Mosaic embryo transfer after oocyte in vitro maturation in combination with non-invasive prenatal testing (NIPT)—first report of a euploid live birth

Naomi Inoue¹ · Rosmary Lopez² · Andrea Delgado³ · Denisse Nuñez¹ · Jimmy Portella¹ · Luis Noriega-Hoces^{1,3} · Luis Guzmán^{1,2}

Received: 21 March 2017 / Accepted: 7 June 2017 / Published online: 24 June 2017
© Springer Science+Business Media, LLC 2017

Abstract

Purposes The purpose of this study is to describe a healthy life birth after a mosaic embryo transfer in oocyte in vitro maturation (IVM).

Methods Patient received minimal stimulation, starting on day 3 after menstrual period. No hCG trigger was administered. Oocyte retrieval was performed and oocytes were matured for 30 h. After denuding, mature oocytes were inseminated by ICSI. Embryos were cultured until blastocyst stage and biopsied.

Results One euploid embryo after array comprehensive genome hybridization (aCGH) was diagnostic. However, the next-generation sequencing (NGS) re-analysis showed that embryo was a mosaic for chromosome 13 and 21. Nevertheless, pregnancy ultrasound scans and non-invasive prenatal test (NIPT—Verifi—Illumina) indicated a normal fetus development. Finally, a healthy baby was born after 38 weeks. Its weight was 4480 g, head circumference 36 cm, and total length of 51 cm. To confirm that the baby was chromosomically normal, an NGS test was performed in buccal cells, a normal profile was obtained.

Conclusions Our finding confirmed that mosaic embryo transfer would bring a healthy offspring.

Keywords Oocyte in vitro maturation (IVM) · Microarray comparative genomic hybridization (aCGH) · Next-generation sequencing (NGS) · Non-invasive prenatal testing (NIPT)

Introduction

The in vitro maturation (IVM) of human oocytes is a promising assisted reproduction technique. IVM is the maturation in culture of immature oocytes obtained from follicles between 2 to 10 mm [1]. During IVM, immature oocytes progress from a germinal vesicle (GV) until the extrusion of the first polar body [2]. Since the first pregnancy report after IVM of human oocytes by Cha [3], it has been suggested as a new clinical strategy. Despite the lack of a unique stimulation protocol and the variability in clinical outcomes [4], several strategies have been developed to maximize the maturation rates and clinical outcomes in selected patients [5–7].

IVM has been suggested as a new alternative in patients with polycystic ovarian syndrome (PCOS) for minimizing the risk of ovarian hyperstimulation syndrome (OHSS). Additionally, IVM is recommended for oncologic patients who require fertility preservation. This group of patients could be treated in follicular phase or luteal phase of menstrual cycle without postponing the oncologic treatment. Additionally, hormone levels (i.e., estrogens) would not be increased since IVM requires either none or minimal gonadotropin stimulation.

For many years, the clinical outcomes of IVM technique have been shown to be variable between IVF laboratories, showing pregnancy rates ranging from 0 [8] to 42% [9]. These findings indicate that hormone stimulation protocol for IVM needs standardization [10]; moreover, the culture media has not been changed substantially [4], and further

✉ Luis Guzmán
lguzmanm@gmail.com

¹ PRANOR. Grupo de Reproducción Asistida, Calle los Olivos 364, San Isidro, Lima, Peru

² Reprogenetics Latinoamerica, Lima, Peru

³ Clínica Concebir, Calle Los Olivos 364 San Isidro, Lima, Peru

prospective studies are needed to resolve the disagreement between fresh and frozen embryo transfer [11].

It is well-known that chromosome aneuploidy is a major cause of IVF failure, establishing that most embryo with aneuploidy do not implant or are miscarried during the first trimester of pregnancy [12]. Since the introduction of pre-implantation genetic diagnosis for aneuploidy screening (PGD-AS), the clinical outcomes and live birth rates have improved and reduced significantly the miscarriage rates [13, 14]. PGD-AS using array comprehensive genome hybridization (aCGH) has been replaced for the next-generation sequencing (NGS) technique which is capable to classify the embryos as euploidy, mosaic, or aneuploidy [15].

