ACIBADEM MEHMET ALİ AYDINLAR UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

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M.Sc. THESIS

DEPARTMENT OF MOLECULAR AND TRANSLATIONAL BIOMEDICINE

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Thesis Title:	Effect of Transferring Isolated Mitochondria From Mononuclear Blood Cell To Mesenchymal Stem Cell on Cellular Chromosome and DNA Content
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This is to certify that I have examined this copy of master thesis. I have found that she/he prepared after fulfilling the specified requirements in the associated legislations before the final examining committee whose signatures are below.

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Jury Member (Thesis Supervisor)	Assist. Prof. Gamze Tümentemur	_____
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DECLARATION

I declare that this thesis work is my own work, I had no unethical behavior at any stages from the planning to the writing of the thesis, I obtained all the information in this thesis in accordance with academic and ethicel rules, I cited all the information and comments that were not obtained with this thesis work, and I provided resources in the list of references. I also declare that there was no violation of any patents and copyrights during the study and writing of this thesis.

21.09.2022
Cemre Su Turhan

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic Acid
MSC	Mesenchymal Stem Cell
PBMC	Peripheral Blood Mononuclear Cell
PCOS	Polycystic Ovary Syndrome
PGT	Preimplantation Genetic Testing
FSH	Follicle Stimulating Hormone
POF	Premature Ovarian Failure
IVF	*In Vitro* Fertilization
ICSI	Intracytoplasmic Sperm Injection
LH	Luteinizing Hormone
AMI	Implementing Autologous Mitochondrial Injection
mtDNA	Mitochondrial DNA
hESCs	Human Embryonic Stem Cells
iPSCs	Induced Pluripotent Stem Cells
HSCs	Hematopoietic Stem Cells
PBS	Phosphate Buffered Saline
DMEM	Dulbecco's Modified Eagle's Medium
EDTA	Ethylene Diamine Tetra Acetic Acid

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ÖZET

Mononükleer Kan Hücresinden İzole Edilen Mitokondrinin Mezenkimal Kök Hücre'ye Transferinin Hücresel Kromozom ve DNA İçeriği üzerine Etkisi

Günümüz dünyasında, kısırlık, bir yıl korunmasız cinsel aktiviteden sonra gebe kalamama ile karakterize edilen sık görülen bir sorundur. Hormonal dengesizlikler veya hipofiz tümörleri, vücut ağırlığı sorunları, polikistik over sendromu (PKOS) ve erken yumurtalık yetmezliği kadın kısırlığının nedenlerinden bazılarıdır. Ayrıca yaş, üreme sistemi güçlükleri, cinsel hastalıklar, tıbbi durumlar, sigara, alkol, uyuşturucu kullanımı, gebe kalma yeteneğini bozabilir. Azalan oosit kalitesi, üreme kapasitesindeki yaşa bağlı düşüşle ilişkilendirilmiştir. Mitokondri, kadın üreme sistemlerinde önemli bir role sahiptir. Embriyo potansiyeli, mitokondriyal oositlerin enerji yaratma yeteneğiyle bağlantılıdır. Bu çalışma, onayı alınmış bir kişinin otolog kan hücresinden izole edilmiş mitokondrinin göbek kordonundan türetilen mezenkimal kök hücrelere (MKH) aktarılması kavramına yeni bir bakış açısı sunmayı amaçlamaktadır. Daha sonraki çalışmalarda bu yöntem kullanılarak tedavi edilen oositlerdeki yan etkilerin ve vücutta oluşabilecek olumsuz tepkilerin ortadan kaldırılmasını umuyoruz. Bu amaçla periferik kan mononükleer hücresinden (PKMH) mitokondri izole edildi. Daha sonra izole edilmiş mitokondriler MKH'a aktarıldı. Mitokondri transfer edilen MKH numuneleri ve kontrol grubu olarak tek başına MSC numuneleri, 48 saatlik inkübasyondan sonra preimplantasyon genetik testi (PGT) uygulanarak analiz edildi. PGT ile birlikte akış sitometri analizi yapıldı. Sonuçlar, mitokondrinin MKH'da kromozom kaybı veya kazanımı üzerindeki etkisini saptamak için karşılaştırıldı. PGT sonuçları kromozomal kayıp ve kazanç açısından kromozomal anöploidiler gösterirken, akış sitometrisi sonuçları Hipodiploidi ve Hiperdiploidi için tepe noktaları göstermiştir. Bu da PGT ve akış sitometrisi sonuçlarının birbirini doğruladığı anlamına gelir. Sonuç olarak, mitokondrinin mezenkimal kök hücreye transferinin hücresel kromozom ve DNA içeriği üzerine etkisi vardır.

