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ORIGINAL ARTICLE ***Reproductive biology***

The mitochondrial DNA content of cumulus granulosa cells is linked to embryo quality

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STUDY QUESTION: Could the mitochondrial DNA (mtDNA) content of cumulus granulosa cells (CGCs) be related to oocyte competence?

SUMMARY ANSWER: The quality of embryos obtained during IVF procedures appears to be linked to mtDNA copy numbers in the CGCs.

WHAT IS KNOWN ALREADY: Oocyte quality is linked to oocyte mtDNA content in the human and other species, and the mtDNA copy number of the oocyte is related to that of the corresponding CGCs. Moreover, the quantification of CGC mtDNA has recently been proposed as a biomarker of embryo viability.

STUDY DESIGN SIZE, DURATION: An observational study was performed on 452 oocyte–cumulus complexes retrieved from 62 patients undergoing ICSI at the ART Center of the University Hospital of Angers, France, from January to May 2015.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The average mtDNA content of CGCs was assessed by using a quantitative real-time PCR technique. The relationship between CGC mtDNA content and oocyte maturity and fertilizability, on one hand, and embryo quality, on the other, was investigated using univariate and multivariate generalized models with fixed and mixed effects.

MAIN RESULTS AND THE ROLE OF CHANCE: No relationship was found between CGC mtDNA content and oocyte maturity or fertilizability. In contrast, there was a significant link between the content of mtDNA in CGCs surrounding an oocyte and the embryo quality, with significantly higher mtDNA copy numbers being associated with good quality embryos compared with fair or poor quality embryos [interquartile range, respectively, 738 (250–1228) and 342 (159–818); $P = 0.006$]. However, the indication provided by the quantification of CGC mtDNA concerning the eventuality of good embryo quality was seriously subject to patient effect (AUC = 0.806, 95%CI = 0.719–0.869). The quantity of CGC mtDNA was influenced by BMI and smoking.

LARGE SCALE DATA: N/A.

LIMITATIONS REASONS FOR CAUTION: The quantification of CGC mtDNA may indicate embryo quality. However, since it is affected by patient specificity, it should be used with caution. It remains to be seen whether this marker could directly predict the implantation capacity of the embryo, which is the main objective in IVF practice.

WIDER IMPLICATIONS OF THE FINDINGS: Our study suggests that the quantification of CGC mtDNA may be a novel biomarker of embryo viability. However, patient specificity makes it impossible to establish a general threshold value, valid for all patients. Nevertheless, further studies are needed to determine whether the quantification of CGC mtDNA may, in combination with the morpho-kinetic method, offer an additional criterion for selecting the best embryo for transfer from a given cohort.

STUDY FUNDING/COMPETING INTEREST(S): This work was supported by the University Hospital of Angers, the University of Angers, France, and the French national research centres INSERM and the CNRS. There were no competing interests.

Key words: cumulus cells / granulosa cells / embryo quality / mitochondria / mitochondrial DNA

Introduction

Despite the progress of Assisted Reproductive Technologies, the overall success rates of IVF remain relatively low. Although there are variations between different countries around the world, the overall delivery rate with IVF is only ~20.3% per fresh embryo transfer (Ishihara *et al.*, 2015).

The choice of the embryo for transfer should allow selection of the embryo with the highest developmental potential so as to increase the chances of pregnancy and limit the risks of multiple pregnancies. Currently, this choice is based on embryo morphology and on the kinetics of development that reflect embryo vitality (Meseguer *et al.*, 2012). The various classifications used by embryologists for choosing embryos for transfer were standardized at an expert meeting of the European Society of Human Reproduction and Embryology (ASRM and ESHRE Special Interest Group of Embryology, 2011).

However, despite the recent standardization and improvement of the criteria of embryo selection, pregnancy rates have remained relatively low, showing that these criteria are rather limited and not completely informative (Bromer and Seli, 2008). Thus, it would be useful to find reliable, non-invasive markers of embryo viability.

