Frontiers in Bioscience Scholar

Front. Biosci. (Schol Ed) **2023**; 15(3): 9
https://doi.org/10.31083/j.fbs1503009

Original Research

Ribosomal, Telomere, and Mitochondrial Repeat Copy Number Variations in Female Genomes during Ovarian Stimulation and the Prediction of *In Vitro* Fertilization Outcome: A Pilot Study

Natalia Nikolaevna Veiko[1], Elizaveta Sergeevna Ershova[1], Lev Nikolaevich Porokhovnik[1,*], Maria Petrovna Klimenko[2], Peter Afanasievich Klimenko[2], Pavel Evgenievich Umriukhin[1,3], Edmund Viktorovich Kostyuk[4], Mark Arkadievich Kurtser[2], Oksana Nikolaevna Agafonova[1], Tatyana Agaragimovna Salimova[1], Sergey Ivanovich Kutsev[1], Vera Leonidovna Izhevskaya[1], Svetlana Viktorovna Kostyuk[1]

[1]Laboratory of Molecular Biology, Research Centre for Medical Genetics (RCMG), 115478 Moscow, Russia
[2]Pirogov Russian National Research Medical University, 117997 Moscow, Russia
[3]Physiology Department, I.M. Sechenov First Moscow State Medical University (Sechenov University), 119991 Moscow, Russia
[4]Moscow Research and Practical Centre for Narcology of the Department of Public Health, 109390 Moscow, Russia
*Correspondence: med-gen@mail.ru (Lev Nikolaevich Porokhovnik)
Academic Editor: Anna Aiello
Submitted: 16 June 2023 Revised: 10 August 2023 Accepted: 7 September 2023 Published: 24 September 2023

Abstract

Introduction: Individual risk assessment of assisted reproductive technologies is essential for personalized treatment strategies. Genetic and genomic indicators of the response to stress by cells could provide individual prognostic indicators for *in vitro* fertilization (IVF) success. Such indicators include the copy number of ribosomal genes (rDNA), which modulates the level of protein synthesis, and the abundance of mitochondrial DNA (mtDNA), which provides the cell with energy, while the content of telomere repeats (TRs) indicate the biological age. **Materials and Methods**: The contents of the three repeats in DNA isolated from blood leukocytes of 40 women before and after ovarian stimulation were assayed prior to IVF. Then, we divided the women into a successful IVF group, IVF+ (N = 17, 7 cases of twins), and a group of failed cases, IVF– (N = 23). The control group included 17 non-pregnant women with natural childbirth in the past. The nonradioactive quantitative hybridization (NQH) method was applied to assay the genome repeat contents. **Results**: The number of rDNA copies in the IVF+ group was significantly higher than in the IVF– group ($p < 10^{-8}$). The number of mtDNA copies in the IVF+ group also exceeded those in the IVF– group ($p < 0.001$), whereas the TR content in the two groups differed, albeit, non-significantly ($p < 0.03$). Following the ovarian stimulation, the rDNA copy numbers did not change, while the contents of the mtDNA and TR varied significantly. **Conclusions**: This pilot study has shown that rDNA abundance in blood leukocytes can be considered a stable and effective predictor. Very low numbers of ribosomal repeat copies (<330) entail a high risk of IVF failure. However, a combination of numerous mtDNA and TRs, provided that rDNA content is not very low, increases the probability of multiple pregnancies.

Keywords: personalized infertility treatment; risk assessment; IVF prognosis; ribosomal genes; telomere repeat; mitochondrial DNA; copy number

1. Introduction

The growing number of infertile couples is a global trend that currently affects approximately 10–15% of couples worldwide. Accordingly, the number of women who resort to *in vitro* fertilization (IVF) is increasing. Infertility is a very complex multifactorial condition, meaning different couples have different chances of success. The individual outcome depends on the health, infertility causes, age, emotional state, lifestyle, eggs and sperm quality, and many other factors relating to the couple. The IVF procedure often fails because of unaccountable reasons. Even in cases where there is a successful conception, an increased risk of pregnancy termination (stopping the development of the embryo, spontaneous abortion, miscarriage) remains. Therefore, uncovering novel factors for infertility and IVF failure is necessary to determine the feasibility of using the patient's own reproductive cells and making an adequate choice of precision-assisted reproductive technology.

IVF is an effective and physiologically stressful procedure [1]. Therefore, indicators of the effectiveness of the cellular response to stress can be considered potential predictors of the success of the IVF procedure. Such indicators include the cellular protein synthesis level, biological age, and ability to sustain a stress response with an adequate ATP

(adenosine triphosphate) supply.

Protein synthesis is limited by, among other factors, the number of available ribosomes. Consequently, the number of ribosomes depends on the ribosomal gene copy number in the genome [2,3]. Previously, we conducted a pilot study to show that the number of ribosomal gene (rDNA) copies in the blood leukocytes of the female allowed for the prediction of IVF success [4]. The number of rDNA copies in the genomes of women with several unsuccessful IVF attempts was significantly lower than in the genomes of women who succeeded in getting pregnant. The ribosomal repeat harbors the genes for 18S, 5.8S, and 28S ribosomal RNA (rRNA) and belongs to the class of moderate tandem repeats located in the p-regions of the human acrocentric chromosomes. There is still no clear answer in the literature to the variability in rDNA copy number under stress. There is evidence for both stable rDNA content in the genome and variation of this parameter under stress [5–7]. The variability of rDNA content in the genome of women during IVF has, to our knowledge, never before been studied.

