Preimplantation genetic diagnosis for aneuploidy testing in women older than 44 years: a multicenter experience

Filippo Maria Ubaldi, M.D., M.Sc.,[a,b] Danilo Cimadomo, M.Sc.,[a,c] Antonio Capalbo, Ph.D.,[a,b] Alberto Vaiarelli, M.D., Ph.D.,[a,d] Laura Buffo, M.D.,[e] Elisabetta Trabucco, M.D.,[f] Susanna Ferrero, M.D.,[a] Elena Albani, M.Sc.,[g] Laura Rienzi, M.Sc.,[a,b] and Paolo E. Levi Setti, M.D.[g]

[a] GENERA Roma, Clinica Valle Giulia, Rome; [b] GENETYX, Molecular Biology Laboratory, Marostica; [c] Università di Roma "Sapienza," Dipartimento di Scienze Anatomiche, Istologiche, Medico-Legali e dell'Apparato Locomotore. Sezione Istologia ed Embriologia Medica, Rome; [d] Dipartimento di patologia umana dell'adulto e dell'età evolutiva "G. Barresi," Università degli Studi di Messina, Messina; [e] GENERA Marostica, Poliambulatorio SALUS, Marostica; [f] GENERA Napoli, Clinica Ruesch, Naples; and [g] Division of Gynaecology and Reproductive Medicine, Department of Gynaecology, Humanitas Fertility Center, Humanitas Research Hospital, Rozzano, Milan, Italy

Objective: To report laboratory and clinical outcomes in preimplantation genetic diagnosis for aneuploidies (PGD-A) cycles for women 44 to 47 years old.
Design: Multicenter, longitudinal, observational study.
Setting: In vitro fertilization (IVF) centers.
Patient(s): One hundred and thirty-seven women aged 44.7 ± 0.7 years (range: 44.0–46.7) undergoing 150 PGD-A cycles during April 2013 to January 2016.
Intervention(s): Quantitative polymerase chain reaction–based PGD-A on trophectoderm biopsies and cryopreserved euploid single-embryo transfer (SET).
Main Outcomes Measure(s): Primary outcome measure: delivery rate per cycle; secondary outcome measures: miscarriage rate, and the rate and reasons for cycle cancelation with subanalyses for female age and number of metaphase 2 oocytes retrieved.
Result(s): In 102 (68.0%) of 150 cycles blastocyst development was obtained, but only 21 (14.0%) were euploid blastocysts. The overall euploidy rate was 11.8% (22 of 187). Twenty-one SET procedures were performed, resulting in 13 clinical pregnancies, of which 1 miscarried and 12 delivered. The delivery rate was 57.1% per transfer, 8.0% per cycle, and 8.8% per patient. The logistic regression analysis found that only female age (odds ratio 0.78) and number of metaphase 2 oocytes retrieved (odds ratio 1.25) statistically significantly correlated with the likelihood of delivery. The delivery rate per cycle was 10.6% (11 of 104) in patients aged 44.0 to 44.9 years and 2.6% in patients aged 45.0 to 45.9 years (n = 1 of 38). No euploid blastocysts were found for patients older than 45.0 years.
Conclusion(s): Extensive counseling based on biological and clinical data should be provided to women older than 43 years who are requesting IVF because of their very low odds of success and high risk for embryonic aneuploidies. Nevertheless, the low miscarriage and good delivery rates reported in this study in women with good ovarian reserve aged 44 should encourage the use of PGD-A in this population. (Fertil Steril® 2017;107:1173–80. ©2017 by American Society for Reproductive Medicine.)
Key Words: Advanced maternal age, IVF, PGD-A, PGS, poor prognosis

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Defining the cutoff age beyond which an in vitro fertilization (IVF) attempt should be discouraged remains a controversial issue in assisted reproductive technology. Several aspects that could affect clinical decision-making should be considered. Indeed, the first consideration is whether the decision should be the patient's or the physician's. The social and legal aspects of this topic go beyond embryologic, genetic, and clinical outcomes, especially because to date no strict guidelines have been issued from the international scientific

Received January 13, 2017; revised February 20, 2017; accepted March 7, 2017.
F.M.U. has nothing to disclose. D.C. has nothing to disclose. A.C. has nothing to disclose. A.V. has nothing to disclose. L.B. has nothing to disclose. E.T. has nothing to disclose. S.F. has nothing to disclose. E.A. has nothing to disclose. L.R. has nothing to disclose. P.E.L.S. has nothing to disclose.
Reprint requests: Filippo Maria Ubaldi, M.D., M.Sc., Clinica Valle Giulia, G.E.N.E.R.A., Via G de Notaris, 2b, Roma 00197, Italy (E-mail: ubaldi.fm@gmail.com).

Fertility and Sterility® Vol. 107, No. 5, May 2017 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2017.03.007

societies. In this regard, it is pivotal to provide patients with specific counseling focused on the evidence reported in the literature so they can make an informed choice.

Very few studies have investigated this topic. Spandorfer et al. (1) reported that approximately 20% of patients older than 44 in their cohort (n = 288) did not even start the treatment due to high follicle-stimulating hormone (FSH) levels and/or the presence of a cyst, and more than 30% were stopped before egg retrieval. The 85.3% pregnancy loss after embryo replacement resulted in a delivery rate per egg retrieval of approximately 3%. Importantly, only patients aged up to 45 years and with a good ovarian reserve could undergo an embryo replacement and try to achieve a full-term pregnancy.

