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## To cite this version:

Amira Sallem, Anne-Lyse Denizot, Ahmed Ziyyat, Audrey L'hostis, Sophie Favier, et al.. A fertilin-derived peptide improves in vitro maturation and ploidy of human oocytes. F&S Science, 2021, F&S Science, 3 (1), pp.21-28. 10.1016/j.xfss.2021.10.004 . hal-03613374

## HAL Id: hal-03613374

## https://paris1.hal.science/hal-03613374v1

Submitted on 18 Mar 2022

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# A fertilin-derived peptide improves in vitro maturation and ploidy of human oocytes

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**Objective:** To analyze the effect of a cyclic fertilin-derived peptide (cFEE) on in vitro maturation of human oocytes.
**Design:** Randomized study.
**Setting:** Fertility center in an academic hospital.
**Patient(s):** Not applicable.
**Intervention(s):** Human immature germinal vesicle-stage oocytes (n = 1,629) donated for research according to French bioethics laws were randomly allocated to groups treated with 1 or 100 $\mu$M of cFEE or to a control group. They were incubated at 37 °C in 6% $CO_2$ and 5% $O_2$, and their maturation was assessed using time-lapse microscopy over 24 hours. In vitro maturated metaphase II oocytes were analyzed for chromosomal content using microarray comparative genomic hybridization, and their transcriptomes were analyzed using Affymetrix Clariom D microarrays.
**Main Outcome Measure(s):** The percentage of oocytes undergoing maturation in vitro was observed. Aneuploidy and euploidy were assessed for all chromosomes, and differential gene expression was analyzed in oocytes treated with cFEE compared with the control to obtain insights into its mechanism of action.
**Result(s):** cFEE significantly increased the percentage of oocytes that matured in vitro and improved euploidy in meiosis II oocytes by the up-regulation of *FMN1* and *FLNA* genes, both of which encode proteins involved in spindle structure.
**Conclusion(s):** cFEE improves human oocyte maturation in vitro and reduces aneuploidy. It may prove useful for treating oocytes before fertilization in assisted reproductive technology and for in vitro maturation in fertility preservation programs to improve oocyte

Received September 13, 2021; revised October 19, 2021; accepted October 29, 2021.
S.P.R. and J.P.W. should be considered similar in author order.
J.P.W. reports grants from Agence Nationale de la Recherche, Assistance Publique-Hôpitaux de Paris, and Société d'Accélération du Transfert Technologique during the conduct of the study and financial support from FREMA SAS, outside the submitted work. In addition, J.P.W. has a patent 1H265600 0013 EP ECT/NIM/AGI) license. A.S. has nothing to disclose. A.L.D. has nothing to disclose. A.Z. has nothing to disclose. A.L. has nothing to disclose. S.F. has nothing to disclose. P.B. has nothing to disclose. J.M.L. has nothing to disclose. S.F.D. has nothing to disclose. C.P. has nothing to disclose. C.S. has nothing to disclose. E.V. has nothing to disclose. J.S. has nothing to disclose. D.V. has nothing to disclose. S.P.R. has nothing to disclose.
Supported by the French National Research Agency (ANR FERTILIVE to J.P.W., A.Z., and P.M.) and a grant from AP-HP *Contrat Amorçage Biothérapie de l'AP-HP*, 2008.
Supported by Contrat Amorçage Biothérapie de l'Assistance Publique-Hôpitaux de Paris, 2008, Essai clinique d'un peptide augmentant les capacités fusiogènes des gamètes humains; Société d'Accélération du Transfert Technologique 2017: "Maturation du projet de développement Fertiline"; Agence Nationale de la Recherche 2017: project ANR-17-CE18-0005 "FERTILIVE": "Amélioration de la ploïdie des ovocytes et embryons humains par le peptide cFEE: intérêt pour la fertilité des femmes"; and FREMA SAS: salary and financial support.
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Fertil Steril Sci® Vol. 3, No. 1, February 2022 2666-335X
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https://doi.org/10.1016/j.xfss.2021.10.004

quality and the chances for infertile couples to conceive. (Fertil Steril Sci® 2022;3:21–8. ©2021 by American Society for Reproductive Medicine.)
**Key Words:** Human oocyte, chromosome segregation, invitro maturation, *FMN1*, *FLNA*

**Discuss:** You can discuss this article with its authors and other readers at https://www.fertstertdialog.com/posts/xfss-d-21-00076

Since the first successful human in vitro fertilization (IVF) in 1978, ovarian stimulation protocols, manipulation of gametes and embryos, culture conditions, and cryopreservation have constantly been improving (1). The 1990s witnessed the development of the intracytoplasmic sperm injection (ICSI) technique for dealing with male factor infertility (2), and preimplantation embryo selection criteria were developed together with single embryo transfer protocols at the blastocyst stage (3). In spite of all these improvements, the percentage of infants born through assisted reproductive technologies (ARTs) attempts did not increase much worldwide, reaching now a mere 25%–30% per attempt, close to the natural fecundability of fertile young couples (4, 5).

