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Effect of next-generation sequencing in preimplantation genetic testing on live birth ratio

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Abstract. The present study analysed live birth ratios in frozen embryo transfer (FET) cycles where embryo ploidy status was determined with preimplantation genetic testing (PGT) using next-generation sequencing (NGS). PGT was performed on trophectoderm cells biopsied at the blastocyst stage. The present prospective cohort study included 112 women undergoing frozen embryo transfer, with NGS PGT. The control group consisted of 85 patients who underwent the IVF procedure with FET planned for a subsequent cycle. The live birth rate per cycle was higher by ~18.5 percentage points in the investigated compared with control group (42.0% vs 23.5% respectively; $P = 0.012$). The differences between the study and control groups were also significant for clinical pregnancy (42.0% vs 23.5% respectively; $P = 0.012$), implantation (41.2% vs 22.2% respectively; $P = 0.001$) and pregnancy loss rates (9.6% vs 28.6% respectively; $P = 0.027$). The results show that PGT NGS is a useful method for embryo selection and it may be implemented in routine clinical practice with propitious results.

Additional keywords: frozen embryo transfer.

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Introduction

Conventionally, most embryos are transferred in a fresh IVF cycle, with the remaining embryos frozen and stored. These embryos may be then used for a subsequent IVF cycle in cases when either the first cycle does not end in a live birth or the couple decides to have another child. Some data suggest that selective embryo cryopreservation followed by frozen–thawed embryo transfer should be recommended for polycystic ovary syndrome patients because of the increased risk of pregnancy complications in fresh cycles (Imudia *et al.* 2013; Massart *et al.* 2013). This leads to the question whether it is possible to move away from fresh embryo transfers in IVF and towards freezing all available embryos for use in subsequent cycles. Thanks to significant improvements in cryopreservation technology (i.e. vitrification) the number of frozen embryo transfer (FET) IVF cycles is increasing (Özgür *et al.* 2015; Basirat *et al.* 2016) and may soon surpass, in terms of both numbers and success rates, fresh stimulated IVF cycles. Preimplantation genetic testing (PGT) in conjunction with FET was previously described by Coates *et al.* (2017). Embryo quality and survival, optimal endometrial preparation and identification of the receptive window for transfer in FET cycles are the most important factors for ensuring optimal FET outcomes.

Aneuploidy in human embryos is the leading cause of implantation failure and early spontaneous abortion in humans (Macklon *et al.* 2002; Kuliev *et al.* 2005). It has been proposed

that testing embryos before transfer increases pregnancy rates, decreases miscarriages and negates the need for multiple IVF cycles for patients with a high incidence of aneuploidy (Scott *et al.* 2010; Treff *et al.* 2013).

Most PGT for aneuploidies is based on low-density array comparative genomic hybridisation (aCGH); however, this is based on <2700 probes and is associated with relatively high costs. Lower-cost methods include aneuploidy screening using quantitative polymerase chain reaction (qPCR), which has been demonstrated to improve pregnancy rates (Wu *et al.* 2014). The qPCR method was based on four markers per chromosome, which severely limits its ability to detect rearrangements or small aberrations, including segmental aneuploidies (Brezina *et al.* 2016; Capalbo *et al.* 2016; Juneau *et al.* 2016).

New technical possibilities, such as next-generation sequencing (NGS) methods, have produced improved genetic diagnostics (Martín *et al.* 2013; Yin *et al.* 2013; Fiorentino *et al.* 2014*a*, 2014*b*; Wang *et al.* 2014; Wells *et al.* 2014; Lukaszuk *et al.* 2015). NGS exploits the linear relationship between the number of sequences aligned to a specific chromosome and copy number. NGS provides the actual DNA sequences, thus significantly reducing both the false positive and false negative rates associated with noise hybridisation signals in aCGH applications (Gutiérrez-Mateo *et al.* 2011; Capalbo *et al.* 2015). Another key advantage of NGS methods is their higher coverage (Martín *et al.* 2013; Lukaszuk *et al.* 2015; 2016).

The present study explored the usefulness of comprehensive chromosomal screening using NGS to analyse material biopsied form blastocyst stage embryos to improve the live birth rate per FET and to consider the implications of using such a strategy in routine clinical practice.

