Autologous mitochondrial transfer as a complementary technique to intracytoplasmic sperm injection to improve embryo quality in patients undergoing in vitro fertilization—a randomized pilot study

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Objective: To study if autologous mitochondrial transfer (AUGMENT) improves outcome in patients with previously failed in vitro fertilization (IVF).
Design: Randomized, controlled, triple-blind, experimental study.
Setting: Private infertility center, Valencian Institute of Infertility (IVI-RMA), Valencia, Spain.
Patient(s): Infertile women $\leq$42 years of age, body mass index $<$30 kg/m^2, antimüllerian hormone $\geq$4 pmol/L, $>$5 million/mL motile sperm, at least one previous IVF with at least five metaphase oocytes (MIIs) collected, and low embryo quality.
Interventions(s): An ovarian cortex biopsy was performed to isolate egg precursor cells to obtain their mitochondria. Sibling MIIs were randomly allocated to AUGMENT (experimental) or intracytoplasmic sperm injection (Control). In AUGMENT, mitochondrial suspension was injected along with the sperm. Viable blastocysts from both groups were biopsied for preimplantation genetic testing for aneuploidy.
Main Outcome Measure(s): Pregnancy, embryo quality.
Result(s): An interim analysis was conducted. The patients' mean age was 36.3 $\pm$ 3.6 years, and they had an average of 2.5 $\pm$ 1.5 previous IVF cycles. Two of the 59 enrolled patients spontaneously conceived (one miscarried). Fifty-seven patients had ovarian biopsies and underwent stimulation. Oocyte retrieval was performed in 56 patients (premature ovulation; n = 1). A total of 253 MIIs were inseminated in AUGMENT and 250 in Control; fertilization rates were 62.7 $\pm$ 30.0% and 68.7 $\pm$ 29.1%, respectively. Statistical differences were observed in day 5 blastocyst formation rates (23.3 $\pm$ 32.0% vs. 41.1 $\pm$ 36.9%). Neither the euploid rate per biopsied blastocyst (43.8 $\pm$ 41.7% vs. 63.8 $\pm$ 44.1%) nor the euploid rate per MII (9.8 $\pm$ 20.5% vs. 11.9 $\pm$ 16.1%) between AUGMENT and Control achieved statistical significance. Moreover, no differences were seen regarding mitochondrial DNA content and relevant morphokinetic variables. Thirty patients were able to undergo embryo transfer. Cumulative live birth rates per transferred embryo were 41.6% in AUGMENT and 41.2% in Control.
Conclusion(s): AUGMENT does not seem to improve prognosis in this population. Therefore, the study has been discontinued.
Clinical Trial Registration Number: NCT02586298. (Fertil Steril® 2019;111:86–96. ©2018 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Received August 6, 2018; revised September 10, 2018; accepted September 27, 2018; published online November 24, 2018.
E.L. reports personal fees and other from Ovascience, during the conduct of the study; grants from Gedeon Richter, Finox; personal fees from Ferring Pharmaceuticals, Angelini, MSD; and non-financial support from Ferring Pharmaceuticals outside the submitted work. M.J.d.l.S. reports personal fees and other from Ovascience, during the conduct of the study, and personal fees from MSD, outside the submitted work. S.H. reports personal fees and other from Ovascience, during the conduct of the study. M.J.E. reports personal fees and other from Ovascience, during the conduct of the study. A.M. reports personal fees and other from Ovascience, during the conduct of the study. A.B. reports personal fees and other from Ovascience, during the conduct of the study. A.P. has nothing to disclose.
Supported by Ovascience, which participated in the study design and authorized the publication of results. The participation of S.H. was partially funded by grant PTQ-16-08222 from the Spanish Ministry of Economy and Competitiveness.
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Fertility and Sterility® Vol. 111, No. 1, January 2019 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2018.09.023

Key Words: Autologous germline mitochondrial energy transfer (AUGMENT), poor embryo quality, IVF failures, euploidy, egg precursor cells (EggPCs)

Embryo quality is a major factor determining the chances of pregnancy in the context of assisted reproductive technologies (ART), and it is critically affected by both chronologic and biologic age, which are not always correlated between each other (1). Regarding the latter, women with premature ovarian aging present lower ovarian reserve and ovarian response to stimulation, as well as lower fertilization rates and higher rates of aneuploidy (2) and pregnancy loss (3).

Among the variables influencing the embryo development competence, oocyte quality plays an important role in determining fertilization and development to high-quality embryos.

All of these initial phases require high energy consumption (4), increasing the synthesis of mitochondrial adenosine triphosphate (ATP), which provides the energy necessary for the rupture of the germinal vesicle and the resumption of meiosis (5, 6), for a normal fertilization (7), and for reducing the incidence of errors during the second meiotic division (8, 9). Moreover, oocytes with high ATP contents seem to enhance implantation of the human embryo (10, 11).

These findings, along with observations that mitochondria in oocytes of women in their 40s frequently exhibit swelling and abnormal cristae (12), support the idea that impaired bioenergetic capacity in oocytes is a primary contributor to declining embryo quality with advancing maternal age (AMA) or even in women with premature ovarian aging. In fact, the mitochondrial DNA (mtDNA) content in oocytes is significantly decreased not only in AMA but also in low ovarian reserve (13). Therefore, an energy deficit due to lower mitochondrial ATP synthesis could be considered as a cause of chromosomal aberration and could determine both the quality of the oocytes and that of the embryo.

Some studies in humans have demonstrated that increasing the oocyte mitochondrial mass improves the embryo quality in poor-prognosis patients. This was first described by Cohen et al. (14), demonstrating that the ooplasmic transfer from donors into oocytes from aged women improved the embryo quality and the success rate of in vitro fertilization (IVF). This was later tested by other groups, and some babies were born from this technique (15–18). However, in 2001 the Food and Drug Administration (FDA) suspended the use of this technique owing to ethical and biological concerns (19) related to the risk of heteroplasmic mitochondrial population (15, 20, 21). Moreover, some cases of Turner syndrome and autism were described (16, 20).

