



Preimplantation genetic testing for aneuploidies does not increase success rates in fresh oocyte donation cycles: a paired cohort study

Carolina Lumertz Martello¹ · Marcos Iuri Roos Kulmann¹ · Luiza Mezzomo Donatti¹ · Adriana Bos-Mikich² · Nilo Frantz¹

Received: 29 July 2021 / Accepted: 1 October 2021 / Published online: 5 October 2021
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract

Purpose To determine whether in vitro fertilization cycles using fresh oocyte donations benefit from preimplantation genetic testing for aneuploidies.

Methods A paired cohort study compared 44 fresh oocyte donation cycles with or without preimplantation genetic testing for aneuploidy (PGT-A). The sibling oocyte study analyzed fertilized oocytes, blastocyst development, and euploidy rate. Only frozen embryo transfers were performed. Pregnancy, implantation, biochemical pregnancy, miscarriage, stillbirth, live birth, and twin pregnancy rates were analyzed between groups.

Results Fresh oocyte donation cycles between PGT-A and non-PGT-A groups were similar in all laboratory and clinical outcomes. A euploidy rate of 74.2% was observed in the PGT-A group. Although a slight trend was observed for implantation rate in the PGT-A group, it was not statistically significant. No difference was observed for live birth between groups.

Conclusion PGT-A associated with fresh oocyte donation cycles does not improve clinical outcomes and can be seen as over-treatment for patients.

Keywords Oocyte donation · PGT-A · Preimplantation genetic testing · Fresh oocyte donation

Introduction

An increasing number of women are delaying pregnancy to focus on their education and careers. Consequently, the proportion of in vitro fertilization (IVF) patients with advanced maternal age (AMA; \geq 35 years old) has increased over the past decades [1]. AMA women typically present with a diminished ovarian reserve (DOR), as ovarian reserves directly correlate with maternal age. Moreover, older patients have a higher chance of developing aneuploid embryos due to meiotic nondisjunction [2].

Assisted reproduction technologies may be able to overcome this issue by using preimplantation genetic testing for aneuploidies (PGT-A) so that euploid embryos can be selected for transfer [3]. The main considerations for

carrying out PGT-A are AMA, recurrent pregnancy loss, repetitive IVF failure, and severe male-factor infertility. However, PGT-A may be used to alleviate insecurities about aneuploidy risk even when the above considerations are not met.

At collection, AMA patients may have very few, low-quality oocytes or no oocytes [1, 4]. Under these circumstances, patients have the option of seeking and using an egg donor, so they can conceive [5]. Oocyte donors need to be young women (< 35 years old) with good ovarian reserves [4]. An oocyte donation (OD) can be performed using fresh or frozen oocytes [6]. Young oocyte donors have a lower chance of developing aneuploid embryos. Furthermore, OD cycles are known to generate good-quality embryos and result in live birth rates higher than 50% and low miscarriage rates [4].

Despite the high success rates of OD cycles, some patients still perform PGT-A on their embryos as they feel it will increase their chances of getting pregnant and delivering a healthy baby. Previous paired cohort studies have shown that genetic testing in oocyte donor-recipient cycles is not associated with increased successful pregnancy and

✉ Carolina Lumertz Martello
carolinamartello1@gmail.com

¹ Nilo Frantz Reproductive Medicine, Porto Alegre, RS, Brazil

² Basic Health Sciences Institute, Federal University of Rio Grande Do Sul, Porto Alegre, RS, Brazil

birth rates [7–10]. However, the results of these studies are exclusively based on cryopreserved OD [8]. In Brazil and the rest of Latin America, OD cycles are usually performed using fresh oocytes.

Therefore, the present retrospective paired cohort analysis was developed to assess the role of PGT-A in fresh OD cycles.

Material and methods

The paired cohort retrospective analysis was performed comparing 44 fresh OD cycles with and without PGT-A. Data was collected between 2015 and 2020 at the Nilo Frantz Reproductive Medicine Clinic in Porto Alegre, Brazil. All participants included in the study provided written informed consent for their data to be used. The same donor population was used in the comparative study to reduce the effect of confounding factors. Groups were divided by fresh OD cycles that either underwent PGT-A or did not. The decision of whether to perform PGT-A was made by the recipient couple in concordance with a physician.