Anahtar Sözcükler: Mitokondri, mezenkimal kök hücre, kısırlık, periferik kan mononükleer hücreleri, aneuploidi.

ABSTRACT

Effect of Transfer of Mitochondria Isolated from Mononuclear Blood Cell to Mesenchymal Stem Cell on Cellular Chromosome and DNA Content

In today's world**,** infertility is a frequent problem characterized by the inability to conceive after a year of unprotected sexual activity. Hormonal imbalances or pituitary tumors, body weight issues, polycystic ovary syndrome (PCOS), and early ovarian failure (POF) are some of the causes of female infertility. Besides, age, reproductive tract difficulties, sexual illnesses, medical conditions, smoking, alcohol, and drug use can all impair the ability to conceive. Reduced oocyte quality has been linked to the age- related decline in reproductive capacity. Mitochondria have an important role in female reproductive systems. Embryo potential is linked to the mitochondrial oocytes' ability to create energy. This study aims to present a novel perspective on the notion of transferring isolated mitochondria from an autologous blood cell of a person who has agreed to participate in the study to umbilical cord-derived mesenchymal stem cells (MSC). In future studies, it is planned to eliminate side effects and adverse bodily responses to oocytes treated using this method. For this purpose, mitochondria were isolated from peripheral blood mononuclear cells (PBMC). Then, isolated mitochondria were transferred into MSC. Mitochondria were transferred to MSC samples and only MSC samples as control groups were analyzed by applying preimplantation genetic testing (PGT) after 48 hours of incubation. Along with PGT, flow cytometry analysis was conducted. Results were compared to detect the effect of mitochondria on chromosomal loss or gain in MSC. PGT results have shown chromosomal aneuploidies in terms of chromosomal loss and gain while flow cytometry results have shown peaks addressing Hypodiploidy and Hyperdiploidy which means that the results of PGT and flow cytometry confirmed each other. In conclusion, the transfer of mitochondria to mesenchymal stem cells affects cellular chromosome and DNA content.

Keywords: Mitochondria, mesenchymal stem cell, fertility, peripheral blood mononuclear cell, aneuploidy.

1 INTRODUCTION

Infertility is a common issue that refers lack of ability to conceive after 1 year of unprotected sexual intercourse. Types of infertility, which aren't based on inheritance, are classified into 2 characteristics; Primary infertility refers to women who have never conceived, and secondary infertility refers to women who have conceived before. Infertility problem has a considerable rate of around 17% among couples in developed countries (1). Causes of female infertility are mentioned as ovulation problems that can be due to luteinizing hormone (LH) and follicle stimulating hormone (FSH) imbalance or due to pituitary tumors, body weight imbalance, and other hormonal conditions that affect thyroid problems, diabetes, polycystic ovary syndrome (PCOS), premature ovarian failure (POF). Adequacy of getting pregnant can also be affected by age, reproductive tract problems, sexual diseases, medical conditions such as sickle cell anemia, smoking, alcohol drinking, drug usage, strong medications such as antidepressants or anti-cancer drugs, and radiation exposure. Treatments for female infertility are mainly composed of medication treatments, surgical manipulations, and assisted reproductive technologies (ART). Various types of treatment modalities falling under the main treatment techniques mentioned previously are weight reducing drugs, induction of ovulation using gonadatrophins – Human Menopausal Gonadotrophin (HMG), artificial insemination, *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI). In this study, it will be conducted mitochondrial transplantation modality which is a kind of *in vitro* fertilization technique (2). It is reported that mitochondria are a key factor in the regulation of female reproductive processes. In a mature human oocyte, mitochondria and mitochondrial DNA are present more compared to other cell types (3).