Klipstein et al. (2) reported a cumulative live-birth rate of about 5% in patients older than 43 years, but no pregnancy was reported in patients aged up to 46. Hourvitz et al. (3) showed only one delivery from their data set in patients older than 43 (1.9% clinical pregnancy rate), thus concluding that IVF should not be proposed in this population of patients, especially if characterized by a scarce ovarian reserve. Bar-Hava et al. (4) instead stressed the importance of extensive counseling and the possibility of an egg donation before attempting an IVF cycle in patients aged 44 to 47. In fact, they reported just one delivery out of 12 embryos transferred, despite 9 of them being high quality.

Gunnala et al. (5) defined the outcomes in patients older than 43 when five to eight cleavage-stage embryos had been transferred. The live-birth rate per transfer was 9.4% and 1.3% in women aged 44 and 45, respectively, with a multiple pregnancy rate of 6.7% in the former group of patients. Gleicher et al. (6) reported live-birth rates of 1.4% and 2.7% in patients aged 44 and 45, respectively. And, finally, Alasmari et al. (7) reported an 8% pregnancy rate in patients of 44 years old after cleavage-stage embryo transfer, which decreased to 6% in patients 45 years old. Because the pregnancy rate was independent of the number of embryos transferred, they concluded that single-embryo transfer (SET) is reasonable in this population of patients. No pregnancies were reported for women aged 46.

Importantly, the miscarriage rate in patients older than 43 in cases of implantation is higher than 70% to 75% (8), and the risk for a full-term chromosomally abnormal pregnancy is about 5%. These are direct consequences of the high aneuploidy rate observed in the blastocysts produced from patients older than 44, which is higher than 80% as reported by Franasiak et al. (9) through the quantitative polymerase chain reaction (qPCR)–based analysis of more than 15,000 trophectoderm biopsies. Thus, careful considerations are required when suggesting an IVF treatment in patients of extremely advanced reproductive age to mediate the risks of implantation failure, miscarriage, and chromosomally abnormal pregnancies and the likelihood of conceiving a chromosomally normal baby.

Since 2013, in our centers a policy that entails the use of blastocyst culture, qPCR-based aneuploidy testing on trophectoderm biopsies, vitrification, and elective SET has been systematically implemented. By retrospectively investigating the outcomes between the period in which these advances were systematically used in comparison with the period when untested cleavage-stage double-embryo transfer was routinely performed, we reported a similar cumulative live-birth rate per cycle together with statistically significantly decreased miscarriage and multiple pregnancy rates (10). Furthermore, it has been extensively reported how miscarriages and trisomic pregnancies can be minimized by the systematic application of blastocyst preimplantation genetic diagnosis for aneuploidies (PGD-A). These data led us to confidently adopt PGD-A in the patient population of extremely advanced maternal age (AMA) with the aim of producing as much evidence as possible to confidently evaluate all the risks and the benefits underlying each IVF treatment. These data are urgently needed and will represent another pivotal tool for properly counseling these patients in making an informed choice.

MATERIALS AND METHODS

Patient Population

Our longitudinal observational study included 137 patients older than 43 years (range: 44.0–46.7) who were undergoing an IVF cycle at the GENERA centers for reproductive medicine (Rome, Naples, and Marostica; n = 87, 19, and 15, respectively) or the Humanitas Fertility Center in Rozzano (n = 16) between April 2013 and January 2016 (Table 1). All these patients underwent ovarian reserve evaluation, coagulation screening (Leyden factor II and V and homocysteine, protein S, protein C, and antithrombin III), autoantibodies (anticardiolipin, lupus anticoagulant, antinuclear antibodies, anti-smooth muscle antibody), and thyroid function analyses as well as hysteroscopy, and they exhibited no specific risk factors. Their ovarian reserve was assessed by FSH, antral follicle count (AFC), and antimüllerian hormone (AMH) evaluation in the early follicular phase (second/third day of the menstrual cycle). Only patients with at least three antral follicles on the day before starting the stimulation protocol and no history of previous no response to the controlled ovarian stimulation were included.

We defined the main exclusion criterion based on the AFC because it is the only valuable predictor of the ovarian reserve that has been consistently reported by several studies in the literature (11–15). The main additional exclusion criteria were positive serology for hepatitis B or C or human immune deficiency virus; monogenic diseases or chromosomal structural abnormalities; maternal diseases that are not clinically stable and are known to impact the ability to conceive and/or bring a pregnancy to term; a body mass index >30; and uncontrolled hypertension.

Patients were provided with an extensive counseling about their putative very low chance of achieving a live birth and the related risks. They made an informed decision to undergo a PGD-A cycle. The institutional review boards of Clinica Valle Giulia and Humanitas Fertility Center approved the study.

We performed 150 PGD-A cycles (Table 2). The applicable infertility factors beyond AMA were male factor infertility, defined as low total number of spermatozoa (<15 million/ejaculate), sperm motility <40%, and normal morphology

TABLE 1

Patient data in study of preimplantation genetic diagnosis for aneuploidy testing cycles.