Embryogenesis stems from oogenesis and embryo scalability largely depends on oocyte competence. However, <7% of the oocytes collected during IVF procedures develop into normal embryos leading to a live birth (Patrizio and Sakkas, 2009). The assessment of oocyte competence may improve embryo selection. Whereas the morphology of oocytes can be directly visualized, it is not sufficiently informative. Within the ovarian follicle, a system of bidirectional signalling between the oocyte and the surrounding granulosa cells ensures the acquisition of oocyte competence (Gilchrist *et al.*, 2008). The study of the cumulus granulosa cells (CGCs) is considered to be one of the best non-invasive approaches for evaluating the metabolic processes underlying oocyte quality and the developmental potential of the embryo. In particular, oocyte–cumulus complex (OCC) interactions orchestrate carbohydrate, lipid and protein metabolism to ensure the appropriate energy balance required for the meiosis and fertilization of the oocyte, and for support during early embryogenesis. Thus, CGC mitochondria, which are central agents of the energetic metabolic pathways, are directly involved in the establishment of oocyte competence during oogenesis (Dumesic *et al.*, 2016).

We have shown in the human that CGCs, through the expression of factors implicated in mtDNA replication and maintenance, play a major role in constituting a sufficiently large mtDNA pool during oogenesis, essential for oocyte competence (Boucret *et al.*, 2015). In a recent study on the pig, the mtDNA content in the oocyte was found to be correlated with that in the CGCs (Pawlak *et al.*, 2015). This implies that, just like the mtDNA of the oocyte (St John, 2013), the mtDNA of the surrounding CGCs may contribute to its competence in supporting normal embryo development. Interestingly, CGC mtDNA copy numbers have recently been reported to be good predictors of embryo quality in IVF procedures, with positive and negative predictive values of 84.4 and 82.1%, respectively (Ogino *et al.*, 2016). Here, we report the results of a study, performed on 452 OCCs retrieved from 62 patients, aimed at assessing the extent to which the quantity of CGC mtDNA may be correlated with oocyte nuclear maturity and fertilizability and embryo quality.

Materials and Methods

Patient characteristics

CGCs were obtained from 62 patients undergoing ICSI at the ART Centre of the University Hospital of Angers, France, from January to May 2015. Couples with male infertility indications are candidates for ICSI and these represent ~50% of the IVF procedures in our facility.

The characteristics of the 62 women as well as the products used for the suppression of pituitary gonadotrophin release [Cétrorelix (Cetrotide®, Merck-Serono, Geneva, Switzerland); Ganirelix (Orgalutran®, Organon, Oss, the Netherlands); Triptoréline (Decapeptyl®, Ipsen Pharma, Paris, France)] and follicular growth stimulation [uFSH + uLH: Ménotropine (Menopur®, Ferring Pharmaceuticals, Copenhagen, Denmark); rFSH: Follitropine alpha (Gonal-f®, Merck-Serono, Geneva, Switzerland) or Follitropine beta (Puregon®, Organon, Oss, the Netherlands)] are summarized in Table I. In each case, ovulation was induced with Ovitrelle® (Choriogonadotropine alfa, Merck-Serono, Geneva, Switzerland), and the oocytes were retrieved transvaginally under ultrasound guidance.

Isolation of oocytes and cumulus cells

OCCs were retrieved 36 h after treatment with human chorionic gonadotropin, and washed in multiple dishes with Flushing medium® (Origio-France, Limonest, France) to eliminate the remaining mural granulosa cells, blood cells and cellular debris. The OCCs were subsequently incubated for 1 h in Ferticult® culture media (Fertipro, Beernem, Belgium). For each patient, the oocytes were individually stripped using hyaluronidase (80 IU,

Table I Characteristics of patients ($n = 62$)

Age (years)	BMI	Smokers	LH suppression			Type of stimulation		Total dose of FSH (UI)
			Decapetyl	Orgalutran	Cetrotide	rFSH	uFSH	
32.7 ± 4.2	25 ± 4.6	23 (37.1%)	9 (14.5%)	44 (71.0%)	9 (14.5%)	47 (75.8%)	15 (24.2%)	2204 ± 923