Donor and recipient populations

A standard donor protocol of controlled ovarian stimulation with GnRH antagonist suppression, recombinant FSH stimulation, and double-dose trigger (GnRH agonist and hCG) was used. Oocyte collection was performed 36 h post-trigger, and oocytes were donated fresh. Recipients who chose to perform PGT-A were included in the PGT-A group, while recipients from the same donor that did not perform PGT-A were included in the non-PGT-A group. The primary infertility factors of the recipient couples included DOR, male-factor infertility, uterine/tubal factor, endometriosis, polycystic ovary syndrome, and unexplained infertility.

Insemination, embryo biopsy, testing, and transfer

Fresh oocytes were inseminated by intracytoplasmic sperm injection (ICSI). Blastocysts were cryopreserved and warmed in accordance with InGamed® (Maringá, Brazil) protocol [11]. Opening of the zona was performed with laser-assisted hatching at the cleavage or blastocyst stage, while embryo biopsy was only performed at the blastocyst stage. PGT-A was performed via array comparative genomic hybridization (aCGH) in 2015 and by next-generation sequencing (NGS) from 2016 onwards. Blastocysts were scored prior to biopsy using the Gardner and Schoolcraft grading system [12]. Good-quality blastocysts were defined

as embryos with a score of BB or higher. Frozen embryo transfer (FET) was performed, and the current study analysis was based on the first FET attempt in both groups. Recipients were prepared for FET by receiving estradiol, starting on the second day of their menstrual cycle, and progesterone for 6 days. On the sixth day of progesterone administration, embryos were transferred approximately 4 h after warming.

Outcome assessment

Pregnancy was determined by a β -hCG test > 25 mIU/mL 14 days after FET. Implantation rate was calculated as the total number of gestational sacs with fetal heartbeat divided by the total number of embryos transferred. Patients with positive β -hCG results that did not present gestational sacs with heartbeats 4 weeks after embryo transfer were classified as having biochemical pregnancies. Miscarriage was determined by pregnancy loss before 20 weeks of gestation. Stillbirth was defined as baby loss after 20 weeks of pregnancy. Live birth was defined as the birth of a healthy baby. Twin pregnancy was determined by two or more gestational sacs with the presence of a fetal heartbeat. These parameters were calculated based on the information of the outcomes provided by the patients.

Statistical analysis

Continuous variables are presented by their mean and standard deviation (SD). Categorical variables are presented as frequency percentages. Inseminated and fertilized oocytes, blastocysts, and embryo transfers were analyzed by paired Student's *t*-test. Paternal and maternal recipient ages were analyzed by unpaired Student *t*-test. Outcomes were analyzed by Fisher's exact test. Data analysis was performed using GraphPad Prism 6, and *p* values lower than 0.05 were considered to be statistically significant.

Results

A total of 22 fresh OD PGT-A cycles and 22 fresh OD non-PGT-A cycles were paired using oocytes from the same donors. The mean donor age was 25.5 years (range of 21–32 years old), and their average BMI and AMH were 22.1 kg/m² and 3.7 ng/mL, respectively (Table 1). The majority of recipients presented with AMA, with the mean recipient age being 42.5 years in the PGT-A group and 41.1 years in the non-PGT-A group. The mean paternal age was 44.5 years in the PGT-A group and 41.7 years in the non-PGT-A group. Both groups presented similar maternal and paternal recipient ages. DOR was the predominant

Table 1 Baseline characteristics

Number of cycles (<i>n</i>)	44		
Oocyte donor age (mean ± SD)	25.5 ± 3.4		
BMI (mean ± SD) kg/m ²	22.1 ± 1.8		
AMH (mean ± SD) ng/mL	3.7 ± 1.2		
	PGT-A	Non-PGT-A	<i>p</i>
Female recipient age (mean ± SD)	42.5 ± 5.0	41.1 ± 5.1	0.5802
Male recipient age (mean ± SD)	44.5 ± 8.8	41.7 ± 6.1	0.2620
Infertility factor*, % (<i>n</i>)			
DOR	75.0 (18)	75.0 (18)	0.9145
Male factor	8.3 (2)	12.5 (3)	
Others	12.5 (3)	12.5 (3)	