Telomere repeats (TRs), which also belong to the class of moderate tandem repeats, are situated at the ends of the chromosomes. A decrease in the number of TR copies in the genome also termed telomere length shortening, is associated with aging and oxidative stress [8–11]. TR is considered one of the cellular aging markers. The IVF success is deemed as depending on age-specific changes in women and the level of oxidative stress. However, published data on the link between the TR content and IVF success are contradictory [12–15].

Mitochondrial repeats (mtDNA) are a component of mitochondria. The mitochondria provide the cell with energy, which is stored in the form of ATP molecules. The ATP synthesis level depends on the number of mitochondria in the cell, i.e., on the mtDNA copy number. Under stress, the mtDNA abundance changes [16,17], meaning a lack of energy may be a cause of IVF failure. Some published reports disprove a positive association between the IVF result and the mtDNA copy number in the female genome, whereas some studies have corroborated the relationship [18–23].

The aim of this study was to investigate the possibility that a successful pregnancy could be predicted in IVF patients by analyzing the contents of three genome repeats in DNA derived from blood leukocytes—ribosomal, telomere, and mitochondrial—as well as monitoring the changes in their contents before and after ovarian stimulation (preparation for IVF).

2. Materials and Methods

2.1 Patients

A total of 40 women (aged 28 to 40) were observed by embryologists for the IVF (In Vitro Fertilization) procedure. Among them, the IVF procedures ended with failure in 23 patients (IVF– group), while in 17 patients, the procedures ended in pregnancy (IVF+ group). Exclusion criteria from the study were (1) aged over 40 years; (2) diseases or pathological conditions accompanied by impaired fertility and/or reproductive function; (3) severe chronic diseases. Blood sampling from women who underwent IVF was conducted prior to beginning hormone therapy (sample 1) and 2–4 weeks later, immediately before the IVF procedure (sample 2).

The control group consisted of 17 non-pregnant women (aged 28 to 40), who were prophylactically observed in the same clinic and had no previous problems with conception or pregnancy. Blood sampling was performed on women in the control group only once in the first 10 days of their menstrual cycle.

2.2 DNA Isolation

Venous blood was collected into test tubes containing heparin. Erythrocyte lysis was performed, and leukocyte sediment was isolated. A solution containing 0.04 M EDTA, 2% sodium lauryl sarcosylate, and 150 µg/mL of RNase A (Sigma, St. Louis, Missouri 68178, USA) was added to the precipitate for 45 minutes at 37 °C. Then, the cell lysate was treated with proteinase K (200 µg/mL, Promega, Madison, Wisconsin 53711, USA) for 24 hours at 37 °C. Protein extraction was carried out using equal volumes of a mixture of phenol/chloroform/isoamyl alcohol (25:24:1), phenol, and a mixture of chloroform/isoamyl alcohol (24:1). Phenol was stabilized with 8-hydroxyquinoline. DNA was precipitated by adding 1/10 volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of ice-cold ethyl alcohol. DNA was collected by centrifugation for 15 minutes at 4 °C and washed with 70% ethanol before being dried and dissolved in water.

2.3 Quantification of the Genomic Repeats in Leukocyte-Derived DNA

The repeat contents in the genomic DNA were assessed using the nonradioactive quantitative hybridization (NQH) technique, which has previously been described in detail [5,7,24]. Membranes (Optitran BA-S85, GE Healthcare) were soaked in a 20-fold SSC buffer pH 7.0. The DNA samples with a concentration of ~75 ng/µL were denatured in 0.1 M NaOH solution for 10 min. Then, the solution was neutralized by adding an equal volume of 20-fold SSC buffer at pH 4.0. The denatured DNA samples were applied to activated membranes in the form of dots (1.5 µL) in 4 repetitions. The membranes were dried at 80 °C for 1.5 hours. Prehybridization was carried out in a buffer (5-fold SSC buffer, 5 mg/mL BSA, 5 mg/mL ficoll, 5 mg/mL polyvinylpyrrolidone, 0.5% SDS, 50% formamide) at 45 °C for 20 min. Hybridization was carried out under the same conditions in the buffer with a biotin-labeled DNA probe. Nonspecific sorption was blocked using a buffer (0.05 M Tris–HCl, pH 7.5, 0.5 M NaCl, 0.1% milk powder, 0.1% gelatin) for 10 minutes with constant stirring. Then, strep-

