Routine use of next-generation sequencing for preimplantation genetic diagnosis of blastomeres obtained from embryos on day 3 in fresh in vitro fertilization cycles

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Objective: To determine the usefulness of semiconductor-based next-generation sequencing (NGS) for cleavage-stage preimplantation genetic diagnosis (PGD) of aneuploidy.
Design: Prospective case–control study.
Setting: A private center for reproductive medicine.
Patient(s): A total of 45 patients underwent day-3 embryo biopsy with PGD and fresh cycle transfer. Additionally, 53 patients, matched according to age, anti-Müllerian hormone levels, antral follicles count, and infertility duration were selected as controls.
Intervention(s): Choice of embryos for transfer was based on the PGD NGS results.
Main Outcome Measure(s): Clinical pregnancy rate (PR) per embryo transfer (ET) was the primary outcome. Secondary outcomes were implantation and miscarriage rates.
Result(s): The PR per transfer was higher in the NGS group (84.4% vs. 41.5%). The implantation rate (61.5% vs. 34.8%) was higher in the NGS group. The miscarriage rate was similar in the 2 groups (2.8% vs. 4.6%).
Conclusion(s): We demonstrate the technical feasibility of NGS-based PGD involving cleavage-stage biopsy and fresh ETs. Encouraging data were obtained from a prospective trial using this approach, arguing that cleavage-stage NGS may represent a valuable addition to current aneuploidy screening methods. These findings require further validation in a well-designed randomized controlled trial.
Clinical Trial Registration Number: ACTRN12614001035617. (Fertil Steril® 2015;103: 1031–6. ©2015 by American Society for Reproductive Medicine.)
Key Words: Next-generation sequencing, preimplantation genetic diagnosis, genotyping, aneuploidy screening, semiconductor-based sequencer

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Received June 25, 2014; revised November 24, 2014; accepted December 26, 2014; published online January 24, 2015.
K.Ł. has nothing to disclose. S.P. has nothing to disclose. D.W. has nothing to disclose. C.C. has nothing to disclose. J.L. has nothing to disclose. Ł.P. has nothing to disclose. W.K. has nothing to disclose. J.Z. has nothing to disclose.
Supported by Invicta Ltd., Gdansk, Poland.
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Fertility and Sterility® Vol. 103, No. 4, April 2015 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.12.123

Estimates indicate that only 30% of natural conceptions result in a successful pregnancy (1). Similarly, the majority of in vitro fertilization (IVF) cycles fail to produce a child (1–3). To maximize the likelihood of success in infertility treatment, the embryos produced undergo a morphologic evaluation,

with the aim of identifying and transferring the embryo(s) that have the greatest potential for forming a viable pregnancy. Unfortunately, standard methods of morphologic assessment are only weakly predictive of implantation rate (4).

Aneuploidy has been well established to be extremely common in human oocytes, as has the fact that such abnormalities are a leading cause of miscarriages and congenital birth defects (5, 6). Additionally, aneuploidy has a significant impact on implantation and pregnancy rate (PR), as many chromosomal abnormalities are observed in material from younger women. For example, at the cleavage stage, aneuploidy rates have been reported to vary from 50% in women aged <35 years to as high as >90% in those aged >42 years (7).

The high frequency of aneuploidy during preimplantation development, and its likely deleterious effects on embryo viability, have led to the suggestion that embryos should be tested for chromosomal abnormalities before a decision is made about which ones to transfer to the uterus (8). However, routine morphologic analysis reveals very little concerning the chromosomal status of an embryo (9–11). For this reason, preimplantation genetic screening (PGS) has been developed. Techniques used for PGS involve the sampling (biopsy) of polar bodies, single blastomeres, or trophectoderm cells, followed by cytogenetic analysis to distinguish chromosomally normal embryos from those affected by aneuploidy. Embryos found to be chromosomally normal can then be prioritized for transfer. Despite earlier controversies concerning the efficiency of PGS, recent data show that use of comprehensive chromosome screening methods has been associated with encouraging improvements in implantation and PRs in randomized controlled trials (12–15).

