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ARTICLE

Autologous endometrial cell co-culture improves human embryo development to high-quality blastocysts: a randomized controlled trial

BIOGRAPHY

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KEY MESSAGE

Autologous endometrial co-culture (AECC) increases the development of good-quality blastocysts compared to conventional culture. This is the first study demonstrating the benefits of AECC in couples undergoing IVF/ICSI with different causes of infertility.

ABSTRACT

Research question: Does autologous endometrial cell co-culture (AECC) improve the number of good-quality blastocysts obtained by IVF/intracytoplasmic sperm injection (ICSI), compared with conventional embryo culture medium in a broad group of patients referred to assisted reproductive technology (ART)?

Design: This interventional, randomized, double-blind study took place at Clinique Ovo from March 2013 to October 2015 and included 207 healthy patients undergoing an IVF or ICSI protocol, of which 71 were excluded before randomization. On the previous cycle, all participants underwent an endometrial biopsy at D5 to D7 post-ovulation, following which the endometrial cells were prepared for AECC.

Results: The data demonstrated that AECC significantly increased the incidence of good-quality blastocysts compared with culture in conventional media (42.6% vs 28.4%, $P < 0.001$). No significant differences were found in pregnancy and live birth rates.

Conclusion: This study demonstrated the benefits of AECC on blastocyst quality compared with conventional embryo culture medium, in a broader category of patients referred to ART as opposed to other studies that concentrated on specific causes of infertility only. However, limitations of the study design should be taken into consideration; the analysis was performed using embryos rather than patients and a follow-up of children born following the treatments could not be conducted.

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KEYWORDS

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INTRODUCTION

One of the key factors in achieving pregnancies in assisted reproductive technologies (ART) is maximizing embryo quality, which is heavily influenced by embryo culture conditions.

In this context, several strategies have been proposed in order to improve embryo culture conditions and pregnancy rates (*Swain et al., 2016*). Many studies have provided evidence related to the impact of culture conditions on the success of IVF and the development of the future offspring, leading to the categorization of embryo culture media as a class III medical device in the European Union and a class II in North America (*Chronopoulou and Harper, 2015; Kleijkers et al., 2015; Mantikou et al., 2013*). The first embryo culture media used were not optimized for blastocyst development. However, sequential media were developed with the purpose of mimicking in-vivo conception and the natural environment of the embryo, which changes in the course of its development, comprising physiological fluids such as tubal or uterine fluid (*Chronopoulou and Harper, 2015*). With the emergence of time-lapse imaging, which allows embryo development to be monitored in real time, single-step media gained popularity (*Chronopoulou and Harper, 2015; Meseguer et al., 2012*). In essence, single-step media is supposed to contain all the nutrients that the embryo needs during its early development (*Meseguer and Pellicer, 2017; Meseguer et al., 2012*). As detailed formulations are not provided by manufacturers, Morbeck *et al*. studied and compared several culture media, showing that they vary considerably in terms of composition (*Ménézo et al., 2013; Morbeck et al., 2014*a,*2014*b). Furthermore, several studies using different sources of cells in their media preparations reported an improvement in terms of embryo quality and blastulation rate (*Feng et al., 1996; Gandolfi and Moor, 1987; Wiemer et al., 1989*).

Several contradictory studies comparing neonatal outcomes such as gestational term, birth weight and congenital malformations between blastocyst stage versus early cleavage stage embryo IVF transfers seemed to be in favour of early cleavage stage transfers. Several of these studies actually showed a negative impact of the embryo's longer incubation in culture medium (*Marianowski et al., 2016*). However, other key opinion leaders (KOL) provided evidence that blastocyst stage transfer leads to a higher rate of clinical pregnancy and live birth (*Papanikolaou et al., 2008; Schwärzler et al., 2004*). Thereby, in order to better support the development of early embryos into blastocysts by simulating the in-vivo environment, KOL developed the co-culture of human embryos (*Bolton et al., 2014; Ménézo, 2004; Ohl et al., 2015*).

Mimicking natural physiological processes, transfers of embryos at the blastocyst stage permit coordination of the dialogue between the embryo and the endometrial cells during the implantation window of the patient undergoing IVF treatment, and thus increases the chance of pregnancy (*Gardner and Lane, 1996; Simón et al., 1999*).

