteria: (i) previous poor ovarian response defined as ≤3 oocytes following a conventional ovarian stimulation protocol or (ii) an abnormal ovarian reserve test defined as antral follicle count (AFC) <5 follicles or serum anti-Mullerian hormone level <0.5 ng/ml. The criteria for poor ovarian response were adapted from Ferraretti *et al.* (2011) taking into account that the age criterion for poor ovarian response was already met by the inclusion criteria for the ESTEEM trial. Patients allocated to PBB and CCS by aCGH were considered the study group; patients allocated to no PB CCS were considered the control group.

Patients were recruited from nine centres in seven countries in Europe and Israel with proven experience in PBB and aCGH analysis. Eligible women were informed about the study and asked for written informed consent to participate. At enrolment, web-based block randomization was performed with a block size of 6, with stratification for centre and age categories 36–37 and 38–40. Both the patient and the clinician as well as the operators performing oocyte retrieval were blinded from the time of enrolment until the day after the research procedure (PBB or no PBB). Participants were not permitted to enrol for a second time. However, if in a first treatment cycle all oocytes were aneuploid on the basis of PB aCGH, a second, non-randomized treatment with PB aCGH was performed to assess the probability of having euploid oocytes. Data were collected in an online platform provided by an independent clinical trial monitoring centre. Sites were monitored for patient safety and data quality by internal monitors, independent from the trial collaborators. Overall organization and procedures of the trial were audited by an external auditor.

Clinical procedures

The synchronization, ovarian stimulation, control of LH surge, ovulation induction, oocyte retrieval, embryology procedures, monitoring, ICSI and luteal support protocols as well as type of protocols for frozen/thawed ET and day of transfer were consistent with the existing procedures in each collaborating centre. These protocols were expected to be the same in both arms of the study in the respective collaborating centres. IVF was performed exclusively by ICSI.

Embryos were transferred at the day of development as decided by the centre policy. The ET policy per centre was to be the same in both arms of the study to avoid the introduction of a bias caused by different days of transfer. Transfer of more than two embryos was not allowed. If not all embryos produced a result on PB CCS, single ET (SET) or double ET (DET) was performed subject to availability of genetically transferable embryos, i.e. if there was only one embryo euploid on the basis of PB CCS, SET was performed and supernumerary embryos without diagnosis were cryopreserved. Embryos derived from oocytes with complete PB diagnosis and embryos derived from oocytes with inconclusive results were never transferred simultaneously. If only embryos derived from aneuploid oocytes and oocytes without diagnosis were available, embryos derived from oocytes without diagnosis were transferred. Clinical outcome was registered according to the ICMART terminology (Zegers-Hochschild *et al.*, 2017). All patients were followed up until 1 year after treatment or until the completion of the pregnancy plus one month. Vital parameters of all children born in this study were registered, as well as parameters of general health including the presence or absence of congenital abnormalities. Abnormalities in the child were registered as an adverse event (AE) or a serious AE (SAE) (admission to NICU or a life-threatening condition) and registered in the eCRF.

Polar body biopsy

Polar body biopsy was scheduled between 6 and 9 h after ICSI (between 42 and 49 h post-HCG injection) using laser or the mechanical procedure. PB1 and PB2 were removed simultaneously and transferred to different tubes for the chromosomal analysis as previously described (Magli *et al.*, 2011). PB1 and PB2 could be distinguished based on their morphology, as PB2 is normally regular in shape and slightly smaller. In addition, PB1 morphology was carefully recorded at the time of ICSI. The laboratory personnel collaborating in the trial were trained by the centres in Bologna or Bonn in performing PBB and tubing before initiation of the trial.