The success of IVF outcomes depends mostly on oocyte quality (6). Indeed, on ovulation, the oocyte must resume meiosis I, complete meiosis II, activate the zygote after fertilization (7), and sustain the expression of the embryonic genome and the development during the first week of embryogenesis from the zygote to the blastocyst stage. Oocyte quality decreases with age, this factor leading to reduced fertility in women aged >35 years and a steep drop in the success rate of ART after 37 years of age (8). Attempts to rejuvenate oocyte quality have been performed, such as mitochondrial replacement (9, 10) and, more recently, NAD+ repletion (11) or coenzyme Q10 supplementation (12). However, they are ethical concerns associated with human fertilization studies, and these approaches have limited clinical impact so far. Nevertheless, improving oocyte quality remains imperative to improve ART outcomes. A simple, noninvasive treatment would be ethically more acceptable and could have a significant clinical impact.

Fertilin is a disintegrin and metalloprotease molecule present on the membrane of spermatozoa that interacts with an integrin on the surface of the oocyte to mediate the primary sperm–oocyte interaction (13). We previously described a synthetic cyclic tripeptide (cFEE) derived from the integrin-binding domain of human fertilin $\beta$. cFEE binds to the oocyte membrane and increases the human gamete fusion index in vitro by 94% using zona-free eggs (14). The equivalent peptide derived from the mouse fertilin cQDE increases the fertilization rate of mouse oocytes in vitro by 88%, and transfer of the resulting embryos into pseudopregnant females provided 3 generations of healthy pups (15). To evaluate the possibility of using the cFEE peptide to improve the success of ART in the clinic, here, we characterized further its effects on human oocyte maturation.

Immature human germinal vesicle (GV)-stage oocytes found in oocyte cohorts retrieved for the purpose of ICSI can be used for research according to French legislation, with the consent of the patient. Once the ICSI procedure was performed for the patients, oocytes that remained at the GV stage can be included in the protocol. Using these GV-stage oocytes, we conducted, over a 5-year period, an in vitro maturation study on more than 1,600 GV-stage oocytes. In vitro maturation to the GV breakdown, metaphase I, and metaphase II stages was recorded for 24 hours, as well as activation or atresia. We compared 1 and 100 $\mu$M of cFEE to a control group and studied the chromosome constitution of metaphase II oocyte using the microarray comparative genomic hybridization (CGH array) on single cell and run a transcriptomic analysis to better understand the mechanism of action of the cFEE peptide.

## MATERIALS AND METHODS

### In Vitro Maturation Study

The randomized in vitro maturation study was performed using immature GV-stage oocytes (n = 1,629) that were donated for research by patients undergoing ART protocols. It was approved by the GERMETHEQUE Biobank (BB-0033–00081) under the number 20160912. The GV-stage oocytes were collected from Cochin Hospital and from the American Hospital in Paris, Jean Verdier Hospital, Pierre-Cherest Clinic, and Foch Hospital facilities. When 1 or several GV-stage oocytes were available in these hospitals, a courier brought them to Cochin facilities. The cFEE peptide was synthesized by SYNPROSIS SA (Fuveau, France) to Good Manufacturing Practice standards for human use. Previous studies were performed using the cFEE peptide at 100 $\mu$M (14, 15), but a recent dose–response study on in vitro human embryo development that we performed (16) showed that probably 1 $\mu$M would give best results, whereas the concentration of 0.1 $\mu$M was ineffective. This is the reason why these 2 concentrations were used. When 1 oocyte was available, it was randomly attributed to 1 group (1 $\mu$M, 100 $\mu$M, or control) using Random.org. Special attention was paid when several GV-stage oocytes were coming from the same patient that they were displayed in the control and cFEE groups in a balanced way. The 100-$\mu$M cFEE concentration was introduced first in the study, followed by the 1-$\mu$M concentration. The study was then focused between these 2 concentrations of cFEE. This is the reason why there were more oocytes in the control group than in any of the cFEE groups.