Abbreviations: *SD* standard deviation, *BMI* body mass index, *AMH* anti-Müllerian, *PGT-A* preimplantation genetic testing for aneuploidies, *DOR* diminished ovarian reserve; others included uterine/tubal infertility factor, endometriosis, polycystic ovary syndrome and unexplained. *Percentage exceeds 100%—multiple infertility factors recorded for some patients. Student *t*-test and Fisher's test was used in the analysis with a significant *p* value < 0.05

infertility factor followed by male-factor infertility. The infertility factors were not statistically different between

groups. The PGT-A group included two cycles that were performed using aCGH and 20 using NGS. Fertilization and blastocyst rates were not significantly different between the groups (Table 2). The proportion of blastocysts that vitrified was 53.1% in the PGT-A group and 59.7% in the non-PGT-A group, presenting no statistical difference. Among the analyzed embryos in the PGT-A group, 74.2% of them were euploid blastocysts. No mosaic embryos were identified. Embryo transfer data and outcomes are summarized in Table 3. Good-quality blastocyst transfer was performed in a similar manner for both groups. PGT-A and non-PGT-A groups transferred an average of 1.2 and 1.4 embryos per cycle, respectively, with no statistically significant difference between them. Three patients in the PGT-A group and eight patients in the non-PGT-A group underwent double-embryo transfer (DET). There was no significant difference in the clinical outcomes of cycles. The pregnancy rate was 77.3% in the PGT-A group and 72.7% in the non-PGT-A group. The live birth rate was 59.1% in the PGT-A group and 45.5% in the non-PGT-A group. Miscarriage and twin pregnancy rates were similar for both groups, 13.6% and 9.1% in the PGT-A group and 9.1% and 13.6% in the non-PGT-A group, respectively.

Table 2 Oocyte and embryo data

	PGT-A	Non-PGT-A	<i>p</i>
Fertilized oocytes, % (<i>n</i>)	75.3 (143)	81.8 (139)	0.1588
Fertilized oocytes per cycle (mean ± SD)	6.5 ± 1.6	6.3 ± 1.3	0.6829
Blastocysts, % (<i>n</i>)	62.9 (90)	70.5 (98)	0.2068
Blastocysts per cycle (mean ± SD)	4.1 ± 1.8	4.5 ± 1.6	0.4818
Blastocysts vitrified, % (<i>n</i>)	53.1 (76)	59.7 (83)	0.2817
Blastocysts vitrified per cycle (mean ± SD)	3.5 ± 1.6	3.8 ± 1.3	0.4632
Analyzed blastocysts, % (<i>n</i>)	86.8 (66)	NA	NA
Euploid blastocysts, % (<i>n</i>)	74.2 (49)	NA	NA

Abbreviations: *SD* standard deviation, *PGT-A* preimplantation genetic testing for aneuploidies. Student *t*-test and Fisher's test was used in the analysis with a significant *p* value < 0.05

Table 3 Embryo transfer and outcome analysis

	PGT-A	Non-PGT-A	<i>p</i>
Embryo transfer cycles, <i>n</i>	22	22	
Transferred embryos, <i>n</i>	25	30	
Good-quality blastocysts, % (<i>n</i>)	96.0 (24)	100 (30)	0.4545
Transferred embryos per cycle (mean ± SD)	1.1 ± 0.4	1.4 ± 0.5	0.0961
Pregnancy, % (<i>n</i>)	77.3 (17)	72.7 (16)	1.0000
Implanted embryos, % (<i>n</i>)	72.0 (18)	60.0 (18)	0.4040
Biochemical pregnancy, % (<i>n</i>)	4.5 (1)	9.1 (2)	0.6012
Miscarriage, % (<i>n</i>)	13.6 (3)	9.1 (2)	1.0000
Stillbirth, % (<i>n</i>)	0 (0)	4.5 (1)	0.4848
Live birth, % (<i>n</i>)	59.1 (13)	45.5 (10)	0.4646
Twin pregnancy, % (<i>n</i>)	9.1 (2)	13.6 (3)	0.6175

Abbreviations: *SD* standard deviation, *PGT-A* preimplantation genetic testing for aneuploidies. Student *t*-test and Fisher's test was used in the analysis with a significant *p* value ≤ 0.05

Discussion

OD is a highly successful treatment for patients with AMA, premature ovarian insufficiency, or poor oocyte quality [6]. Oocytes from young women usually develop good-quality embryos, leading to high pregnancy and birth rates. OD cycles can be associated with PGT-A, although it is not generally recommended. PGT-A technology increases pregnancy and live birth rates by enabling the selection of ideal embryos for transfer [13]. Therefore, the premise of the technology is most relevant for women with AMA [2, 14]. In the current study, the clinic where the data was gathered does not have a policy for offering PGT-A in OD cycles. However, oocyte-recipient couples sometimes choose to perform PGT-A during the IVF cycle. This decision normally involves the desire to have a healthy baby, reduce birth defects, and decrease miscarriage risk. Therefore, the current study only presents results from a small number of cases, as a combination of these procedures is not recommended.