Materials and methods

Registration and ethics approval

The study was approved on 20 May 2014 by the Independent Ethics Committee at the Regional Medical Chamber (Komisja Bioetyczna przy Okręgowej Izbie Lekarskiej) in Gdansk, Poland. The prospective study has been registered with the Australian and New Zealand Clinical Trials Registry (www.anzctr.org.au, accessed 20 August 2017; ID: ACTRN12614001037695). The authors confirm that all ongoing and related trials for this intervention are registered.

Written informed consent was obtained from each couple undergoing IVF treatment.

Patients

The prospective cohort study was performed from 1 August 2014 to 1 June 2015 at INVICTA Fertility Clinic, a private fertility centre. The mean (± s.d.) age of the patients in the study and control groups was 35.8 ± 3.7 and 35.2 ± 2.1 years respectively (Table 1). In all, 197 patients who underwent intracytoplasmic sperm injection (ICSI) cycles were included in the study. Patients with endometriosis or adenomyosis were excluded from the study. To avoid negative selection bias that could affect the results, only first and second IVF cycles were considered. In addition, only transfers of one or two blastocysts (depending on patient decision) were investigated.

The PGT NGS study group ($n = 112$) consisted of patients who had at least one embryo that reached the blastocyst stage. Following the PGT NGS, 96 patients had at least one euploid embryo. The remaining patients had no euploid embryos, as determined by PGT NGS. All 96 patients who had at least one euploid embryo underwent transfer of thawed embryos.

The control group included 85 patients who underwent the IVF procedure with embryo transfer (ET) planned to be performed in a subsequent cycle. In all cases in the control group, the ET was postponed for one of the following reasons: progesterone concentrations >2.0 ng mL^{-1} on the day of human chorionic gonadotropin (hCG) administration ($n = 45$); endometrial thickness <6 mm on the day of hCG administration ($n = 33$), or risk of ovarian hyperstimulation ($n = 7$).

Stimulation protocol

All women were treated with a long agonist protocol (Lukaszuk *et al.* 2013). Follicular growth was monitored on Day 8 using transvaginal ultrasound and assays evaluating serum oestradiol (E2), progesterone (P4) and LH concentrations. Oocyte retrieval was performed 36 h after the administration of 5000 IU hCG (Choragon; Ferring).

Embryo culture, biopsy and vitrification

All embryos were cultured in sequential G1 and G2 media (Vitrolife) to the blastocyst stage under 6% CO_2 and 5% O_2 at 37°C. On Day 5 or Day 6 after fertilisation, the blastocysts were graded according to the criteria of Gardner *et al.* (2000). In the control group, only blastocysts classified as 2AA or better morphology were frozen. The same morphological criteria were used to determine qualification of blastocysts for PGT biopsy in the study group. Approximately 5–10 trophectoderm (TE) cells located opposite the inner cell mass were aspirated with a biopsy pipette and dissected with a laser (Saturn 3 system; Research Instruments). Biopsied TE cells were washed in phosphate-buffered saline (PBS)–polyvinylpyrrolidone (PVP) and stored at −20°C for subsequent PGT. Blastocysts were vitrified immediately after the biopsy using the vitrification kit (Kitazato) according to the manufacturer's instructions.

Next-generation sequencing

The sequencing procedure was performed as described previously by Lukaszuk *et al.* (2016) with the exception that TE cells instead of blastomeres were analysed. Aspirated TE cells were subjected to lysis and whole-genome amplification (WGA; PicoPlexWGA kit; New England BioLabs). For DNA fragmentation, an Ion Xpress Plus Fragment Library Kit (Life Technologies) was used according to the manufacturer's instructions for 10–100 µg genomic DNA. An Ion Xpress Barcode Adapters 1–96 Kit (Life Technologies) was used for barcoding and an Ion Xpress Equalizer Kit (Life Technologies) was used for library input normalisation, both according to the manufacturer's instructions. For library enrichment, an Ion PGM Template OT2 200 Kit (Life Technologies) was used according to the manufacturer's instructions. Sequencing was performed using the Ion PGM Sequencing 200 Kit v2 (Life Technologies) on an Ion 316 chip (Life Technologies). Up to 32

Table 1. Characteristics of the control group and the group that underwent preimplantation genetic testing (PGT) using next-generation sequencing (NGS)