The first report using IVM with PGD-AS by FISH was performed by Ao et al. in 2006 [16]. This case demonstrated that sufficient number of embryos could be developed after IVM procedure followed by PGD-AS analysis in patients with PCO/PCOS. Likewise, other studies have mentioned successful pregnancies after IVM procedures where embryo biopsy was performed on day 3 and analyzed for fragile X syndrome and chromosome translocation [17, 18].

In the present report, we described an IVM treatment followed by trophoectoderm cell biopsy with aCGH analysis, NGS re-analysis, and a follow-up genetic assessment.

Case report

A 34-year-old woman was referred with a desire to conceive. She presented three negative results of intrauterine insemination (IUI). Two consecutive conventional IVF cycles were performed: the first cycle without success and the second cycle with a miscarriage during the first trimester. She only had one ovary, and it was polycystic on ultrasound scan; additionally, the patient presented high anti-müllerian hormone levels (15.4 ng/ml). Patient opted to undergo a minimal ovarian stimulation protocol for IVM technique followed by a PGD-AS analysis.

On day 3 of her menstrual cycle, minimal ovarian stimulation began with 150 IU per day of hp-HMG (Menopur, Ferring). After 3 days of stimulation, an ultrasound scan was performed and it showed that follicles were below than 10 mm [19]. Oocyte retrieval was performed 42 h after the last dose of gonadotropins [20]. Then, eight immature cumulus oocyte complexes were retrieved and cultured for 30 h in a rich media supplemented with FSH 75 mIU/ml and hCG 75 mIU/ml. After the IVM culture, the oocytes were denuded with hyaluronidase (LifeGlobal 80 IU/ml). Resulting in four (50%) mature oocytes, two (25%) metaphase I, one (12.5%) germinal vesicle, and one (12.5%) atretic oocyte.

Partner semen sample was obtained by masturbation; motile sperms were selected through density gradient of 95 and 45% of Isolate® (Irvine, US). Sperms were

selected physiologically by using SpermSlow (Origio, Denmark) which consist in hyaluronic acid (HA) attached to the base of the culture dish for better sperm selection. The HA binding sites on sperm plasma membrane indicate sperm maturity, having better fertilization rates, and less sperm DNA fragmentation [21]. Mature oocytes underwent intracytoplasmic sperm injection.

The inseminated oocytes were cultured for 20 h in Global® Total fertilization media. Three zygotes were obtained after insemination and cultured in Global® Total. On day 4, three embryos developed and assisted hatching was performed with a laser (Lykos; Hamilton Thorne, Beverly, ME, USA) on multipulse function (pulse/s = 7; pulse duration = 150 µs); extended culture was done until day 6 where two fair quality blastocysts were biopsied (Fig. 1). Embryo grading was done according to SART classification [22].

Biopsied cells were collected according to the genetic laboratory protocol. Blastocysts were vitrified within 1 h after TE biopsy using a Cryotech vitrification kit (Cryotec, Japan) following manufacture's protocols. Each blastocyst was vitrified individually in an open system using Cryotech.

Biopsied trophoectoderm cells were processed for aCGH analysis and were sent to Reprogenetics Latinoamerica (Lima, Peru). Briefly, biopsies were collected in Eppendorf tubes containing 2 µl of non-sticking buffer and referred to the genetic laboratory. Samples were lysed and amplified using the SurePlex kit®. Amplified samples were processed following the protocol of BlueGnome Cyto-Chip. Microarray chips were scanned, analyzed, and quantified as previously described by Gutierrez-Mateo [23]. Copy number ratios were analyzed using Cyto-Chip algorithm fixed setting in BlueFuse software (BlueGnome). aCGH results shown that embryo showed in Fig. 1a, c was considered euploid while embryo showed in Fig. 1b, d was aneuploid for chromosome X (Fig. 2).