The majority of embryo aneuploidies have a maternal origin and depend on maternal age [2, 14, 15]. The aneuploidy rate increases during the ages of 31 to 41, occurring at lower rates between the ages of 26 and 30 [2]. No difference in euploidy rate among the interval of donor ages was observed in this study [16]. Furthermore, IVF cycle outcomes of younger women with no PGT-A indications do not seem to benefit from having it performed [2, 9, 17, 18]. This lack of benefit also applies to young patients in the same age range as donors using autologous oocyte cycles. However, the impact of paternal age on embryo ploidy in OD cycles is still controversial. Studies have demonstrated that a paternal age over 50 increases fresh OD embryo aneuploidies and recommend PGT-A in such cases [19, 20]. Conversely, others have suggested that paternal age has no influence on the aneuploidy rate in donor oocyte embryos [21, 22].

The euploidy rate in this study (74.2%) was higher than reported for OD embryos in previous studies [9, 16, 17]. Conversely, some studies have demonstrated high aneuploidy rates in donated oocytes embryos [15, 18]. A variation in the euploidy rate of donated oocyte IVF cycles is expected as it is center-dependent [22]. Only 86.8% of the morphologically ideal biopsied embryos were analyzed. Therefore, the euploidy rate of 74.2% does not reflect the totality of the embryos biopsied. Moreover, ten cycles (45%) did not present aneuploid embryos.

Live birth rates were similar between groups, as previously demonstrated in studies examining OD cycles [8–10, 18, 23]. Ozgur et al. [17] found that in young women (under 35 years old) who had at least two good-quality blastocysts (\geq BB), PGT-A did not increase live

birth rates. Doyle et al. [8], using a paired cohort model, demonstrated no increase in live birth rates after PGT-A following cryopreserved/warmed oocyte donation. The current study found similar results in fresh OD. Only one previous study found lower rates of live birth following PGT-A for OD IVF cycles [7]. However, this study did not discriminate between cleavage and blastocyst stages during embryo biopsy, and it is known that cleavage-stage embryo biopsy decreases the implantation competence of the embryo [24].

Implantation rates were similar between groups. Although there was a trend towards a higher implantation rate in the PGT-A group, it was not statistically significant. Additionally, miscarriage rates were similar between groups, which differs from the results of a previous study [9]. However, this might be due to the smaller sample size of the current study. Coates et al. [23] found higher rates of live birth when DET was performed in the PGT-A group. The global recommendation for OD embryos is the single-embryo transfer (SET) [5]. However, some patients opt for a DET to increase their pregnancy rate. In the current study, patients in the non-PGT-A group underwent more DETs compared to patients in the PGT-A group. Although it is a limitation of the study, live birth rates were similar between SET and DET [25], and twin pregnancies were similar in both groups.

PGT-A has been described as cost-effective in all age groups except for patients under 35 years old that have 8 embryos or more [26]. The average number of blastocysts per cycle was less than 4 in both groups. However, as full oocyte cohorts were not donated to one patient, it is not plausible to conclude that oocyte donors were impaired in the PGT-A cost-effective group. Moreover, Antero et al. [27] showed that PGT-A is not cost-effective in IVF cycles when fresh oocyte donors in the same age range as the current study were used. PGT-A cost varies in different countries, and technology tends to be more expensive in developing countries [28]. Although it has been demonstrated that blastocyst biopsy does not lead to embryo harm depending on the experience and technique of embryologists [24], the possibility of decreased embryo potential following PGT-A must be considered. The use of both aCGH and NGS is not thought to impact the results of the current study, as their main difference is in chromosomal mosaicism and segmental aneuploidy identification [29].

This single-center study has limitations intrinsically related to its retrospective, non-randomized design and small sample size. The greatest strength of this study is that the comparison model used the same oocyte donor population, which reduced the effect of confounding factors. Live birth rate did not increase after PGT-A use in OD cycles. Therefore, PGT-A can be seen as over-treatment in patients using fresh OD. This work aims to provide better counseling to oocyte-recipient patients.

Declarations

Consent to participate All patients had previously given informed consent for the use of their data.

Conflict of interest The authors declare no competing interests.