The dogma that the oocytes of female mammals are fixed after birth and decrease after puberty until they are depleted was challenged by revolutionary studies showing postnatal oogenesis in the adult ovary (22, 23). Although still questionable, the discovery that ovarian stem cells are present in ovaries, not just of adult mice but also of reproductive-age women (22, 24, 25), opened a new alternative for ooplasmic transfer. This was considered an autologous source of germline mitochondria, because they have been suggested to be of high quality (26).

This observation was the basis of the so-called autologous germline mitochondrial energy transfer (AUGMENT), a promising proprietary technology launched in 2014 by Ovascience involving the injection of autologous mitochondria into the patient's own oocyte at the time of intracytoplasmic sperm injection (ICSI) (27). These mitochondria were isolated from egg precursor cells (EggPCs) present in ovarian cortical tissue and found through the use of antibodies specific to the human Vasa analogue DDX4, a cell surface protein found on these cells (22). This technique was proposed as a solution to resolve the ethical objections associated with heteroplasmy without the theoretical risk of mitochondrial disease inheritance.

Some groups sought to investigate the clinical efficacy of EggPC–derived autologous mitochondrial injection to improve oocyte quality in women with multiple IVF failures (28, 29). However, the preliminary results must be interpreted with caution, because they were not randomized trials and there were certain deficiencies in their designs.

In this context, and as part of the global research on ovarian rejuvenation, our objective was to analyze the impact of AUGMENT in poor-prognosis patients with previous IVF failures and well documented poor embryo quality, with the use of sibling intracohort oocytes as control, thereby avoiding potential interpatient or intercycle variability biases.

MATERIALS AND METHODS

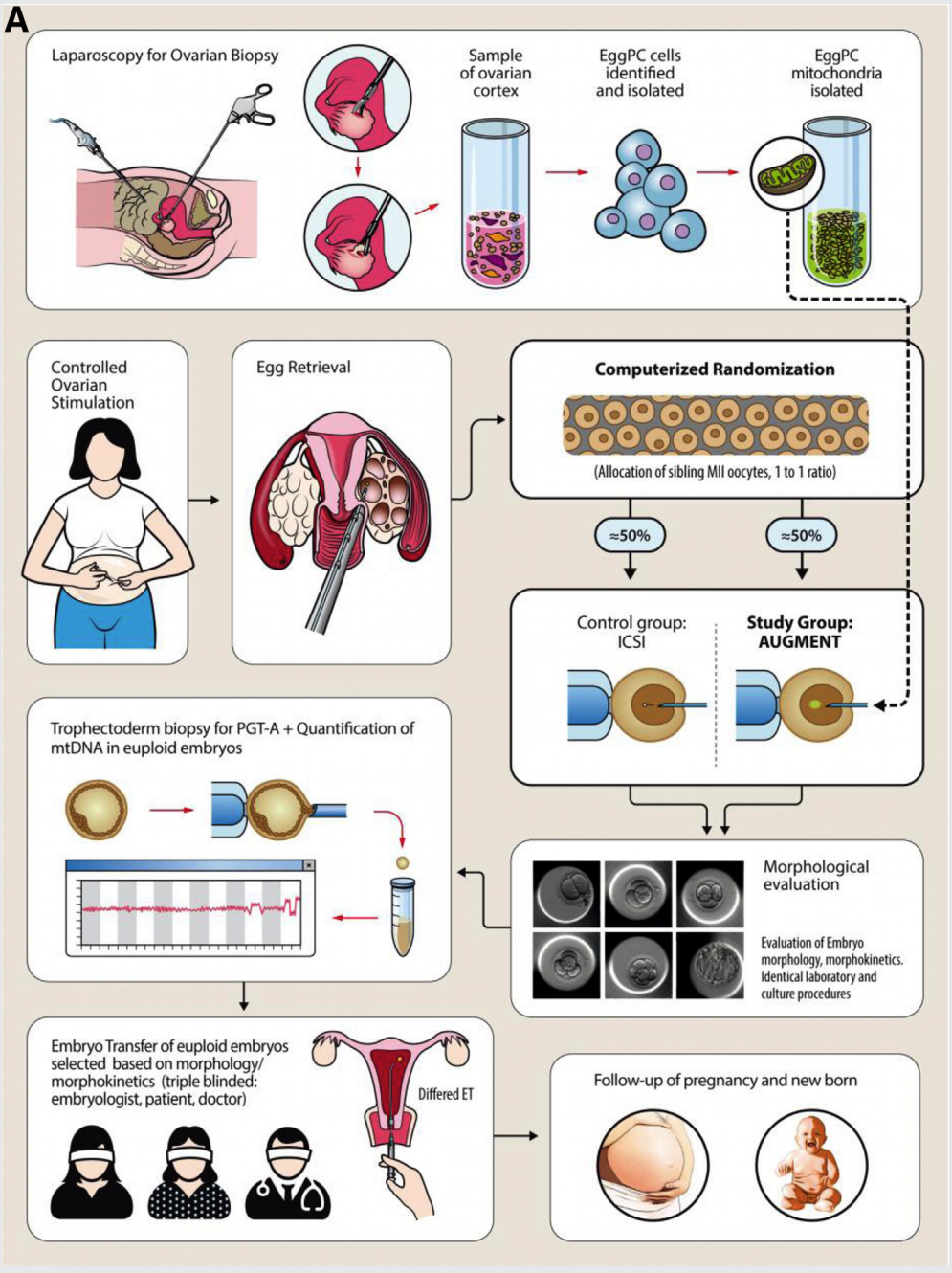
Ethical Approval

This study was approved by the Institutional Review Board of IVI-RMA Valencia (1501-VLC-005-AP) and registered at ClinicalTrials.gov (NCT02586298).