Co-culture systems, originally developed in the 1990s, used several cell types from non-human cells to human reproductive cells (*Simón et al., 1999*). Consequently, in 2002, the FDA limited the use of co-culture in human IVF treatments in order to avoid the risk of disease transmission from non-human or heterologous human cell lines (*Swain et al., 2016*). Rather, autologous endometrial co-culture (AECC) was developed, using the patient's own endometrial cells.

The establishment of autocrine and paracrine communications and cell-to-cell interactions between the endometrial feeder cells and the embryo should contribute to the detoxification of the culture medium, facilitating blastocyst development and improving the implantation rate (*Bochev et al., 2016; De los Santos et al., 1996; Guérin et al., 2001; Mercader et al., 2006; Simón et al., 1999*). Many teams concentrating their studies more specifically on patients with IVF failure such as poor ovarian reserve, history of poor-quality embryos or repeated implantation failure, demonstrated the benefits of AECC in in-vitro treatments (*Eyheremendy et al., 2010; Spandorfer et al., 2002a,2004*). Recently a European, multicentre, prospective and randomized controlled trial assessed the efficacy of AECC, comparing the pregnancy rate after single embryo transfer of D5 blastocysts on AECC to a D3 embryo transfer, cultured in a conventional medium (*Ohl et al., 2015*). Intermediate analysis of this study showed that AECC improved early embryo and blastocyst quality and significantly increased the clinical pregnancy rate per transfer (*Ohl et al., 2015*). However, this study compared blastocysts subjected to co-culture with early cleavage stage embryos grown in conventional medium, hence limiting the scope of the given conclusion. Based on the hypothesis that all women undergoing IVF/intracytoplasmic sperm injection (ICSI) can and should benefit from AECC, the present study aimed to validate the efficacy of a single blastocyst (D5 or D6) transfer co-cultured on AECC compared to in a conventional medium in a cohort including a broad category of women.

MATERIALS AND METHODS

Study design

This was an interventional, single-site, randomized, double-blind controlled study evaluating the benefits of AECC versus conventional medium in IVF or ICSI treatments.

Embryo quality and blastulation rate were evaluated as primary endpoints comparing D5/D6 blastocyst transfer rates between the AECC and conventional medium groups. Biochemical and clinical pregnancy rates and live birth rates were then analysed as secondary endpoints.

In order to detect a 10% increase in the rate of usable embryos (namely the embryos of sufficient quality to be transferred or vitrified) in the AECC versus conventional medium groups, 770 D2 embryos were required for inclusion, based on an average of 7 embryos per patient at Clinique Ovo (Montréal, Quebec, Canada). Factoring in a 15% dropout rate, a minimum of 128 patients were required, or 64 patients per group.

Ethics statement

This trial, approved by an independent ethics committee on 14 December 2012 (reference: OVO-12-24), was registered at ClinicalTrials.gov (NCT01886118) and was conducted in accordance with the Declaration of Helsinki. Informed consents were obtained from all participants prior to the performance of any study-related procedures.

Participants

Between April 2013 and March 2015, 207 healthy women aged between 18 and 38, undergoing an IVF or ICSI protocol with ovarian stimulation at Clinique Ovo, were screened for eligibility. Inclusion criteria included normal uterine cavity, regular menstrual cycles, basal FSH levels <10 IU/l, anti-Müllerian hormone (AMH) >1 ng/ml and/or antral follicle count (AFC) ≥12 within 12 months prior to entering the study and could not have previously undergone more than three IVF cycles with embryo transfer. The following patients were excluded: aged ≥39; amenorrhoea; anovulatory cycles; menstrual cycle >40 days; chronic endometritis; severe endometriosis (stages III and IV); hydrosalpinx; Asherman's syndrome or uterine synechia; uterine polyp, fibroids or other uterine anomalies; use of anticoagulant; male partner with secretory azoospermia and multiple embryo transfer.

Based on the inclusion and exclusion criteria above, 136 patients were randomized to receive either AECC (n = 63) or conventional medium (n = 73).