Clinical and experimental data show that decreased oocyte quality is correlated with the age-related regression of reproductive capacity. Besides, embryo potential is related to the ability of mitochondrial oocytes to generate energy. Therefore, improving mitochondrial capacity in women has been tried with the treatment of assisted reproductive technologies including transferring mitochondria from autologous germ line precursor cells (4). Mitochondrial dysfunction has significantly

important for age related decrease in oocyte quality. Related studies show that transferring cytoplasm of young oocytes to aged oocytes has been successful, but it rises heteroplasmy concerns so that it has been not preferred anymore. Later, they obtained results indicating that using oogonial precursor cells (OPCs) as an autologous source of healthy mitochondria and implementing autologous mitochondrial injection (AMI) rather than the *in vitro* fertilization (IVF) showed enhancement in the results however the outcomes aren't consistent enough to say that the experiment completely accomplished the desired consequent (5). Considering this information, here it is conferred a different perspective with the principle of transferring isolated mitochondria from autologous blood cells from the person to mesenchymal stem cell (MSC) obtained from the umbilical cord. Based on the analysis of the results that contain the effects of mitochondria on MSC, it can be taught whether the principle used in this study might be applied to oocytes in future studies. This approach, it is aimed to eliminate side effects and negative body responses against treated oocytes. From this perspective, the study to be carried out will be a starting point for further studies focusing on enhancing the quality of oocytes.

2 BACKGROUND

This experimental procedure using mesenchymal stem cells analyzes the effect of mitochondrial transfer on the cellular chromosome and DNA content and in the future aims to improve the quality of oocytes incapable of fertilization or subjected to previous miscarriages.

2.1 Infertility

Infertility is commonly defined as the inability to conceive after one year of unprotected sexual activity. Most practitioners begin an infertility evaluation after a year of trying to conceive, however, for females who are 35 years old or older than 35 years, most specialists begin a clinical examination after 6 months of incapability to conceive. A standard infertility evaluation consists of semen analysis, ovulation assessment, a hysterosalpingogram, and if indicated, ovarian reserve and laparoscopy. Practitioners assign a diagnosis of unexplained infertility when the results of a standard infertility evaluation are normal. Even though numbers range, the chances that many such experimental findings for an infertile couple are around 15% to 30% (6).

Fertility in women peaks between the ages of 20 and mid-20s, drops noticeably in the early 30s and then drops dramatically between the mid-30s and early 40s. In men, sterility increases dramatically in their late 30s and then increases further after the age of 40. Infertility can be caused by a variety of biological factors, including female complications like tubal causes, ovarian instability, reduced ovarian reserve, endometriosis, and vaginal characteristics, as well as male factors like irregular insemination, semen or varicocele, and testicular factors. These biological influences, nevertheless, are no longer as limited as elevated childbearing age thanks to improvements in infertility treatments. The decision of couples to continue career improvement and thus postpone childbirth may pose the greatest risk of unavoidable childlessness (7).

Defects in oocyte quality, across many cases, provide perspective on why some partners experience fertility problems even after getting normal fertility diagnoses. Poor oocyte quality in most women can be described by the natural degeneration that appears with the aging process. Notably, for a significant number of early age women who utilize IVF, oocyte quality is lower than usual and may represent an increased process of aging. Others, such as when severe anomalies such as the oocyte maturation process exists, would have an innate genetic abnormality. Because these abnormalities are commonly sporadic and are not passed on to offspring due to the generated sterility generated, developing genetic tests for particular genetic variations in identified genes might be difficult (8).

Besides genetic abnormalities, mitochondria is the main source of oxidative phosphorylation throughout early embryonic progress and play crucial roles in oocyte activities. Oocyte mitochondrial dysfunction is thought to be a key factor in oocyte developmental competence in older infertility patients. In oocytes, the mtDNA content of reproductive-aged women or women with diminished ovarian reserve is substantially lower than in younger individuals with usual ovarian reserve (9).

Treatment is determined by the cause, length of time of the occurring condition, and the patient's age. It is categorized into two types: the first is the use of medications or surgery to restore fertility, and the second is the use of assisted reproductive technologies. Women with ovulation disorders are treated with fertility medicines (clomiphene citrate, FSH, and LH hormone injections). These treatments can also be used in conjunction with an intrauterine sperm inoculation (IUI), which involves injecting sperm specifically into the uterus. When infertility is caused by clogged fallopian tubes or endometriosis, surgical treatment might be required. The other treatment option is assisted reproductive technology (ART). Egg and fertilized egg donation, *in vitro* fertilization, and intracytoplasmic sperm infusion are examples of these technologies (10). In elderly infertile patients, oocyte mitochondrial dysfunction is thought to have a significant role in oocyte developmental competence (9).