Study Design and Patient Population

This triple-blind, single-center, randomized, controlled experimental pilot study was conducted at IVI-RMA Valencia (Spain) from October 2015 to June 2017. Patients voluntarily agreed to enroll in this study after being duly informed and after signing a written informed consent form. Infertile patients undergoing an IVF cycle with preimplantation genetic testing for aneuploidy (PGT-A), aged $\leq$42 years, body mass index $<$30 kg/m^2, antimüllerian hormone (AMH) $\geq$4 pmol/L, with $>$5 million/mL motile sperm, at least one unsuccessful previous IVF cycle with at least 5 metaphase II eggs (MIIs) collected, and extremely low embryo quality were enrolled.

FIGURE 1

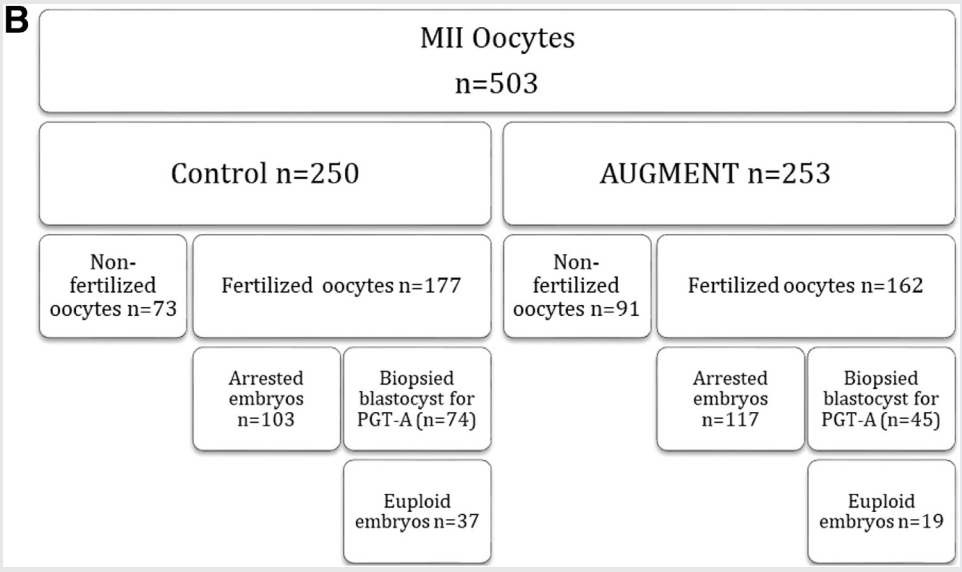


(**A**) Schematic drawing representing the complete procedure. (**B**) Study flow diagram. Oocytes from the same patient were randomized to receive either conventional ICSI or AUGMENT. Comparisons were made between embryos coming from both groups. AUGMENT = autologous mitochondrial transfer; ET = embryo transfer; MII = metaphase II; ICSI = intracytoplasmic sperm injection; mtDNA = mitochondrial DNA; PGT-A = preimplantation genetic testing for aneuploidy.

Labarta. Autologous mitochondrial transfer in IVF. Fertil Steril 2018.

Low embryo quality was understood as >70% of the embryos obtained being included in the worst prognosis category according to: 1) previous low fertilization rate (<20% of MIIs correctly fertilized); 2) deficient-quality embryos according to morphologic criteria established by the Association for the Study of Reproductive Biology (ASEBIR) (30); 3) embryos of deficient quality according to morphokinetic criteria established (31) for Embryoscope time-lapse when used; 4) no embryo transfer occurred owing to the absence of euploid embryos after PGT-A; or 5) arrested embryos. We excluded patients with uterine pathology, any medical contraindication for ovarian cortex biopsy or oocyte retrieval, or any characteristic incompatible with carrying out a new IVF cycle at IVI-RMA Valencia. A

FIGURE 1 Continued



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stepwise diagram of the experimental design is shown in Figure 1A.

End Points

The main end point of the study was to compare the ongoing pregnancy and live birth rates, and the in vitro embryo development, between embryos coming from AUGMENT and Control groups.

The in vitro embryo development end point was compared in terms of: 1) fertilization rate; 2) blastocyst rate; 3) embryo quality established according to classic morphologic ASEBIR classification; 4) morphokinetic analysis of in vitro embryo development by means of assessing the direct (time of the second polar body extrusion [tPB2], timing for pronuclear appearance [tPNa] and fading [tPNf], time of blastomere cleavage to the 2- [t2], 3- [t3], 4- [t4], 5- [t5], and 8-cell [t8] stages, timing to reach the morula [tM], starting blastocyst [tsB], and expanded blastocyst [tEB] stages) and indirect variables (duration of the second [cc2] and third [cc3] cell cycle, calculated as t3 − t2 and t5 − t3, respectively, and time for blastomere synchrony to reach the 4-cell stage, calculated as t4 − t3); 5) euploidy rate, calculated as the percentage of euploid embryos per biopsied blastocyst, zygote, or microinjected MII; and 6) mtDNA content in euploid embryos.

Ovarian Tissue Procurement

First, all patients underwent a surgical procedure via two-puncture laparoscopy under general anesthesia to obtain three fragments of the ovarian cortex (6 × 6 mm each). Fragments were placed in Multipurpose Handling Medium with Gentamicin (Irvine Scientific) and then cryopreserved by means of slow freezing with the use of a propylene glycol–based cryopreservation medium (Irvine Scientific).