Fertipro, Beernem, Belgium) and gentle pipetting with a 125-µm diameter stripper pipette (Origio-France, Limonest, France). Metaphase II oocytes were used for the ICSI procedure and immature oocytes were discarded. The CGCs associated with each oocyte were recovered and isolated. A total of 452 isolated CGCs were individually collected (325 from mature oocytes, i.e. metaphase II oocytes, and 127 from immature oocytes; Fig. 1). CGCs were recovered in 500 µl of physiological serum, and centrifuged at 10 000*g* for 5 min. The supernatant was removed and the CGC pellets were immediately frozen at −80°C until nucleic acid extraction.

DNA extraction

Total DNA extraction, from isolated fractions of CGCs, was carried out using the Nucleospin Tissue Kit® (Macherey-Nagel, Düren, Germany) according to the manufacturer's recommendations. Lysis was achieved by incubation of the sample material in a proteinase K/SDS solution. Appropriate conditions for DNA binding to the silica membrane in the NucleoSpin tissue columns were obtained by the addition of chaotropic salts and ethanol to the lysate. The binding process was reversible and specific to nucleic acids. Contamination was removed by washing with two different buffers. Genomic DNA was finally eluted in 60 µl of pre-warmed (72°C) elution buffer.

Quantification of mtDNA

The mean mtDNA copy number in CGCs was determined by real-time quantitative PCR (Q-PCR) using SYBR green DNA intercalator on the Chromo4 System® (Biorad, Hercules, CA, USA) in a 20 µl reaction volume containing a final concentration of 0.5 µM of each gene-specific primer and 3 µl of template. The pairs of primers selected were, respectively, MT-CO1 (mtDNA nucleotide positions 7017–7036 and 7205–7224) to quantitate mtDNA, and B2M (beta two microglobulin, exon 3 −112 to −93 and exon 3 +84 to +113) to quantitate nuclear DNA in follicular cells. The reactions were performed as follows: initial denaturing at 95°C for 10 min, and 40 cycles at 95°C for 45 s, 54°C for 1 min and 72°C for 45 s. A melting curve was analysed in order to check the specificity of the PCR product. The DNA copy number was determined with standard curves from 10^2 to 10^6 copies of each gene.

The average CGC mtDNA was determined by calculating the ratio between the mtDNA copy number and twice the nuclear DNA copy number (reflecting the copy number per diploid genome).

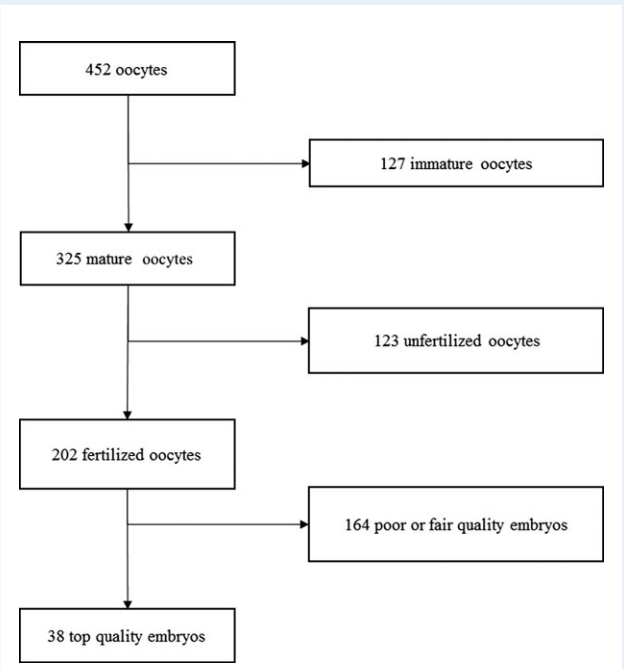

Figure 1 Oocyte and embryo flow-chart.

