Variables associated with mitochondrial copy number in human blastocysts: what can we learn from trophectoderm biopsies?

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Objective: To study the potential variables that affect the mitochondrial DNA (mtDNA) content of trophectoderm (TE) cells in blastocysts that have undergone TE biopsy.
Design: Observational retrospective single-center analysis.
Setting: University-affiliated private in vitro fertilization center.
Patient(s): A total of 465 consecutive preimplantation genetic screening (PGS) cycles of 402 women undergoing preimplantation genetic testing.
Intervention(s): Trophectoderm biopsy performed on blastocysts of women undergoing preimplantation genetic testing-aneuploidy (PGT-A).
Main Outcome Measure(s): The mtDNA content in trophectoderm cells.
Result(s): We checked the possible influence of patient characteristics, ovarian stimulation variables, embryo morphology, and embryo culture conditions on mtDNA values. Of all the analyzed variables, some such as body mass index (BMI), serum progesterone (P4), aneuploidy, and trophectoderm quality had an effect on mtDNA content in blastocysts. Body mass index had a small but positive effect on the mtDNA copy number; as the BMI values increased, the probability of women producing blastocysts with an mtDNA content above the median increased by 6%. For P4 serum concentration, an increase in P4 lowered the probability of blastocysts having values above the median by 39%. Embryo-associated variables such as TE quality and aneuploidy status appeared to affect the mtDNA copy number. For the aneuploid blastocysts, the probability of being above the median increased by 42%. Finally, blastocysts with poor quality TE had more chances of carrying higher mtDNA values.
Conclusion(s): Summarizing, larger quantities of mtDNA in blastocysts are associated with the condition of aneuploidy and low quality TE, as well as being from women with high BMI values. Understanding the biological meaning of mtDNA content in human blastocysts and what factors may interfere with their values is fundamental. Other key gaps, such as whether a correlation exists between mtDNA content and mitochondrial mass and biogenesis in human TE cells, and whether this correlation can be extended to the inner cell mass, need to be further addressed. These questions are currently being investigated. (Fertil Steril® 2018;109:110–7. ©2017 by American Society for Reproductive Medicine.)
Key Words: Blastocyst quality, body mass index, mtDNA, progesterone, trophectoderm cells

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The possibility of analyzing trophectoderm (TE) cells from human blastocysts during preimplantational genetic testing is an opportunity to analyze the chromosomal constitution of trophoblastic cells (1, 2), and, by extension, to infer with no certain degree of uncertainty the chromosomal content of the inner cell mass (ICM) (3–5) and the entire embryo, and to investigate other interesting cellular physiology related aspects such as mitochondrial DNA (mtDNA) content (6–8). Mitochondrial copy numbers vastly vary among oocytes from different patients and even among oocytes from the same cohort. It is believed that this initial mtDNA content number correlates with oocytes' ability to fertilize and

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reach the blastocyst stage (9–11). Whatever the initial concentration of mtDNA a given oocyte may have, it is expected to equally segregate into a number of blastomeres during embryo development. So the mtDNA copy number per daughter cell will be transiently lower, with the smallest number in the blastocyst stage (12–14). Studies of various mammalian species (15–17), including humans (12, 18), have shown that mtDNA replication does not occur before the morula stage. Hence, the mitochondrial content of oocytes should be enough to sustain embryo development before implantation occurs (19).

The net amount of mitochondrial DNA exponentially increases in the blastocyst stage to coincide with increased oxygen consumption (12, 20, 21). Despite the mtDNA copy number being higher in the ICM compared with the TE cells (22), the proportion of moderate- and high-activity mitochondria is higher in TE cells (12). This also agrees with mitochondrial morphologic changes in TE cells, where the mitochondria are much more elongated, are less matrix-electrodense, and have more cristae (23–26) in the ICM, which are rounded and more electrodense (12).

Mitochondrial organelle complement, measured by mtDNA, has been proposed as a marker of embryo viability (6–8). It has been correlated with a woman's age, aneuploidy status, and embryo implantation potential. This is somewhat unexpected because embryos with greater implantation potential—and, therefore, of better quality—are not those with a larger mtDNA copy number, as observed in cumulus cells (27, 28). However, the lower values have led us to hypothesize that stressed blastocysts—and thus less viable ones—increase mitochondrial biogenesis or mtDNA replication. Such a correlation has not been found by other research groups (29, 30), so the real biological value of this idea and its eventual use in assisted reproduction as an additional marker of embryo competence needs to be established.