Assays

The PB DNA amplification, hybridization and analysis techniques were performed according to the procedures described for the ESHRE Task Force on the ESTEEM pilot study (Geraedts *et al.*, 2011; Magli *et al.*, 2011). As in the pilot study, all lab personnel were trained by Bluegnome/Illumina to perform all stages of the array procedure (Sureplex amplification, 24sure fluorescent labelling, hybridization, washing, scanning) and analysis. Following study initiation, array results for the first five cases from each centre were monitored and validated by Bluegnome. The BlueFuse Multi-software 'one button-'automated image analysis was used to estimate copy number on a per chromosome basis. Only whole chromosome aneuploidies (gains and losses) were scored. Results were classified as euploid, aneuploid, euploid compensated (aneuploid balanced results from PB1 and PB2 taken together indicate a euploid oocyte), inconclusive (aCGH analysis produced incomplete results) and no evaluation possible (amplification failure or no available result). The entire protocol was completed in <24 h, such that ET and vitrification of surplus euploid embryos could take place up to Day 5.

Data analysis

The primary outcome was live birth per participant within 1 year from the first follicle aspiration after enrolment in the study. As a primary measure of the effectiveness of PB CCS, we calculated the relative live birth rate, i.e. the live birth in the PGT-A group relative to that in the control group, with a 95% CI. This evaluation was based on the ITT principle, in which all randomized women were included in the analysis, in the group to which they had been allocated. In addition to calculating a measure of effectiveness, we tested the alternative hypothesis of a difference in live birth rate against the null hypothesis of no effect using a two-sided Cochran–Mantel–Haenszel statistic, adjusting for the stratified randomization, at a 5% significance level. Assuming a 20% live birth rate at 1 year in the control group, a group size of 266 participants per study arm was aimed for to have a power of more than 90% in detecting an absolute increase of 15 points or more in the live birth rate.

Results

Between June 2012 and December 2016, 205 consenting women were assigned to ICSI treatment with PGT-A (study group) and 191 were assigned to ICSI without PGT-A (control group). The study was stopped prior to reaching the targeted sample size because of suboptimal recruitment. With the included sample size of 396, we still had 90% power to detect the targeted increase. Table I summarizes the baseline characteristics in the two groups, which were comparable. Figure 1 shows the flow of the participants through the study. In total, 15 patients never started ICSI treatment: eight in the PGT-A group and seven in the control group. In the PGT-A group, two patients withdrew while in the six remaining cases discontinuation was due to clinical decisions including the risk of hyperstimulation syndrome and absent fertilization. In the control group, three women had a pregnancy before treatment, one participant withdrew, while in three cases discontinuation was due to clinical decisions.

Table I Baseline characteristics.

Characteristic	PGT-A (*N* = 205)	No PGT-A (*N* = 191)
Age (years)[a]	38.6 ± 1.4	38.6 ± 1.4
BMI[a,b]	23.2 ± 2.8	23.2 ± 3.1
Nulligravid	124 (61%)	107 (56%)
Nulliparous	174 (86%)	153 (80%)
Duration of infertility (months)[a]	38.9 ± 33.5	37.7 ± 29.0
No previous IVF treatment	117 (58%)	110 (58%)
Causes of infertility		
Male	54 (26%)	50 (26%)
Female	45 (22%)	39 (20%)
Both	16 (8%)	7 (4%)
Unknown	90 (44%)	95 (50%)

[a]Means ±SD.
[b]Weight in kilograms divided by the square of the height in metres.

Array CGH results

Seventeen patients in the PGT-A group who had undergone ICSI did not have PBB performed (9%) for technical or logistical reasons. In the remaining 180 patients (91%), between 1 and 17 oocytes were evaluated, with a median of 5 oocytes. The total number of oocytes evaluated with PGT-A was 1023. In 1006 (98%) oocytes, the biopsy of the first or the second PB, or both, were successful; in 17 (1.7%) both biopsies were unsuccessful. The similar rate of embryo development between the two groups (53 versus 50% morphologically transferable), irrespective of the outcome of the PB CCS, points to the absence of a detrimental effect of the PBB procedure. In total, 242 (24%) oocytes were found to be euploid, 525 (51%) aneuploid, 84 (8%) were inconclusive, 128 (13%) had no result while 44 (4%) were scored as euploid compensated (Table II). The aneuploidy rate observed in this study was 525 out of 811 oocytes (65%) with an available result. Of the 180 participants who had one or more PBB, 65 (36%) were found to have zero euploid embryos, 53 participants (29%) had one euploid embryo, while 35 had two (19%), and 27 (16%) more than two, with a maximum of eleven euploid embryos. There were 11 participants who started a second cycle, of which 10 had ICSI in a second cycle, and PBB was performed for 9 of them. For these, 42 oocytes were analysed, of which 3 produced inconclusive results. Of the remaining 39 oocytes, 5 (13%) were euploid and 34 (87%) aneuploid. Of the nine evaluated participants, six (67%) did not have any euploid embryos in this second cycle.