Embryo culture and classification

After sperm injection, embryos were cultivated in Global medium® (Life Global, Guilford, CT, USA) under an atmosphere of CO_2. They were observed 18 h post-injection to observe the presence of the two pronuclei, and 48 h post-injection to observe embryo cleavage. Among the 325 mature oocytes, 202 led to embryos 48 h post-injection. The embryos were scored at Day 2 (48 h post-injection) according to the ESHRE consensus (ASRM and ESHRE Special Interest Group of Embryology, 2011). We took into account the cell number, the stage-specific cell size, the percentage of fragmentation and the existence of multinucleation to define two groups of embryos, i.e. 'good quality' and 'fair or poor quality' embryos (Supplementary Table S1).

Among the 202 embryos retrieved, 38 were good quality embryos and 164 were fair or poor quality embryos (Fig. 1).

Statistical analysis

All continuous variables (age, BMI, total dose of FSH and mtDNA) were described with mean values and standard deviations [or with median values and the interquartile range (IQR), if appropriate] and were compared using Student's *t*-test (or the Mann–Whitney test, if applicable). All binary and qualitative variables (smoking status, type of LH suppression, type of stimulation and the three outcomes: oocyte nuclear maturity, oocyte fertilizability and embryo quality) were summarized with their counts or percentages and were compared using Pearson's X^2 test (or Fisher's exact test, if appropriate). For the purposes of statistical analysis, the association between outcomes and the factors studied (age, BMI, total dose of FSH, smoking status, type of LH suppression, type of stimulation and mtDNA content) were investigated using multivariate generalized linear models, via maximum likelihood, with successively fixed and mixed effects (an additional random effect was introduced to take into account the cluster of oocytes belonging to individual mothers), and the *P* values corresponding to the factors studied were reported. Receiver operating characteristic (ROC) curves were drawn to assess the discriminating ability of the fitted logistic model, and the AUC was determined. The 95%CI was calculated using the bootstrap procedure. All analyses were computed using R, the lme4 and ROCR packages (Sing *et al.*, 2005; Bates *et al.*, 2015; R Core Team, 2016).

Ethical approval

All participants gave their written informed consent, and the study was approved by the Ethical Committee of the University Hospital of Angers, France (Number DC-2014–2224).

Results

Mitochondrial DNA copy number in CGCs

The median quantity and the IQR of mtDNA per CGC was 379 (IQR: 173–963) in the oocytes overall, 405 (IQR: 185–986) in the mature oocytes, 383 (IQR: 190–990) in the fertilized oocytes and 738 (IQR: 250–1228) in the good quality embryos (Fig. 2). Factors such as the BMI and addiction to tobacco were significantly associated with CGC mtDNA content: patients with a higher BMI had a smaller quantity of CGC

mtDNA than those with a lower BMI ($P = 0.003$), whereas smokers had a larger quantity of CGC mtDNA than non-smokers ($P = 0.0002$).

Oocyte nuclear maturity

In univariate analysis, oocyte nuclear maturity was associated with a lower BMI and the use of rFSH (Table II and Supplementary Figures S1 and S2). After adjustment for age, smoking, LH suppression, total dose of FSH and mtDNA content, only the type of stimulation used remained significantly associated. The association with BMI did not appear in the multivariate models, particularly in the mixed model that takes into account the individual patient effect. No link was found between oocyte nuclear maturity and the CGC mtDNA content (Table II and Fig. 2).

CGC mtDNA and oocyte fertilizability

No link was found between oocyte fertilizability and the CGC mtDNA content or any other parameter related to the patients or their treatment (Table III and Fig. 2).