At this point, and far from discussing the utility of mtDNA complement as a marker of embryo viability, we wished to explore and discover what the distribution and values are of the mtDNA copy numbers of human blastocysts generated in in vitro fertilization (IVF) laboratories, and what variables may affect their final count to better understand to what extent intrinsic and extrinsic factors can condition mtDNA values. This may eventually help to define more accurate mtDNA number values as a marker of embryo viability.

MATERIALS AND METHODS

Patient Population

Our retrospective study was approved by our institutional review board, the ethics committee of Clinical Research IVI Valencia, which complies with Spanish law on assisted reproductive technologies (14/2006). The study included 1,641 biopsied blastocysts from 465 consecutive cycles preimplantation genetic screening (PGS) cycles (402 couples, among whom 3 had three cycles and 19 had two cycles) performed from June 2016 to January 2017. The indications for PGS were advanced maternal age (n = 265 cycles, 218 couples), repetitive implantation failure (n = 54 cycles, 45 couples), severe male factor infertility (n = 47, 47 couples), abnormal fluorescent in situ hybridization results in sperm (n = 10 cycles, 10 couples), recurrent miscarriage (n = 41, 41 couples), previous pregnancy with aneuploidy (n = 17 cycles, 17 couples), monogenic disease (n = 23 cycles, 16 couples), and other reasons (n = 8 cycles, 8 couples). The study population characteristics are described in Table 1.

Ovarian Stimulation Protocols

The patients who started ovarian stimulation on cycle day 1 or 2 received a starting dose of recombinant follicle-stimulating hormone (FSH; Gonal-F, Serono; or Puregon, MSD) that ranged from 150 IU to 225 IU. Gonadotropin-releasing hormone (GnRH) antagonist (0.25 mg of ganirelix, Orgalutran; Organon) was administered daily starting on day 5 or 6 after FSH administration or when the follicles reached a diameter of 14 mm, and human chorionic gonadotropin (hCG, Ovitrelle; Serono Laboratories) was administered subcutaneously for final oocyte maturation when at least three follicles or more had a mean size of 18 mm.

Ovum Pickup, Intracytoplasmic Sperm Injection, and Embryo Culture Conditions

Oocyte retrieval was performed 36 hours after hCG with ultrasound guidance. Follicles were aspirated, and the oocytes were washed in gamete medium (Cook). After the oocytes had been washed, intracytoplasmic sperm injection (ICSI) was performed on all the metaphase II oocytes in a medium that contained HEPES (gamete medium), at ×400 magnification under an Olympus IX7 microscope.

The embryos involved in the retrospective study were cultured under two different conditions. One group of 816 embryos was cultured in Sydney IVF cleavage media (Cook Medical, France) until day 3 of development, and CCM (Vitrolife AB) was used from day 3 onward in 5.5% CO_2 in air using Sanyo incubators. The other group of 825 embryos was incubated under low oxygen tension (in the following proportion: 5% O_2, 5.5% CO_2, and 85% nitrogen) using

TABLE 1

Demographic characteristics of the patient study population.

Characteristic	Mean ± SD
Age (y)	38.8 ± 3.2
BMI (kg/m^2)	23.2 ± 3.6
AMH (pmol/L)	16.3 ± 13.8
Days of ovarian stimulation	10.7 ± 2.3
Days of hCG administration	
Estradiol (pg/mL)	2,346.8 ± 1,425.8
Progesterone (ng/mL)	0.7 ± 0.6
Ovarian sensitivity index	5.2 ± 6.3
No. of aspirated oocytes	11.4 ± 7.4
No. of fertilized oocytes	6.6 ± 4.6
No. of embryos biopsied	3.23 ± 2.7
Sperm concentration (mill/mL)	36.4 ± 25.1

Note: AMH = antimüllerian hormone; BMI = body mass index; hCG = human chorionic gonadotropin; SD = standard deviation.

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