A single vitrified-warmed embryo was transferred in an artificial endometrial priming cycle after priming of the endometrium with oral estradiol valerate (2 mg administered three times a day) (Progynova; Bayer Schering Pharma). When an endometrial thickness of >7 mm was reached, luteal phase support was started with the use of intravaginal micronized progesterone tablets (200 mg three times a day; Utrogestan; Biopas Laboratories). Single embryo transfer was scheduled 5 days later. Embryo transfer (ET) was performed under ultrasound guidance with the use of a soft catheter.

Twelve days after ET, a β -hCG blood test was performed confirming a positive biochemical pregnancy. Six weeks later after ET, the ultrasound scan confirmed a single gestational sac and a heartbeat.

Due to a technology transition, some euploid aCGH were re-analyzed by NGS. Briefly, the same amplified sample was processed following the protocol of Veriseq

Fig. 1 **a, b** Blastocyst on day 5. **c, d** Blastocyst on day 6 before blastocyst biopsy. **e** The embryo showed on Fig. 1c was euploid and it was thawed for embryo transfer. **f** Euploid blastocyst was cultured for 2 h before embryo transfer

PGD-AS (Illumina, US). NGS run was performed in a MiSeq. Copy number ratios were analyzed using BlueFuse Multi analysis software (BlueGnome). However, NGS re-analysis indicated that the transferred embryo considered as a euploid for aCGH was mosaic for a monosomy in chromosome 13 and 21; mosaicism was 24 and 34% for each chromosome, respectively (Fig. 2).

At 12 weeks of pregnancy, a 4D genetic ultrasound scan was performed to evaluate the nuchal translucency or other abnormal structures in the fetus due to chromosomal abnormalities. Results showed a normal value of nuchal translucency, and no abnormal structures were found.

At 17 weeks of pregnancy, a non-invasive prenatal test (Verifi®) which analyzes cell-free fetal DNA circulating in maternal blood for chromosome 9, 13, 16, 18, and 21, and sex chromosome was performed. Results from this test confirmed a euploid fetus development.

Finally, a healthy baby boy was born at 38 weeks; its weight was 4480 g, head circumference 36 cm, and total length of 51 cm. Additionally, NGS test was performed in buccal cells to confirm the existence of mosaic cells. This resulted in a normal NGS profile (Fig. 3).

Discussion

In vivo, oocytes are arrested at GV stage because of different regulatory factors and signaling pathway which keep the oocyte immature [24, 25]. However, during an IVM treatment, immature oocytes are retrieved from the follicle environment allowing the spontaneous meiotic resumption [2, 26].

Conventional IVF necessarily requires controlled ovarian hyperstimulation; patients who suffer from PCOS are at risk of development of OHSS, whereas in IVM cycles, these risks

Fig. 2 **a** aCGH profile from the euploid embryo. **b** NGS re-analysis showed that aCGH embryo was mosaic for chromosomes 13 and 21. **c** aCGH profile from aneuploidy embryo. **d** NGS re-analysis confirmed the aneuploidy and showed a mosaicism for chromosome 19

Fig. 3 An NGS normal profile obtained from buccal cells

decrease [27]. Despite IVM offers certain benefits, this treatment has not been widely used at fertility centers because of the large variation in minimal-stimulation protocols. Some studies have reported the use of hCG trigger prior oocyte retrieval to obtain mature and immature oocytes [28, 29]. However, other clinicians indicate no or low doses of gonadotrophins instead of hCG trigger [30, 31] resulting in the recruitment of a homogeneous cohort of oocytes at GV stage. For this case report, hCG triggering was not administrated.

IVM of human oocytes is a promising treatment for selected patients [32] and should be considered as an alternative for fertility preservation. However, more prospective studies are needed to provide strategies to optimize the clinical outcomes. The extension of the embryo culture at blastocyst stage permits a better embryo selection process [33], because of their post-embryonic genome activation and better development potential [34]. Likewise, numerous studies had indicated higher implantation rates and ongoing pregnancy following frozen-thawed embryo transfer instead of fresh cycles [34–36], this strategy increases the synchronization between embryos and the endometrium. Additionally, embryo cryopreservation prevents OHSS and allows a time frame to perform genetic analyses [20].