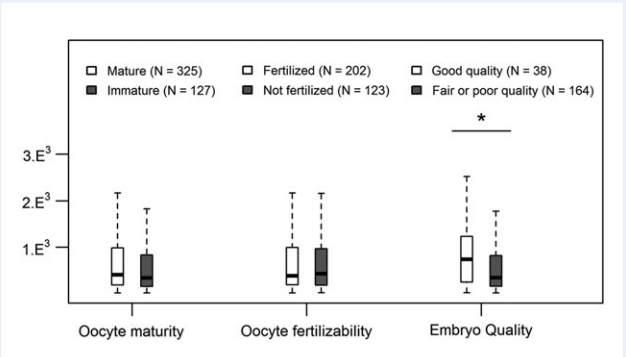

Figure 2 IQR of mtDNA per CGC according to oocyte maturity, oocyte fertilizability and embryo quality. IQR, interquartile range.

mtDNA and embryo quality

Embryo quality was associated with a greater quantity of CGC mtDNA in both univariate and multivariate analyses (Table IV and Fig. 2). The ROC curves (Fig. 3) illustrate the effect of CGC mtDNA on embryo quality with the univariate and mixed logistic models (AUC = 0.806, 95%CI = 0.719–0.869).

Moreover, as shown in Fig. 4, the subgroup analysis of smokers and non-smokers, using a mixed multivariate model, shows that the relationship between embryo quality and the mtDNA content of CGCs is significant for non-smokers ($P = 0.011$) whereas there is no relationship between mtDNA content of CGCs and embryo quality for smokers ($P = 0.55$).

The total dose of FSH was found to be associated with embryo quality after adjustment for age, BMI, use of tobacco, LH suppression and mtDNA, with and without the random patient effect, and the patient's age was the major determinant of this relationship with a strong correlation between the dose of FSH received and age [R^2 = 38.8% (30.7–46.4%); $P < 0.0001$].

Discussion

Oocyte quality is linked to oocyte mtDNA content in the human and other species (Reynier *et al.*, 2001; El Shourbagy *et al.*, 2006). A relationship between mtDNA in the oocyte and in the surrounding CGCs was first indirectly suggested in two successive articles showing increased quantities of mtDNA in the oocytes (Wang *et al.*, 2009) and in the cumulus cells (CCs) (Wang *et al.*, 2010) of diabetic mice. Recently, a similar correlation has been demonstrated in the pig (Pawlak *et al.*, 2015). In the human, for a given OCC, we showed that the mtDNA content of the CCs was positively correlated with that of the corresponding oocyte (Boucret *et al.*, 2015). The quantification of CGC mtDNA was subsequently proposed as a biomarker of embryo viability following a study of 60 OCCs (Ogino *et al.*, 2016).

Table II Mitochondrial DNA copy number in CGCs and oocyte nuclear maturity

Oocyte maturity	Mature (325)	Immature (127)	*P* values		
			Univariate analysis	Multivariate analysis	Mixed multivariate analysis
Age (years)	32.2 ± 4.3	32.7 ± 4.0	0.3144	0.9573	0.9859
BMI	24.9 ± 4.3	26.3 ± 5.3	0.0086*	0.0545	0.2632
Tobacco	109 (29.9%)	38 (33.5%)	0.5312	0.2990	0.5383
LH suppression					
Decapetyl	47 (14.5%)	24 (18.9%)	0.4991	0.7174	0.9960
Orgalutran	221 (68%)	81 (63.8%)			
Cetrotide	57 (17.5%)	22 (17.3%)			
Stimulation					
rFSH	274 (84.3%)	93 (73.2%)	0.0100*	0.0167*	0.0239*
uFSH	51 (15.7%)	34 (26.8%)			
Total dose of FSH (UI)	2045.1 ± 840.4	2242.4 ± 1063.1	0.0624	0.2609	0.3935
mtDNA (median) (IQR)	405 (185–986)	339 (151–825)	0.6844	0.9245	0.8200

IQR, interquartile range.
*Significant results.