Embryo transfer

Table III shows the mean numbers of oocytes and embryos in each study arm. Of the patients who had undergone ICSI, 13 out of 184 (7%) in the control group and 48 out of 197 (24%) in the PGT-A group

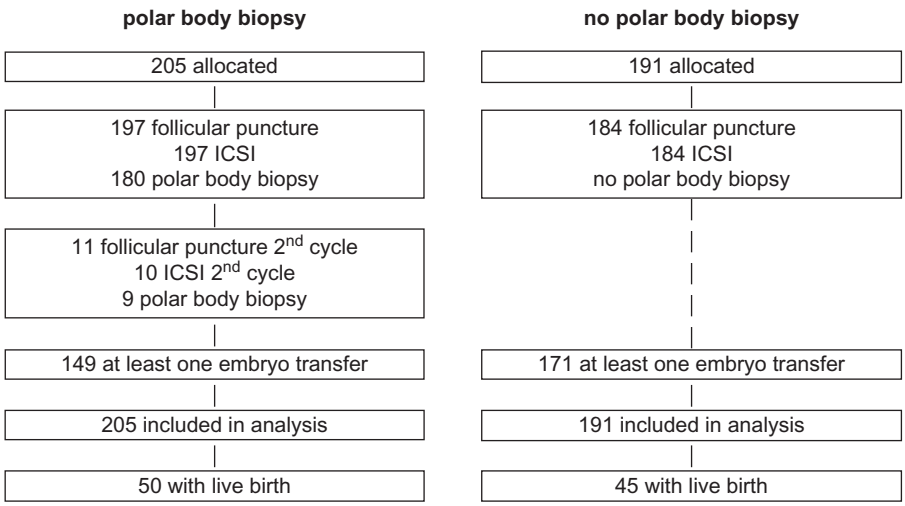

Figure 1 Trial profile.

Table II Oocytes and embryos.

Outcomes	PGT-A	No PGT-A
Number of cumulus oocyte complexes	1893	1925
Number of oocytes in MII	1488	1561
Number of oocytes with polar body biopsy	1023	–
– Euploid	242 (24%)	–
– Aneuploid	525 (51%)	–
– Euploid compensated	44 (4%)	–
– Inconclusive[a]	84 (8%)	–
– No evaluation possible[b]	128 (13%)	–
Number of embryos with morphological evaluation[c]	1411	1476
– Transferable	745 (53%)	734 (50%)
– Not transferable	666 (47%)	742 (50%)
Embryos with known outcome[d]	1416	1480
– Transfer	198 (14%)	306 (21%)
– Cryopreservation	148 (10%)	418 (28%)
– Discarded	1070 (76%)	756 (51%)

First cycle results only; absolute numbers.
[a]Inconclusive means the array analysis produced incomplete results.
[b]No evaluation possible: amplification failure or no available result.
[c]Embryos were morphologically scored independently of their genetic status and according to the usual policy in the individual centres; 20 results missing.
[d]11 results missing.

did not have ET. In the control group, 171 had a minimum of one ET (90% of total) versus 149 in the PGT-A group (73% of total) (Fig. 1).