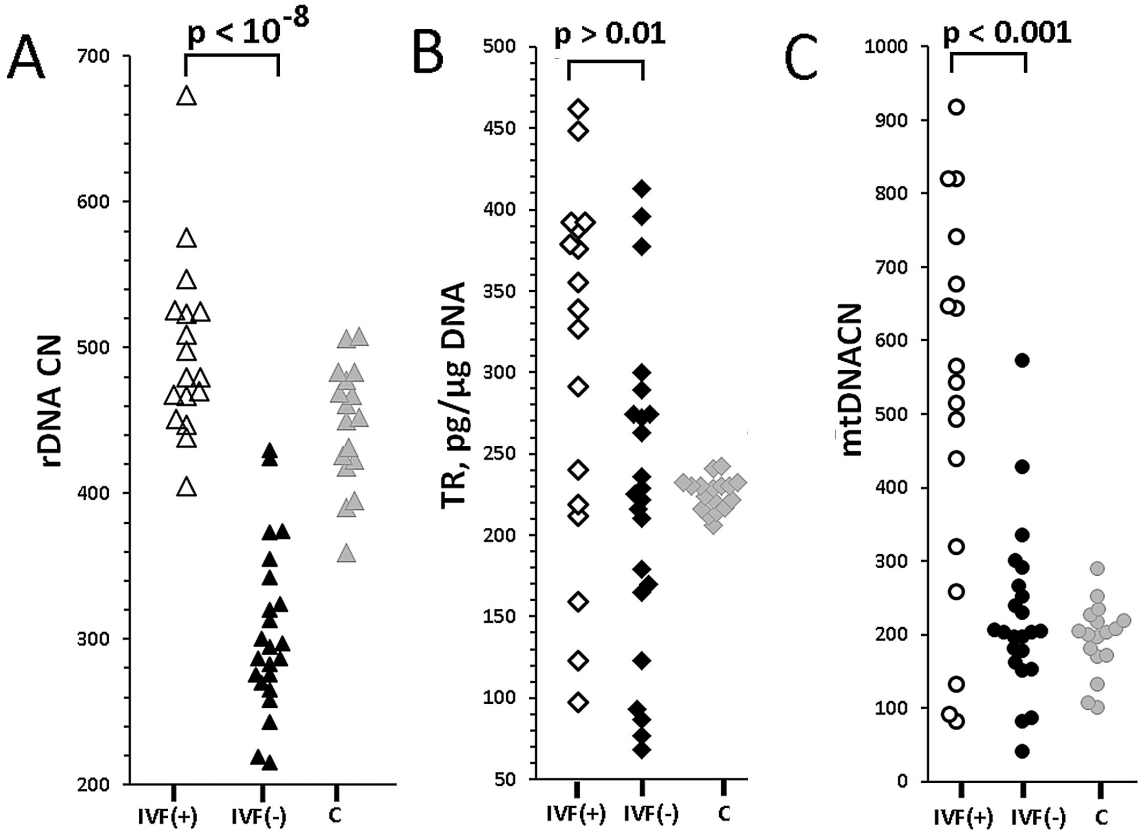

Fig. 1. Comparisons of the groups by the contents of the three repeats: rDNA (A), TR (B), and mtDNA (C) in the genomic DNA; Mann–Whitney test.

tavidin conjugate with alkaline phosphatase (Merck) was added to the buffer "AP 7.5 oligo" (0.05 M Tris–HCl, 0.5 M NaCl, 0.05 M $MgCl_2$). The membranes were washed in "AP 7.5 oligo" (2 × 5 min) buffer. Visualization was performed by adding BCIP and NBT to "AP 9.5 oligo" buffer (0.05 M Tris–HCl, 0.2 M NaCl, 0.05 M $MgCl_2$) at a final concentration of 0.165 and 0.33 mg/mL, respectively, and incubated for 2–14 hours in the dark. The filters were dried and scanned, and their images were analyzed using «Imager 6a» software, which had been specially designed by us for this purpose. To obtain a calibration dependence, which linked the signal and the amount of the target repeat in the DNA, several standard samples with known repeat contents were applied to the filter.

2.4 DNA Probes Labeled with Biotin and Used for Hybridization

DNA probes were labeled with biotin using nick translation. The pBR322–rDNA plasmid was used for rDNA analysis. The pBR322 vector contains an insert of the rDNA EcoR1 fragment (5836 b, between –515 and 5321 nucleotides from the transcription start site, "GenBank" No. U13369). Ribosomal DNA content is represented as the number of rDNA copies per diploid genome. Biotinylated mtDNA was used for mtDNA quantification. MtDNA content is represented as a number of mtDNA copies per diploid genome. TR quantification was performed using biotin-labeled oligonucleotide biotin-(TTAGGG) 7 (synthesized by Syntol, RF). TR content is presented in units of mass: pg TR/µg DNA.

2.5 Statistics

Statistical data processing was performed using Microsoft Office Excel, StatGraph, and StatPlus2007 Professional software. Nonparametric Mann–Whitney (U-test) and Kolmogorov–Smirnov tests were applied. The differences were considered statistically significant at $p < 0.01$.

3. Results

3.1 The Contents of the Three Repeats in DNA Samples Isolated from Blood Leukocytes

The contents of the three genome repeats (ribosomal, mitochondrial, and telomeric) were determined from the blood leukocytes in each group. Then, the IVF procedure was performed. Next, the women were divided into two groups, according to the outcome: IVF+ (pregnancy occurred, N = 17) and IVF– (negative result, N = 23). Of the 17 women who underwent a successful IVF, 7 gave birth to twins. Fig. 1 shows the abundance of the three repeats in the genomes from leukocytes in the three groups: IVF+, IVF–, and C. Groups were compared by nonparametric statistics

Table 1. Descriptive statistics for the three repeat contents in DNA isolated from female blood leukocytes.