Culture dishes were prepared, covered with mineral oil (Irvine Scientific, Ireland), warmed, and pregassed before in vitro maturation. Cumulus cells had been removed from the oocytes by brief treatment with hyaluronidase IV-S (Sigma-Aldrich, St. Louis, Missouri) at 37 °C. These decoronized oocytes were incubated in either Continuous Single Culture Complete medium (CSCM-C; Irvine Scientific, Santa Ana, California) or Global medium (LifeGlobal Group, Brussels,

Belgium) or incubated in these same media supplemented with the cFEE peptide (1 or 100 $\mu$M) in a time-lapse system (Geri; Genea Biomedx Pty Ltd., Box Hill, Australia) at 37 °C with 6% $CO_2$ and 5% $O_2$ to record the maturation process. Nuclear maturation was evaluated 24 hours later. Oocytes were considered "matured" when the first polar body was present.

## Single Cell Chromosomal Content Assessment by Microarray-Based CGH of Matured Human Oocytes

To determine whether individual in vitro matured oocytes contained chromosome anomalies such as trisomies and monosomies, we used microarray-based CGH (aCGH) to determine chromosome numbers. The zona pellucida of the matured oocytes was removed by gentle pipetting after 30-second incubation in acidified Tyrode's solution (Sigma-Aldrich) on ice to preserve chromosome integrity. They were then washed 3 times with culture medium and placed in a 3-$\mu$L sterile polymerase chain reaction (PCR) microtube containing a homemade lysis buffer composed of equal volumes of 50 mM of dithiothreitol (Promega) and 200 mM of KOH (Sigma-Aldrich) (17).

For whole genome amplification, multiple displacement amplification reactions were performed using the TruePrime kit (SYG380100; Ozyme, Saint-Cyr-l'École, France) according to the manufacturer's instructions. As a quality control, a PCR on the basis of amelogenin amplification (QIAGEN Multiplex PCR Kit, Stockach, Germany) was performed for each sample.

Samples that were properly amplified from the single oocyte deoxyribonucleic acid (DNA) were fluorescently labeled with Cy5 d-CTP using the SureTag DNA labeling kit (Agilent Technologies, Santa Clara, California). Male reference DNA provided with the labeling kit was fluorescently labeled with Cy3 d-CTP. Both DNA samples were then cleaned up by Microcon YM-30 columns (Millipore, Billerica, Massachusetts). The DNA concentration was adjusted after purification and dosage using a NanoDrop spectrophotometer (NanoDrop ND-2000; Thermo Fisher Scientific, Waltham, Massachusetts), and whole genome amplification DNA yields were hybridized on oligonucleotide-based 8 × 60,000 array chips (Agilent customer array; Necker-Enfants Malades Hospital, Paris, France). Microarrays were washed and scanned into image files using an Agilent Microarray Scanner (Scanner SurScan Dx; Agilent Technologies) and analyzed with Agilent CytoGenomics software (Agilent Technologies) for entire chromosome loss (monosomy) or gain (trisomy) across 23 chromosomes. Hybridizing the female sample (oocyte DNA) against a male reference DNA (sex mismatch) had to show a consistent gain of chromosome X and was considered as an additional quality control step, which had to be verified before the interpretation of the aCGH profile. According to Agilent recommendations for aCGH experiments on single cell, we used a threshold of 0.35 for the log2 ratio of gains of chromosomal content and −0.45 for loss of chromosomal content.

## Single Oocyte Transcriptomic Analysis

To obtain insight into the mechanism of action of the cFEE peptide, we analyzed differential gene expression in oocytes that matured to metaphase II with and without the peptide. Before transcriptomic analysis, the zona pellucida of each in vitro matured oocyte was removed in acidic Tyrode's solution, washed twice with CSCM-C medium, and transferred directly into the lysis buffer provided with the Ovation One-Direct System kit (NuGEN Technologies, Inc., San Carlos, California). Double-stranded complementary (cDNA) synthesis was then performed in 5 $\mu$L of lysate, and cDNA was purified before linear amplification by T7 ribonucleic acid polymerase. After a second round of purification on a QIAGEN MinElute column, the concentration of the amplified cDNA products was determined. The cDNA was then fragmented, labeled with biotin, and hybridized to Clariom D human microarrays (Affymetrix, Santa Clara, California) for 17 hours at 45 °C. These microarrays present 6,765,500 probes of 25 bases long (targeting >4,781,200 exons and >1,984,300 exon–exon splice junctions), which cover the whole genome. Samples were then stained with streptavidin–phycoerythrin using a biotin-targeted antibody step, washed in a GeneChip Fluidics Station 450, and scanned in a GeneChip Scanner 3000 7G according to the Affymetrix recommendations. The scanned images were converted into intensities using Affymetrix GeneChip Command Console Software, and transcriptomic data were analyzed using Transcriptome Analysis Console software. STRING was used to determine potential networks linking significantly modified genes (18).