In the PGT-A group, 124 (63%) participants had only one ET, 23 (12%) had two and two had three or more ETs. In the control group these figures were 116 (63%), 40 (23%) and 15 (8%), respectively. As shown in Table IV, 177 transfers were performed in the PGT-A group and 249 were performed in the control group. Of the 177 transfers in

Table III Oocytes and embryos (per patient).

Outcomes	PGT-A (*N* = 205)	No PGT-A (*N* = 191)
Cumulus oocyte complexes	9.2 ± 5.7	10.1 ± 6.5
Oocytes in MII	7.3 ± 4.5	8.2 ± 5.3
Morphologically transferable embryos	3.6 ± 3.5	3.8 ± 3.2
Embryos cryopreserved	0.7 ± 1.7	2.2 ± 2.9
Discarded embryos	5.2 ± 3.6	4.0 ± 3.4

Table III shows the results for the first cycle only. The mean is given per participant (±SD).

Table IV Embryo transfers.

Outcomes	PGT-A (*N* = 205)	No PGT-A (*N* = 191)
Number of embryo transfers	177	249
– SET	105 (59%)	58 (23%)
– DET	72 (41%)	191 (77%)
Number of embryos transferred	249	440
– Fresh	215 (86%)	306 (70%)
– Frozen	34 (14%)	134 (30%)
Number of positive pregnancy tests	72	87
Number of clinical pregnancies	64	72
Number of miscarriages	14	27
Number of live births	50	45
Number of live born children	57	57
– Number of singletons	43	33
– Number of twins	14	24

Table IV gives the results of the embryo transfers per participant. Absolute numbers are given.

the PGT-A group, 72 (41%) were double ETs versus 191 out of 249 (77%) in the control group ($P < 0.001$) (Table IV). Fewer embryos were cryopreserved in the study group (10 versus 28%).

Pregnancies

The proportion of women with a live birth within one cycle followed by delivery was not significantly different: 50 of the 205 with chromosome screening (24%) versus 45 of 191 without chromosome screening (24%; Table V). The corresponding difference in live birth rate was 0.83% in favour of PGT-A (95% CI: −7.60 to 9.18%). The relative risk (RR) for live birth was 1.06 following PGT-A (95% CI: 0.75–1.50; $P = 0.75$). The live births were those recorded following ET, thus, excluding deliveries following coincidental spontaneous pregnancies.

Significantly more participants had a minimum of one positive pregnancy test in the control group (45 versus 34%, $P = 0.03$), whereas clinical pregnancy rates were not significantly different (37 versus 31%, $P = 0.25$; Table V). There were significantly fewer participants with a miscarriage in the PGT-A group (7 versus 14%; $P = 0.02$). The RR of miscarriage per pregnancy was 0.58 following PGT-A (95% CI: 0.34–1.01). The RR per participant, in an ITT analysis, was 0.48 (95% CI: 0.26–0.90; $P = 0.02$).

There were 57 children born in each group (Table IV). In the control group, there were more twin deliveries: 12 of 45 (27% of live births) versus 7 of 50 in the PGT-A group (14% of live births), but this difference did not reach statistical significance (RR = 0.48, 95% CI: 0.23–1.22).

After the first ET, there were more live births in the PGT-A group compared to the control group (30 versus 22%; RR = 1.33; 95% CI: 0.91–1.93) but this difference was not significant (Table VI). For euploid embryos, the live birth rate was significantly higher than for embryos without a diagnosis or no PGT-A (Table VII). As shown in Fig. 2, the curves showing time to live birth were largely overlapping, and there was no significant difference in time to pregnancy between the study and control group (Fig. 2).

Discussion

The study was performed in nine centres in seven countries, which allows an optimum analysis of a screening method and provides a realistic view on best clinical practice. A major strength of this study is the ITT design reflecting clinical practice in normal responders to ovarian stimulation in the tested advanced maternal age (AMA) population.