Repeat	Group	N	Mean	SD	Range, min–max	Median	C_V
rDNA, copy number	IVF	40	388	113	215–673	374	0.29
	IVF+	17	499	62	405–673	480	0.12
	IVF–	23	305	57	215–430	295	0.19
	C	17	447	42	359–508	452	0.09
mtDNA, copy number	IVF	40	345	236	40–917	253	0.69
	IVF+	17	511	262	80–917	541	0.51
	IVF–	23	223	113	40–572	202	0.51
	C	17	193	49	99–289	202	0.25
TR, pg/µg, DNA	IVF	40	259	110	68–462	252	0.43
	IVF+	17	306	112	97–462	339	0.37
	IVF–	23	224	97	68–413	225	0.43
	C	17	225	10	206–242	229	0.04

(U-test). Table 1 represents the descriptive statistics for the parameters studied in each group.

The IVF+ and IVF– groups differed significantly ($p < 10^{-8}$) by rDNA CN (copy number), with a mean rDNA CN of 305 $\pm$ 57 copies in the IVF– group, which was significantly lower than in the IVF+ group (499 $\pm$ 62 copies) and C group (447 $\pm$ 42 copies). The IVF+ group contained significantly more rDNA copies than the control group ($p < 0.01$). Additionally, there were no genomes in this group that contained less than 405 rDNA copies. Comparatively, fractions of the same genomes were 18% and 24% in control group C, respectively, and as much as 91% in the IVF– group. Women in the C group who had conceived naturally contained at least 359 rDNA copies of the genome.

The IVF+ group differed significantly from the other three groups ($p < 0.001$) with the mtDNA copies more abundant in number. The genomes of the women who underwent a successful IVF procedure contained a higher mean number of mtDNA copies of 511 $\pm$ 262, compared to the IVF– group (223 $\pm$ 113 copies) and the C group (193 $\pm$ 43 copies). Moreover, 41% of the DNA samples in the IVF+ group contained higher mtDNA numbers (more than 572 copies), compared with the maximum numbers present in the IVF– and C groups.

In the groups of women who experienced problems with conception and had resorted to IVF, we found significantly greater telomere repeat variability than in the control C group. The maximum mean values of the TR content were observed in the IVF+ group (306 $\pm$ 112 pg/µg DNA) compared to the IVF– group (224 $\pm$ 97 pg/µg DNA) and the C group (225 $\pm$ 10 pg/µg DNA). According to this parameter, the IVF+ group significantly differed from the other three groups ($p < 0.03$), although the differences were smaller than for the rDNA and mtDNA copy numbers.

Fig. 2A–C shows the pairwise dependencies linking the analyzed parameters. The table in Fig. 2D shows the results of the correlation analysis (only significant indicators are shown). For the IVF+ group, we found a positive correlation between the number of mtDNA copies and the TR content. Indeed, the highest was the TR content, whereby the longer the TRs were, the more mitochondria the cell contained. A similar correlation was observed across the entire sample.

An interesting trend was revealed (Fig. 2A–C), whereby for the 10 women in the IVF+ group with the highest mtDNA copy counts (more than 500) and elevated TR contents (more than 240 pg/µg of DNA), 7 gave birth to twins. Additionally, the rDNA CN ranged from 438 to 576 in these women.

3.2 Changes in the Three DNA Repeat Contents during Ovarian Stimulation (Preparation for IVF)

The IVF procedure was performed 2–4 weeks after the first blood sampling. Preparation for the IVF procedure included hormonal ovarian stimulation. Fig. 3A shows data reflecting the changes in the three genome repeat contents during ovarian stimulation. For the IVF+ and IVF– groups, significant changes were observed in the TR and mtDNA contents. The content of these repeats in the DNA of some participants increased, while in others, it did not change or even decrease. For the IVF+ group, a negative correlation was observed between the initial repeat contents in the DNA (sample 1) and the relative content change after preparation for IVF (sample 2). During the observation period, the content of the repeats in the leukocytes increased in the women who had initially contained low repeat copy numbers. In the sample leukocytes with higher TRs and mtDNA contents (1), a decrease was observed in the repeat copy numbers in the sample (2) (Fig. 3B,C).

The ribosomal repeats in samples (1) and (2) varied in number, yet were within the experimental error, i.e., remained stable, suggesting it is a constant trait over a lifetime.

4. Discussion

In the present study, we analyzed, for the first time, the copy numbers of the three repeats in the female genome in blood leukocytes. All three repeats perform essential cel-