## Statistical Analysis

Statistical analyses of the data on in vitro maturation and chromosome content were performed by the Clinical Research Unit of Lariboisière Hospital (Paris, France) using SAS v9.4 (SAS Institute Inc., Cary, North Carolina). Continuous variables are expressed as means with standard deviations, and categorical variables are summarized as counts and percentages. The associations between groups of categorical parameters were tested using the $\chi^2$ and Fisher exact tests. The Wilcoxon–Mann–Whitney U test was used to determine whether there were significant differences between groups. The Pearson correlation coefficients were used to evaluate correlations between variables. For analyses involving multiple oocytes per patient, generalized estimating equations were used to take into account the nonindependence of the statistical units. The Student paired $t$ test was used to compare the transcriptomes of oocytes from the 2 groups and evaluate whether the ratios of aneuploidies/euploidies for all chromosomes in the control and treated groups were significantly different. Multivariate analyses were performed using multivariate logistic regression. For all statistical tests, a $P$ value of $<.05$ was considered significant.

# RESULTS

## cFEE Improves the Human Oocyte In Vitro Maturation Rates

One group of GV-stage oocytes was treated with 1 $\mu$M of cFEE (424 oocytes), a second group was treated with 100 $\mu$M of cFEE (473 oocytes), and the control group (732 oocytes) was simply incubated in medium. To investigate whether the

## TABLE 1

**Confounding factors and detailed evolution of in vitro matured oocytes according to cFEE concentrations.**

| | | | Day 0 | | Day 1, n (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | Women age[a] | GV per cohort[a] | GV (N) | GV | MI | MII | Atretic | Activated |
| Control | 33.9 ± 4.7 | 2.5 ± 1.8 | 732 | 119 (16.3) | 232 (31.7) | 293 (40.0) | 84 (11.5) | 4 (0.005) |
| 1 μM | 34.3 ± 4.9 | 2.1 ± 1.8 | 424 | 60 (14.1) | 103 (24.2) | 220 (51.9)[c] | 36 (8.5) | 5 (1.2) |
| 100 μM | 33.5 ± 4.8 | 2.0 ± 1.5 | 473 | 70 (14.8) | 116 (24.5) | 221 (46.7)[b] | 61 (12.9) | 5 (1.1) |

*Note:* Attention was paid not to include the germinal vesicle, which represented more than 20% of the cohort, to prevent any bias related to ovarian dysfunction. The numbers in parentheses represent the percentage by condition. GV = germinal vesicle; MI = Metaphase I; MII = Metaphase II.
[a] Mean ± standard deviation.
[b] $P$= .020.
[c] $P$= .002.

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results depended on a special medium or not, different media (Global and CSCM-C) were used. No supplementation with gonadotropin was performed to prevent any bias of analysis. Table 1 shows the maturation stage reached by oocytes in each group after 24 hours of incubation. As shown in Figure 1, 40.0% of the oocytes in the control group had matured (N = 293/732), compared with 51.9% in the 1-μM cFEE group (N = 220/424) and 46.7% in the 100-μM cFEE group (N = 221/473). Regression analysis using a generalized estimating equation and considering the correlation between oocytes originating from the same puncture (i.e., collected at the same time from the same woman) showed that cFEE significantly increased the percentage of matured oocytes, at both 1 μM (odds ratio [OR], 1.53; $P$=.002) and 100 μM (OR, 1.32; $P$=.020) (Table 2). Other factors, including the incubation medium (Global or CSCM-C) and age of the patient at the time of puncture, had no significant impact on the outcome. As incubation with 1 μM of cFEE gave the best results, the aCGH and transcriptomic analyses were performed using oocytes that had been incubated with this cFEE concentration.