Parameter	Value
No. of patients	137
Female partner age (y)	44.7 ± 0.7 (44.0–46.7)
Male partner age (y)	45.4 ± 4.9 (31.0–60.0)
Kind of infertility, n (%)	
Primary	90 (65.7)
Secondary	47 (34.3)
Main infertility factor, n (%)	
Only female age	109 (79.6)
Female age + male factor	15 (10.9)
Female age + tubal factor	6 (4.4)
Female age + multiple miscarriages	7 (5.1)
Duration of infertility (y)	3.6 ± 2.7 (0–13)
BMI	20.9 ± 3.6 (17.3–26.6)
Hormones	
FSH (mIU/mL)	9.0 ± 5.7 (3.2–39.0)
LH (mIU/mL)	6.1 ± 3.8 (1.2–20.0)
AMH (ng/mL)	1.2 ± 0.7 (0.3–6.7)
Previous live term births	0.1 ± 0.3 (0–2)
Previous IVF cycles	1.0 ± 1.0 (0–5)
Previous ETs	1.0 ± 1.1 (0–7)
Previous miscarriages	0.4 ± 0.8 (0–4)
Previous implantation failures	0.5 ± 1.0 (0–6)
Sperm factor, n (%)	
Normozoospermic	86 (62.8)
Single/double defect	36 (26.3)
OAT or surgical	15 (10.9)
Indication to PGD-A, n (%)	
Only AMA	120 (87.6)
AMA + RIF	9 (6.6)
AMA + RPL	8 (5.8)

Note: Values are mean ± standard deviation (range) unless otherwise indicated. AMA = advanced maternal age; AMH = antimüllerian hormone; BMI = body mass index; ET = embryo transfer; FSH = follicle-stimulating hormone; IVF = in vitro fertilization; LH = luteinizing hormone; OAT = oligoasthenoteratozoospermia; PGD-A = preimplantation genetic diagnosis for aneuploidy testing; RIF = recurrent implantation failure; RPL = recurrent pregnancy loss.

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TABLE 2

Clinical data in patients undergoing preimplantation genetic diagnosis for aneuploidy testing.

Parameter	Value
PGD-A cycles	150
COCs[a]	1,141; 7.5 ± 5.3 (1–26)
MII oocytes[a]	876; 5.8 ± 4.4 (0–24)
Zygotes[a]	631; 4.3 ± 3.3 (0–19)
Biopsied blastocysts[a]	187; 1.0 ± 1.3 (0–6)
PGD-A results	
Aneuploid blastocysts	165 (88.2, 83.6–92.8)
Euploid blastocysts	22 (11.8, 7.2–16.4)
No. of PGD-A cycles canceled	
Reason for cancellation	129 (86.0, 80.5–91.6)
None MII oocytes produced	5 (3.3, 0.44–6.16)
None zygotes produced	5 (3.3, 0.44–6.16)
None blastocysts produced	38 (25.3, 18.3–32.3)
None euploid blastocysts produced	81 (54.0, 46.0–61.8)
Frozen single euploid blastocyst transfers (n)	21
Clinical pregnancies (n)	13
Per transfer	13/21 (61.9, 41.1–82.7)
Per cycle	13/150 (8.7, 4.7–14.3)
Per patient	13/137 (9.5, 5.2–15.7)
No. of miscarriages (<20 wk)	1 (7.7, 0–22.2)
No. of deliveries	12
Per transfer	12/21 (57.1, 35.9–78.3)
Per cycle	12/150 (8.0, 3.7–12.3)
Per patient	12/137 (8.8, 4.1–13.5)

Note: Values are number, percentage, and 95% confidence interval unless otherwise indicated. COC = cumulus-oocyte complexes; MII = metaphase 2; PGD-A = preimplantation genetic diagnosis for aneuploidy testing.

[a] Values are number, mean ± standard deviation (range).

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<4%; tubal factor infertility, defined as blocked or damaged fallopian tubes; and multiple miscarriages, defined as at least three previous pregnancy losses.

Ovarian Stimulation

Before starting the controlled ovarian stimulation, we performed an ultrasound scan to check the number of follicles smaller than 10 mm. If we observed at least three follicles, a flexible gonadotropin-releasing hormone antagonist protocol with 300 IU of recombinant FSH + 75–150 IU recombinant luteinizing hormone (LH) was performed (16, 17). We administrated the gonadotropin-releasing hormone antagonist either when at least one of the follicles reached 12 mm of diameter or depending on the level of estrogen (≥200 pg/mL) (18).

Laboratory Procedures

Oocyte collection and denudation were performed as already described elsewhere (19). Metaphase 2 (MII) oocytes were subjected to intracytoplasmic sperm injection (ICSI) between 36 and 38 hours after human chorionic gonadotropin administration, as described by Rienzi et al. (20). At 16 to 18 hours after ICSI was performed, the pronuclei presence was assessed. Zygotes displaying two pronuclei were cultured further in separate 25-μL microdrops (CSCM; Irvine, Australia) up to the blastocyst stage (days 5–7) in a humidified atmosphere containing 5% O_2 and 6% CO_2. Embryos underwent biopsy of trophectoderm cells once they reached the fully expanded blastocyst stage, through a previously described method that does not entail any hatching procedure in day 3 (21), and were vitrified soon after according to a validated protocol (22–24). Comprehensive chromosome testing was conducted by qPCR (25–27) at the GENETYX molecular biology laboratory. Euploid blastocysts were selected for SET, and were warmed and cultured at 37°C (6% CO_2 and 5% O_2) 2 hours before replacement. Endometrial preparation and transfer procedures were performed as previously described elsewhere (28).