Several IVM reports had shown low clinical efficiency, not only for the reduced oocyte quality, but also for insufficient endometrial receptivity. In IVM cycles, embryo cryopreservation had increased implantation and pregnancy rates [31, 37–39]. The majority of the IVM cycles reported had a predilection for cleavage-stage embryo transfer on day 3 [4]. Even though, there is increasing evidence of improving clinical outcomes of IVM cycles when two modifications were applied. First, the reduction of time oocyte culture from 40 to 48 to 30 h, and second, the extension of embryo culture till blastocyst stage [9, 30]. In this report, these two modifications were followed, together with the addition of PGD-AS analysis where the incidence of aneuploidy after IVM cycles has been reported to be similar to conventional IVF in arrested [40] and good quality embryos [41].

Embryo morphology is still the standard method for embryo selection. However, it has been reported that good quality morphology and euploidy are not well correlated [42]. aCGH analysis to perform PGD-AS represents a good tool for embryo selection. Nevertheless, aCGH is not able to discriminate mosaic embryos. On the contrary, NGS detects partial or segmental aneuploidies improving the analysis resolution [43].

Embryo mosaicism is a frequent event in pre-implantational embryo development where the majority of aneuploidies detected are coming from mitotic error [44, 45]. Mosaicism has been observed at all developmental stages of human embryos [46], occurring in 15–90% of cleavage stage [47] and 30–40% of blastocysts in human embryos [47, 48]. In prenatal stages, the incidence of mosaicism is

reduced up to 1–2% [47]. This reduction could be explained by the self-repairing mechanisms throughout the development process and thus, making mosaicism undetectable at the time of NIPT test [49, 50].

Several studies have focused on the implantation potential of mosaic embryos, based on the recent evidence reported about pregnancies and healthy live births after the transfer of mosaic blastocysts [51, 52]. Implantation and ongoing pregnancy rates of mosaic embryos are less in comparison between euploid embryos (15.4 and 30.1% in mosaic embryos vs. 46.2 and 55.8% in euploid embryos, respectively) [53]. Likewise, mosaic embryos could be considered for embryo transfer according to the chromosome involved and type of chromosome abnormality. Genetic guidelines have suggested that mosaic embryos with monosomies should be preferentially transferred over trisomies [54, 55], mainly due to some trisomies that are compatible with pregnancy, and children could develop severe congenital abnormalities [56].

This report, based on the several studies conducted through all developmental stages, increases the available data of safety transfer of mosaic embryos, and it is in agreement with genetic guidelines where mosaic embryos could be considered for embryo transfer when monosomies are detected [55].

Finally, the current report describes an IVM treatment which resulted in a healthy baby born. It is important to highlight that this case report involves multiple analysis procedures at different time of embryo development which should be interpreted with caution. Nevertheless, further studies should be conducted to understand the potential development of a mosaic embryo in ART.

References

1. Smits JE, Thompson JG, Gilchrist RB. The promise of in vitro maturation in assisted reproduction and fertility preservation. *Semin Reprod Med*. 2011;29(1):24–37.
2. Edwards RG. Maturation in vitro of human ovarian oocytes. *Lancet*. 1965;2(7419):926–9.
3. Cha KY, Koo JJ, Ko JJ, Choi DH, Han SY, Yoon TK. Pregnancy after in vitro fertilization of human follicular oocytes collected from nonstimulated cycles, their culture in vitro and their transfer in a donor oocyte program. *Fertil Steril*. 1991;55(1):109–13.
4. Son WY, Tan SL. Laboratory and embryological aspects of hCG-primed in vitro maturation cycles for patients with polycystic ovaries. *Hum Reprod Update*. 2010;16(6):675–89.
5. Chian RC, Gulekli B, Buckett WM, Tan SL. Priming with human chorionic gonadotropin before retrieval of immature oocytes in women with infertility due to the polycystic ovary syndrome. *N Engl J Med*. 1999;341(21):1624–6.
6. Chian RC, Buckett WM, Tulandi T, Tan SL. Prospective randomized study of human chorionic gonadotrophin priming before immature oocyte retrieval from unstimulated women with polycystic ovarian syndrome. *Hum Reprod*. 2000;15(1):165–70.