This large RCT on PGT-A via PBB shows that the cumulative live birth rate at 1 year of follow-up is not substantially increased with PGT-A by aCGH on PBs of oocytes harvested after ovarian stimulation for ICSI in AMA patients. In both study arms, 24% of patients had one live birth, and the 95% CI around the difference excludes an increase of 10% or more. It is now widely accepted that PGT-A will not increase live birth rates, so the emphasis has turned towards improving secondary outcomes such as decreased miscarriages and reduced time to pregnancy (Sermon *et al.*, 2016).

Of the 180 participants who had one or more oocytes with PBB, 65 (36%) were found to have zero euploid oocytes. We observed a higher number of ETs and positive pregnancy tests in the control arm, but a similar live birth rate. The incidence of twin pregnancies was higher in the control group, although not significantly so, and was a direct

Table V Outcomes per participant.

Outcomes	PGT-A (*N* = 205)	No PGT-A (*N* = 191)	Relative risk (95% CI)	*P*
At least one embryo transfer	149 (73%)	171 (90%)	0.81 (0.74–0.89)	<.0001
At least one positive pregn test	69 (34%)	85 (45%)	0.77 (0.60–0.99)	0.03
At least one clinical pregnancy	63 (31%)	70 (37%)	0.85 (0.65–1.12)	0.25
Miscarriage	14 (7%)	27 (14%)	0.48 (0.26–0.90)	0.02
Live birth	50 (24%)	45 (24%)	1.07 (0.75–1.51)	0.71

Table V shows the outcome per participant following ITT analysis, including only one outcome per participant; outcome is patients with at least one embryo transfer, and possibly ensuing positive pregnancy test, clinical pregnancy and live birth. Relative risks and *P*-values are based on Mantel–Haenszel statistics, adjusting for stratified randomization.

Table VI Outcomes from first transfer.

Outcomes	PGT-A (*N* = 149)	No PGT-A (*N* = 171)	Relative risk (95% CI)	*P*
Single embryo transfer	83 (56%)	32 (19%)	2.91 (2.08–4.06)	<.0001
Positive pregnancy test	60 (40%)	67 (39%)	1.00 (0.96–1.05)	0.93
Clinical pregnancy	57 (38%)	54 (32%)	1.15 (0.85–1.55)	0.36
Miscarriages	12 (8%)	17 (10%)	0.77 (0.37–1.60)	0.49
Live birth	44 (30%)	38 (22%)	1.32 (0.91–1.93)	0.15
Twins	5 (3%)	10 (6%)	0.50 (0.18–1.44)	0.19

Percentages are calculated over number of transfers in each group.

Table VII Outcomes by euploidy status.

Groups	Transfers	Live birth	Relative risk (95% CI)
No polar body biopsy	249	45 (18%)	Ref.
Polar body biopsy			
– Euploid embryos	115	38 (33%)	1.83 (1.26–2.65)
– Embryos without diagnosis	57	11 (17%)	0.97 (0.59–1.93)

Table VII shows the differences in live birth rate when transferring embryos diagnosed as coming from an euploid oocyte versus when transferring embryos where no diagnosis was obtained in the oocyte. Embryos without diagnosis in the oocyte were allowed to be transferred if no euploid oocyte/embryo was available. The reason for failed diagnosis could be failed biopsy, failed amplification of the PB DNA, or failure of the microarray. In three transfers, an euploid embryo was transferred with an embryo without a diagnosis (results not included).
First cycle only; Chi-square test: $P = 0.005$.

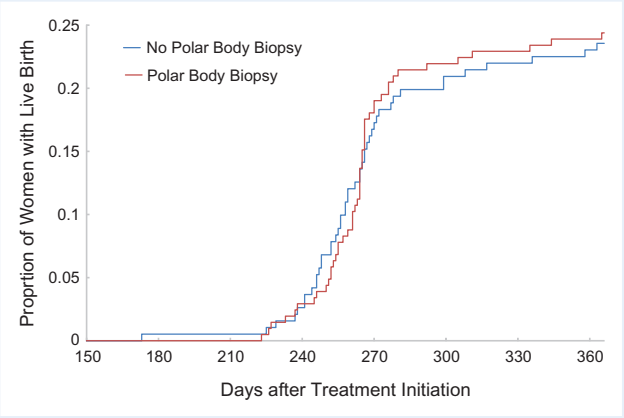

Figure 2 Cumulative live birth rate (Log-rank test: $P = 0.82$).