2.2 Mitochondria

Mitochondria is a cell organelle that ranges in size from 0.2 to 5 microns. Their forms range from oval to tool-like. Large mitochondria are abundant in most cells. Their number is determined by the cell's energy requirements. There are numerous mitochondria, particularly in cells that require energy, such as muscle and nerve cells. Mitochondria have the ability to multiplication and dividing. They produce ATP through aerobic respiration. Mitochondria are observed in aerobically respiring eukaryotic cells. It is not found in mitochondria, prokaryotic cells, and mammalian red blood cells. However, it is found in both animal and plant cells. It is where the oxidative phase of cellular respiration takes place. They play a role in obtaining ATP from chemical bond energy (11).

2.2.1 Mitochondrial DNA

Mitochondrial DNA (mtDNA) is the unique piece of DNA found in the mitochondria, which, unlike the DNA found in the nucleus of the cell, performs most of the cell's energy production. The mtDNA of mitochondria produces its proteins. mtDNA lacks protective histone proteins and has a nuclear DNA- independent replication mechanism. Therefore, the mutation rate is very high. mtDNA synthesizes 2-3% of mitochondrial proteins and 98% of nuclear DNA, and the synthesized proteins are transported to the mitochondria. The inner membrane of mitochondria is impermeable, so there are some mechanisms for transporting proteins synthesized by nuclear DNA into the matrix. Amino acids, carbohydrates, and fatty acids come to the inner membrane, where they undergo oxidative destruction, high- energy ATP is synthesized, and the energy needs of the cell are provided. (7) mtDNA has its replication and transcription mechanism. However, since the enzymes required for replication and transcription of mtDNA are synthesized by nuclear DNA, mtDNA does not show complete autonomy. mtDNA replication and transcription occur in the S and G2 phases of the cell cycle. However, mitochondrial protein synthesis doubles during interphase and remains constant throughout the cell cycle (13).

2.2.2 Mitochondrial fission and fusion

Mitochondrial fission is the separation of two membranes, while fusion is the complete union of the inner and outer membranes of the two mitochondria. Fission-fusion is an integrative mechanism required for mitochondrial homeostasis. Fission and fusion in healthy cells ensure a healthy life for both mitochondria and cells. The role of mitochondria in calcium balance, the aging process, various diseases, and cell vitality and death is inevitable. Mitochondrial fission and fusion have an important role in these processes. The balance between fission and fusion prevents problems with mitochondrial morphology and mitochondrial DNA/proteins. Some studies have shown that oxidative phosphorylation increases with fusion and decreases with fission (14).

2.2.3 Mitochondrial dysfunction

Infertility and developmental abnormalities are linked to mitochondrial impairments in the oocyte and embryo. Mitochondrial dysfunction, along with sub-optimal mitochondrial function, is linked to meiotic spindle abnormalities. Mitochondrial functions are required for the configuration of meiotic spindles as well as the preservation of the MII spindle before fertilization (15).

Aneuploidy, an impairment in which chromosomal separation errors are frequent, is associated with impaired ATP synthesis. In humans, oocyte chromosomal separation is prone to errors, which are estimated to account for 15–20 percent of all pregnancies, with aneuploid pregnancies accounting for 5% of all pregnancies. In oocytes from obese and type 2 diabetic females, as well as aging oocytes, the intensity of aneuploidy rises. While intracellular transmission from normal, healthy oocytes with perfectly functioning mitochondria has successfully overcome mitochondrial abnormalities in quality-compromised oocytes, the procedures have raised ethical concerns, but new studies have clarified the potential risks, such as the introduction of heteroplasmy or mitochondrial genetic disorder (16).

2.2.4 Mitochondrial transplantation

Mitochondrial transfer therapy is a therapeutic method with successful results in genetic diseases. In some diseases with mtDNA damage and mitochondrial dysfunction, there is a great need to transplant mitochondria as organelles. Many studies on this subject have shown that mitochondria isolated from various cell sources (mesenchymal stem cells, cell culture, patients' tissue samples) have successful results in damaged tissues, organs, or cells (17).

Horizontal transfer of mitochondria in different cell types *in vitro and in vivo*, which defines a new cellular feature, has been reported by some scientists for natural mitochondrial transfer (18). In horizontal transfer, mitochondria or mtDNA can be transported naturally between cells and this active transfer from stem cells/somatic cells can provide aerobic respiration in cells with dysfunction. Most studies on mitochondria transfer from one cell to another are damaged by mitochondria obtained from a healthy cell source such as mesenchymal stem cells, which have been related to the repair of vision cells (19). Many studies have been conducted on the transfer of intercellular material (mitochondria) (20).