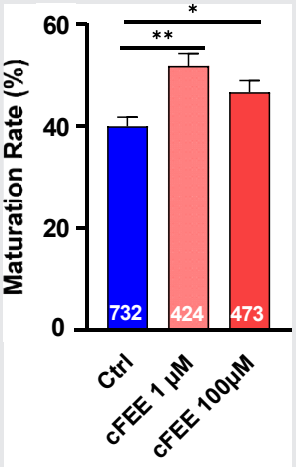
The delay between GV breakdown and polar body extrusion was 14.7 ± 1.8 hours for the control group, 14.5 ± 1.9 hours for the group treated with 1 μM of cFEE, and 14.9 ± 1.6 hours for the group treated with 100 μM of cFEE. A comparison of these delays found no significant differences when analyzed with either the Wilcoxon two-sample test (Z = 0.000, $P$=1.000) or Kruskal–Wallis test ($\chi^2$ test, 0.0003; $P$=.9866; df = 1), suggesting that the cell cycle and course of meiosis were unaffected by the treatment.

## cFEE Improves Human Oocyte Ploidy In Vitro

The ploidy of the mature oocytes in metaphase II was analyzed using CGH arrays. As a control of the technique, 18 single human lymphocyte samples with known chromosome abnormalities were hybridized, resulting in full concordance of the aCGH technique with the expected chromosomal status (Supplemental Fig. 1, available online). We then performed aCGH on oocytes that had matured to metaphase II and had passed the quality control criteria (Supplemental Fig. 2). A total of 112 oocytes from 103 women aged between 21 and 43 years (median, 34 years) were included in this ploidy study. Half of these oocytes had been treated with 1 μM of cFEE, and half were from the control group (N = 56 per group). The concentration of 1 μM was chosen for this study because it provides the best percentage of euploid oocytes. Attention was paid not to induce any bias in the infertility factors of women who gave the oocytes (Table 3).

The results of aCGH demonstrated the performance of cFEE peptide in improving oocyte ploidy (Fig. 2A and Table 4). All types of aneuploidies, including trisomy, monosomy, or both, were observed in both treated and untreated groups of oocytes, and all chromosomes could be affected by aneuploidy (Fig. 2B), but a statistically significant improvement in the proportion of euploid metaphase II oocytes was observed in the cFEE-treated group (46.4%)

## FIGURE 1




cFEE peptide improves in vitro maturation human germinal vesicle (GV)-stage oocytes. Human oocytes at the GV stage (N = 1,629 from 755 women aged 18– years) were incubated with either Global or CSCM-C medium (control, 732 oocytes), 1 μM of cFEE (424 oocytes) or 100 μM of cFEE (473 oocytes). Oocyte maturation was determined by the observation of polar body extrusion and expressed as a percentage. A regression analysis using a generalized estimating equation (shown in Table 2) and taking into account the correlation between oocytes from the same puncture shows that the cFEE peptide significantly increased the maturation rate at 1 μM (odds ratio, 1.53; **$P$=.002) and 100 μM (odds ratio, 1.32; *$P$=.020).

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## TABLE 2

**Factors associated with the probability of maturation.**

| Parameters | Estimates | Standard error | OR | 95% CI low | 95% CI up | *P* value |
|---|---|---|---|---|---|---|
| cFEE 1 μM vs. 0 | 0.422 | 0.133 | 1.53 | 1.18 | 1.98 | .002 |
| cFEE 100 μM vs. 0 | 0.279 | 0.1201 | 1.32 | 1.04 | 1.67 | .020 |
| Global vs. CSCM-C | −0.160 | 0.1349 | 0.85 | 0.65 | 1.11 | .234 |
| Age >35 vs. <30 | 0.031 | 0.1655 | 1.03 | 0.75 | 1.43 | .850 |
| Age 30–35 vs. <30 | 0.128 | 0.1651 | 1.14 | 0.82 | 1.57 | .437 |
| 2 oocytes vs. 1 | −0.221 | 0.1452 | 0.80 | 0.60 | 1.07 | .128 |
| 3 oocytes vs. 1 | −0.207 | 0.1733 | 0.81 | 0.58 | 1.14 | .233 |
| 4 oocytes vs. 1 | −0.327 | 0.2039 | 0.72 | 0.48 | 1.07 | .108 |
| 5+ oocytes vs. 1 | −0.392 | 0.1774 | 0.68 | 0.48 | 0.96 | .027 |