consequence of the fact that DET was allowed. As more embryos were available for transfer in the control group, DET was more often performed (191 (77%) versus 72 (41%)). On one hand, it could be argued that this may have introduced a bias and favoured the control group by increasing the pregnancies in the fresh transfer cycle, while on the other hand it may have been a disadvantage to the control group, because SET would have allowed for good quality embryos to be cryopreserved and transferred later only to add to the cumulative birth rate. Ultimately, however, DET did not make a difference in live birth rates and rather disfavoured the control group with a higher rate of iatrogenic twin pregnancies. Furthermore, in the PGT-A group fewer embryos were cryopreserved, fewer ETs were performed and fewer miscarriages were observed to obtain the same live birth rate as in the control group, despite a lack of significant difference in time to pregnancy. At the end of the study, we calculated that 97 embryos were still cryopreserved in the PGT-A group versus 284 in the control group.

The aneuploidy rate observed in this study was 525 out of 811 oocytes (65%) with an available result. This figure is consistent with aneuploidy rates observed in similar patient groups of advanced maternal age, for instance, Babariya *et al.* (2017) found 71% of oocytes analysed to carry whole chromosomal aneuploidy. As expected, this figure is lower than the 78.6% aneuploidy rate observed in a comparable age group undergoing blastomere CCS (Rubio *et al.*, 2017) and higher than 35–55% in TE biopsy (Franasiak *et al.*, 2014). Remarkably, 4% of oocytes analysed showed compensated euploidy and embryos derived from these oocytes were not transferred on the basis of insufficient evidence on safety at the time of the initiation of the ESTEEM trial (Kuliev *et al.*, 2011). Later it was shown by Forman *et al.* (2013) that compensated euploid oocytes mostly lead to euploid embryos, but the protocol was not changed, hence, these embryos were not transferred. Patients who had no embryos available derived from euploid oocytes were allowed a non-randomized second cycle with PB CCS, performed on only 11 patients of whom 67% had exclusively aneuploid oocytes in the second cycle. This means that the majority tend to repeat the same outcome of the previous cycle, although due to the small number of patients in this group, no firm conclusions can be drawn.

Recruitment for this study was suboptimal predominantly because patients were reluctant to be randomized for this type of procedure. This was anticipated by lifting blinding to treatment allocation after the time of the research procedure (PB biopsy or no PB biopsy) in order to provide optimum guidance to the patient. We acknowledge this is a potential source of bias, however, this approach was chosen to minimize the risk of recruitment problems of and drop-out by the patient for subjective reasons. Though the targeted sample size was not reached, the included sample size of 396 allowed 90% power to detect the targeted 15% increase. The confidence interval around the difference in live birth rate, ranging from −7.6 to 9.2%, excludes a substantial increase in live birth between the study and control groups, of 10% or higher, yet a smaller difference may still exist.

The results are in line with a recent smaller RCT on PGT-A in cleavage stage embryos, which showed that in spite of a higher pregnancy rate per first embryo transferred and lower miscarriage rate following PGT-A, cumulative pregnancy rates per treatment cycle did not improve (Rubio *et al.*, 2017). In contrast to the study by Rubio, the ESTEEM trial did not show a shorter time to pregnancy. This may be explained by the earlier stage of screening which does not detect postmeiotic aneuploidy, leading to a lower level of selection in the study arm, as well as the slightly higher age in the Rubio study (38–41 versus 36–40 years) which might render PGT-A more effective. Moreover, the study by Rubio *et al.* had more stringent patient and embryo selection criteria, for instance a minimum of five MII oocytes had to be available, sometimes requiring multiple cycles, which may have favoured live birth rates, while only one cycle was allowed in the ESTEEM study. Additionally, the ESTEEM study evaluated live birth rate per participant which is different from LBR per ET in the Rubio study (Griesinger, 2016).