Unless indicated otherwise, values are given as the mean ± s.d. AFC, antral follicle count; AMH, anti-Müllerian hormone; BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; E2, oestradiol; IQR, interquartile range; SHBG, sex hormone-binding globulin

	PGT NGS	Control (no PGT)	*P*-value
No. subjects	112	85	–
Age (years)			0.176
Mean ± s.d.	35.8 ± 3.7	35.2 ± 2.1	
Median (IQR)	37 (33–39)	35 (34–36)	
BMI (kg/m^2)	21.9 ± 2.7	22.6 ± 2.3	0.431
Cause of infertility (*n*)			
History of miscarriage	26	25	0.681
Advanced maternal age (set at 35 years)	47	29	
Male factor	11	6	
Anovulation	5	4	
Unexplained	23	21	
Duration of infertility (years)	3.3 ± 1.4	3.0 ± 1.2	0.101
AMH (ng mL^{-1})			0.280
Mean ± s.d.	2.5 ± 2.1	2.8 ± 1.9	
Median (IQR)	1.8 (1.1–3.4)	2.5 (1.1–4.3)	
Inhibin B (pg mL^{-1})			0.306
Mean ± s.d.	62.6 ± 49.1	70.6 ± 43.5	
Median (IQR)	59.4 (17.0–90.1)	70.4 (44.6–90.0)	
Basal FSH (mIU mL^{-1})			0.361
Mean ± s.d.	7.5 ± 8.5	7.2 ± 2.7	
Median (IQR)	6.1 (4.8–8.1)	6.3 (5.3–8.4)	
Basal LH (mIU)			0.241
Mean ± s.d.	6.8 ± 4.7	7.9 ± 5.1	
Median (IQR)	6.3 (4.6–8.0)	6.8 (5.4–9.1)	
Basal E2 (pg mL^{-1})			0.315
Mean ± s.d.	89.1 ± 94.2	76.3 ± 96.4	
Median (IQR)	37.0 (23.0–56.5)	37.0 (30.0–68.1)	
DHEAS (μg dL^{-1})			0.592
Mean ± s.d.	244.6 ± 116.9	234.0 ± 101.9	
Median (IQR)	227 (166–291)	226 (159–286)	
Testosterone (ng mL^{-1})			0.394
Mean ± s.d.	1.1 ± 0.6	1.1 ± 0.4	
Median (IQR)	1.0 (0.6–1.3)	1.0 (0.8–1.2)	
SHBG (nM)			0.567
Mean ± s.d.	79.7 ± 42.2	86.7 ± 47.8	
Median (IQR)	75 (45–105)	77 (44–131)	
AFC (n)			0.303
Mean ± s.d.	14.9 ± 8.7	13.5 ± 8.7	
Median (IQR)	12 (9–18)	11 (7–18)	

samples per 316 chip were processed. Aneuploidies were detected using sample results compared with baseline values obtained from 85 male and 83 female euploid single-cell samples processed beforehand with the established protocols described above. Control samples served as a positive control for the entire process. In addition, a negative control sample was processed to exclude the possibility of contamination. The NGS protocol described was designed by the INVICTA Fertility Clinic Molecular Biology Laboratory and validated using human cell lines.

The INVICTA Bioinformatics Team created a C++ builder application that was used to integrate data from Torrent Suite Software (Life Technologies) with baseline values and perform further computational calculations. The C++ standard template library was used for general data manipulation and the DBXJSON library (Embarcadero) was used to import data from JSON files supplied by the Torrent Suite Server. The study was performed between 1 August 2014 and 1 June 2015 and followed on our previously published work performed between 1 August 2013 and 1 July 2014 (Lukaszuk *et al.* 2015). Both studies were performed with the same laboratory workflow to maintain methodical consistency. The protocol chosen for these studies in 2013 was based on low-resolution PGT providing 100 000–250 000 mapped reads per sample. In the present study, questions of mosaicism, as defined in the Preimplantation Genetics Diagnosis International Society (PGDiS)

recommendations published on 19 July 2016 (http://www.pgdis.org/docs/newsletter_071816.html, accessed 20 August 2017) , were not addressed. Given that the NGS platform is flexible in scalability, resolution and cost-effectiveness, the design of the protocol can be adjusted depending on the targets of a study. The present protocol was designed to detect aneuploidies; therefore, because of insufficient resolution, the data from the present study cannot be used to draw conclusions in terms of mosaicism determined by each 5% or 10% change in the rate of cells affected by aneuploidy or segmental aneuploidies <50% of a chromosome. If we were to consider the current embryo mosaicism classification criteria that define as mosaic embryos with 20–80% of aneuploid cells for a given chromosome, then one would conclude that, in the present study, embryos with 20–50% mosaicism were reported as euploid and embryos with between >50% and 80% mosaicism were as aneuploid. Segmental aberrations are reported as aneuploid when they cover >50% of a chromosome.