Endometrial biopsy

For all enrolled participants, an endometrial biopsy was performed and treated in accordance with the Endocell® AECC protocol (Laboratoires Genévrier, Sophia Antipolis, Antibes, France). Briefly, prior to the IVF/ICSI stimulation cycle, participants were asked to perform basal body temperature curve monitoring as well as urinary LH testing (First Response; Church and Dwight Canada Corp., Mississauga, Ontario, Canada). Between D5 and D7 following ovulation (D7 to D9 following a positive urinary LH test), participants underwent an endometrial biopsy at Clinique Ovo with Pipelle de Cornier® endometrial sampling (Surgi-Pharm Avancee Inc., Canada). The biopsy was then prepared for cryopreservation using the provided Endocell® kit, according to protocols and procedures from Laboratoires Genévrier. All participants were tested for *Mycoplasma hominis*, *Chlamydia trochomatis* and *Ureaplasma urealyticum* by vaginal swab prior to the biopsy. Patients with positive results were treated with antibiotics; these patients continued their IVF cycle but were excluded from the present study.

Ovarian stimulation protocols

Gonadotrophins were prescribed at the physician's discretion between the gonadotrophin-releasing hormone (GnRH) antagonist, short GnRH agonist or SMART (stimulation with minimal adverse effects, retrieval and transfer) protocols, according to the age and ovarian reserve test results (AMH, FSH and AFC). The FSH doses were then adjusted according to follicular growth until the day of ovulation triggering by the human chorionic gonadotrophin (HCG) hormone (5000 IU) (Ferring Canada, North York, Canada).

Randomization

Participants were randomized 1:1 to either the AECC group (study group) or conventional medium group (control group) by opening a randomization envelope on the day of HCG administration, 36 h prior to oocyte retrieval. Randomization was performed using an Excel file. Only the research associates and the embryologists were aware of the randomization group; physicians and participants were kept blinded until all vitrified embryos were transferred or the end of the study. In the case of a negative pregnancy test, all patients randomized in the conventional medium group were offered the option to use AECC in their subsequent treatment attempt.

Autologous endometrial co-culture

Endometrial tissue was kept in liquid nitrogen until the participant's randomization. The day prior to oocyte retrieval, endometrial tissue was carefully thawed in order to proceed to cell preparation for the endometrial culture. All solutions were provided in the Endocell® kit (Laboratoires Genévrier). Briefly, cryovials were warmed in a 37°C water bath. Tissue was then processed to isolate epithelial and stromal cellular fractions. Endometrial cells were then seeded in a monolayer, on IVF grade tissue culture plates (Corning, USA), in a 1/75 epithelial gland/stromal cell ratio. Cells were cultured in CCM-30 (Vitrolife Inc., CO, USA) and incubated at 37°C with 5% CO_2 and 20% O_2. After in-vitro oocyte fertilization either by regular IVF or ICSI, D2 healthy developing embryos were transferred, one per well containing 1 ml blastocyst culture medium, over the endometrial culture, to pursue their development until D5 or D6. A maximum of eight embryos were placed in co-culture due to restrictions in terms of the plate's dimension: the first eight embryos were arbitrarily assigned to co-culture, in a non-selective way. Hence, supernumerary embryos from the study group were placed in the conventional medium. Blastocysts qualifying as high quality were either transferred on D5 or vitrified for future use and good-quality blastocysts on D6 were vitrified.

Early embryo culture in conventional media

Fertilized oocytes confirmed by the presence of two pronuclei were cultured in 20 µl drops of Global Total (Global, Toronto, Canada) under oil (Global) in 35 mm Falcon Primaria™ dishes (Fisher Scientific, Canada). Embryos were assessed daily for development, except D4, and media was changed at the end of D2. Embryos were cultured in IVF-specific incubators (G185, K-Systems, Denmark) at 37°C, 6% CO_2 and 5% O_2. Blastocyst assessment was carried out on D5 and D6 using the modified Gardner's system (Veeck *et al.*, 2003) and blastocysts of BB score or higher were considered for transfer or vitrification (*Liebermann, 2017*).

Embryo grading

Embryos were scored according to the criteria described in the *ESHRE-ALPHA consensus (2011)*. Transferable embryos were defined on D3 as having between 6 and 8 cells and grade 2 or better. For D5 and D6, usable blastocysts were defined as blastocysts with a BB score or higher. The blastocysts obtained were either transferred fresh into the uterus at D5 or vitrified for further transfer.

Pregnancy outcomes

Biochemical pregnancy was determined by serum β-HCG test and clinical pregnancy was determined by the presence of a fetal heart visualized on ultrasound at 8 weeks. A miscarriage was defined with a positive β-HCG test but no fetal heart activity observed on ultrasound, as well as clinical pregnancies that do not result in a live birth.