*Note:* The analysis was conducted on all oocytes, at both cFEE concentrations and in both media. These calculations were made using a generalized estimating equation, taking into account the number of oocytes obtained from the same puncture. Human oocytes at the germinal vesicle stage (N = 1,629 from 755 women aged 18–45 years) were included in the in vitro maturation study (either Global or CSCM-C medium). There were 732 oocytes incubated in the control group, 424 oocytes in the 1-μM cFEE group, and 473 oocytes in the 100-μM cFEE group. A regression analysis using a generalized estimating equation model and taking into the correlation between oocytes originating from the same puncture shows that the cFEE peptide significantly increased the maturation rate of oocytes, at both 1 μM (odds ratio, 1.53; *P*= .002) and 100 μM (odds ratio, 1.32; *P*= .020). CI = confidence interval; CSCM-C = Continuous Single Culture Complete medium; OR = odds ratio.

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compared with the control (26.8%) (*P*=.031) (Fig. 2A). The distribution of difference showed that, on average, there were more than twofold more aneuploid chromosomes in the control group than in the cFEE-treated group (mean, 2.1818; standard deviation, 2.5567; *P*=.0006) (Fig. 2B). Similarly, a Wilcoxon two-sample test indicated that cFEE treatment significantly decreased the number of aneuploid chromosomes per oocyte from 1.8 ± 1.8 in the control group to 1.1 ± 1.5 in the cFEE group (Z = 2.4091, *P*=.0160).

Treatment with cFEE also significantly reduced the number of oocytes containing 3 or more aneuploid chromosomes, 30.4% vs. 14.3% (*P*=.041), respectively (Fig. 2A). Analysis by logistic regression confirmed that for an oocyte, being into the control group was a predictive factor significantly associated with an increased risk of aneuploidy (OR, 2.84 [1.22–6.61]; *P*=.0155).

The comparison of the percentage of aneuploidy associated with each individual chromosome found no significant differences between the cFEE-treated and control groups because of the limited number of events for each individual chromosome. The smaller number of cases of trisomy and monosomy together observed in aneuploid oocytes incubated with cFEE (5.4%) compared with the control (16.1%) almost reached statistical significance (*P*=.068) but was limited by the small number of events.

## TABLE 3

**Infertility factors in the aCGH control and cFEE groups.**

| Infertility factors | Control group (n = 56) | cFEE group (n = 56) | *P* value[b] |
|---|---|---|---|
| Female | 11 (19.6%) | 20 (35.7%) | .40 |
| Male | 22 (39.3%) | 16 (28.6%) | |
| Mixed | 145 (25.0%) | 13 (23.2%) | |
| Idiopathic | 6 (10.7%) | 4 (7.1%) | |
| Others[a] | 3 (5.4%) | 3 (5.4%) | |

*Note:* The confounding factors for patients whose oocytes were involved in the ploidy study were analyzed (*P* = .40, $\chi^2$ test).
[a] Includes fertility preservation and oocyte donor.
[b] $\chi^2$ *P* value.

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### cFEE Up-Regulates Genes Encoding Spindle and Cytoskeleton Proteins

To obtain insight into the mechanism of action of the cFEE peptide, we analyzed the transcriptome of the control (n = 11) and 1-μM cFEE-treated (n = 11) oocytes from 8 patients that matured to metaphase II stage, using Clariom D microarray. A nonsupervised hierarchical clustering clearly shows 2 distinct profiles of gene expression between the control and cFEE-treated oocytes (Supplemental Fig. 3).