Although PGT-A by CCS is nowadays more commonly performed on TE biopsy, comparison to available studies on TE PGT-A is difficult due to significant differences in methodology, more specifically the lack of ITT analysis in highly selected good prognosis study populations (Chen *et al.*, 2015; Dahdouh *et al.*, 2015; Lee *et al.*, 2015). Larger studies will be needed to establish the benefit of this or other types of PGT-A in cases of high versus low ovarian response or in patients with recurrent miscarriage or failed implantation. A recent multicentre RCT did not show a benefit either in ongoing pregnancy or in miscarriage rates when performing PGT-A by next generation sequencing on TE

cells for patients of all ages (25–40 years) with a minimum of two blastocysts for analysis (Munné et al., 2017b).

Despite its state-of-the-art methodology, this study has a number of limitations that may affect its clinical implications. The number of oocytes retrieved and embryos analysed is in line with other studies (Franasiak et al., 2014; Rubio et al., 2017). Unlike these studies, PBB was performed in our study, which may have influenced the outcomes in three ways.

Firstly, there may be a potentially negative effect of the PBB on the oocyte and the further development of the embryo. Two smaller studies, one on PGD embryos (Levin et al., 2012) and one on 1PN embryos (Macas et al., 2011) showed a clear negative effect specifically of laser PBB; however, both had significant technical shortcomings. Generally, the PBs are considered as a useless by-products that are not necessary for further development. If care is taken not to biopsy the PBs when they are still connected with spindle strands to the oocyte, they can be removed without negative impact (Montag et al., 2013). The fact that further preimplantation development as assessed by morphology alone was identical in both study groups, strengthened us in our conclusion that PBB was not more harmful to the embryo than biopsy at other developmental stages.

Secondly, PBB does not analyse mosaicism present at the later development stage and therefore non-viable mosaic embryos cannot be excluded. Studies have argued that the predictive value of PB analysis was lower than blastomere or trophectoderm analysis (Salvaggio et al., 2014). PGT-A is currently mostly carried out at the blastocyst stage, which seems to show far less mosaicism than Day 3 embryos (Munné and Wells, 2017; Vera-Rodriguez and Rubio, 2017). However, several studies have now demonstrated that mosaic blastocysts are able to implant and give rise to healthy newborns (Greco et al., 2015; Munné et al., 2017a). Moreover, it is not yet clear how well a TE biopsy is able to predict aneuploidy in the inner cell mass, which is to become the embryo proper (Vera-Rodriguez and Rubio, 2017). Until these points are clarified, comparison between PGT-A by PBB and TE biopsy remains qualitative at best.

Thirdly, the diagnostic rate of 811/1023 (79%) in the ESTEEM trial may have contributed negatively to the eventual clinical result. In 21% of oocytes that underwent PBB, there was either no result available or the result was inconclusive, which is higher than the results obtained from multicellular blastocyst biopsies (Wells et al., 2014), as can be expected from a method in which two cells with sometimes degraded DNA are analysed. The efficiency is also lower than that obtained in the pilot study (195/226, 86% oocytes with a result) and can be explained by the learning curve and the fact that some participating centres did not contribute a large number of cycles. As a consequence, the embryos derived from oocytes without diagnosis were excluded from transfer, except in cases where no embryos derived from euploid oocytes were available. In these cases, embryos derived from oocytes producing an inconclusive result were allowed to be transferred as part of the commitment of the treating physicians to maximize reproductive chances for the patient. The results of the transfer of these embryos were included in the overall results as part of the ITT design of the study. To estimate the effect of the transfer of undiagnosed embryos, we analysed live birth rates for both groups separately (Table VII). For euploid embryos specifically, the live birth rate was significantly higher than for embryos without diagnosis, either with or without having undergone PBB.