References

- Ubaldi FM, Cimadomo D, Vaiarelli A, Fabozzi G, Venturella R, Maggiulli R, et al. Advanced maternal age in IVF: still a challenge? The present and the future of its treatment. *Front Endocrinol (Lausanne)* [Internet]. 2019. 10. Available from: <https://www.frontiersin.org/article/>, <https://doi.org/10.3389/fendo.2019.00094/full>
- Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, Treff NR, et al. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. *Fertil Steril* [Internet]. 2014. 101:656–663.e1. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0015028213032573>
- Viotti M. Preimplantation genetic testing for chromosomal abnormalities: aneuploidy, mosaicism, and structural rearrangements. *Genes (Basel)* [Internet]. 2020. 11:602. Available from: <https://www.mdpi.com/2073-4425/11/6/602>
- Crawford NM, Steiner AZ. Age-related Infertility. *Obstet Gynecol Clin North Am*. 2015;42:15–25.
- Daar J, Benward J, Collins L, Davis J, Francis L, Gates E, et al. Oocyte or embryo donation to women of advanced reproductive age: an Ethics Committee opinion. *Fertil Steril* [Internet]. 2016. 106:e3–7. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S001502821661405X>
- Kushnir VA, Gleicher N. Fresh versus cryopreserved oocyte donation. *Curr Opin Endocrinol Diabetes Obes*. 2016;23:451–7.
- Barad DH, Darmon SK, Kushnir VA, Albertini DF, Gleicher N. Impact of preimplantation genetic screening on donor oocyte-recipient cycles in the United States. *Am J Obstet Gynecol* [Internet]. 2017. 217:576.e1–576.e8. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0002937817308657>
- Doyle N, Gainty M, Eubanks A, Doyle J, Hayes H, Tucker M, et al. Donor oocyte recipients do not benefit from preimplantation genetic testing for aneuploidy to improve pregnancy outcomes. *Hum Reprod* [Internet]. 2020. 35:2548–55. Available from: <https://academic.oup.com/humrep/article/35/11/2548/5923786>
- Masbou AK, Friedenthal JB, McCulloh DH, McCaffrey C, Fino ME, Grifo JA, et al. A comparison of pregnancy outcomes in patients undergoing donor egg single embryo transfers with and without preimplantation genetic testing. *Reprod Sci* [Internet]. 2019. 26:1661–5. Available from: <http://journals.sagepub.com/doi/>, <https://doi.org/10.1177/1933719118820474>
- Peysers A, Brownridge S, Rausch M, Noyes N. The evolving landscape of donor egg treatment: success, women's choice, and anonymity. *J Assist Reprod Genet* [Internet]. 2021. Available from: <https://link.springer.com/>, <https://doi.org/10.1007/s10815-021-02262-6>
- Almodin CG, Minguetti-Camara VC, Paixao CL, Pereira PC. Embryo development and gestation using fresh and vitrified oocytes. *Hum Reprod* [Internet]. 2010. 25:1192–8. Available from: <https://academic.oup.com/humrep/article-lookup/doi/>, <https://doi.org/10.1093/humrep/deq042>
- Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. *Fertil Steril* [Internet]. 2000. 73:1155–8. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0015028200005185>
- Dahdouh EM, Balayla J, García-Velasco JA. Comprehensive chromosome screening improves embryo selection: a meta-analysis. *Fertil Steril* [Internet]. 2015. 104:1503–12. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0015028215018841>
- Rubio C, Rodrigo L, Garcia-Pascual C, Peinado V, Campos-Galindo I, Garcia-Herrero S, et al. Clinical application of embryo aneuploidy testing by next-generation sequencing. Gardner DK, editor. *Biol Reprod* [Internet]. 2019. 101:1083–90. Available from: <https://academic.oup.com/biolreprod/article/101/6/1083/5306439>
- Sills ES, Li X, Frederick JL, Houry CD, Potter DA. Determining parental origin of embryo aneuploidy: analysis of genetic error observed in 305 embryos derived from anonymous donor oocyte IVF cycles. *Mol Cytogenet* [Internet]. 2014. 7:68. Available from: <http://molecularcytogenetics.biomedcentral.com/articles/>, <https://doi.org/10.1186/s13039-014-0068-5>
- Hoyos LR, Cheng CY, Brennan K, Hubert G, Wang B, Buyalos RP, et al. Euploid rates among oocyte donors: is there an optimal age for donation? *J Assist Reprod Genet* [Internet]. 2020. 37:589–94. Available from: <http://link.springer.com/>, <https://doi.org/10.1007/s10815-020-01694-w>
- Ozgur K, Berkkanoglu M, Bulut H, Yoruk GDA, Candurmaz NN, Coetsee K. Single best euploid versus single best unknown-ploidy blastocyst frozen embryo transfers: a randomized controlled trial. *J Assist Reprod Genet* [Internet]. 2019. 36:629–36. Available from: <http://link.springer.com/>, <https://doi.org/10.1007/s10815-018-01399-1>
- Haddad G, Deng M, Wang CT, Witz C, Williams D, Griffith J, et al. Assessment of aneuploidy formation in human blastocysts resulting from donated eggs and the necessity of the embryos for aneuploidy screening. *J Assist Reprod Genet* [Internet]. 2015. 32:999–1006. Available from: <http://link.springer.com/>, <https://doi.org/10.1007/s10815-015-0492-4>
- García-Ferreira J, Luna D, Villegas L, Romero R, Zavala P, Hilario R, et al. High aneuploidy rates observed in embryos derived from donated oocytes are related to male aging and high percentages of sperm DNA fragmentation. *Clin Med Insights Reprod Heal* [Internet]. 2015. 9:CMRH.S32769. Available from: <http://journals.sagepub.com/doi/>, <https://doi.org/10.4137/CMRH.S32769>
- García-Ferreira J, Hilario R, Dueñas J. High percentages of embryos with 21, 18 or 13 trisomy are related to advanced paternal age in donor egg cycles. *JBRA Assist Reprod* [Internet]. 2018. Available from: <http://www.gnresearch.org/doi/>, <https://doi.org/10.5935/1518-0557.20180004>
- Carrasquillo RJ, Kohn TP, Cinnioglu C, Rubio C, Simon C, Ramasamy R, et al. Advanced paternal age does not affect embryo aneuploidy following blastocyst biopsy in egg donor cycles. *J Assist Reprod Genet* [Internet]. 2019. 36:2039–45. Available from: <http://link.springer.com/>, <https://doi.org/10.1007/s10815-019-01549-z>
- Munné S, Alikani M, Ribustello L, Colls P, Martínez-Ortiz PA, McCulloh DH. Euploidy rates in donor egg cycles significantly differ between fertility centers. *Hum Reprod* [Internet]. 2017. 32:743–9. Available from: <https://academic.oup.com/humrep/article/32/4/743/3059568>
- Coates A, Bankowski BJ, Kung A, Griffin DK, Munne S. Differences in pregnancy outcomes in donor egg frozen embryo transfer (FET) cycles following preimplantation genetic screening (PGS): a single center retrospective study. *J Assist Reprod Genet* [Internet]. 2017. 34:71–8. Available from: <http://link.springer.com/>, <https://doi.org/10.1007/s10815-016-0832-z>