Blastocyst warming and transfer

For the FET cycle, no more than two blastocysts were transferred to each patient. Blastocysts were warmed using the Kitazato thawing kit (Kuwayama 2007) and then transferred to G2 medium and cultured for 2–3 h. Each transfer was preceded by endometrial preparation. The luteal phase was supplemented by transvaginal E2 and P4 administration (3 × 2 mg Estrofem (NovoNordisk) and 3 × 100 mg Lutinus (Ferring)). Following the transfer, E2, P4 and hCG concentrations were verified every 3–4 days.

Clinical outcomes

Biochemical pregnancy was defined as a positive pregnancy test. Implantation was assessed by transvaginal ultrasound at 5 weeks and 1 day (±2 days) after ET. Clinical pregnancy (defined as detection of a heartbeat on ultrasound by 6 weeks and 1 day (±2 days)) and live birth (defined as at least one infant born alive after 24 weeks gestation and surviving longer than 1 month) were determined for patients in both groups. Follow-up analyses also included determination of spontaneous miscarriage rates.

Statistical analysis

Data were analysed using STATISTICA version 10 (StatSoft). Mean values of continuous variables for the two patient groups were compared using the Mann–Whitney rank-sum test. Variables representing proportions were compared using Fisher's exact test for a contingency table. Two-sided $P < 0.05$ was considered significant.

Results

Patient characteristics in the study and control groups

The control group matched the PGT group in terms of age, cause and duration of infertility, body mass index, anti-Müllerian hormone concentrations and antral follicle count. The groups did not differ in other routinely verified hormone parameters (Table 1).

Treatment-related parameters, such as mean duration of stimulation, dose of human menopausal gonadotrophin, mean number of oocytes retrieved, normal fertilisation rate and number of embryos transferred (Table 2) were also similar for both groups.

The 112 cycles performed in the PGT group resulted in the retrieval of a mean of 7.2 oocytes per patient. Two hundred and seventy-four of the embryos obtained after fertilisation were classified as 2AA or better and underwent biopsy (2.4 per cycle). Following NGS PGT, 148 embryos were found to be euploid, 120 were abnormal and six were non-diagnostic (i.e. the euploid/aneuploidy status could not be determined). Summary information about the number of monosomies, trisomies and complex aneuploidies is given in Table 3. The vitrification survival rate was 92% and, of the 148 available euploid embryos, 136 were ultimately transferred to 96 patients.

In the control group, 85 cycles were performed. The mean number of oocytes retrieved was 6.7 per patient. Two hundred and twenty-one of the embryos obtained after fertilisation were classified as 2AA or better and vitrified. Of these, 126 with the best morphology were selected for thawing and transfer (the vitrification survival rate was 93%). Finally, 117 successfully thawed embryos were transferred to 85 patients (Table 2).

Biochemical pregnancy rates

Comparisons between biochemical pregnancy rates in the study and control groups are given in Table 2 and shown in Fig. 1. In the PGT group, in which blastocysts were screened against aneuploidy and only euploid blastocysts were selected for transfer, 54.2% ($n = 52$) of transfers and 46.4% ($n = 52$) of cycles resulted in a biochemical pregnancy. In contrast, in the control group, the clinical pregnancy rates per embryo transfer and per cycle were 32.9% ($n = 28$) and 32.9% ($n = 28$) respectively, indicating decreased rates for both ($P < 0.004$ and $P < 0.047$ respectively).

Implantation rates

Clinical implantation rates in the PGT and control groups are compared in Table 2 and Fig. 1. In the control group, 22.2% of ETs led to successful implantation In contrast, in the study group, 41.2% of ETs resulted in the formation of gestational sac ($P < 0.001$ vs control group; Fig. 1; Table 2). These observations indicate that using NGS PGT increases the implantation rate almost twofold in equivalent patient groups.