Outcome Measures and Statistical Analysis

This is a longitudinal observational study with the live-birth rate per started treatment as the primary outcome measure. The main secondary outcomes were biochemical pregnancy loss, miscarriage, and chromosomally abnormal pregnancy rates. Clinical pregnancy was defined as the presence of a gestational sac and fetal heartbeat at week 7 after transfer.

A pregnancy loss between weeks 7 and 20 was defined as a miscarriage. The delivery rate was expressed on a per-transfer, per-cycle, and per-patient basis. Subanalyses of both the embryologic and clinical outcomes per female age at oocyte retrieval (range: 44.0–44.9, 45.0–45.9, and 46.0–46.9) and number of MII oocytes retrieved (range: 1–5, 6–10, and >10) were also performed.

Categorical variables are shown as percentages with 95% confidence interval (CI), and continuous variables as mean ± standard deviation. Logistic regression analysis was used to investigate basal and cycle variables related to the likelihood of achieving a live birth per started treatment. The statistical analysis was conducted through a two-tailed Fisher's exact test. $P \leq .05$ was considered statistically significant.

RESULTS

One hundred and thirty-seven patients older than 44 started a PGD-A cycle, and 13 patients tried a second time after the failure of the first cycle. The patients' basal characteristics are reported in Table 1. In total, 150 PGD-A cycles were performed. A mean number of 5.8 ± 4.4 MII oocytes (range: 0–24) were retrieved, and 1.0 ± 1.3 blastocysts were obtained (range: 0–6). Specifically, in 102 (68.0%) of 150 cycles (95% CI, 60.5%–75.5%) at least one blastocyst was obtained; however, in only 21 (14.0%) of 150 cycles was a transferable embryo obtained (95% CI, 85%–19.6%) (Tables 2 and 3). The overall euploidy rate of the 187 blastocysts obtained was 11.8% (n = 22 of 187; 95% CI, 7.2%–16.4%) (Table 2). Twenty-one euploid blastocysts were transferred that resulted in one miscarriage (7.7%; 95% CI, 0–22.2%) and 12 deliveries of healthy babies (57.1%, 8.0%, and 8.8%, on a per transfer, per cycle, and per patient basis, respectively; 95% CI, 35.9%–78.3%, 3.7%–12.3%, and 4.1%–13.5%) (Table 2).

A logistic regression analysis was performed to investigate how predictive the basal and cycle variables on the primary outcome were as measures—that is, the delivery rate per treatment. The IVF center was used as a covariate to adjust for specific intrinsic confounding factors related to each setting. Female age and number of MII retrieved were the only factors statistically significantly associated with the likelihood of achieving a live birth per started cycle. Specifically, female age was a negative predictor with an odds ratio of 0.78 (95% CI, 0.69–0.86; $P=.04$), and the number of MII collected at oocyte pickup was a positive predictor with an odds ratio of 1.24 (95% CI, 1.0–1.54; $P=.05$) (Supplemental Table 1). With this evidence we performed a post hoc subanalysis of the data per female age at egg retrieval and per number of MII oocytes collected.

Laboratory and clinical outcomes per female age at egg retrieval (range: 44.0–44.9, 45.0–45.9, 46.0–46.9) are shown in Table 3. The fertilization rate was statistically significantly related to female age (44.0–44.9 vs. 46.0–46.9: $P=.003$; 45.0–45.9 vs. 46.0–46.9: $P=.01$; Table 3); no differences have been instead reported for the blastocyst formation, euploidy, and delivery rates. Specifically, no euploid blastocyst was found in the eight PGD-A cycles undertaken by patients older than 46, whereas the euploidy rate was 14.4% (n = 20 out of 139 blastocysts; 95% CI, 8.6%–20.2%) and 4.5% (n = 2 out of 44 blastocysts; 95% CI, 0–10.6%) in the group of patients aged 44.0–44.9 and 45.0–45.9, respectively (not statistically significant [NS]; Table 3). Eleven of the 12 deliveries occurred in the former group of patients, which translates as a 10.6% (n = 11 out of 104; 95% CI, 4.7%–16.5%) delivery rate per cycle versus 2.6% in the latter group (n = 1 out of 38; 95% CI, 0–7.7%) (NS; Table 3).

Table 4 shows the main embryologic and clinical outcomes per the number of MII oocytes retrieved (range: 1–5, 6–10, and >10). The mean age in the three groups was almost the same (Table 4). No differences were found in the fertilization, blastocyst, or euploidy rates (Table 4). Indeed, the rates for cycles in which a blastocyst (53.2%, 90.2%, and 92.0% in the ranges 1–5, 6–10, and >10 MII oocytes, respectively; 95% CI, 42.2%–64.2%, 81.1%–99.3%, 81.4%–100%; 1–5 MII oocytes vs. 6–10 or >10 MII oocytes: $P<.001$), a euploid blastocyst (7.6%, 17.1%, and 32.0%; 95% CI, 1.8%–13.4%, 5.6%–28.6%, 13.7%–50.3%; 1–5 MII oocytes vs. >10 MII oocytes: $P=.004$), and a delivery (3.8%, 7.3% and 24.0%; 95% CI, 0%–8.0%, 0–15.3%, 7.3%–40.7%; $P=.05$) was obtained statistically significantly increased as a function of the number of mature oocytes retrieved (Table 4).