Statistical analysis

Descriptive statistics were produced for all study variables, including the mean, standard deviation, and 95% confidence interval (CI) for continuous variables, and frequency distributions for categorical variables. Between-group differences with respect to demographic characteristics, oocyte characteristics and IVF/ICSI outcomes were assessed using one-way

analysis of variance (ANOVA), the chi-squared test or Fisher's exact test, as appropriate.

Multivariate logistic regression was used to ascertain predictors of fertilization, blastulation and pregnancy outcomes for multiple covariates such as smoking, infertility causes, IVF rank protocol types, stimulation days, total number of oocytes and number of metaphase II (MII) oocytes retrieved (TABLE 1). Within each group, pregnancy outcomes were stratified by fresh versus vitrified-warmed embryo transfer.

Furthermore, a predictive model was constructed in order to ascertain the probability of biochemical pregnancy, clinical pregnancy and live birth in the remaining vitrified embryos.

Statistical analysis was performed using IBM SPSS Statistics (version 24). Statistical significance was accepted when $P < 0.05$.

RESULTS

Participant flow

A participant flow chart is presented in FIGURE 1. From 207 patients enrolled in the study, 71 were excluded before randomization due to either failure in ovulation detection using urinary LH detection kit ($n = 20$), cancelled stimulation cycle ($n = 7$), spontaneous pregnancy ($n = 4$), personal reasons ($n = 19$) and other reasons ($n = 21$). A total of 1406 mature oocytes were recovered from the 136 randomized patients. These oocytes had been fertilized either by classical IVF or ICSI.

Of these mature oocytes, a total of 507 were withdrawn from consideration in the pregnancy outcome analyses due to unsuccessful fertilization ($n = 475$) or double embryo transfer ($n = 32$). Because the AECC group could only allow a maximum of 8 embryos per participant due to restrictions from the Endocell® culture plates, the supernumerary embryos ($n = 115$) were placed in the conventional medium (control group) and analysed as controls. The study group (AECC) was then composed of 326 embryos co-cultured on autologous endometrial cells from D2. The remaining 115 supernumerary embryos initially randomized in the AECC group were then added to the 458 control group embryos and were grown in conventional medium following Clinique Ovo's standard culture protocols (FIGURE 1).

TABLE 1 BASELINE DEMOGRAPHIC AND CYCLE CHARACTERISTICS

Parameter	Group	
	AECC[a]	CM[a]
Demographics	*n* = 63	*n* = 73
Age, female, years (mean ± SD)	32.8 ± 3.5	32.6 ± 4.2
Age, male, years (mean ± SD)	36.6 ± 5.9	35.7 ± 9.7
BMI, kg/m^2 (mean ± SD)	23.9 ± 5.3	24.2 ± 5.4
Smoker, yes, *n* (%)	5 (7.9)	10 (13.7)
Infertility cause		
Female factor, *n* (%)	20 (31.7)	18 (24.7)
Male factor, *n* (%)	20 (31.7)	27 (37.0)
Mixed, *n* (%)	6 (9.5)	9 (12.3)
Idiopathic, *n* (%)	17 (27.0)	19 (26.0)
IVF/ICSI attempt number		
1, *n* (%)	32 (50.8)	46 (63)
2, *n* (%)	20 (31.7)	19 (26)
3, *n* (%)	8 (12.7)	5 (6.8)
4, *n* (%)	2 (3.2)	2 (2.7)
5, *n* (%)	1 (1.6)	1 (1.4)
Oocytes	*n* = 56	*n* = 70
Stimulation, days (mean ± SD)	11.6 ± 1.4	11.6 ± 1.4
Number of oocytes collected (mean ± SD)	13.5 ± 5.8	14.1 ± 8.2
Number of metaphase II oocytes (mean ± SD)	10.1 ± 4.6	10.7 ± 5.8
Number of fertilized oocytes (mean ± SD)	6.8 ± 4.3	6.6 ± 4.3
Stimulation protocol		
Antagonist GnRH, *n* (%)	56 (88.9)	69 (94.5)
Agonist GnRH, *n* (%)	7 (11.1)	3 (4.1)
SMART, *n* (%)	0 (0.0)	1 (1.4)

No significant between-group differences were identified using one-way analysis of variance (ANOVA) and the chi-squared test for continuous and categorical variables, respectively.