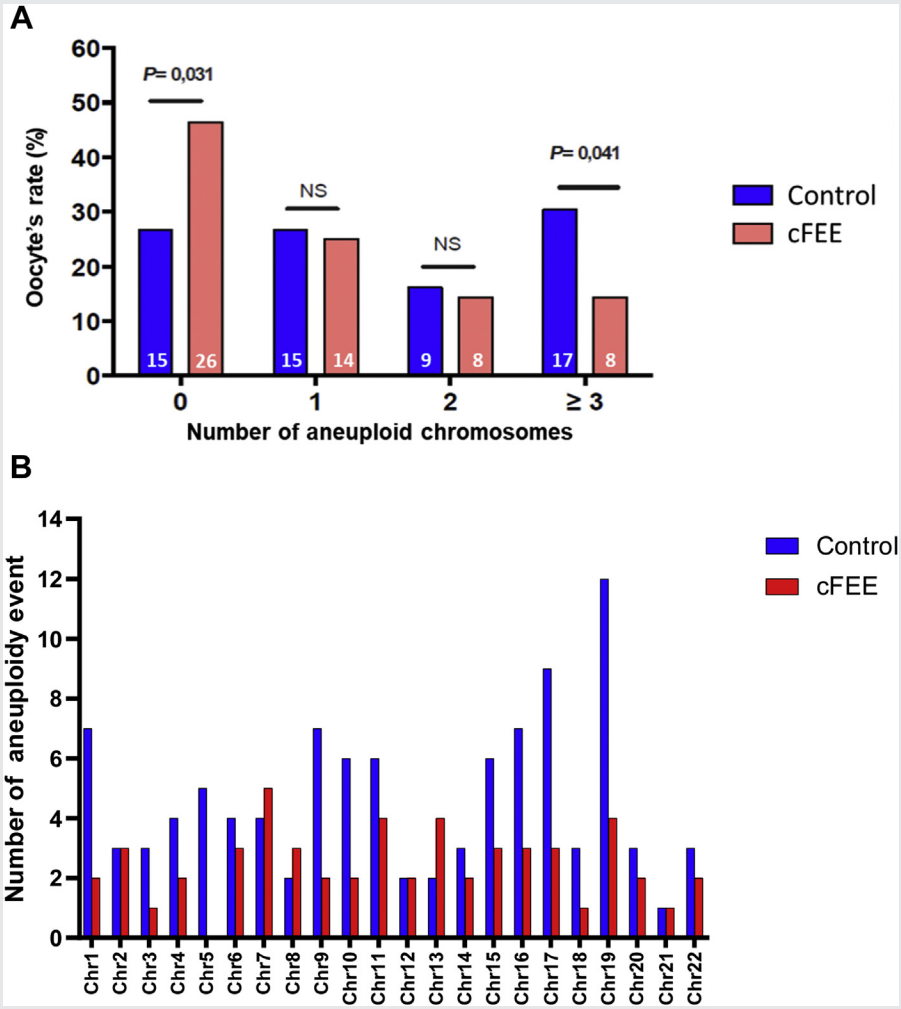
Transcriptomic data were also analyzed by paired *t* test between patients of both groups. Gene expression analysis showed that 623 genes were differentially expressed between the 2 groups with a *P* value of.05 and a fold change (FC) of ≤−1.5 or ≥1.5, 364 being up-regulated and 259 down-regulated in the cFEE-treated oocytes compared with the control group. The most highly up-regulated gene was FMN1 (FC, 8.68; *P*=.012), which encodes for formin-1. Formins are multidomain proteins that are key regulators of actin and microtubule cytoskeletal dynamics (19, 20). There was also an increase in the *FLNA* gene expression (FC, 1.38; *P*=.005), which encodes filamin A. It is an actin-binding protein that is also implicated in spindle migration during meiosis (21). Hence, cFEE treatment improves the expression of genes that are crucial for spindle and cytoskeleton.

## DISCUSSION

A reduced efficiency of chromosome segregation during meiosis (22, 23) is one of the main causes for the loss of fertility in women aged >30 years (24). In the present study, we precisely demonstrate that it is improved by cFEE during the first meiosis division, which is responsible for most of aneuploidies (25, 26).

Indeed, cFEE improves the percentage of euploid metaphase II oocytes and reduces the percentage of "chaotic" oocytes containing 2 or more chromosome abnormalities. The timing of polar body extrusion was not modified, suggesting that the cell cycle was not affected by the molecule. The percentage of aneuploid oocytes was high in the control group,

## FIGURE 2




cFEE peptide improves human matured oocyte ploidy. Human oocytes at metaphase II stage (n = 56 per group) were assessed for chromosomal status using aCGH. (**A**) Number of oocytes according to the number of aneuploid chromosomes in the control and 1-$\mu$M cFEE group. Statistical significance is indicated above the bars. NS = no significant difference. The number of oocytes tested is indicated in each bar. (**B**) Comparison of the number of aneuploidy event for each chromosome per oocyte between the control and cFEE groups. A concentration of 1 $\mu$M of the cFEE peptide significantly reduced the occurrence of aneuploidy for each chromosome ($P$=.0007, Student paired $t$ test).

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approximately 70%, probably because these GV-stage oocytes came from too small follicles that were punctured. Hence, the GV-stage oocytes should most probably present a cytoplasmic immaturity. The fact that cFEE was efficient on these oocytes suggests that it could even be more efficient on cytoplasmic matured oocytes.