Figure 1 is a dispersion graph that displays the distribution of aneuploid and euploid blastocysts and pregnancies with respect to both the woman's age at oocyte retrieval and the number of MII oocytes collected. It clearly shows that even though blastocysts can be obtained beyond the age of 45.0 and starting from a single/few MII oocyte(s), none of them was euploid. Indeed, most of the pregnancies cluster in the range of 44.0–45.0 years and are highly correlated to the number of MII oocytes collected.

DISCUSSION

The treatment of very poor prognosis patients, such as women older than 43, is a controversial issue for which a general consensus has yet to be reached (29). However, some guidelines in this regard are needed; the American Society for Reproductive Medicine ethics committee has recently stated that futile treatments should not be provided "solely for clinic's own financial benefit" (30). Our study produces solid evidence for the realistic possibility of pregnancy and associated gestational risks in patients older than 43 undergoing PGD-A. This may be particularly useful for guiding the counseling of couples toward treatment. Whether it is a clinician, a patient, or an ethics committee making the decision, it should be based on reliable, up-to-date biological and clinical data.

In the AMA patient population, the influence of maternally derived aneuploidies on infertility, miscarriage, or a chromosomally abnormal pregnancy is massive, and both the safety of the procedure and the reduction of the time to pregnancy—if attempting conception with the patient's own eggs—are of foremost importance. In this respect, the goal of using PGD-A is to avoid the useless transfer of aneuploid embryos and to limit the incidences of miscarriage and chromosomally abnormal pregnancies. Embryo-selection parameters other than aneuploidy testing are limited. In fact, even though excellent/good quality embryos can be obtained in patients older than 44 as well, this is still not proof of high

TABLE 3

Embryologic and clinical data per age of women at egg retrieval.

	44.0–44.9 y 104 PGD-A cycles		45.0–45.9 y 38 PGD-A cycles		46.0–46.9 y 8 PGD-A cycles		Overall 150 PGD-A cycles		
Variable	**n (%)**	**95% CI**	**n (%)**	**95% CI**	**n (%)**	**95% CI**	**n (%)**	**95% CI**	***P* value**
Rate of cycles with MII oocytes retrieved (no. of cycles with MII oocytes retrieved/cycles)	99/104 (95.2)	91.1–99.3	38/38 (100.0)		8/8 (100.0)	–	145/150 (96.7)	93.8–99.6	NS
Fertilization rate (no. of zygotes/MII oocytes)	440/596 (73.8)	70.3–77.3	176/249 (70.7)	65.1–76.4	15/31 (48.4)	30.8–66.0	631/876 (72.0)	69.0–75.0	.003[a] .01[b] NS[c]
Rate of cycles with zygotes (no. of cycles with zygotes/cycles)	96/104 (92.3)	87.2–97.4	37/38 (97.4)	92.3–100	7/8 (87.5)	64.6–100	140/150 (93.3)	89.3–97.3	NS
Blastocyst formation rate (no. of blastocysts/ zygotes)	139/440 (31.6)	27.6–36.0	44/176 (25.0)	18.6–31.4	4/15 (26.7)	4.3–49.0	187/631 (29.6)	26.0–33.2	NS
Rate of cycles with blastocysts (no. of cycles with blastocysts/cycles)	71/104 (68.3)	59.4–77.2	28/38 (73.7)	59.7–87.7	3/8 (37.5)	4.0–71.1	102/150 (68.0)	60.5–75.5	NS
Euploidy rate (no. of euploid blastocysts/blastocysts)	20/139 (14.4)	8.56–20.2	2/44 (4.5)	0–10.6	0/4 (0)	–	22/187 (11.8)	7.2–16.4	NS
Rate of cycles with euploid blastocysts (no. of cycles with euploid blastocysts/ cycles)	19/104 (18.3)	10.9–25.7	2/38 (5.3)	0–12.4	0/8 (0)	–	21/150 (14.0)	8.5–19.6	NS
Rate of cycles with a delivery (no. of cycles with a delivery/cycles)	11/104 (10.6)	4.7–16.5	1/38 (2.6)	0–7.7	0/8 (0)	–	12/150 (8.0)	3.7–12.3	.04

Note: CI = confidence interval; MII = metaphase 2; NS = not statistically significant; PGD-A = preimplantation genetic diagnosis for aneuploidy testing.
[a] 44.0–44.9 y, 104 PGD-A cycles versus 46.0–46.9 y, 8 PGD-A cycles.
[b] 45.0–45.9 y, 38 PGD-A cycles versus 46.0–46.9 y, 8 PGD-A cycles.
[c] 44.0–44.9 y, 104 PGD-A cycles versus 45.0–45.9 y, 38 PGD-A cycles.