95% CI = 95% confidence interval for mean; AECC = autologous endometrial co-culture group; BMI = body mass index; CM = conventional medium group; GnRH = gonadotrophin-releasing hormone; ICSI = intracytoplasmic sperm injection; SMART = stimulation with minimal adverse effects, retrieval and transfer.

[a] Baseline characteristics assessed in randomized patients.

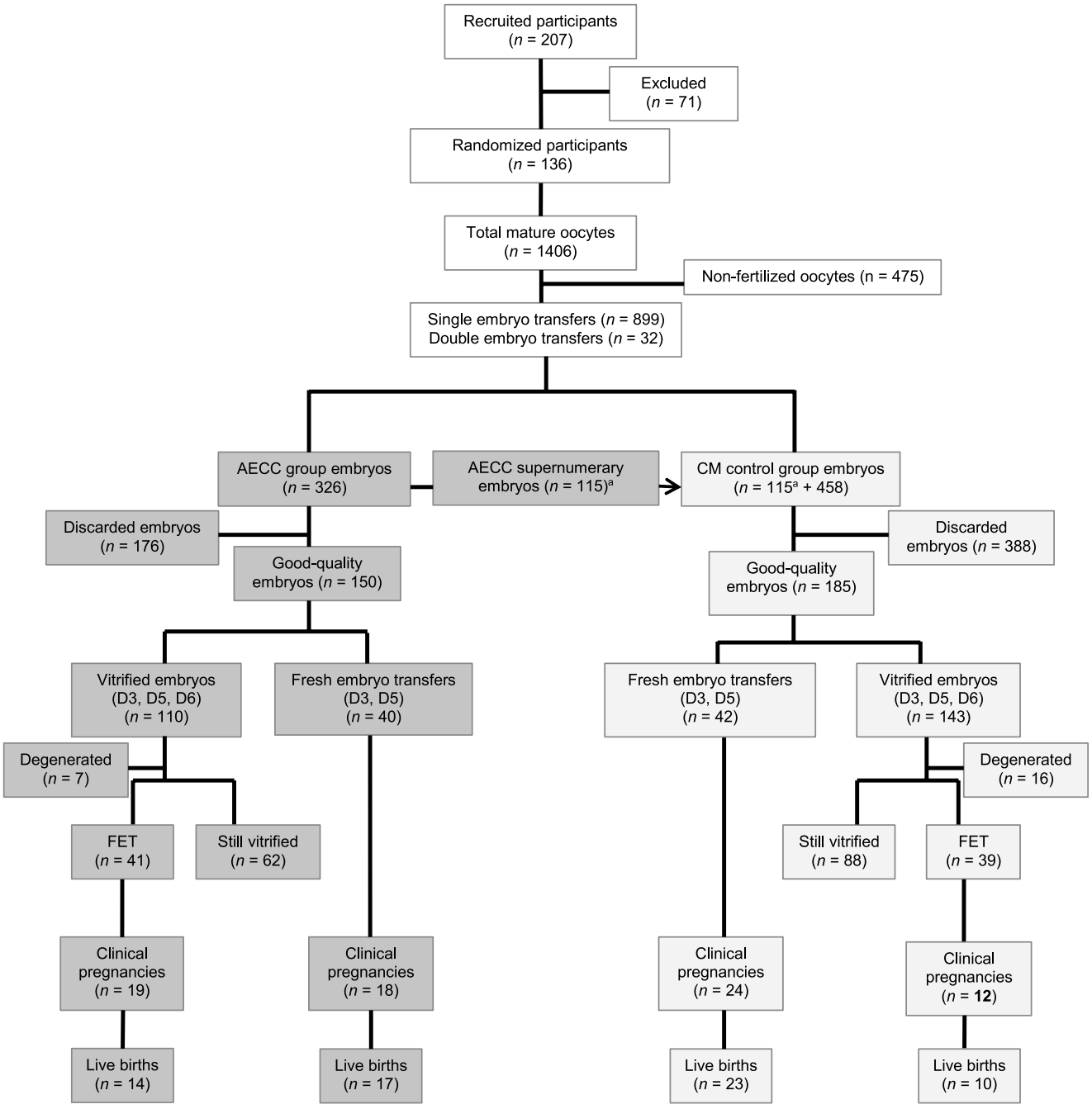

FIGURE 1 Participant flow chart. Embryos included in double embryo transfers were excluded. [a]Because the AECC group could only allow a maximum of eight embryos per participant due to restriction from the culture plates' dimension, the supernumerary embryos (*n* = 115) were placed in the conventional medium (control group) and analysed as controls. AECC group = autologous endometrial co-culture group; CM group = conventional medium group; FET = frozen embryo transfers.