Table III CGC mtDNA copy number and oocyte fertilizability

Oocyte fertilizability	Fertilized (202)	Not fertilized (123)	*P* values		
			Univariate analysis	Multivariate analysis	Mixed multivariate analysis
Age (years)	32.2 ± 4.2	32.3 ± 4.6	0.7565	0.6329	0.7231
BMI	25 ± 4.4	24.9 ± 4.2	0.8258	0.9475	0.6104
Tobacco users	66 (32.7%)	43 (35.0%)	0.7625	0.9847	0.9272
LH suppression					
Decapetyl	22 (10.9%)	25 (20.3%)	0.0639	0.0796	0.1832
Orgalutran	143 (70.8%)	78 (63.4%)			
Cetrotide	37 (18.3%)	20 (16.3%)			
Stimulation					
rFSH	172 (85.1%)	102 (82.9%)	0.7063	0.5092	0.6023
uFSH	30 (14.9%)	21 (17.1%)			
Total dose of FSH (UI)	2021.3 ± 879.4	2084.3 ± 773.8	0.4996	0.8904	0.8142
mtDNA (median) (IQR)	383 (190–990)	428 (179–970)	0.6432	0.7963	0.6945

IQR, interquartile range.

Table IV CGC mtDNA copy number and embryo quality

Embryo quality	Good (*n* = 38)	Fair or poor quality (*n* = 164)	*P* values		
			Univariate analysis	Multivariate analysis	Mixed multivariate analysis
Age (years)	31.5 ± 3.8	32.3 ± 4.2	0.2641	0.0564	0.1035
BMI	25.5 ± 4.3	24.9 ± 4.4	0.3976	0.4884	0.5140
Tobacco use	15 (39.5%)	51 (31.1%)	0.4237	0.6824	0.5412
LH suppression					
Decapetyl	3 (7.9%)	19 (11.6%)	0.687	0.1398	0.2299
Orgalutran	29 (76.3%)	114 (69.5%)			
Cetrotide	6 (15.8%)	31 (18.9%)			
Stimulation					
rFSH	33 (86.8%)	139 (84.8%)	0.9421	0.1509	0.2840
uFSH	5 (15.2%)	25 (13.2%)			
Total dose of FSH (UI)	2271.7 ± 1203.7	1963.2 ± 779	0.1385	0.0114*	0.0215*
mtDNA (median) (IQR)	738 (250–1228)	342 (159–818)	0.0408*	0.0044*	0.0064*

IQR, interquartile range.
*Significant results.

In our study, we focused on the totality of 452 CGCs obtained from 62 patients. We found no relationship between CGC mtDNA content and the nuclear maturity or fertilizability of oocytes. The nuclear maturity of oocytes was related only to the BMI of patients, as already described in the literature (Zhang *et al.*, 2015), and to the type of gonadotropins used for the stimulation of patients, which are chosen according to the patient profile including the BMI. However, this relationship with the BMI was not evidenced in the multivariate models, particularly the mixed model which takes into account the individual patient effect. In this case, the model may have generated an over-adjustment, as the BMI is a variable that is equivalent for all the oocytes of a given cohort. Moreover, our findings suggest that although the BMI affects oocyte maturity, it does not modify embryo quality. This reflects observations in mice in which fertility is compromised due to ovulation failures and not to embryo development (Bermejo-Alvarez *et al.*, 2012).

In contrast, we found a significant link between CGC mtDNA content and embryo quality, with higher mtDNA copy numbers being associated with good quality embryos rather than with fair or poor quality embryos [IQR, respectively, 738 (250–1228) and 342 (159–818)]. Interestingly, the indication of a good quality embryo provided by the quantification of CGC mtDNA was subject to a patient effect (Fig. 3). In other words, the informativeness of CGC mtDNA with regard to oocyte competence would be appropriate for a given patient, without offering the possibility of establishing a general threshold value, valid for all patients. Indeed, some factors specific to a given