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TABLE 4

Embryologic data and clinical data per number of metaphase 2 oocytes retrieved.

	1–5 MII oocytes 79 PGD-A cycles mean age (y): 44.8 ± 0.8		6–10 MII oocytes 41 PGD-A cycles mean age (y): 44.7 ± 0.6		>10 MII oocytes 25 PGD-A cycles mean age (y): 44.6 ± 0.5		Overall 145 PGD-A cycles		
Variable	**n (%)**	**95% CI**	**n (%)**	**95% CI**	**n (%)**	**95% CI**	**n (%)**	**95% CI**	***P* value**
Fertilization rate (no. of zygotes/MII oocytes)	173/234 (73.9)	68.3–79.5	229/310 (73.9)	69.0–78.8	229/332 (69.0)	64.0–74.0	631/876 (72.0)	69.0–75.0	NS
Rate of cycles with zygotes (no. of cycles with zygotes/cycles)	74/79 (93.7)	88.3–99.1	41/41 (100.0)	–	25/25 (100.0)	–	140/145 (96.6)	92.0–98.7	NS
Blastocyst formation rate (no. of blastocysts/ zygotes)	58/173 (33.5)	26.5–40.5	73/229 (31.9)	25.9–38.0	56/229 (24.5)	18.9–30.1	187/631 (29.6)	26.0–33.2	NS
Rate of cycles with blastocysts (no. of cycles with blastocysts/cycles)	42/79 (53.2)	42.2–64.2	37/41 (90.2)	81.1–99.3	23/25 (92.0)	81.4–100	102/145 (70.3)	62.5–77.2	<.001[a] <.001[b] NS[c]
Euploidy rate (no. of euploid blastocysts/blastocysts)	7/58 (12.1)	3.7–20.5	6/73 (8.2)	1.9–14.5	9/56 (16.1)	6.5–25.7	22/187 (11.8)	7.2–16.4	NS
Rate of cycles with euploid blastocysts (no. of cycles with euploid blastocysts/ cycles)	6/79 (7.6)	1.8–13.4	7/41 (17.1)	5.6–28.6	8/25 (32.0)	13.7–50.3	21/145 (14.5)	9.6–21.2	NS[a] .004[b] NS[c]
Rate of cycles with a delivery (no. of cycles with a delivery/cycles)	3/79 (3.8)	0–8.0	3/41 (7.3)	0–15.3	6/25 (24.0)	7.3–40.7	12/145 (8.3)	4.7–14.0	.05

Note: CI = confidence interval; MII = metaphase 2; NS = not statistically significant; PGD-A = preimplantation genetic diagnosis for aneuploidy testing.

[a] 1–5 MII oocytes, 79 PGD-A cycles versus 6–10 MII oocytes, 41 PGD-A cycles.

[b] 1–5 MII oocytes, 79 PGD-A cycles versus >10 MII oocytes, 25 PGD-A cycles.

[c] 6–10 MII oocytes, 41 PGD-A cycles versus >10 MII oocytes, 25 PGD-A cycles.

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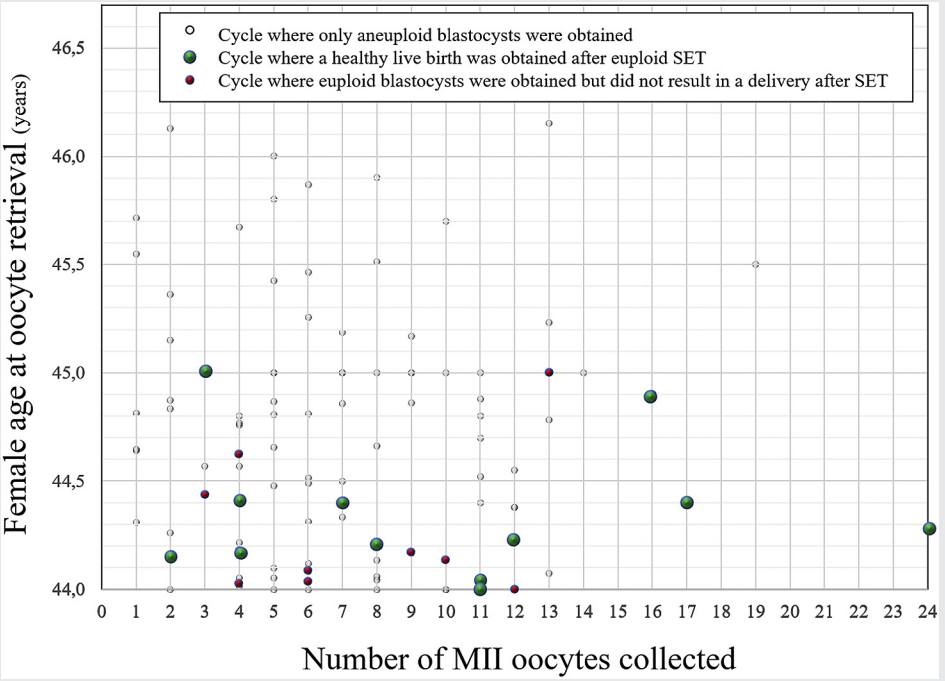
FIGURE 1



Dispersion graph from a multicenter, longitudinal observational study correlating the possibility of obtaining blastocysts, euploid blastocysts, and healthy babies after euploid single-embryo transfer (SET) with both female age at oocyte retrieval and number of metaphase 2 (MII) oocytes collected. No euploid blastocysts were obtained beyond the age of 45, and all the full-term pregnancies clustered between the ages of 44 and 45 years. The probability of euploid blastocysts increased together with the number of MII oocytes collected.