Embryos that did not develop according to the defined criteria were discarded (*n* = 176 in the study group vs *n* = 388 in the control group). Among the quality D3 embryos and blastocysts obtained from the two groups, one selected embryo per participant was transferred in a fresh embryo transfer (*n* = 40 in the study group vs *n* = 42 in the control group). Supernumerary good embryos were vitrified for further use. One hundred and ten (110) early cleavage stage embryos and blastocysts were vitrified in the AECC group. Of these, 41 were transferred after warming, 7 degenerated post-warming and 62 were still cryopreserved at the time of the analysis. In the conventional medium group, from the 143 vitrified embryos, 39 were transferred; 16 degenerated post-warming and 88 were still cryopreserved (FIGURE 1).

Baseline characteristics

Population homogeneity between the two studied groups was assessed with the results provided in TABLE 1. Patient demographic characteristics, assessed in randomized patients, found between-group similarity with respect to patient age (32.8 ± 3.5 and 32.6 ± 4.2 years in the AECC and control group, respectively), partner age (36.6 ± 5.9 years vs 35.7 ± 9.7 years)

and body mass index (23.9 ± 5.3 kg/m^2 vs 24.2 ± 5.4 kg/m^2). In addition, no significant between-group differences were observed between oocyte characteristics with respect to stimulation duration (11.6 ± 1.4, AECC and control group, respectively), number of collected oocytes (13.5 ± 5.8 vs 14.1 ± 8.2 in the AECC and control groups, respectively), and number of MII (10.1 ± 4.6 and 10.7 ± 5.8) and fertilized oocytes (6.8 ± 4.3 vs 6.6 ± 4.3) (TABLE 1).

No between-group differences in smoking status, infertility cause, IVF/ICSI attempt number or stimulation protocol were observed (TABLE 1). Ninety-two per cent (92%) of the participants were treated using an antagonist protocol; 7% followed a short agonist protocol. Sixty-three per cent (63%) of the patients had their oocytes fertilized by ICSI, 26% by classical IVF, 11% had half of their oocytes fertilized by ICSI and half by classical IVF.

Embryo quality evaluation

The results showed that the quality of the 931 embryos (early cleavage stage and blastocysts), including the double embryo transfers (326 embryos in the study group and 605 embryos in the control group), was significantly improved by culturing them on AECC vs conventional culture medium (46% and 36% usable embryos, respectively, $P = 0.003$) (TABLE 2). Multivariable logistic regression analysis, conducted on the 931 fertilized oocytes, showed that an embryo is four times more likely to be transferable, regardless of the embryo stage (data for blastocysts and early cleaved embryos combined), when co-cultured (OR [95% CI]: 3.794 [2.847, 5.057], $P < 0.001$). Additionally, the blastulation rate was twice as high in embryos from the AECC group versus those placed in conventional culture medium (OR [95% CI]: 2.185 [1.603, 2.980], $P < 0.001$).

D5 and D6 embryos were three times (OR [95% CI]: 2.983 [2.185, 4.071], $P < 0.001$) and eight times (OR [95% CI]: 8.199 [3.466, 19.396], $P < 0.001$) more likely to develop into usable blastocysts when cultured on AECC compared with conventional medium, respectively.