24. Scott RT, 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. *FertilSteril* [Internet]. 2013. 100:624–30. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0015028213005530>
25. Racca A, Drakopoulos P, Van Landuyt L, Willem C, Santos-Ribeiro S, Tournaye H, et al. Single and double embryo transfer provide similar live birth rates in frozen cycles. *Gynecol Endocrinol* [Internet]. 2020. 36:824–8. Available from: <https://www.tandfonline.com/doi/full/>, <https://doi.org/10.1080/09513590.2020.1712697>
26. Neal SA, Morin SJ, Franasiak JM, Goodman LR, Juneau CR, Forman EJ, et al. Preimplantation genetic testing for aneuploidy is cost-effective, shortens treatment time, and reduces the risk of failed embryo transfer and clinical miscarriage. *Fertil Steril* [Internet]. 2018. 110:896–904. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0015028218304977>
27. Facadio Antero M, Singh B, Pradhan A, Gornet M, Kearns WG, Baker V, et al. Cost-effectiveness of preimplantation genetic testing for aneuploidy for fresh donor oocyte cycles. *F&S Reports* [Internet]. 2021. 2:36–42. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S2666334120301240>
28. Scriven PN. Towards a better understanding of preimplantation genetic screening for aneuploidy: insights from a virtual trial for women under the age of 40 when transferring embryos one at a time. *Reprod Biol Endocrinol* [Internet]. 2017. 15:49. Available from: <http://rbej.biomedcentral.com/articles/>, <https://doi.org/10.1186/s12958-017-0269-y>
29. Lai H-H, Chuang T-H, Wong L-K, Lee M-J, Hsieh C-L, Wang H-L, et al. Identification of mosaic and segmental aneuploidies by next-generation sequencing in preimplantation genetic screening can improve clinical outcomes compared to array-comparative genomic hybridization. *Mol Cytogenet* [Internet]. 2017. 10:14. Available from: <https://molecularcytogenetics.biomedcentral.com/articles/>, <https://doi.org/10.1186/s13039-017-0315-7>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.