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reproductive competence (4, 21). In this scenario and to our knowledge, ours is the first study to be focused on blastocyst stage PGD-A cycles conducted in this population of patients.

In our data set, approximately 70% of the patients obtained at least one blastocyst, also among those aged 46.0–46.9, but only approximately 15% had transferable embryos after PGD-A. However, if one euploid blastocyst was transferred, then the live-birth rate per SET was >50%, as reported in the literature for the female patient population across the age board (young or AMA, good or poor prognosis) after PGD-A cycles (31). These data overall translated into a live-birth rate per cycle of 8%, which represents the efficacy of IVF in this population of patients even in previous reports (1–7). Even so, if an analysis is then performed by clustering the couples in three groups per woman's age at egg retrieval, it becomes evident that most of these deliveries (11 of 12) derive from patients aged 44.0–44.9, even if no other difference has been registered in terms of fertilization and blastocyst formation rates, versus patients aged 45.0–45.9. Notably, some statistically significant differences were reported in the fertilization rate between patients aged 46.0–46.9 and younger ones, suggesting a putative fall of egg quality after the threshold of 45.9 years. It is important that, when clustering the cycles instead per the number of MII oocytes retrieved, some interesting statistically significant differences emerged. Accepting that the embryologic parameters remain constant, the possibility of finding a blastocyst, a euploid blastocyst, and finally obtaining a live birth increase as a function of the number of mature eggs obtained (Table 4).

The ovarian reserve thus represents the most important limiting factor in the decision of whether to perform an IVF treatment, especially in this population of patients. Specifically, we found that when more than 10 oocytes were retrieved in women aged 44, the delivery rate per cycle and per transfer were as high as 24.0% (n = 6 of 25) and 75.0% (n = 6 of 8), respectively. These findings suggest that oocyte accumulation could be beneficial for this population of patients. In this regard, either multiple oocyte retrievals after consecutive conventional ovarian stimulation cycles (32) or the DuoStim strategy (oocyte retrievals after both follicular and luteal phase stimulations within the same menstrual cycle) (33) may be adopted. However, the cost-effectiveness of both these approaches still needs to be investigated. Nevertheless, no euploid blastocysts were found after the age of 45, which is strong evidence that this as a proper specific maximum threshold for egg genetic (and thus reproductive) competence and fertility in women.

To conclude, this study provides important biological and clinical data from chromosomal embryo assessments and related IVF outcomes in cycles performed in women older than 43. We found a very high risk for embryonic aneuploidies and the minimal/null odds of success in patients

aged 45 and 46. On the other hand, the low miscarriage and good delivery rates we found in women with good ovarian reserve aged 44 encourages the use of PGD-A in this population.