## TABLE 4

**Percentage of euploid/aneuploid oocytes in the 1-$\mu$M cFEE and control in vitro matured groups of oocytes.**

| Variable | Total | cFEE | Control | P value[a] |
|---|---|---|---|---|
| Aneuploidy, N (%) | 71 (63.4%) | 30 (53.6%) | 41 (73.2%) | .0310 |
| Euploidy, N (%) | 41 (36.6%) | 26 (46.4%) | 15 (26.8%) | |
| Total | 112 (100.0%) | 56 (100.0%) | 56 (100.0%) | |

*Note:* A total of 112 oocytes from 103 women aged 21–43 years (median, 34 years) were analyzed for aneuploidy using aCGH (see the Materials and Methods section).
[a] $\chi^2$ $P$ value.

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Its action also appears to be independent of the medium since different media were used without any significant difference in the result. This peptide is, therefore, a strong candidate molecule for improving ART outcome, the 1-$\mu$M concentration being the most efficient. It may be particularly useful in protocols of in vitro maturation for patients with polycystic ovary syndrome or for fertility preservation programs when ovarian stimulation is contraindicated. The supplementation of medium with gonadotropin, as regularly performed in protocols of in vitro maturation, may further improve these results (27).

There are 3 main causes of aneuploidy: spindle assembly checkpoint defects (28); loss of cohesion between sister chromatids (29); and spindle instability (30). Using CGH and transcriptomic analysis, we showed that the cFEE peptide stimulates gene pathway involved in the meiotic process. The absence of any differential expression of genes associated with cell division checkpoint or with the expression of genes encoding for sister chromatid cohesion mediators suggests that neither the checkpoint nor the cohesins are direct targets of cFEE.

The third major cause of aneuploidy is spindle defects. The meiotic spindle of human oocytes is highly unstable compared with those of mouse oocytes (31). Because of the large size of mammalian oocytes and asymmetry of the meiotic divisions (32), the spindle has an atypical structure compared with those of mitotic cells. Notably, oocyte spindles have atypical centrosomes and lack astral microtubules (20, 32). To compensate for this, oocytes use an actin-based mechanism for spindle positioning, which transmits force over long distances during oocyte meiosis (20). The formation of the meiotic spindle and its migration near the cortex are, therefore, crucial events to allow most maternal components to stay within the oocytes for preimplantation embryo development (32).

Consistent with this, our transcriptomic data found a significant increase in the expression of *FMN1* and *FLNA* in oocytes. *FMN1* encodes formin-1, belonging to the formin homology family, which consists of actin nucleators that play a significant role in actin filament assembly during cell division (19, 20). *FLNA* encodes filamin A, another essential actin regulator that controls spindle migration and asymmetric division during oocyte meiosis (21). This increase in the expression of *FMN1* and *FLNA* in the transcriptome of human oocytes, which may be due to the stabilization of the corresponding messenger ribonucleic acid (e.g., by polyadenylation), suggests that cFEE promotes spindle formation and migration to the membrane and reduces spindle defects. This appears to be the most probable explanation for its effect in reducing aneuploidy during meiosis I.

Several studies attempting to improve human oocyte maturation have been performed (33). Some succeeded in improving the maturation rate (34). Only supplementation with coenzyme Q10 to restore mitochondrial functions also succeeded in reducing postmeiotic aneuploidies, with apparently another mechanism of action, and mostly in oocytes from older women (12).

cFEE appears to have the potential to actually rejuvenate oocytes and significantly improve the ART outcome. It may provide a safe and ethically acceptable way to improve ART outcomes in humans and in a program of in vitro maturation for fertility preservation.

**Acknowledgments:** We thank the medical and paramedical staff of Cochin Hospital, the American Hospital of Paris, Jean Verdier Hospital, Pierre-Cherest Clinic, and Foch Hospital for collecting GV-stage oocytes; the technicians of the aCGH platform at the Necker-Enfants Malades Hospital for their help with aCGH experiments; and S. Jacques (GENOM'IC Platform, Cochin Institute) for technical help with transcriptomic analysis. The study of in vitro maturation of human GV-stage oocytes was performed in the IVF unit of Cochin Hospital with the collaboration of the Laboratories of Genetics and Cytogenetics at Necker-Enfants Malades Hospital for genomics analysis. Life Science Editors provided professional scientific editing services during the preparation of the manuscript.

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