Although preimplantation genetic diagnosis (PGD) for aneuploidy is emerging as a powerful tool for embryo selection, methods that are even more efficient are still required. Ideally, new approaches should give reliable information on chromosome copy number at lower cost than existing methods, and allow more detailed genetic information to be obtained, thereby improving patient access to aneuploidy screening, and shedding more light on embryo viability. The introduction of next-generation sequencing (NGS) into routine clinical use, as described in this article, represents an important step toward these goals.

MATERIALS AND METHODS

A prospective study was performed at a private fertility clinic. The study population consisted of patients treated by intracytoplasmic sperm injection between August 2013 and July 2014. A total of 45 patients decided to undergo PGD for aneuploidy, because of repeated implantation failures. Each had previously undergone ≥2 unsuccessful IVF attempts. A control group of 53 patients was created, matched to the PGD patients in terms of infertility etiology, number of failed cycles, and age and range of hormonal and other prognostic markers (Table 1). Patient selection was conducted using our proprietary database and medical software (Invictus, Version 3.1.62, Invicta, Ltd).

TABLE 1

Characteristics of the treatment and control groups.

Variable	PGD NGS group	Control group—no PGD	*P* value[a]
No. subjects	45	53	
Age (y)	34.0 (4.0)	34.4 (1.2)	< .22
Median (quartiles)	32 (31–38)	34 (33–35)	
BMI (kg/m^2)	21.7 (3.4)	22.4 (3.3)	.57
Duration of infertility (y)	4.2 (2.8)	4.3 (2.9)	.82
IVF cycles done previously	2.56 (0.96)	2.41 (0.73)	.41
AMH (ng/ml)	2.7 (1.7)	2.7 (0.5)	.56
Median (quartiles)	2.2 (1.3–3.7)	2.6 (2.3–3.1)	
Inhibin B	53.4 (44.1)	54.6 (30.7)	.66
Basal FSH	7.4 (7.2)	7.5 (7.3)	.58
Basal LH	7.3 (6.18)	7.2 (5.26)	.74
Basal E_2	45.2 (32.7)	46.3 (32.4)	.69
DHEAS	203.1 (91.4)	202.6 (73.5)	.57
Testosterone	1.5 (1.2)	1.6 (1.7)	.34
SHBG	72.2 (35.4)	69.7 (36.1)	.43
AFC	17.3 (9.4)	16.2 (3.6)	.59
Median (quartiles)	14.5 (10.5–21)	16 (13–19)	

Note: Values are mean (standard deviation), unless otherwise indicated. AFC = antral follicles count; AMH = anti-Müllerian hormone; BMI = body mass index; DHEAS = dehydroepiandrosteron-sulfate; E_2 = estradiol; FSH = follicle-stimulating hormone; LH = luteinizing hormone; SHBG = sex hormone–binding globulin.

[a] *P* value compared with control group.

Łukaszuk. Sequencing for preimplantation genetic diagnosis in fresh cycle transfers at 3 days. Fertil Steril 2015.

Control and PGD patients underwent treatment during the same time interval. Assisted hatching after day-3 embryo biopsy was performed for embryos in both groups because of its known positive effect on implantation rates and PRs in patients with repeated failures (16, 17). In all cases, in both groups, day-5 fresh embryo transfer (ET) was performed.

Stimulation Protocol

All women were treated on a long agonist protocol starting with oral contraceptive pills (Ovulastan, Adamed) taken on days 2–5 of the cycle. Triptorelin acetate 0.1 mg (Gonapeptyl, Ferring Pharmaceuticals) was administered 14 days after patients began taking the contraceptive. Fourteen days later (7 days after the oral contraceptive was stopped), the administration of urinary gonadotropins (Menopur, Ferring Pharmaceuticals) for ovarian stimulation was initiated; the dosage was dependent on the anti-Müllerian hormone level (150–300 international units [IU] daily) (18).

Monitoring of follicular growth was carried out on day 8 by ultrasonographic scan and assays of serum estradiol (E_2), progesterone (P), and luteinizing hormone (LH). Oocyte pickup was performed 36 hours after the administration of 5,000 IU of human chorionic gonadotropin hCG (Choragon, Ferring Pharmaceuticals). The luteal phase was supplemented by transvaginal P (100 mg, three times a day; Lutinus, Ferring Pharmaceuticals) and E_2 (2 mg, three times a day vaginally; Estrofem NovoNordisk); hormone levels (E_2, P, and hCG) were checked every 3 to 4 days.