Clinical pregnancy rates

In the PGT group, 49% ($n = 47$) of transfers and 42% ($n = 47$) of cycles resulted in a clinical pregnancy. In the control group, the clinical pregnancy rates per ET and per cycle were 23.5% ($n = 20$) and 23.5% ($n = 20$) respectively, which were significant lower than in the PGT group ($P < 0.002$ and $P < 0.012$ respectively).

The difference in the likelihood of achieving pregnancy with the transfer of a euploid embryo compared with the transfer of an embryo with good morphology was statistically significant ($P < 0.004$). The odds ratio (OR) in the group with euploid ET was 1.55, compared with 0.64 in the non-NGS group.

Table 2. Characteristics of the IVF programs for the control group and the group that underwent preimplantation genetic testing (PGT) using next-generation sequencing (NGS)

Unless indicated otherwise, values are given as the mean ± s.d. or as *n* (%). ET, embryo transfer; hMG, human menopausal gonadotrophin; OHSS, ovarian hyperstimulation syndrome

	PGT NGS	Control (no PGT)	*P*–value
No. cycles	112	85	–
No. transfers	96	85	–
Duration of stimulation (days)	10.2 ± 4.4	10.4 ± 5.4	0.735
hMG dose (IU)	2205 ± 638	2228 ± 675	0.864
No. oocytes retrieved	7.2 ± 4.0	6.7 ± 3.4	0.419
Fertilisation rate (%)	80.8	76.7	0.153
Mean no. embryos biopsied	274	–	–
Mean no. embryos per cycle	2.4		
Euploid	148	–	–
Abnormal	120	–	–
Non-diagnostic[B]	6	–	–
Total no. vitrified embryos	274	221	–
Total no. embryos available for transfer	148	126	–
Vitrification survival rate (%)	92	93	0.892
Total no. embryos transferred	136	117	0.600
No. embryos transferred per ET	1.4 ± 0.5	1.4 ± 0.5	
Biochemical pregnancies (%)			
Per cycle	52 (46.4)	28 (32.9)	0.047
Per ET	52 (54.2)	28 (32.9)	0.004
Total no. embryos implanted	56	28	–
Implantation rate[A] (%)	41.2	22.2	0.001
Rate of multiple pregnancies (%)	4.0	0	–
Ectopic pregnancy (%)	0	0	–
OHSS (%)	0	0	–
Spontaneous abortion rate (%)	5 (9.6)	8 (28.6)	0.027
Clinical pregnancies (%)			
Per cycle	47 (42.0)	20 (23.5)	0.012
Per ET	47 (49.0)	20 (23.5)	0.002
Live births (%)			
Per cycle	47 (42.0)	20 (23.5)	0.012
Per ET	47 (49.0)	20 (23.5)	0.002

[A]Determined as the number of intrauterine gestational sacs.
[B]Non-diagnostic meaning cases when we could not determine if embryo was euploid or aneuploid.

Table 3. Number of monosomies, trisomies and complex aneuploidies among the aneuploid embryos (*n* = 120) on next-generation sequencing analysis

Data are given as *n* (%)

Monosomy	35 (29.2)
Trisomy	26 (21.6)
Two abnormal chromosomes	36 (30.0)
Three abnormal chromosomes	18 (15.0)
Complex chromosomal abnormalities	5 (4.2)

This demonstrates that the chance of achieving clinical pregnancy is twofold higher when an embryo confirmed as euploid is transferred.

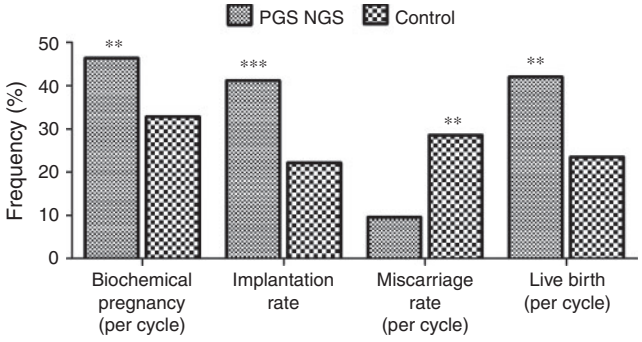

Fig. 1. Comparison of clinical outcomes in the control group and the group that underwent preimplantation genetic testing (PGT) using next-generation sequencing (NGS). $^{**}P < 0.0$x compared with the control group.