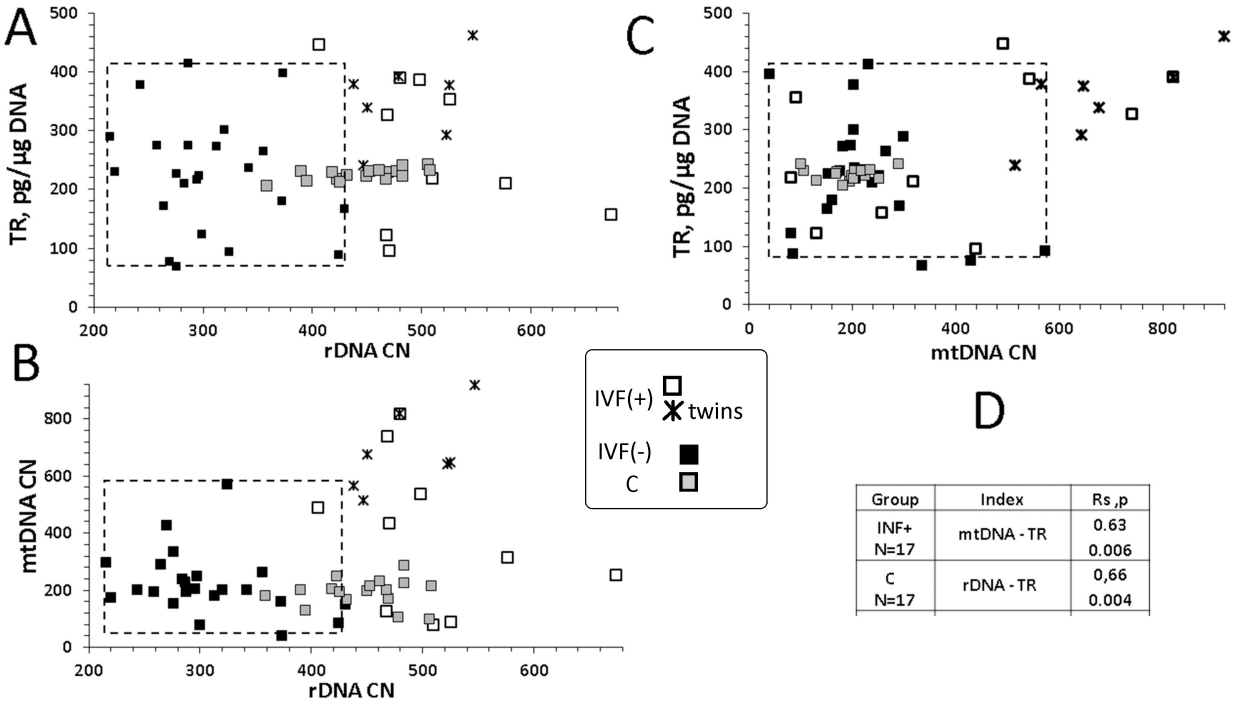

Fig. 2. Correlations between the three repeat copy numbers. (A–C) Pairwise correlations between the three repeat contents. The dotted lines surround an area of points corresponding to the IVF– group. (D) Three DNA repeat correlation analyses.

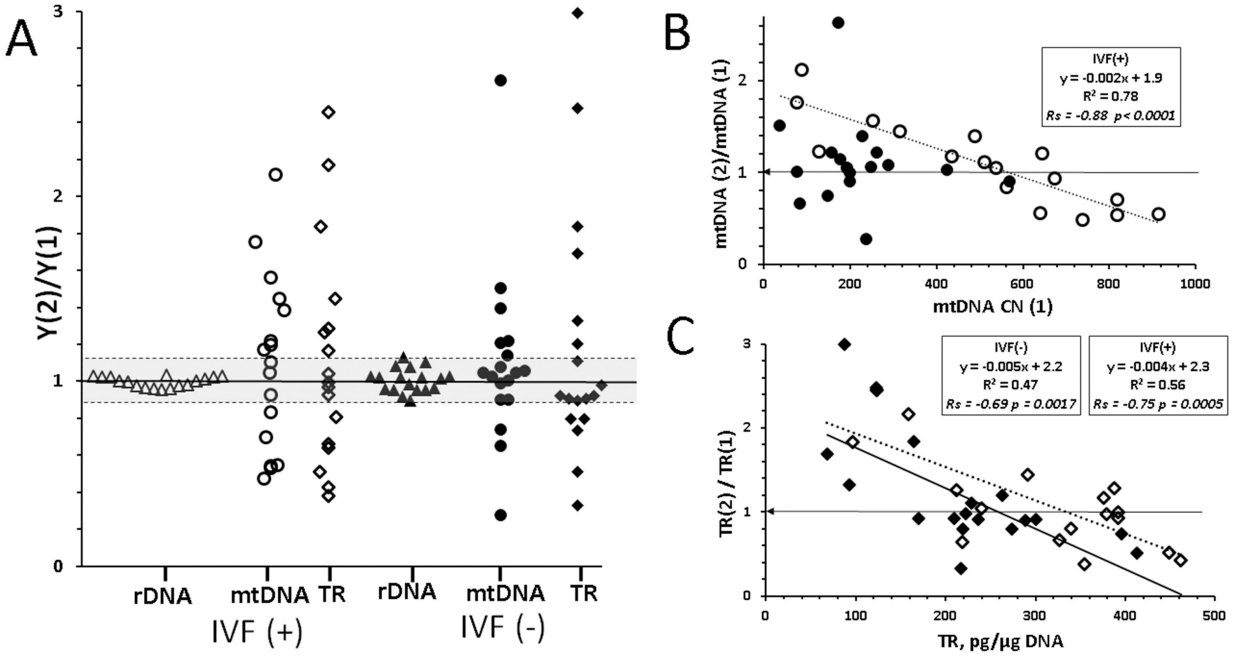

Fig. 3. Changes in the three genome repeat copy numbers. (A) Changes in the three genome repeat contents in women undergoing IVF during a 2–4-week preparation period. Blood samples (1) were taken during the first inspection. Blood samples (2) were also taken shortly before the IVF procedure. (B) The dependence of mtDNA content changes on the mtDNA content in the sample (1). (C) The dependence of TR changes on the TR content in the sample (1). The data analyses on correlation are presented in the boxes.

lular functions, such as modulating the level of protein synthesis (rDNA), controlling the biological age (TR), and supplying energy to the cell (mtDNA). A distinctive feature of the female genomes in women who had not been able to conceive naturally and had resorted to IVF was a higher variability in the contents of the three repeats compared to the control group (Table 1).