The first transfer mechanism is the transport of mitochondria by micro-vesicle. This transport especially increases the phagocytic properties of immune cells and saves the cell from damage. Transport by micro-vesicles is usually seen in mitochondrial transmission by mesenchymal stem cells (21). The second transfer route is nanotube tunnel transfer. It has the ability to deliver mitochondria to diseased cells to heal cellular damage. Such mitochondria transfer is associated with metabolic reprogramming, immune enhancement as well as differentiation (22).

Mitochondria transfer from one cell to another is part of a process necessary for the maintenance and development of homeostasis in multicellular organisms. Mitochondria can move from one cell to another via intracellular structures such as nanotube tunnels and micro-vesicles (23). Although there are different perspectives among researchers on mitochondrial transplantation, Clark and Shay's "mitochondrial

transformation", the first example of mitochondrial transfer from one cell to another in 1982, pioneered in all transplantation techniques (24). In this study, they used the antibiotics chloramphenicol and efrapeptin to inhibit the ATPase function and protein synthesis of mitochondria. Chloramphenicol-resistant cells had mutations in their mtDNA located in an area of the mitochondrial large subtype rRNA gene. They proved that mitochondria transfer from chloramphenicol and efrapeptin resistant fibroblasts preserves the viability of recipient cells sensitive to these antibiotics. Clark and Shay's observations and questions about the mitochondrial transfer mechanism paved the way for future studies in this area. Exogenous mitochondria microinjection was applied to reach healthy mitochondria content of oocytes carrying mitochondrial diseases (25). The photothermal nanoblade enabled the efficient transfer of isolated mitochondria into the cell, providing a shortcut to endocytosis and cell fusion. However, its application is limited to a small number of cells (26).

Two different approaches have been developed to facilitate mitochondria entry in recipient cells. The first is Pep-1 and the other is magnetic beads designed to bind to the mitochondrial membrane receptor complex (TOM22). (Magnetomitotransfer). MitoCeption technique is a standardized transplantation method by adding thermal shock and centrifugation steps to the coincubation method in the transfer of isolated mitochondria to cultured cells. This method improves the uptake of mitochondria into the cell. This technique, which was first applied in cancer cells, initiated the metabolic reprogramming of the cells (27).

2.3 Stem Cell

Stem cells are described as undifferentiated cells obtained from the embryo, fetus, or adult that have a distinct ability to form diverse differentiated tissue cells *in vitro* and *in vivo* when exposed to the right biochemical, hormonal, and biomechanical signals (28). Stem cells have a unique capability for self-renewal. During early life and development, they can evolve into a variety of types of cells. Many distinct types of stem cells are studied by researchers. The "pluripotent" stem cells (embryonic stem cells and induced pluripotent stem cells) and "adult" stem cells (often referred to as

nonembryonic or somatic stem cells) are the two primary types. Pluripotent cells have the capacity to differentiate into any of the adult body's cells. Adult stem cells present in a tissue or organ and have the ability to differentiate into the tissue or organ's specific cell types (29).

Pluripotent (Embryonic) Stem cells: At the blastocyst stage, early mammalian embryos comprise two cell types: inner cell mass cells of the blastocyst and trophectoderm cells. The placenta is supported by trophectodermal cells. The inner cell mass will eventually grow into specific cell kinds, tissues, and organs across the organism's complete body. Because of the legal and ethical concerns shaped around the use of embryonic stem cells, a method for extracting stem cells out from the inner cell mass of preimplantation human embryos and growing human embryonic stem cells (hESCs) in the laboratory was developed in 1998. Researchers discovered conditions in 2006 that allowed several adult human cells that can be reprogrammed into a state similar to embryonic stem cells. Induced pluripotent stem cells are stem cells that have been reprogrammed (iPSCs) (29,30).

Adult Stem cells: Adult stem cells act as an intrinsic repair mechanism for the organism throughout its life, generating replacements for cells lost due to routine maintenance, injury, or disease. Adult stem cells can be present in a variety of organs and tissues. They are usually linked to specific anatomical regions. These stem cells may continue to be dormant (non-dividing) for a long time until a natural demand for additional cells to preserve and repair tissues activates them (29,30).