REFERENCES

1. Spandorfer SD, Bendikson K, Dragisic K, Schattman G, Davis OK, Rosenwaks Z. Outcome of in vitro fertilization in women 45 years and older who use autologous oocytes. Fertil Steril 2007;87:74–6.
2. Klipstein S, Regan M, Ryley DA, Goldman MB, Alper MM, Reindollar RH. One last chance for pregnancy: a review of 2,705 in vitro fertilization cycles initiated in women age 40 years and above. Fertil Steril 2005;84:435–45.
3. Hourvitz A, Machtinger R, Maman E, Baum M, Dor J, Levron J. Assisted reproduction in women over 40 years of age: how old is too old? Reprod Biomed Online 2009;19:599–603.
4. Bar-Hava I, Ferber A, Ashkenazi J, Dicker D, Ben-Rafael Z, Orvieto R. Natural-cycle in vitro fertilization in women aged over 44 years. Gynecol Endocrinol 2000;14:248–52.
5. Gunnala V, Reichman DE, Meyer L, Davis OK, Rosenwaks Z. Beyond the American Society for Reproductive Medicine transfer guidelines: how many cleavage-stage embryos are safe to transfer in women ≥43 years old? Fertil Steril 2014;102:1626–32.e1.
6. Gleicher N, Kushnir VA, Weghofer A, Barad DH. The "graying" of infertility services: an impending revolution nobody is ready for. Reprod Biol Endocrinol 2014;12:63.
7. Alasmari NM, Son WY, Dahan MH. The effect on pregnancy and multiples of transferring 1–3 embryos in women at least 40 years old. J Assist Reprod Genet 2016;33:1195–202.
8. Heffner LJ. Advanced maternal age—how old is too old? N Engl J Med 2004; 351:1927–9.
9. 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 2014;101:656–63.e1.
10. Ubaldi FM, Capalbo A, Colamaria S, Ferrero S, Maggiulli R, Vajta G, et al. Reduction of multiple pregnancies in the advanced maternal age population after implementation of an elective single embryo transfer policy coupled with enhanced embryo selection: pre- and post-intervention study. Hum Reprod 2015;30:2097–106.
11. Al-Azemi M, Killick SR, Duffy S, Pye C, Refaat B, Hill N, et al. Multi-marker assessment of ovarian reserve predicts oocyte yield after ovulation induction. Hum Reprod 2011;26:414–22.
12. Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. Hum Reprod Update 2006;12:685–718.
13. Rustamov O, Smith A, Roberts SA, Yates AP, Fitzgerald C, Krishnan M, et al. Anti-Mullerian hormone: poor assay reproducibility in a large cohort of subjects suggests sample instability. Hum Reprod 2012;27:3085–91.
14. Rustamov O, Smith A, Roberts SA, Yates AP, Fitzgerald C, Krishnan M, et al. The measurement of anti-Mullerian hormone: a critical appraisal. J Clin Endocrinol Metab 2014;99:723–32.
15. Nelson SM. Biomarkers of ovarian response: current and future applications. Fertil Steril 2013;99:963–9.
16. Alviggi C, Mollo A, Clarizia R, De Placido G. Exploiting LH in ovarian stimulation. Reprod Biomed Online 2006;12:221–33.
17. Lehert P, Kolibianakis EM, Venetis CA, Schertz J, Saunders H, Arriagada P, et al. Recombinant human follicle-stimulating hormone (r-hFSH) plus recombinant luteinizing hormone versus r-hFSH alone for ovarian stimulation during assisted reproductive technology: systematic review and meta-analysis. Reprod Biol Endocrinol 2014;12:17.
18. Tarlatzis BC, Fauser BC, Kolibianakis EM, Diedrich K, Rombauts L, Devroey P. GnRH antagonists in ovarian stimulation for IVF. Hum Reprod Update 2006; 12:333–40.
19. Capalbo A, Bono S, Spizzichino L, Biricik A, Baldi M, Colamaria S, et al. Sequential comprehensive chromosome analysis on polar bodies, blastomeres and trophoblast: insights into female meiotic errors and chromosomal segregation in the preimplantation window of embryo development. Hum Reprod 2013;28:509–18.
20. Rienzi L, Ubaldi F, Anniballo R, Cerulo G, Greco E. Preincubation of human oocytes may improve fertilization and embryo quality after intracytoplasmic sperm injection. Hum Reprod 1998;13:1014–9.
21. Capalbo A, Rienzi L, Cimadomo D, Maggiulli R, Elliott T, Wright G, et al. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts. Hum Reprod 2014;29:1173–81.
22. Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. Theriogenology 2007;67: 73–80.
23. Kuwayama M, Vajta G, Ieda S, Kato O. Comparison of open and closed methods for vitrification of human embryos and the elimination of potential contamination. Reprod Biomed Online 2005;11:608–14.
24. Cobo A, de los Santos MJ, Castello D, Gamiz P, Campos P, Remohi J. Outcomes of vitrified early cleavage-stage and blastocyst-stage embryos in a cryopreservation program: evaluation of 3,150 warming cycles. Fertil Steril 2012;98:1138–46.e1.
25. Capalbo A, Treff NR, Cimadomo D, Tao X, Upham K, Ubaldi FM, et al. Comparison of array comparative genomic hybridization and quantitative real-time PCR-based aneuploidy screening of blastocyst biopsies. Eur J Hum Genet 2015;23:901–6.
26. Treff NR, Scott RT Jr. Four-hour quantitative real-time polymerase chain reaction-based comprehensive chromosome screening and accumulating evidence of accuracy, safety, predictive value, and clinical efficacy. Fertil Steril 2013;99:1049–53.
27. Treff NR, Tao X, Ferry KM, Su J, Taylor D, Scott RT Jr. Development and validation of an accurate quantitative real-time polymerase chain reaction-based assay for human blastocyst comprehensive chromosomal aneuploidy screening. Fertil Steril 2012;97:819–24.
28. Ubaldi F, Anniballo R, Romano S, Baroni E, Albricci L, Colamaria S, et al. Cumulative ongoing pregnancy rate achieved with oocyte vitrification and cleavage stage transfer without embryo selection in a standard infertility program. Hum Reprod 2010;25:1199–205.
29. Klitzman RL. How old is too old? Challenges faced by clinicians concerning age cutoffs for patients undergoing in vitro fertilization. Fertil Steril 2016; 106:216–24.
30. Ethics Committee of American Society for Reproductive Medicine. Fertility treatment when the prognosis is very poor or futile: a committee opinion. Fertil Steril 2012;98:e6–9.
31. Lee E, Illingworth P, Wilton L, Chambers GM. The clinical effectiveness of preimplantation genetic diagnosis for aneuploidy in all 24 chromosomes (PGD-A): systematic review. Hum Reprod 2015;30:473–83.
32. Cobo A, Garrido N, Crespo J, Jose R, Pellicer A. Accumulation of oocytes: a new strategy for managing low-responder patients. Reprod Biomed Online 2012;24:424–32.
33. Ubaldi FM, Capalbo A, Vaiarelli A, Cimadomo D, Colamaria S, Alviggi C, et al. Follicular versus luteal phase ovarian stimulation during the same menstrual cycle (DuoStim) in a reduced ovarian reserve population results in a similar euploid blastocyst formation rate: new insight in ovarian reserve exploitation. Fertil Steril 2016;105:1488–95.e1.