Miscarriage rates

Table 2 and Fig. 1 show comparisons of miscarriage rates in the PGT and control groups. Among the NGS PGT-screened embryos with a euploid status confirmed before transfer, the spontaneous abortion rate was 9.6% (5/52; $P < 0.027$ vs control group). In the control group, in which embryos were selected for transfer solely on the basis of morphology, miscarriages occurred in 28.6% of cases (8/28). These observations suggest that NGS PGT lowers the spontaneous abortion rate for implanted embryos.

Analysis of miscarriage rates also differed significantly between the PGT and control groups ($P < 0.004$), with ORs of 0.42 and 2.37 respectively, indicating a fivefold increase in the risk of miscarriage in the non-NGS group.

Live birth rates

The live birth rates in the PGT and control groups are given in Table 2 and shown in Fig. 1.

In the PGT group, in which embryos were screened by NGS PGT, 49% ($n = 47$) of transfers and 42% ($n = 47$) of cycles resulted in a live birth. In contrast, in the control group, live birth rates per ET and per cycle were 23.5% ($n = 20$) and 23.5% ($n = 20$) respectively, both being significantly lower than in the PGT group ($P < 0.002$ and $P < 0.012$ respectively).

The difference in the likelihood of achieving a live birth with the transfer of an embryo confirmed as euploid compared with the transfer of an embryo with good morphology was significant ($P < 0.004$). The ORs for the NGS PGT and control groups were 1.55 and 0.64 respectively. This indicates that the chance of achieving a live birth was twofold higher after screening by NGS PGT.

Discussion

It is thought that 32–85% of blastocyst stage embryos are chromosomally abnormal (Harton *et al.* 2013). Because most aneuploidies are lethal, it has been argued that IVF outcomes could be improved if embryos were tested for aneuploidy and those embryos found to be chromosomally normal were given priority for transfer (Munné *et al.* 1993). Poor correlation of embryo morphology and chromosomal complement led to the development of PGT. PGT is a method that evaluates the

numerical chromosomal constitution of embryos before transfer, and PGT may provide a practical method to reduce the risk of adverse reproductive outcomes caused by the transfer of chromosomally abnormal embryos.

Although genetic screening of embryos can be performed at various stages of oocyte or embryo development, analysis at the blastocyst stage combined with FET has become increasingly popular in recent years. Biopsy at this stage has the benefit of allowing more cells to be sampled (5–10 cells), making comprehensive aneuploidy screening more powerful (Schoolcraft *et al.* 2010; Forman *et al.* 2012). This trend is due primarily to the lower mosaicism rate at the blastocyst compared with cleavage stage. The largest increase in implantation and live birth rates was reported after blastocyst ET (Ly *et al.* 2011; Glujovsky *et al.* 2016). Analysis of TE biopsy material using 24 chromosome screening platforms requires the freezing of the embryos. Patients then undergo FET in a subsequent cycle.

The chance of becoming pregnant after frozen blastocyst transfer is comparable to those after transfer of fresh embryos (Wennerholm *et al.* 2009; Roque *et al.* 2013). Some studies have even reported increased pregnancy rates after FET compared with fresh ET, arguing that this is due to the impaired endometrial receptivity after ovarian stimulation in fresh cycles (Shapiro *et al.* 2011). We routinely perform vitrification, which, unlike slow freezing, is more efficient and results in higher survival rates (Cao *et al.* 2009). Properly implemented, embryo freezing procedures in the IVF laboratory in conjunction with NGS PGT for aneuploidies can lead to propitious treatment results, as reported herein.