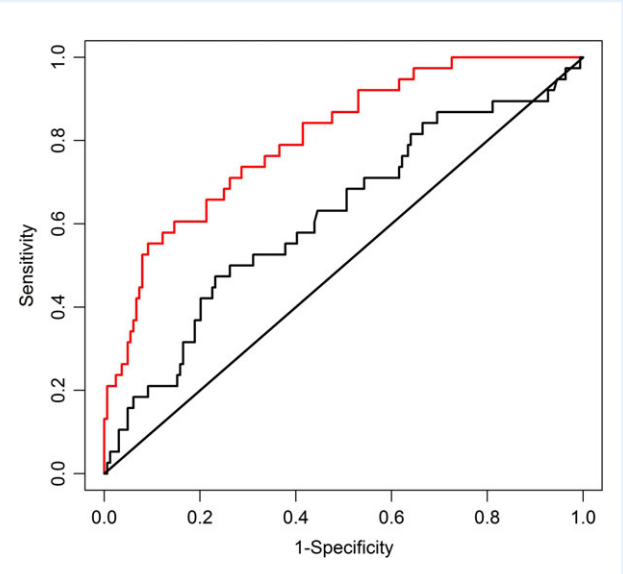

Figure 3 ROC curves of CGC mtDNA content for predicting the eventuality of obtaining a good quality embryo. The ROC curves were obtained by plotting the relationship between the specificity and the sensitivity of the test. The AUC is 0.618 (black) and 0.806 (red) when the patient effect is taken into account.

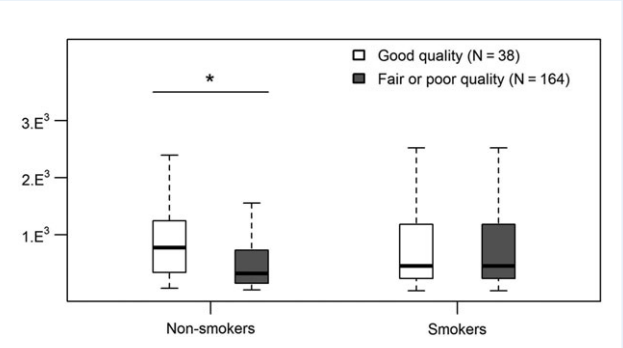

Figure 4 IQR of mtDNA per CGC according to embryo quality, in smokers and non-smokers. IQR, interquartile range.

woman could influence the mean mtDNA content of her own granulosa cells. Thus, we found that the CGC mtDNA content varied according to the BMI and smoking. Although the underlying mechanism is not yet fully understood, high values of the BMI have been associated with mitochondrial dysfunction and decreased mtDNA copy numbers in various tissues (Ritov *et al.*, 2005; Lee *et al.*, 2014). Regarding tobacco consumption, we have shown that smoking increases the mtDNA content in CGCs. The relationship between embryo quality and the mtDNA content in CGCs is impacted by this phenomenon since this relationship is only highlighted in non-smokers (Fig. 4). Cigarette smoke contains a number of chemical compounds many of which are oxidants or free radicals that lead to oxidative stress and DNA damage. In murine granulosa cells, exposure to smoke was shown to lead to mitochondrial damage and dysfunction (Gannon *et al.*, 2013). Moreover, smoking has been shown to impair ovarian vascularization and lead to follicular stress during the maturation of the human oocyte (Motejlek *et al.*, 2006). Taking these findings into account, it is likely that the action of tobacco compounds on the ovarian follicle, particularly on CGCs, leads to an increase in mtDNA copy numbers in these cells in order to compensate for mtDNA injuries, mitochondrial dysfunction and oxidative stress, as shown in other cells (Pavanello *et al.*, 2013). Thus, the increase in the mtDNA content in CGCs in smokers may lessen the relationship between embryo quality and CGC mtDNA content.