7. Reavey J, Vincent K, Child T, Granne IE. Human chorionic gonadotrophin priming for fertility treatment with in vitro maturation. The Cochrane database of systematic reviews. 2016;11:CD008720.
8. Mikkelsen AL, Lindenberg S. Benefit of FSH priming of women with PCOS to the in vitro maturation procedure and the outcome: a randomized prospective study. *Reproduction*. 2001;122(4):587–92.
9. Junk SM, Yeap D. Improved implantation and ongoing pregnancy rates after single-embryo transfer with an optimized protocol for in vitro oocyte maturation in women with polycystic ovaries and polycystic ovary syndrome. *Fertil Steril*. 2012;98(4):888–92.
10. Fadini R, Dal Canto MB, Mignini Renzini M, Brambillasca F, Comi R, Fumagalli D, et al. Effect of different gonadotrophin priming on IVM of oocytes from women with normal ovaries: a prospective randomized study. *Reprod BioMed Online*. 2009;19(3):343–51.
11. Ortega-Hrepich C, Stoop D, Guzman L, Van Landuyt L, Tournaye H, Smits J, et al. A “freeze-all” embryo strategy after in vitro maturation: a novel approach in women with polycystic ovary syndrome? *Fertil Steril*. 2013;
12. Hyde KJ, Schust DJ. Genetic considerations in recurrent pregnancy loss. *Cold Spring Harb Perspect Med*. 2015;5(3):a023119.
13. Scott RT Jr, Ferry K, Su J, Tao X, Scott K, Treff NR. Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. *Fertil Steril*. 2012;97(4):870–5.
14. Grifo JA, Hodes-Wertz B, Lee HL, Amperloquio E, Clarke-Williams M, Adler A. Single thawed euploid embryo transfer improves IVF pregnancy, miscarriage, and multiple gestation outcomes and has similar implantation rates as egg donation. *J Assist Reprod Genet*. 2013;30(2):259–64.
15. Fiorentino F, Bono S, Biricik A, Nuccitelli A, Cotroneo E, Cottone G, et al. Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. *Hum Reprod*. 2014;29(12):2802–13.
16. Ao A, Jin S, Rao D, Son WY, Chian RC, Tan SL. First successful pregnancy outcome after preimplantation genetic diagnosis for aneuploidy screening in embryos generated from natural-cycle in vitro fertilization combined with an in vitro maturation procedure. *Fertil Steril*. 2006;85(5):1510 e9–11.
17. Nayot D, Chung JT, Son WY, Ao A, Hughes M, Dahan MH. Live birth following serial vitrification of embryos and PGD for fragile X syndrome in a patient with the premutation and decreased ovarian reserve. *J Assist Reprod Genet*. 2013;30(11):1439–44.
18. Ao A, Zhang XY, Tan SL. First successful pregnancy following PGD for chromosome translocation on embryos generated from in-vitro matured oocytes: a case report. *Reprod BioMed Online*. 2011;22(4):371–5.
19. Son WY, Chung JT, Herrero B, Dean N, Demirtas E, Holzer H, et al. Selection of the optimal day for oocyte retrieval based on the diameter of the dominant follicle in hCG-primed in vitro maturation cycles. *Hum Reprod*. 2008;23(12):2680–5.
20. Ortega-Hrepich C, Stoop D, Guzman L, Van Landuyt L, Tournaye H, Smits J, et al. A “freeze-all” embryo strategy after in vitro maturation: a novel approach in women with polycystic ovary syndrome? *Fertil Steril*. 2013;100(4):1002–7.
21. Parmegiani L, Cognigni GE, Bernardi S, Troilo E, Ciampaglia W, Filicori M. “Physiologic ICSI”: hyaluronic acid (HA) favors selection of spermatozoa without DNA fragmentation and with normal nucleus, resulting in improvement of embryo quality. *Fertil Steril*. 2010;93(2):598–604.
22. Racowsky C, Vernon M, Mayer J, Ball GD, Behr B, Pomeroy KO, et al. Standardization of grading embryo morphology. *Fertil Steril*. 2010;94(3):1152–3.