EggPCs and Mitochondrial Isolation

On the day of ICSI, EggPCs and mitochondria from each patient were isolated in a laboratory set up by the sponsor company (Ovascience), following a protocol previously described by White et al. (24). After thawing them, ovarian sections were placed in a standard serum-free holding medium and dissociated by means of continuous mechanical tissue fractionation and enzymatic digestion. The ovarian cells were then resuspended in an IVF-compatible buffer, and the single-cell suspension was filtered and incubated with a proprietary specific antibody for DEAD-Box Helicase 4, after which the EggPC population was positively selected by means of flow cytometry. The EggPCs were placed on ice until mitochondrial isolation.

Mitochondrial isolation was performed, following a previously described protocol with minor modifications (16), to achieve the established minimum threshold (500 mt) to improve ICSI results in oocytes (32). Briefly, the EggPC suspension was pelleted by means of centrifugation and lysed to release the mitochondria. The lysed cells were then centrifuged to separate the mitochondria from other cellular components and debris. After centrifugation, mitochondria were washed and harvested after a second centrifugation step. The obtained pellet was kept on ice until ICSI was performed.

If necessary, remaining mitochondria were cryopreserved in an IVF buffer supplemented with 10% dimethyl sulfoxide with the use of a Mr. Frosty device and stored in a liquid nitrogen tank.

Ovarian Stimulation

One to three menstrual cycles after the laparoscopic surgery, patients underwent controlled ovarian stimulation (COS) with the use of a GnRH antagonist protocol, with starting doses of recombinant FSH (Gonal-f; Merck) and hp-hMG (Menopur; Ferring) ranging from 225 IU to 300 IU. Ooocyte retrieval was carried out 36 hours after GnRH agonist triggering (0.2 mg triptorelin; Decapeptyl; Ipsen Pharma) by means of conventional follicle aspiration.

IVF Procedures

Approximately 4 hours after oocyte retrieval, sibling MIIs, obtained from the same patient, were randomly allocated to the two groups: AUGMENT (experimental or study group) or conventional ICSI (Control group). This randomization was computerized, with a 1:1 ratio of AUGMENT to Control, always ensuring that at least one MII had undergone the AUGMENT technique (Fig. 1B). Before use, mitochondria were concentrated by centrifugation at 10,000*g* for 20 minutes at +4°C. In the experimental group, ICSI was carried out as follows: ~1-2 pL mitochondrial suspension was injected along with the spermatozoon, without polyvinylpyrolidone (PVP). As is our routine practice during ICSI, the MII was positioned and maintained by gentle aspiration applied with the use of a holding pipette, and the autologous mitochondria, along with the sperm, were microinjected together in one act. Thus, there was not any additional microinjection. In the Control group, ICSI was carried out following conventional procedures, with PVP injected along with the sperm into the oocyte cytoplasm.

After microinjection, eggs were placed in an Embryoslide and cultured in a time-lapse incubator (Embryoscope; Fertilitech) until the blastocyst stage, in accordance with the IVI-RMA Valencia laboratory's embryo culture protocols. The allocation of microinjected oocytes, pertaining to study or control group, was blinded to the embryologist team.

On day 5 or 6 of development, resulting blastocysts were biopsied by means of the pulling method and individually cryopreserved until genetic aneuploidy screening results with the use of the next-generation sequencing (NGS) technique were available (33).

Quantification of Mitochondrial DNA

The relative quantity of mitochondrial DNA was assessed by means of real-time polymerase chain reaction, with the use of beta-actin as a housekeeping gene and indicator of the amount of nuclear DNA as previously described (33).

Embryo Transfer and Clinical Outcome

Euploid embryos were warmed and transferred to the patient in a subsequent cycle, with the use of artificial endometrial preparation, as described elsewhere (34).

On the day of embryo transfer, neither the doctor, the patient, nor the embryologist who selected the embryo for warming and transfer, were aware of its study arm. Single-embryo transfer (SET) was strongly recommended, but double-embryo transfer (DET) was also authorized. When DET was performed with embryos from both groups, a fingerprinting analysis was planned to trace the babies or products of conception (35). Fingerprinting consisted of obtaining the DNA of the successfully implanted embryos either from tissue from remains of conception in the case of spontaneous miscarriage or from buccal swabs in the case of live birth when DET had been performed. If the sex of the embryos was different (XX and XY), fingerprinting was not needed, because we could thereby know the origin of the embryos (from Control or AUGMENT).

Clinical outcome was evaluated in terms of: positive β-hCG tests (serum levels of beta-hCG >10 mIU/mL 11 days after embryo transfer); implantation rate (number of gestational sacs seen on ultrasound scan per number of transferred embryos per patient); clinical pregnancy (presence of at least one gestational sac on ultrasound); clinical miscarriage rate (any clinical pregnancy lost before 12 weeks); ongoing pregnancy rate (OPR) (presence of at least one viable fetus beyond 12 weeks of pregnancy on ultrasound); and live birth rate (LBR; number of deliveries that resulted in at least one live-born neonate). Follow-up of the newborn was conducted with all patients for 6 months. The cumulative outcome of all these variables was analyzed both per patient and per transferred embryo.

Sample Size Calculation and Statistical Analysis

Sample size was calculated to detect a 20% difference in OPR (from 15% in the control group to 35% in the group of oocytes that received the AUGMENT technique) in a two-tailed test with a statistical power of 80% (beta error 0.2) and a 95% confidence interval (CI) (alpha error 0.05). Under these criteria, 166 patients needed to be recruited.

Estimating a study loss percentage of ~15% (cases in which the laparoscopic procedure, obtaining of mitochondria, or embryo transfer are not performed), 190 patients were needed.

As an experimental study, an interim analysis was initially planned after inclusion of ~60 patients to decide about adding the other 130 patients. This was justified to evaluate whether it was ethically acceptable to continue recruiting patients if the results were already clear.