Pregnancy and live birth outcomes

Pregnancy and live birth outcomes were assessed in the population of transferred embryos ($n = 162$). Overall, rates of biochemical pregnancies, clinical pregnancies and live births were comparable between AECC and conventional medium groups (TABLE 3). When assessed by type of embryo transferred, in the vitrified-warmed embryo transfer subgroup ($n = 80$), apparently higher rates of biochemical pregnancy (AECC = 58.5% vs conventional medium = 46.2%), clinical pregnancy (AECC = 46.3% vs conventional medium = 30.8%) and live births (AECC = 34.1% vs conventional medium = 25.6%) were found following AECC compared with conventional medium. Conversely, in the fresh embryo transfer ($n = 82$), apparently higher pregnancy rates (biochemical: AECC = 60.0% vs conventional medium = 66.7%; clinical: AECC = 45.0% vs conventional medium = 57.1%) and live birth rates (AECC = 42.5% vs conventional medium = 54.8%) were found following conventional medium compared with AECC (TABLE 3). Although none of the differences in rates in pregnancy and live birth rates across type of embryo transfer were statistically significant, the magnitude of the difference observed is nonetheless of clinical importance.

Miscarriage rate

No difference between the two groups has been observed for the miscarriages that happened during the study.

Predictive model

A predictive model was generated for the embryos that were still vitrified at the time of the analysis in order to estimate the pregnancy outcomes that would have been obtained with higher statistical power. The statistical coefficients obtained on the 150 vitrified embryos remaining were applied, respecting their randomization group. Cumulative probabilities showed a significant improvement in clinical pregnancy rates (57.03% in the AECC group vs 45.40% in the conventional medium group, $P = 0.04$) in favour of the AECC co-cultured embryos.

DISCUSSION

The primary objective of this monocentric interventional study was to assess whether the use of AECC would improve embryo development and provide more usable blastocysts for all patients undergoing IVF/ICSI, independent of their history of infertility.

Because the two populations in this study did not present any differences in terms of demographics or oocyte characteristics, it was possible to demonstrate that AECC significantly improved by 50% the number of quality blastocysts compared with the use of conventional culture medium.

TABLE 2 EMBRYO QUALITY (PRIMARY OUTCOME)

	Group		
	AECC	**CM**	***P*-value[a]**
Number of total embryos	*n* = 326	*n* = 605	
Total of good-quality embryos[b], *n* (%)	150 (46)	217 (36)	0.003
D3 embryos, *n* (%)	11 (3.4)	45 (7.4)	<0.001
D5 embryos, *n* (%)	99 (30.4)	150 (24.8)	<0.001
D6 embryos, *n* (%)	40 (12.3)	22 (3.6)	<0.001
Good-quality blastocysts, *n* (%) (D5 and D6)	139 (42.6)	172 (28.4)	<0.001

AECC = autologous endometrial co-culture group; CM = conventional medium group.

[a] Between-group differences assessed with the chi-squared test.

[b] Embryo quality assessed in fertilized embryos at early cleavage or blastocyst stage; includes double embryo transfers.

TABLE 3 PREGNANCY AND LIVE BIRTH OUTCOMES FOLLOWING FRESH VERSUS VITRIFIED-WARMED EMBRYO TRANSFERS (SECONDARY ENDPOINTS)

	Group	
	AECC[a]	CM[a]
Embryo transfers		
Fresh transfers, *n*	40	42
Frozen embryo transfers, *n*	41	39
All transfers, *n*	81	81
Biochemical pregnancies		
Fresh transfers, *n* (%)	24 (60.0)	28 (66.7)
Frozen embryo transfers, *n* (%)	24 (58.5)	18 (46.2)
All transfers, *n* (%)	48 (59.3)	46 (56.8)
Clinical pregnancies		
Fresh transfers, *n* (%)	18 (45.0)	24 (57.1)
Frozen embryo transfers, *n* (%)	19 (46.3)	12 (30.8)
All transfers, *n* (%)	37 (45.7)	36 (44.4)
Single live births		
Fresh transfers, *n* (%)	17 (42.5)	23 (54.8)
Frozen embryo transfers, *n* (%)	14 (34.1)	10 (25.6)
All transfers, *n* (%)	31 (38.3)	33 (40.7)

No significant between-group differences were identified with the chi-squared statistic.

AECC = autologous endometrial co-culture group; CM = conventional medium group.

[a] Pregnancy outcomes assessed in transferred embryos.

Thus, the AECC appears to enhance embryo development to blastocyst stage. These results were confirmed by the multivariate logistic regression analysis because the blastulation rate obtained with the autologous co-culture was twice as high as with the conventional medium. Indeed, D5 and D6 blastocysts co-cultured have three and eight times more chance, respectively, of being suitable for transfer, based on their quality criteria. A limitation of the design of this study is that the analysis was performed using embryos rather than patients. Because specific patient characteristics such as ovarian reserve and oocyte quality will impact on the overall results, this should be taken into consideration when assessing the findings. In addition, because the study was conducted within a private clinical setting and without access to a national registry, a follow-up of children born as a result of these treatments was not conducted. However, as with the introduction of any technology, it is an important step that should be carried out.