In FET cycles, there is time to perform additional diagnostics after previous unsuccessful attempts of IVF treatment, as well as time for the patient to recover psychologically. Moreover, this seems to be a good choice for patients with low ovarian reserve, for whom a low number of oocytes may be retrieved after the ovarian induction process with consequently fewer embryos created in each cycle. Frozen cycles allow for the collection of embryos from multiple cycles, and the analysis and selection of the best ones for transfer. Another advantage of performing PGT at the blastocyst stage is the exclusion of the risk of embryos biopsied on Day 3 not developing further. Finally, the longer turnaround time allows pooling of up to 384 samples for NGS PGT from different patients, thereby reducing the provider's costs. Efficiency in scheduling PGT can be improved when the testing laboratory is less dependent on biopsy dates. This, together with the longer turnaround time, makes it easier to accommodate equipment downtime due to maintenance without affecting patients, thus lowering the investment needed in the PGT platform. For patients, the option to freeze embryos enables them to use the strategy of 'collecting' enough material for a single test run on the NGS PGT platform, allowing them to pay for one test instead of multiple tests performed on samples with smaller numbers.

The present study examined the feasibility of using emerging NGS methods for the detection of aneuploidy in blastocyst stage embryos. NGS technology is very promising and because of its high data availability, flexibility and lower costs has the potential to supersede currently available methods (Martín *et al.* 2013; Fiorentino *et al.* 2014*a*, 2014*b*; Deleye *et al.* 2015; Capalbo *et al.* 2016). The advantages of NGS over aCGH have been discussed recently (Lukaszuk *et al.* 2015). In addition, thanks to the possibility of lowering testing costs, NGS methods could open up the opportunity of using PGT for an increasing number of patients. With lower false positive rates and lower costs, NGS PGT could be suggested for younger women, in whom PGT is currently not recommended, despite some studies suggesting that even 31.7% of their blastocysts are aneuploid (Harton *et al.* 2013).

Furthermore, as sequencing costs are further reduced, allowing a major read depth per sample for the same or reduced price, the NGS approach may allow for simultaneous evaluation of single-gene disorders (Treff *et al.* 2013) and translocations (Deleye *et al.* 2015) with versatile aneuploidy screening performed on the material from a single biopsy without the need for multiple platforms (Fiorentino *et al.* 2004, 2014*a*, 2014*b*; Zheng *et al.* 2015).

The clinical outcomes obtained in the present study from PGT cycles performed with NGS were very encouraging, resulting in a clinical pregnancy rate per cycle of 42.0% (mean ($\pm$ s.d.) age 35.8 $\pm$ 3.7 years) and an implantation rate of 41.2%. All pregnancies went to term and 47 healthy babies were delivered. These higher values in the PGT group are comparable with recent results from other studies in which NGS PGT was used (Fiorentino *et al.* 2014*a*, 2014*b*; Tan *et al.* 2014; Yang *et al.* 2015; Liu *et al.* 2016).

As expected, the number of embryos available for transfer following NGS was much lower compared with cycles without PGT. Therefore, to achieve more reliable calculations we maintained similarities between the groups being compared. In the control group, only patients with at least one morphologically good embryo available for transfer were included.

In the group undergoing NGS PGT, the procedure enabled improved prioritisation of embryos, resulting in 96 transfers of a total of 136 embryos. Although approximately 15% of patients did not achieve a transfer, the transfer of abnormal embryos and their implantation were prevented, thus avoiding the associated very high risk of miscarriage or carrying a child with a birth defect. The karyotypes of the five miscarried fetuses from the PGT group were assumed to be normal, suggesting other reasons for the miscarriages. Karyotype analysis for the eight miscarriages in the control group were not performed, hence we are unable to address whether miscarriage in the control group was higher due to aneuploidy.

The results of the present study suggest that NGS PGT leads to improved selection of viable embryos and an increased chance of achieving pregnancy and live birth. Pregnancy and implantation rates achieved in cycles that used NGS PGT suggest that this approach improves the identification of viable embryos. However, the relatively small size of the group investigated and the lack of randomisation are acknowledged weaknesses of the present investigation.

Conclusion

The data indicate that TE biopsy of blastocysts followed by frozen transfer of euploid embryos is associated with higher implantation, pregnancy and live birth rates per transfer. These findings show that NGS PGT is a useful method for embryo

selection, enabling the preferential transfer of euploid embryos, and that it can be implemented in routine clinical practice with favourable results.

However, further investigations, including more patients and randomisation, should be conducted to confirm the findings of the present study. Confirmation of the results could lead to changes in the indications for PGT in the general IVF population.

Conflicts of interest

The authors declare no conflicts of interest.

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