Continuous variables were presented as arithmetic mean ± SD or median and interquartile value (IQR), based on normal or abnormal sample distribution, respectively. Chi-square and nonparametric Mann-Whitney *U* tests were used for statistical comparisons of categoric and continuous variables, respectively. For the intrasubject comparison between both groups, a paired-samples *t* test was done.

A *P* value of $<.05$ was considered to be statistically significant.

RESULTS

Patient Follow-Up

A total of 59 patients were enrolled in the study. Their mean age was 36.3 ± 3.6 years, body mass index 22.9 ± 3.1 kg/m^2, and with a mean of 2.5 ± 1.5 previous failed IVF cycles. Antral follicle counts (AFC) showed a median of 13 (IQR 10–

20) and serum AMH levels a median of 17.2 (IQR 10.9–29.4) pmol/L (measured by means of the Elecsys automated assay). Two of the 59 enrolled patients spontaneously conceived (one ended up in a miscarriage) and therefore withdrew from the study. Therefore, a total of 57 ovarian biopsies were performed, being able to find EggPCs in all cases except in one patient from whom a suboptimal number of EggPCs were isolated in the first surgical attempt. After performing a second ovarian cortex biopsy, sufficient EggPCs were obtained to include that patient in the study. The mean size of ovarian cortex fragments was 113.6 ± 19.7 mm^2. One month after the ovarian biopsy, AFC showed a median of 13 (IQR 8.8–18.3) whereas the AMH was 12.1 [IQR: 6.9–23.5] pmol/L. A median of 10 (IQR 7–15) oocytes were obtained after stimulation, a median of 8 (IQR 5–12) of them MIIs.

Figure 1B shows the study flow diagram. Following this strategy, we were able to analyze the net impact of the AUGMENT technique on the oocytes in terms of intracohort comparison of treated oocytes with control oocytes coming from the same harvest.

In Vitro Embryo Development

Results regarding in vitro embryo development are presented in Table 1. A total of 253 MII were assigned to the AUGMENT group and 250 MII oocytes to the Control group. The fertilization rates were similar in the two groups.

Day 5 blastocyst formation rates per zygote were significantly reduced in the AUGMENT group (23.3 ± 32.0%) compared with the Control group (41.1 ± 36.9%; P=.0001; Fig. 2A).

Moreover, significant differences were observed in the distribution of embryo-quality phenotypes between the groups (P=.005; Fig. 2B).

No statistically significant differences in terms of the euploidy rate between AUGMENT and Control were found (43.8 ± 41.7% and 63.8 ± 44.1%, respectively; P=.412; Table 1). In addition, the net euploidy rates calculated as the percentage of euploid blastocysts per microinjected MIIs between AUGMENT and Control were similar (9.8 ± 20.5% vs. 11.9 ± 16.1%; P=.541).

Regarding mtDNA content in euploid embryos, no difference was observed between AUGMENT (21.8 [IQR 14.6–24.7]) and Control groups (16.9 [IQR 13.8–23.9]; P=.56).

Morphokinetic variables were analyzed in a time-lapse imaging incubator. The tPNa parameter occurred earlier in the AUGMENT group than in the Control group (9.4 ± 2.9 h vs. 10.1 ± 3.8 h), but it did not reach statistical significance. Further along embryo development, AUGMENT oocytes achieved synchrony at 4-cell stage later than the Control oocytes (3.89 ± 8.3 h vs. 2.7 ± 4.3 h; P=.006). Slightly, though significant, longer cc3 (11.7 ± 9.5 h vs. 11.6 ± 12.6 h; P=.032) was found in the AUGMENT group compared with Control. More detailed results are presented in Supplemental Table 1 (available online at fertstert.org).

Embryo Transfers

Twenty-seven patients did not undergo an embryo transfer for the following reasons: premature ovulation (n = 1); no fertilization (n = 1 patient); no blastocysts available for biopsy (n = 16 patients); all embryos aneuploid (n = 8 patients); and no survival after warming (n = 1 patient).

Thirty patients had at least one euploid embryo available for transfer. Twelve patients had embryos coming only from the Control group, eight patients had embryos only coming from AUGMENT group, and ten patients had embryos coming from both groups. Detailed information about results after each embryo transfer is presented in Supplemental Table 2 (available online at www.fertstert.org).

IVF Pregnancy Outcomes

A total of 30 patients received at least one embryo transfer. All embryo transfers were pure (embryos coming from only one group) except for four cases in which a mixed ET (one embryo from AUGMENT and one embryo from Control) was performed: Three of those four ended up in a twin pregnancy, with one newborn coming from each group, and the remaining one in a single pregnancy which was later confirmed as coming from the AUGMENT group.

There was no need to perform fingerprinting analysis in any of the cases, because in the all cases the oocyte origin could be determined by sex tracking from the blastocysts to the newborn.

Among the 26 patients with pure ET, two of them underwent a first embryo transfer with AUGMENT embryos without achieving pregnancy, followed by a second embryo transfer with Control embryos.

The cumulative OPR per transferred embryo was 41.2% (7/17) in the AUGMENT group and 41.7% (10/24) in the Control group (P=.97). LBRs were exactly the same as OPR. Two embryos from the Control group did not survived after the warming process; therefore, to date, there are two cryopreserved embryos left in the AUGMENT group and ten in the Control group from patients who achieved pregnancies in this trial (Table 2).

A total of 17 children have been born from the included patients. In the Control group, one case of esophageal atresia and another one of hypospadias were recorded, whereas no congenital malformations were reported in the AUGMENT group.

DISCUSSION

The results of our experimental pilot study demonstrate that AUGMENT technique does not seem to improve embryo quality in infertile patients with premature ovarian aging and a background of poor embryo quality in previous IVF cycles.