The maximum differences ($p < 10^{-8}$) between groups IVF+ and IVF– were found in the rDNA copy number index (Fig. 1). In recent decades, we have obtained a growing amount of experimental data indicating the possibility of using this index as a marker of human life duration and quality [7,24,25]. Indeed, in a group of centenarians, there were no genomes with less than 330 rDNA copies. However, in the general population of people under 50, such genome numbers occurred only in 15% of the analyzed cases [7]. In the group of women who had been unable to conceive naturally, 43% of the tested DNA samples contained less than 330 rDNA copies. The genomes of 74% of the women with failed IVF protocols contained the same low number of rDNA copies (Fig. 1). Thus, a small number of rDNA genomic copies is not only associated with a relatively shorter life expectancy [7], it is also associated with an impaired female reproductive function. The small number of rDNA copies determines that there is a reduced level of ribosomes in the cell [2,3]. In conditions where stress is associated with aging or pregnancy; such cells are unable to raise the ribosomal biosynthesis. This results in an insufficient increase in protein production in response to the stress stimuli. We have previously shown that a small number of rDNA copies in the genome blunts the effectiveness of the oxidative stress response to genotoxic environmental factors in cultured human cells [26]. Presumably, subjects with lower rDNA counts in their genomes (less than 330 copies per diploid genome) suffer from various impacts due to the inadequate level of ribosomal biogenesis. For example, the ribosomal number has been shown to be reduced in elderly people with cognitive impairments [27], thereby indicating a small number of rDNA copies in their genomes.

The IVF+ and IVF– groups also differed in mtDNA CN (copy number), although the differences were less pronounced ($p < 0.001$) than for the rDNA CN. Previously, the authors of a previous study [13] investigated the possibility of using this parameter to predict IVF success. They showed that the mtDNA CN in the IVF+ and IVF– groups did not differ significantly. However, other researchers [18,23,28] identified a positive correlation between the chance of pregnancy, resulting from IVF, and the number of mtDNA copies in the leukocytes of women, which is corroborated by our findings. The analysis of the entire sample of women (N = 57), including all three groups, showed a positive correlation between the leukocyte mtDNA and rDNA copy numbers (Fig. 2C). Obviously, an intensive level of protein synthesis must be supported by an elevated production of mitochondria and the resultant energy flush. For IVF groups, separately, no such dependence was detected.

Previously, a number of authors have explored the possibility of using telomere repeat numbers to predict the IVF outcome since failed IVF attempts were found to be associated with a lower TR number in the genome [11,12,14,15]. However, these results contradict the data presented in the previous study [13]. In our study, we found no significant differences according to TR content in the IVF+ and IVF– groups ($p < 0.03$), which corroborates the previously obtained data [13]. The TR content in the leukocyte genomes is not a good predictive marker of IVF success. We found a positive correlation between the TR numbers and mtDNA contents in the IVF+ group and in the general sample (N = 57). The longer the telomere, the more mitochondria are contained in the white blood cells of the women unable to conceive after IVF treatment. An interesting fact was discovered, whereby twins were born by those women who had longer telomeres and higher mtDNA contents along with a moderately elevated ribosomal gene count (438–576 copies) prior to beginning the ovarian stimulation. Previously, a positive correlation has been reported in several studies between the mtDNA and TR contents in the blood leukocytes of healthy controls and healthy subjects undergoing an affective stress response [29,30].

An important requirement for an index to reflect the chances of a successful IVF treatment is its stability over long periods of a lifespan. The TR and mtDNA contents cannot be deemed as stable genetic traits. Cells are heterogeneous in the abundance of these repeats over the same cell culture. The amount of mtDNA can increase or decrease under conditions of stress of varying intensities [16,17]. The TR amount in blood cells is also influenced by psychoemotional (affective) stress [5]. Oxidative stress reduces the TR content in some cells in the cell pool, which manifests as a TR count decrease in the DNA isolated from the cells. However, the exaggerated clearance of cells with short telomeres via apoptosis following the induction of the stress response impacts the results of an increase in TR abundance.

We found high variability in the TR and mtDNA contents in the DNA isolated from the leukocytes of the same women before and after (2–4 weeks) preparation for the IVF procedure (Fig. 3). Preparation for IVF had a modulating effect on the contents of these repeats in DNA and was most pronounced in the IVF+ group. Moreover, changes in the TR and mtDNA repeat copy numbers can be attributed to both hormonal ovarian stimulation and the affective stress experienced by the women during this difficult period.

Unlike TR and mtDNA, the genomic ribosomal repeat content was stable and did not depend on the hormonal preparation for IVF (Fig. 3). Previously, we obtained the same result (variations in the TR content, albeit at a stable number of rDNA copies) in blood leukocyte DNA isolated from medical students, when studying the response to the affective stress caused by passing their exams [5]. The study of replicative aging of cultured skin fibroblasts also showed that the rDNA content stability was in contrast to a decrease in the TR count [7,31]. We found few cases of exception in aging cultures when analyzing late passages since these cells had lost hypermethylated rDNA copies, which were functionally inactive, and located outside the nucleo-

lar structures, meaning they were a problem for replication. This process resulted in a reduction in the rDNA CN. However, the number of such cells in the total cell pool did not exceed 5–10% [7].