23. Gutierrez-Mateo C, Colls P, Sanchez-Garcia J, Escudero T, Prates R, Ketterson K, et al. Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos. *Fertil Steril*. 2011;95(3):953–8.
24. Ball CB, Rodriguez KF, Stumpo DJ, Ribeiro-Neto F, Korach KS, Blackshear PJ, et al. The RNA-binding protein, ZFP36L2, influences ovulation and oocyte maturation. *PLoS One*. 2014;9(5):e97324.
25. Prunskaitė-Hyyryläinen R, Shan J, Railo A, Heinonen KM, Miinalainen I, Yan W, et al. Wnt4, a pleiotropic signal for controlling cell polarity, basement membrane integrity, and antimüllerian hormone expression during oocyte maturation in the female follicle. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2014;28(4):1568–81.
26. Edwards RG. Maturation in vitro of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes. *Nature*. 1965;208(5008):349–51.
27. Das M, Son WY, Buckett W, Tulandi T, Holzer H. In-vitro maturation versus IVF with GnRH antagonist for women with polycystic ovary syndrome: treatment outcome and rates of ovarian hyperstimulation syndrome. *Reprod BioMed Online*. 2014;29(5):545–51.
28. Son WY, Chung JT, Dahan M, Reinblatt S, Tan SL, Holzer H. Comparison of fertilization and embryonic development in sibling in vivo matured oocytes retrieved from different sizes follicles from in vitro maturation cycles. *J Assist Reprod Genet*. 2011;28(6):539–44.
29. Shalom-Paz E, Holzer H, Young Son W, Levin I, Tan SL, Almog B. PCOS patients can benefit from in vitro maturation (IVM) of oocytes. *Eur J Obstet Gynecol Reprod Biol*. 2012;165(1):53–6.
30. Walls M, Junk S, Ryan JP, Hart R. IVF versus ICSI for the fertilization of in-vitro matured human oocytes. *Reprod BioMed Online*. 2012;25(6):603–7.
31. De Vos M, Ortega-Hrepich C, Albuz FK, Guzman L, Polyzos NP, Smits J, et al. Clinical outcome of non-hCG-primed oocyte in vitro maturation treatment in patients with polycystic ovaries and polycystic ovary syndrome. *Fertil Steril*. 2011;96(4):860–4.
32. Guzman L, Ortega-Hrepich C, Polyzos NP, Anckaert E, Verheyen G, Coucke W, et al. A prediction model to select PCOS patients suitable for IVM treatment based on anti-müllerian hormone and antral follicle count. *Hum Reprod*. 2013;28(5):1261–6.
33. Gardner DK, Schoolcraft WB, Wagley L, Schlenker T, Stevens J, Hesla J. A prospective randomized trial of blastocyst culture and transfer in in-vitro fertilization. *Hum Reprod*. 1998;13(12):3434–40.
34. Ozgur K, Humaidan P, Coetzee K. Segmented ART—the new era in ART? *Reprod Biol*. 2016;16(2):91–103.
35. Keane KN, Mustafa KB, Hinchliffe P, Conceicao J, Yovich JL. Higher beta-HCG concentrations and higher birthweights ensue from single vitrified embryo transfers. *Reprod Biomed Online*. 2016;
36. Gomaa H, Baydoun R, Sachak S, Lapana I, Soliman S. Elective single embryo transfer: is frozen better than fresh? *JBRA Assist Reprod*. 2016;20(1):3–7.
37. Guzman L, Ortega-Hrepich C, Albuz FK, Verheyen G, Devroey P, Smits J, et al. Developmental capacity of in vitro-matured human oocytes retrieved from polycystic ovary syndrome ovaries containing no follicles larger than 6 mm. *Fertil Steril*. 2012;98(2):503–7. e2
38. Nogueira D, Sadeu JC, Montagut J. In vitro oocyte maturation: current status. *Semin Reprod Med*. 2012;30(3):199–213.
39. Coticchio G, Dal-Canto M, Guglielmo MC, Mignini-Renzini M, Fadini R. Human oocyte maturation in vitro. *Int J Dev Biol*. 2012;56(10–12):909–18.
40. Benkhalifa M, Demirel A, Menezo Y, Balashova E, Abduljalil AK, Abbas S, et al. Natural cycle IVF and oocyte in-vitro maturation in polycystic ovary syndrome: a collaborative prospective study. *Reprod BioMed Online*. 2009;18(1):29–36.