Moreover, despite the fact that no differences were observed in fertilization rates between the groups, the blastocyst formation rate per zygote was significantly lower in the AUGMENT group than in the Control group. Regarding the ratio of euploid embryos obtained per injected MII and per fertilized oocyte, no differences were seen between groups, suggesting that the injection the extra volume of mitochondria suspension during ICSI did not damage oocyte membrane integrity. Despite pregnancy rates being similar, the sample size was too small to draw any conclusions regarding LBRs. An interim analysis was conducted after including 59 eligible

TABLE 1

Comparison of IVF lab results in Control and AUGMENT group.

Result	Control	AUGMENT	Risk difference (95% CI)	*P* value
MIIs microinjected	250	253		
Correctly fertilized oocytes (n)	177	162		
Fertilization rate (%)	68.7 ± 29.1	62.7 ± 30.0	−6.1 (−15.5 to 3.3)	.198
Day 5 blastocyst formation rate per zygote (%)	41.1 ± 36.9	23.3 ± 32.0	−17.8 (−27.1 to −8.5)	.0001
Biopsied blastocysts (day 5 + day 6)	74	45		
Euploid blastocyst rate per biopsied blastocyst (%)	63.8 ± 44.1	43.8 ± 41.7	−20.0 (−74.2 to 34.2)	.412
Euploid blastocyst rate per zygote (%)	19.7 ± 24.4	15.7 ± 26.6	−4.0 (−14.4 to 6.4)	.442
Euploid blastocyst rate per MII (%)	11.9 ± 16.1	9.8 ± 20.5	−2.1 (−9.1 to 4.8)	.541

Note: Data are expressed as n or mean ± SD. Paired *t* test was performed, and $P < .05$ considered to be statistically significant. AUGMENT = autologous mitochondrial transfer; MII = metaphase II oocyte.

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patients to evaluate continuation of the study, considering its invasive nature. To be able to see if there is any improvement in OPR, 190 patients should have been recruited, but the initial results indicated a premature discontinuation of the study.

OPR and LBR had to be considered as the main outcomes of the study, but the first step was to demonstrate an improvement in embryo quality when using the autologous mitochondrial transfer.

The time-lapse analysis showed that pronuclei appearance after ICSI tended to occur earlier in the AUGMENT group than in the Control group. This could indicate a certain degree of effect of the technique in some molecular mechanisms driving the dynamics of fertilization events, such as a possible energy boost during both male and female nuclear envelop formation, but it rapidly vanished as development continued and was not able ameliorate the immediate kinetic direct variables, i.e., nuclear fading, t2, and t3, or indirect kinetic ones, such as synchrony at 4-cell stage. In fact, this last variable was significantly shorter, and thus better, in the embryos from the Control group. We think that the unexpected and very small shortening of the cc3 in the AUGMENT group could be just an artifact because neither t3 nor t5 reached statistical significance and, more interestingly, did not have a positive impact on the blastocyst formation rates.

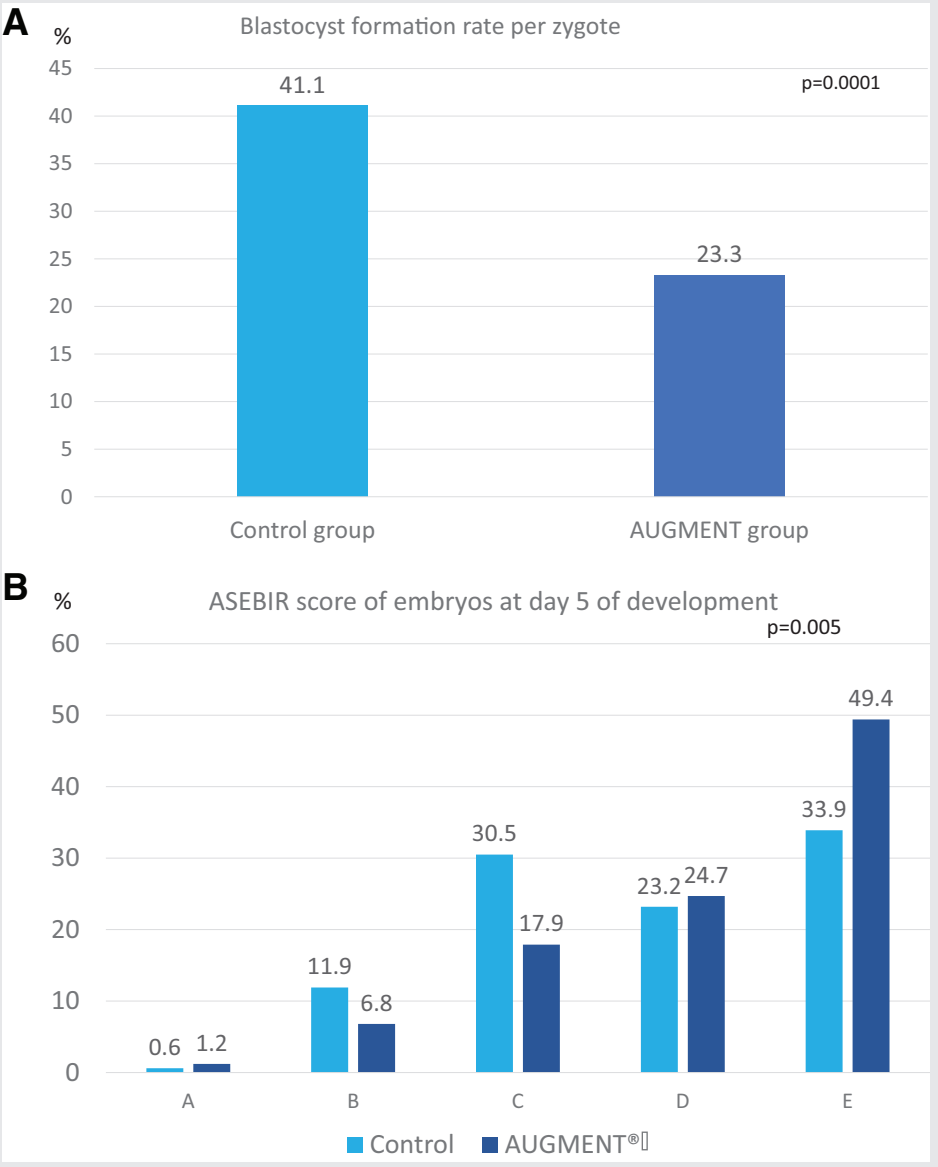
Studies on different mammalian species (36–38), including humans (39, 40), have shown that mitochondrial biogenesis is silenced at the MII stage and is not reactivated in the embryo until the periimplantation period, and mtDNA replication does not occur before the morula or blastocyst stage. The AUGMENT treatment was not able to modify the dynamics of mtDNA content of the human blastocysts, with no differences observed compared with the Control group, at least at the trophectoderm cell level.