Cumulative outcome data were limited to 1 year, which may have introduced a negative bias to the control group. Only 11 patients with exclusively aneuploid results underwent a second treatment cycle in the chromosome screening group, which does not allow to draw strong conclusions on this issue. Per ITT and with a similar distribution of embryological development in each study arm, a difference in live birth rate was not observed. This does not exclude differences per embryo transferred or per developmental stage of the embryo.

A significant improvement in live birth in ICSI performed with or without PGT-A of PB in women of AMA with an anticipated normal ovarian response and without a history of repeated implantation failure or repeated miscarriage was not detected in this study. There is, however, a clinical benefit for the PGT-A group from a significant reduction of interventions and miscarriages. The observation that the similarity in live birth rates was achieved with less embryo cryopreservation, fewer transfers, fewer double ETs and fewer miscarriages points to a greater efficiency of transfers with PGT-A. Whether these benefits outweigh drawbacks such as the cost for the patient, the higher workload for the IVF lab and the potential effect on the children born after prolonged culture and/or cryopreservation remains to be shown.

Acknowledgements

The authors would like to acknowledge the work of Yves Vandeskelde, Johan Sterckx and Geertrui Bocken at UZ Brussel and Alessandra Pomante at SISMER, Bologna, for their significant contribution. We thank Raphael Ron-El, Strassburger Dvora and Esti Kasterstein from the Assaf Harofeh Medical Center, Tel Aviv University, Israel, for contributions to the study. We thank Dr Manuela Ropeter for her contribution in the IVF laboratory in Goettingen, especially for skillfully performing the PBB, as well as Mukulika Mitra for data management and Dr Jessica Nolte for monitoring the study. We thank Fina Gomez for her contribution in the IVF laboratory in Barcelona, especially for skillfully performing the PBB. We thank Professor Jan Traeger-Synodinos for her role and support as the principal investigator for the Athens group. We especially thank ESHRE and Illumina for their financial support of the ESTEEM trial, as well as CTCM for their professional, efficient and unrelenting support for the whole process, from RCT design to data analysis. We thank Emeritus Professor John Collins at McMaster University, Canada, for his pivotal role in the conception of this trial and Dr Markus Montag, Ilabcomm, Germany, for his support in the training in PBB and his work during the pilot study of this trial.

Authors' roles

W.V., the coordinating investigator for ESTEEM, authored the final protocol and protocol amendments, contributed to the acquisition of data and interpretation of data and co-authored this article. P.M.B. contributed to the analysis and interpretation of data and drafting of the article and approved the final version before publication. C.S., G.A., M.B., M.D., T.E.G., L.G., V.G., G.G., G.Ka., G.Ko., J.L., M.P., M.T., K.V. contributed to the acquisition of data and interpretation of data, revised the article critically for important intellectual content and approved the final version before publication. J.G., M.C.M. and A.S. contributed to conception and design of the study and to the acquisition of data and the analysis and interpretation of data, revised the

article critically for important intellectual content and approved the final version before publication. K.S. coordinated the ESTEEM RCT, contributed to the acquisition of data and the analysis and interpretation of data, drafted the article and approved the final version before publication.

Conflict of interest

M.B.'s institution (UZBrussel) has received educational grants from IBSA, Ferring, Organon, Schering-Plough, Merck and Merck Belgium. M. B. has received consultancy and speakers' fees from Organon, Serono Symposia and Merck. G.G. has received personal fees and non-financial support from MSD, Ferring, Merck-Serono, Finox, TEVA, IBSA, Glycotope, Abbott and Gedeon-Richter as well as personal fees from VitroLife, NMC Healthcare, ReprodWissen, BioSilu and ZIVA. W.V., C. S., P.M.B., V.G., G.A., M.D., T.E.G., L.G., G.Ka., G.Ko., J.L., M.C.M., M. P., A.S., M.T., K.V., J.G. and K.S. declare no conflict of interest

Funding

European Society for Human Reproduction and Embryology. Illumina provided microarrays and other consumables necessary for aCGH testing of polar bodies.

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