Although the mean age of women is lower in the group of good quality embryos, it is not significantly different from that of the group of lower quality embryos group. This lack of significance may be related to the small number and the low amplitude of age in our population. This may also be due to the fact that the chronological age is not sufficient to describe a woman's fertility potential or the quality of her oocytes. Indeed, ovarian ageing varies between individuals under the influence of environmental and genetic factors not yet well defined, and is not influenced by the chronological age alone (Levi *et al.*, 2001). In our study, the total dose of FSH administered was not associated with embryo quality under univariate analysis, but after adjustment for the age of the patients, there was a significant positive relationship between the dose of FSH and embryo quality. More precisely, at a constant age, the total dose of FSH administered was higher in patients with good embryo quality. This result is not in accordance with the literature and notably with the recent study of Baker *et al.* showing that the gonadotrophin dose is negatively correlated with success of ART cycles (Baker *et al.*, 2015). However, in that study, the total dose of FSH administered at which a decrease is observed in the live birth rate, i.e. 3000 UI, is above the mean dose used in our study. Overall, with due caution considering the number of participants in our study, these results could mean that for a given woman, at a given age, there would be a critical dose of FSH, both necessary and maximal, to obtain the best response to ovarian stimulation in terms of embryo quality.

The role played by mitochondria in embryo and gamete quality has aroused growing interest in recent years (Ramalho-Santos *et al.*, 2009; Van Blerkom, 2011; Otten and Smeets, 2015). For a long time, the idea has prevailed that mtDNA does not gainfully replicate before implantation (Piko and Taylor, 1987; St John *et al.*, 2010) and that the mitochondrial mass of the fertilizable oocyte must be sufficient for its distribution among the several embryonic blastomeres to ensure the optimal functioning of each cell until the blastocyst stage (Van Blerkom, 2011; St John, 2013). However, recently, it has been proposed that mitochondrial proliferation may sometimes occur during early embryogenesis. Thus, the increased mtDNA levels observed in abnormal embryos (Wells *et al.*, 2014; Fragouli *et al.*, 2015) or in embryos with poor implantation potential (Diez-Juan *et al.*, 2015) could be a response to embryonic stress and a sign of developmental abnormality (Diez-Juan *et al.*, 2015). In particular, the abnormal increase in mtDNA during early embryogenesis may compensate for an insufficient or faulty mitochondrial pool at the end of oogenesis (May-Panloup *et al.*, 2016). The link revealed in our study, as in that of other authors (Ogino *et al.*, 2016), between CGC mtDNA content and embryo quality may reflect the ability of granulosa cells to boost the energetic capability of the oocyte so as to support adequate embryonic development.

In conclusion, our results show that CGC mtDNA content is linked to embryo quality. Subject to the validation of these results on an

independent group, the quantification of mtDNA copy numbers in CGCs during the IVF procedure may allow the selection from a given cohort of oocytes those that are more likely to lead to good quality embryos. It would be particularly interesting, from an ethical point of view, to choose the oocytes with the greatest potential for fertilization, thus limiting the production of supernumerary embryos.

One of the highlights of our study is that it shows that the quantification of CGC mtDNA is not only linked to embryonic quality but is also affected by patient specificity, making it impossible to establish a general threshold value valid for all patients. Nevertheless, the weakness of this work is that it focuses on embryo quality, which is subjective and only a surrogate endpoint. Indeed, the main objective now in IVF is the search for markers that would indicate embryos with the best chances of implantation leading to pregnancy and live birth. To determine whether the quantification of CGC mtDNA offers an advantage over the use of the classic morpho-kinetic criteria, further studies will need to be carried out on CGC mtDNA from implanted and non-implanted embryos.

Supplementary data

Supplementary data are available at *Human Reproduction* Online.

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Authors' roles

P.M.-P., V.D.-D., A.C., V.P. and P.R., the principal investigators, take primary responsibility for the paper. P.M.-P., V.D.-D., A.C. and P.R. contributed to the conception, design and coordination of the research. P.E.B. and P.D. recruited the patients. P.M.-P., V.D.-D., A.C., V.S., L.B. and V.F-L. contributed to the collection and analysis of data. P.M.-P., V.D.-D., A.C., V.S. and P.R. contributed to writing the manuscript. P.M.-P., V.S., V.P. and P.R. contributed to the revision of the article, and the final version was approved by all the authors.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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