At this point, lack of improvement was observed in terms of embryo morphology, most of the morphokinetic variables, euploid status, or mtDNA content when this technique was applied.

Before conducting our study, AUGMENT was offered as an adjunct treatment to improve IVF outcomes, by eventually restoring the possible energy deficiencies of the oocytes retrieved from poor-prognosis patients. However, only low-grade evidence existed to support its efficacy, because previous studies were not randomized trials, but rather prospective cohort (28) or descriptive (29) studies with small numbers of patients. Moreover, in the study of Fakih et al. (28), there was a difference in egg allocation toward AUGMENT compared with IVF only (171 vs. 106, respectively). Finally, the embryologist was unblinded in embryo selection and transfer. Fakih et al. (28) reported marked improvement in pregnancy rates above the historic IVF success rate for these patients (e.g., 11- and 18-fold increases in OPR in the United Arab Emirates and Canada, respectively). However, some commenters stated that this was somehow flawed and these few pregnancies could be explained by the intercycle variability in IVF (41). Although Ovascience triumphantly announced the results of these two publications and the first healthy baby born in Canada, voices in the reproductive science community urged caution and expressed great concern when AUGMENT leapt suddenly from the laboratory to the clinic setting (42). Moreover, the FDA made the technology unavailable in the United States, although the company was based in Boston, and this led to reproductive tourism to countries in which the technique was allowed, such as Canada. While some scientists were still asking questions about the technique (43), some women were already receiving treatment and the media was frequently publicizing information about it, creating hopes for patients as a "last chance" therapy (42). In this confusing scenario, we considered a well designed study as urgent to analyze the net impact of the technique.

Compared with the two previous clinical studies, the strength of our study is that intrapatient and intracycle comparison were performed, through allocation of sibling oocytes to receive either AUGMENT or conventional ICSI, thus avoiding bias related to other factors that can affect the quality of the oocytes even in the same patient (intercycle variation). Another reason to support this design was that such bad-prognosis patients, with a mean of 2.5 previous failed IVF cycles, may not volunteer for a study if they are possibly randomized into standard treatments that have previously failed them. Moreover, this design allowed us to maintain the blindness of the patient, the doctor, and the embryologist selecting the best embryo for transfer. If we had randomized patients into these two different groups, the control group would not have undergone a laparoscopy, therefore both

FIGURE 2



(**A**) Day 5 blastocyst formation rate per zygote obtained in Control and AUGMENT groups. (**B**) ASEBIR score of embryos on day 5 of development. ASEBIR = Association for the Study of Reproductive Biology; other abbreviations as in Figure 1.

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the patient and the doctor would have been unblinded. And last but not least, if AUGMENT would have increased the quality of the embryos significantly, these patients with previous failed attempts would have benefited from this invasive technique.

It is important to point out that our pregnancy rates were relatively high for such a bad-background population, once a euploid embryo was transferred. In fact, our results were even higher than those previously published by other groups testing the AUGMENT technique: Oktay et al. (29) obtained an LBR of 9% in 11 patients, whereas Fakih et al. (28) published the results from two groups with an OPRs of 18% in a sample of 59 patients and 26% in another of 34 patients. Our cumulative LBR with the use of AUGMENT was 41.2%. The fact that this pregnancy outcome was good, regardless of whether the embryo came from the study or the control group, demonstrates that AUGMENT was not able to improve the prognosis, at least in this sample of patients. However, we have to bear in mind that 45.6% of this poor-prognosis patient population did not have any available embryo for transfer.

There has been a lot of debate about the existence or not of EggPCs in the human ovary. Although some authors have been able to find them (22, 24, 44–46), others could not replicate this in nonhuman animals (47, 48) or in humans (49). In our study, the ovarian cortex was frozen and transported to an Ovascience laboratory, where a company employee isolated the putative EggPCs and extracted mitochondria from them.

TABLE 2

Cumulative pregnancy outcome after transferring embryos from Control and AUGMENT groups in a total of 30 patients.