Our study has three limitations. Firstly, the sample size was restricted. Secondly, only Caucasian patients were involved. Finally, we explored the three repeats in the genomes of lymphocytes, without studying other tissues and organs, including reproductive. Thus, the question remains open as to what extent our findings can be extrapolated to additional tissues and organs and to different ethnicities. Our previous decades-long studies of phenotypic manifestations relating to the rDNA copy numbers suggest that the ribosomal genes, as universal "housekeeping" genes, manifest similarly in every cell and tissue (except for malignant), and their dosage effects do not depend on the racial origin. Moreover, we have already verified this assumption in recent studies on the different effects of rDNA copy numbers. For instance, we found elevated rDNA copy numbers in the lymphocytes of Caucasian patients with schizophrenia, a multifactorial disease, by still as yet obscure causes [24]. Recently, these findings were corroborated by Sen Li and co-authors in East Asian (Japanese) patients, from a direct assay of their brain cells [32].

5. Conclusion

This pilot study has shown that the very low number of ribosomal repeat copies in the DNA isolated from blood leukocytes can be considered a stable and effective predictor of the failure of the IVF procedure. The combination of high mitochondrial DNA and telomere repeat content along with a moderately elevated ribosomal repeat number can be a potential predictor of multiple pregnancies. The major limitation of our pilot study was the use of a small sample size. Therefore, to validate these conclusions, further studies on larger samples and racially different populations (e.g., Asians) are warranted.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

ESE, NNV and VLI designed the study; MPK, PAK and MAK selected and described patients and conducted blood sampling, ESE and EVK worked with cell cultures and performed DNA isolation; TAS determined the ribosomal repeat count and performed fluorescence microscopy; ONA determined the mtDNA and telomere repeat abundance; LNP and NNV wrote the text; LNP translated the text to English language; EVK and VLI statistically processed the data obtained; LNP, PEU, SIK and SVK made substantial contributions to data analysis and interpretation; SIK and SVK supervised the research. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was carried out in accordance with the latest version of the Declaration of Helsinki and was approved by the Independent Interdisciplinary Ethics Committee on Ethical Review for Clinical Studies (Protocol No. 12 dated October 27, 2021). All participants provided written informed consent to participate in the study after the procedures had been completely explained.

Acknowledgment

Not applicable.

Funding

FGFF-2022-0007/State assignment of the Ministry of Science and Higher Education (changes in TR and mtDNA repeat content) supported this research.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Czamanski-Cohen J, Sarid O, Cwikel J, Levitas E, Lunenfeld E, Douvdevani A, *et al*. Decrease in cell free DNA levels following participation in stress reduction techniques among women undergoing infertility treatment. Archives of Women's Mental Health. 2014; 17: 251–253.

[2] Laferté A, Favry E, Sentenac A, Riva M, Carles C, Chédin S. The transcriptional activity of RNA polymerase I is a key determinant for the level of all ribosome components. Genes & Development. 2006; 20: 2030–2040.

[3] Larson DE, Zahradka P, Sells BH. Control points in eucaryotic ribosome biogenesis. Biochemistry and Cell Biology. 1991; 69: 5–22.

[4] Porokhovnik LN, Veiko NN, Ershova ES, Poletkina AA, Shmarina GV, Dolgikh OA, *et al*. Copy number of ribosomal genes in woman's genome is associated with IVF outcome and pregnancy complications. Medical genetics. 2019; 18: 14–25. (In Russian)

[5] Umriukhin PE, Ershova ES, Filev AD, Agafonova ON, Martynov AV, Zakharova NV, *et al*. The Psychoemotional Stress-Induced Changes in the Abundance of SatIII (1q12) and Telomere Repeats, but Not Ribosomal DNA, in Human Leukocytes. Genes. 2022; 13: 343.

[6] Lou J, Yu S, Feng L, Guo X, Wang M, Branco AT, *et al*. Environmentally induced ribosomal DNA (rDNA) instability in human cells and populations exposed to hexavalent chromium [Cr (VI)]. Environment International. 2021; 153: 106525.

[7] Malinovskaya EM, Ershova ES, Golimbet VE, Porokhovnik LN, Lyapunova NA, Kutsev SI, *et al*. Copy Number of Human Ribosomal Genes With Aging: Unchanged Mean, but Narrowed Range and Decreased Variance in Elderly Group. Frontiers in Genetics. 2018; 9: 306.

[8] Turner KJ, Vasu V, Griffin DK. Telomere Biology and Human Phenotype. Cells. 2019; 8: 73.

[9] Blackburn EH, Epel ES, Lin J. Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection. Science. 2015; 350: 1193–1198.