41. Spits C, Guzman L, Mertzaniidou A, Jacobs K, Ortega-Hrepich C, Gilchrist RB, et al. Chromosome constitution of human embryos generated after in vitro maturation including 3-isobutyl-1-methylxanthine in the oocyte collection medium. *Hum Reprod*. 2015;30(3):653–63.
42. Fragouli E, Katz-Jaffe M, Alfarawati S, Stevens J, Colls P, Goodall NN, et al. Comprehensive chromosome screening of polar bodies and blastocysts from couples experiencing repeated implantation failure. *Fertil Steril*. 2010;94(3):875–87.
43. Zheng H, Jin H, Liu L, Liu J, Wang WH. Application of next-generation sequencing for 24-chromosome aneuploidy screening of human preimplantation embryos. *Mol Cytogenet*. 2015;8:38.
44. Mertzaniidou A, Wilton L, Cheng J, Spits C, Vanneste E, Moreau Y, et al. Microarray analysis reveals abnormal chromosomal complements in over 70% of 14 normally developing human embryos. *Hum Reprod*. 2013;28(1):256–64.
45. Mertzaniidou A, Spits C, Nguyen HT, Van de Velde H, Sermon K. Evolution of aneuploidy up to day 4 of human preimplantation development. *Hum Reprod*. 2013;28(6):1716–24.
46. Katz-Jaffe MG, Trounson AO, Cram DS. Chromosome 21 mosaic human preimplantation embryos predominantly arise from diploid conceptions. *Fertil Steril*. 2005;84(3):634–43.
47. Taylor TH, Gitlin SA, Patrick JL, Crain JL, Wilson JM, Griffin DK. The origin, mechanisms, incidence and clinical consequences of chromosomal mosaicism in humans. *Hum Reprod Update*. 2014;20(4):571–81.
48. Fragouli E, Alfarawati S, Daphnis DD, Goodall NN, Mania A, Griffiths T, et al. Cytogenetic analysis of human blastocysts with the use of FISH, CGH and aCGH: scientific data and technical evaluation. *Hum Reprod*. 2011;26(2):480–90.
49. Mantikou E, Wong KM, Repping S, Mastenbroek S. Molecular origin of mitotic aneuploidies in preimplantation embryos. *Biochim Biophys Acta*. 2012;1822(12):1921–30.
50. Gleicher N, Metzger J, Croft G, Kushnir VA, Albertini DF, Barad DH. A single trophectoderm biopsy at blastocyst stage is mathematically unable to determine embryo ploidy accurately enough for clinical use. *Reproductive biology and endocrinology : RB&E*. 2017;15(1):33.
51. Greco E, Minasi MG, Fiorentino F. Healthy babies after intrauterine transfer of mosaic aneuploid blastocysts. *N Engl J Med*. 2015;373(21):2089–90.
52. Gleicher N, Vidali A, Braverman J, Kushnir VA, Barad DH, Hudson C, et al. Accuracy of preimplantation genetic screening (PGS) is compromised by degree of mosaicism of human embryos. *Reproductive biology and endocrinology : RB&E*. 2016;14(1):54.
53. Munne S, Wells D. Detection of mosaicism at blastocyst stage with the use of high-resolution next-generation sequencing. *Fertil Steril*. 2017;107(5):1085–91.
54. Abstracts: 15th international conference on preimplantation genetic diagnosis. *Reprod Biomed Online*. 33(2):215–331.
55. Sachdev NM, Maxwell SM, Besser AG, Grifo JA. Diagnosis and clinical management of embryonic mosaicism. *Fertil Steril*. 2017;107(1):6–11.
56. Munne S, Grifo J, Wells D. Mosaicism: “survival of the fittest” versus “no embryo left behind”. *Fertil Steril*. 2016;105(5):1146–9.