The benefits of AECC in in-vitro treatments have been evaluated in several controlled studies. Improvements in terms of the number of blastomeres (*Barmat et al., 1999; Eyheremendy et al., 2010; Liu et al., 1999; Simón et al., 1999*) and a decrease in fragmentation (*Spandorfer et al., 2002b*) leading to higher pregnancy rates were in favour of the co-culture. *Eyheremendy et al. (2010)* also showed the benefits of AECC on embryo development and clinical pregnancies in patients with repeated implantation failures. Furthermore, one trial had reported pre-embryo development improvement in patients with history of poor-quality embryos (*Spandorfer et al., 2002a*). AECC secretome analysis showed an enhanced expression of several growth factors that correlated with implanted blastocysts (*Dominguez et al., 2010; Liu et al., 1999; Spandorfer et al., 1998*). A meta-analysis also established that the use of co-culture (human cells and other species combined) improved embryo morphology and implantation rate as well as clinical and ongoing pregnancy rates (*Kattal et al., 2008*).

Although it was not possible to show a significant difference between the two groups in terms of pregnancy and live birth outcomes, it is clinically relevant to note that higher pregnancy and live birth rates were observed with the co-culture in case of frozen embryo transfer (FET) and higher rates with the conventional medium in the case of fresh embryo transfers (TABLE 3).

However, based on the predictive model, according to the cumulative probabilities extracted from the embryos that were still vitrified at the time of the analysis, it was possible to achieve statistical power. Significantly, a 26% increase in clinical pregnancy rate and a 29% increase in live birth rate could be hypothetically predicted with the co-culture compared with the conventional medium. These results are together very interesting because they were corroborated by *Ohl et al. (2015)*. In this study, it was shown that AECC significantly ($P < 0.05$) increased the pregnancy rate per blastocyst transfer by 16% compared with the transfer of D3 embryos cultured on a conventional culture medium (*Ohl et al., 2015*). The present work allowed confirmation of the benefits of AECC on blastocyst development and more specifically in the context of vitrified embryos. Indeed, freeze-all cycles gained in popularity particularly because some authors demonstrated the advantages associated

with FET when compared with fresh transfers, such as a reduction in the pre-term delivery (*Maheshwari et al., 2012*) and a decrease in low birth weight and perinatal mortality (*Marianowski et al., 2016*). Additionally, freeze-all cycles avoid the detrimental effects of ovarian stimulation that have been observed (*de Carvalho et al., 2017; Fatemi and Popovic-Todorovic, 2013; Gardner et al., 1998; Roque et al., 2015*) and combined with the results from endometrial receptivity tests (*Haouzi et al., 2011; Simón et al., 1999*) allow the transfer of quality blastocysts at a more propitious time.

In addition, it has been demonstrated that lower oxygen concentrations benefit blastocyst development (*Bontekoe et al., 2012; Meseguer and Pellicer, 2017*) and this represents a difference in these study groups, however the co-culture group did not benefit from lower O_2 concentrations and it could be projected that this would have only increased the efficiency of the AECC.

As the literature has shown, blastocysts are more likely to lead to ongoing pregnancies and live births (*Gardner and Lane, 1996; Papanikolaou et al., 2008; Schwärzler et al., 2004; Simón et al., 1999*). The rate of quality blastocysts obtained is thus an important factor for ART, consequently contributing to elective single embryo transfer (eSET) success combined with the use of vitrification and preimplantation genetic testing (*Practice Committee of the American Society for Reproduction Medicine, 2018*).

Data from this study suggest that the use of co-culture exploiting the patient's own endometrial cells could favour the development of embryos into good-quality blastocysts, and may be specifically beneficial to freeze-all cycles. While several teams have concentrated their works more specifically on patients with IVF failure (*Eyheremendy et al., 2010; Spandorfer et al., 2002a,2004*), data from this study suggest that all patients could benefit from AECC. However, a confirmatory study using patients as the denominator rather than embryos would help to confirm this proposal, as well as a follow-up of children born as a result of these treatments.

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