[10] Shay JW. Telomeres and aging. Current Opinion in Cell Biology. 2018; 52: 1–7.
[11] Rossiello F, Jurk D, Passos JF, d'Adda di Fagagna F. Telomere dysfunction in ageing and age-related diseases. Nature Cell Biology. 2022; 24: 135–147.
[12] Anifandis G, Samara M, Simopoulou M, Messini CI, Chatzimeletiou K, Thodou E, *et al*. Insights into the Role of Telomeres in Human Embryological Parameters. Opinions Regarding IVF. Journal of Developmental Biology. 2021; 9: 49.
[13] Li Piani L, Reschini M, Somigliana E, Ferrari S, Busnelli A, Viganò P, *et al*. Peripheral mitochondrial DNA, telomere length and DNA methylation as predictors of live birth in in vitro fertilization cycles. PLoS ONE. 2022; 17: e0261591.
[14] Keefe DL, Franco S, Liu L, Trimarchi J, Cao B, Weitzen S, *et al*. Telomere length predicts embryo fragmentation after in vitro fertilization in women–toward a telomere theory of reproductive aging in women. American Journal of Obstetrics and Gynecology. 2005; 192: 1256–1261.
[15] Czamanski-Cohen J, Sarid O, Cwikel J, Douvdevani A, Levitas E, Lunenfeld E, *et al*. Cell-free DNA and telomere length among women undergoing in vitro fertilization treatment. Journal of Assisted Reproduction and Genetics. 2015; 32: 1697–1703.
[16] Liu CS, Tsai CS, Kuo CL, Chen HW, Lii CK, Ma YS, *et al*. Oxidative stress-related alteration of the copy number of mitochondrial DNA in human leukocytes. Free Radical Research. 2003; 37: 1307–1317.
[17] Lee HC, Wei YH. Mitochondrial biogenesis and mitochondrial DNA maintenance of mammalian cells under oxidative stress. The International Journal of Biochemistry & Cell Biology. 2005; 37: 822–834.
[18] Bonomi M, Somigliana E, Cacciatore C, Busnelli M, Rossetti R, Bonetti S, *et al*. Blood cell mitochondrial DNA content and premature ovarian aging. PLoS ONE. 2012; 7: e42423.
[19] Kim J, Seli E. Mitochondria as a biomarker for IVF outcome. Reproduction. 2019; 157: R235–R242.
[20] Zhou X, Liu X, Shi W, Ye M, Chen S, Xu C. Mitochondrial DNA Content May Not Be a Reliable Screening Biomarker for Live Birth After Single Euploid Blastocyst Transfer. Frontiers in Endocrinology. 2021; 12: 762976.
[21] Kristensen SG, Humaidan P, Coetzee K. Mitochondria and reproduction: possibilities for testing and treatment. Panminerva Medica. 2019; 61: 82–96.
[22] Fragouli E, McCaffrey C, Ravichandran K, Spath K, Grifo JA, Munné S, *et al*. Clinical implications of mitochondrial DNA quantification on pregnancy outcomes: a blinded prospective non-selection study. Human Reproduction. 2017; 32: 2340–2347. Erratum in: Human Reproduction. 2019; 34: 782.
[23] Ogino M, Tsubamoto H, Sakata K, Oohama N, Hayakawa H, Kojima T, *et al*. Mitochondrial DNA copy number in cumulus cells is a strong predictor of obtaining good-quality embryos after IVF. Journal of Assisted Reproduction and Genetics. 2016; 33: 367–371.
[24] Chestkov IV, Jestkova EM, Ershova ES, Golimbet VE, Lezheiko TV, Kolesina NY, *et al*. Abundance of ribosomal RNA gene copies in the genomes of schizophrenia patients. Schizophrenia Research. 2018; 197: 305–314.
[25] Kondratyeva EI, Ershova ES, Voronkova AY, Shmarina GV, Krasovsky SA, Zhekaite EK, *et al*. Variation of the ribosomal genes copy number in the genomes of patients with cystic fibrosis. Medical genetics. 2021; 20: 49–60. (In Russian)
[26] Veĭko NN, Terekhov SM, Shubaeva NO, Simirnova TD, Ivanova SM, Egolina NA, *et al*. Early and late responses to oxidative stress in human dermal fibroblasts of healthy donors and rheumatoid arthritis patients. Relationship between the cell death rate and the genomic dosage of active ribosomal genes. Molekuliarnaia Biologiia. 2005; 39: 264–275. (In Russian)
[27] Ding Q, Markesbery WR, Chen Q, Li F, Keller JN. Ribosome dysfunction is an early event in Alzheimer's disease. The Journal of Neuroscience. 2005; 25: 9171–9175. Erratum in: The Journal of Neuroscience. 2006; 26: 3077.
[28] Seli E. Mitochondrial DNA as a biomarker for in-vitro fertilization outcome. Current Opinion in Obstetrics & Gynecology. 2016; 28: 158–163.
[29] Tyrka AR, Parade SH, Price LH, Kao HT, Porton B, Philip NS, *et al*. Alterations of Mitochondrial DNA Copy Number and Telomere Length With Early Adversity and Psychopathology. Biological Psychiatry. 2016; 79: 78–86.
[30] Tyrka AR, Carpenter LL, Kao HT, Porton B, Philip NS, Ridout SJ, *et al*. Association of telomere length and mitochondrial DNA copy number in a community sample of healthy adults. Experimental Gerontology. 2015; 66: 17–20.
[31] Ershova ES, Malinovskaya EM, Konkova MS, Veiko RV, Umriukhin PE, Martynov AV, *et al*. Copy Number Variation of Human Satellite III (1q12) With Aging. Frontiers in Genetics. 2019: 10: 704.
[32] Li S, Otsuka I, Tanifuji T, Okazaki S, Horai T, Takahashi M, *et al*. Ribosomal DNA gene copies are increased in blood and brain of Japanese schizophrenia patients. PLoS ONE. 2023; 18: e0280694.