Outcome	Control	AUGMENT
Pure embryo transfer		
No. of patients with transfer	16	12
No. of transfers	18	12
Transfer type	(16 SET + 2 DET)	(11 SET + 1 DET)
Total embryos transferred	20	13
Positive β-hCG tests	10	6
Per transfer	55.5%	50%
Implantation rate	35.0% (7/20)	30.8% (4/13)
Clinical miscarriage rate	0% (0/7)	25% (1/4)
Live births (LB)	7	3
LB per embryo transferred	35.0% (7/20)	23.1% (3/13)
LB per patient	43.8% (7/16)	25.0% (3/12)
Embryos left	7 (+2[a])	2
Mixed embryo transfer		
No. of patients with transfer	4	
No. of transfers	4	
Transfer type	(4 DET; 1:1 ratio)	
Total embryos transferred	8	
Positive β-hCG tests	4	
Per transfer	100%	
Implantation rate	87.5% (7/8)	
Clinical miscarriage rate	0% (0/4)	
Live births (LB)	7	
LB per embryo transferred	87.5% (7/8)	
LB per patient	100% (4/4)	
Embryos left	3 (from Control)	

Note: Four patients underwent a mixed embryo transfer (one embryo from Control and one embryo from AUGMENT). Two of the 26 patients with pure embryo transfer (ET) first underwent an ET with embryos from AUGMENT and second an ET with embryos from Control. All ongoing pregnancies ended in live births in both groups. AUGMENT = autologous mitochondrial transfer; DET = double-embryo transfer; embryos left = surplus embryos that were vitrified; SET = single-embryo transfer.

[a] Two embryos from Control were already warmed for a second transfer and did not survive.

Labarta. Autologous mitochondrial transfer in IVF. Fertil Steril 2018.

This means that we were not able to see these procedures, and there is a lack of information about the quality and quantity of mitochondria finally injected.

In any case, the aim of this trial was not to question the existence of EggPCs, in fact, we assumed its presence once the AUGMENT technique was launched and available for their use. Our aim was to analyze the net impact of this new technology on the quality of the embryos before deciding to implement it into clinical practice. When the study started, some babies had been born with the use of this technique, suggesting that the AUGMENT protocol did not pose an additional risk to the patient or the babies. In fact, no congenital malformations were described in pregnancies coming from AUGMENT embryos. In any case, at this moment no long-term follow-up could be performed because the study was terminated 1 year ago.

Although mitochondria from EggPCs were reported to be in better bioenergetic fitness than mitochondria isolated from other human cell lineages (27), we were not able to demonstrate a direct causative association between the developmental competency of oocytes from our patient population and the AUGMENT treatment.

In ART, there are important concerns about the use of adjunct treatments in IVF laboratories that have not been proven in terms of safety and efficacy (50), and the AUGMENT treatment is one of them.

In summary, this study demonstrates that injecting autologous mitochondria into the patient's own oocyte at the time of ICSI does not benefit the developmental capacity of treated oocytes, the euploidy status of the embryo, nor the pregnancy rate. In conclusion, the AUGMENT approach should not be considered as a novel way of ovarian rejuvenation in poor-prognosis patients with a background of bad embryo quality.

Acknowledgments: The authors are very grateful to Leslie Atkinson for her helpful assistance during the development of this study, especially in the patient recruitment process and for reviewing the English language of the manuscript.

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Transferencia de mitocondrias autólogas como técnica complementaria a la microinyección espermática para aumentar la calidad embrionaria en pacientes que se realizan un ciclo de fecundación in vitro- estudio piloto aleatorizado

Objetivo: Estudiar si la transferencia de mitocondrias autólogas (AUGMENT) mejora los resultados en pacientes con fallos previos de fecundación in vitro (FIV).

Diseño: Aleatorizado, controlado, triple ciego, estudio experimental.

Lugares: Centro privado de reproducción asistida, Instituto Valencia de Infertilidad (IVI-RMA), Valencia, España.

Pacientes: Mujeres infértiles $\leq$ 42 años, índice de masa corporal $<$30 kg/m2, Hormona antimülleriana $\geq$ 4pmol/L, $>$ 5 millones/mL de espermatozoides móviles, al menos un ciclo previo de FIV con al menos 5 metafase (MIIs) obtenidos y baja calidad embrionaria.

Intervenciones: Se realizó una biopsia de corteza ovárica para aislar células precursoras de ovocitos y obtener de ellas las mitocondrias. MIIs de la misma cohorte fueron asignados aleatoriamente para AUGMENT (experimental) o para microinyección espermática (Control). En AUGMENT, una suspensión de mitocondrias fue inyectada junto con el esperma. Los blastocistos viables de ambos grupos fueron biopsiados para la realización de un test genético preimplantacional de Aneuploidías.

Medidas principales de resultado: Embarazo y calidad embrionaria

Resultado(s): Se realizó un análisis interino. La edad media de las pacientes fue de 36.3 $\pm$ 3.6 años, y con una media de 2.5 $\pm$ 1.5 ciclos previos de FIV. Dos de las pacientes incluidas gestaron de manera espontánea (una de ellas abortó). A 57 pacientes se les realizó una biopsia ovárica y una estimulación. La captación ovocitaria se realizó en 56 pacientes (ovulación prematura; n=1). Un total de 253 MIIs fueron inseminados con AUGMENT y 250 fueron el Control; las tasas de fecundación fueron 62.7 $\pm$ 30.0% y 68.7 $\pm$ 29.1%, respectivamente. Se observaron diferencias estadísticas en la tasa de formación de blastocistos en día 5 (23.3 $\pm$ 32.0% vs. 41.1 $\pm$ 36.9%). Ni la tasa de euploides por blastocistos biopsiados (43.8 $\pm$ 41.7% vs. 63.8 $\pm$ 44.1%) ni la tasa de euploide por MII (9.8 $\pm$ 20.5% vs. 11.9 $\pm$ 16.1%) entre AUGMENT y el grupo Control alcanzó significancia estadística. Sin embargo, no se vieron diferencias en el contenido de DNA mitocondrial y en las variables relevantes de morfocinética. En 30 pacientes fue posible la transferencia embrionaria. La tasa de recién nacido vivo acumulada por transferencia embrionaria fue de 41,6% en AUGMENT y un 42,1% en el grupo Control.

Conclusión (es): No parece que AUGMENT mejore el pronóstico en esta población. Así pues, el estudio fue interrumpido.