Pluripotent stem cells derived from human fetal cells have recently been cultivated and proven to be capable of giving rise to a range of specialized cell types. After trauma, sickness, or aging, many adult tissues have stem cells that can regenerate. The cells might be found in the tissue itself or in some tissues that act as stem cell repositories. On the other hand, Adult hematopoietic stem cells (HSCs) that regenerate circulating blood components, for example, can be found in a variety of organs other than bone marrow. Mesenchymal stem cells (MSCs) are found in the adult bone

marrow and participate in the renewal of mesenchymal tissues such as skin cartilage, ligament, adipose, storm, tendon, bone, and muscle (31, 32).

2.4 Mitochondria and Mesenchymal Stem Cell Relation

MSCs are multilineage stromal cells that can self-renew and differentiate in multiple directions. MSCs can be extracted from a wide range of tissues, including adipose tissue, endometrial tumors, menses blood, bone marrow, and umbilical cord. Recently been isolated from novel sources, including endometrium and menstrual blood. Due to the simplicity with which these sources can be harvested, and the quantity of material obtained, MSCs are ideal for both experimental and clinical use (33). Nevertheless, the rotation differentiation of transplanted MSCs is difficult to handle, and the cells are likely to be vulnerable to aging and apoptosis in the injured cells, resulting in a loss of function and making it challenging for the damaged MSCs to efficiently impose their activities and achieve the desirable renewal and reconstruction abilities. Thus, increasing MSC capabilities under clinical conditions, such as promoting directional differentiation, suppressing the aging process and apoptotic process, and stimulating local tissue regeneration, is critical in stem cell therapies (34).

Over the past several years, it has been discovered that mitochondria, the major organelles in cells involved in energy metabolism and play a vital role in regulating MSC functions via a variety of pathways such as aging, apoptosis, differentiation, proliferation, and immune regulation. Many studies have found that mitochondrial transfer, biogenesis, distribution dynamics, mitophagy, membrane potential, and ROS generation all play a role in the maintenance of MSC activities in local injured tissues. Mitochondrial energy metabolism impact factors such as HIF-1, PGC-1, sirtuin, SOD2, AMPK, and UCP may regulate MSC activity (35). Furthermore, as the age–related issues are becoming more serious, one of the considerations that need to be explored here is to control the biogenesis, dynamics, mitophagy, and membrane potential of mitochondria to minimize mitochondrial damage, inhibit the aging process of MSCs, and boost remodeling of tissues to help organisms to minimize agaging36).

The efficiency of MSCs can indeed be influenced by changes in energy metabolism and determined by the major variables that govern the energy metabolism of mitochondria in MSCs. With that being the case, studies on how to manage mitochondrial energy metabolism to regulate MSC differentiation, proliferation, and other functions and enhance MSC activities, is getting more and more relevant.

2.5 Mitochondrial Effect on Cell Cycle

Mitochondrial development necessitates the cytoplasmic uptake of nuclear-encoded proteins and the synthesis of polypeptides expressed by the organelle genetic material. Lipid incorporation and mtDNA replication are also important aspects of mitochondrial biogenesis. Over the years, scientists have looked at the molecular basis of mitochondrial dynamics in cells, notably, mitochondrial shape and inheritance, cellular distribution, and mitochondrial division during cell division. Mitochondrial morphology and distribution are frequently modified to fit a cell's specific energy requirements (37). In most cell types, the cytoskeleton is essential for polarized mitochondrial motions and mitochondrial heredity, as well as for determining these various mitochondrial morphologies and distributions (38).

2.5.1 Mechanism of mitochondria during mitosis and meiosis

Gene products necessary for mitochondrial protein synthesis, electron transport, and oxidative phosphorylation are encoded by mitochondrial DNA (mtDNA). Other metabolic activities that take place in the mitochondrial fraction, such as tricarboxylic acid (TCA) cycle events and amino acid and lipid production, are, however, critical (39).

Mitochondrial reproduction occurs when a piece of the network reaches into the emerging daughter cell or bud early during the cell cycle (late G1/early S phase). More mitochondrial membranes are brought in from the mother cell as the bud expands (S/G2 phase). During this time, mitochondria are said to migrate in a vertical and polarized pattern. A transitory aggregation of mitochondria is also detected at the bud

tip, implying that mitochondria can be trapped and held soon after transferring to inhibit their return to the mother cell. These immobile mitochondria are dispersed throughout the bud before cytokinesis (39, 40).

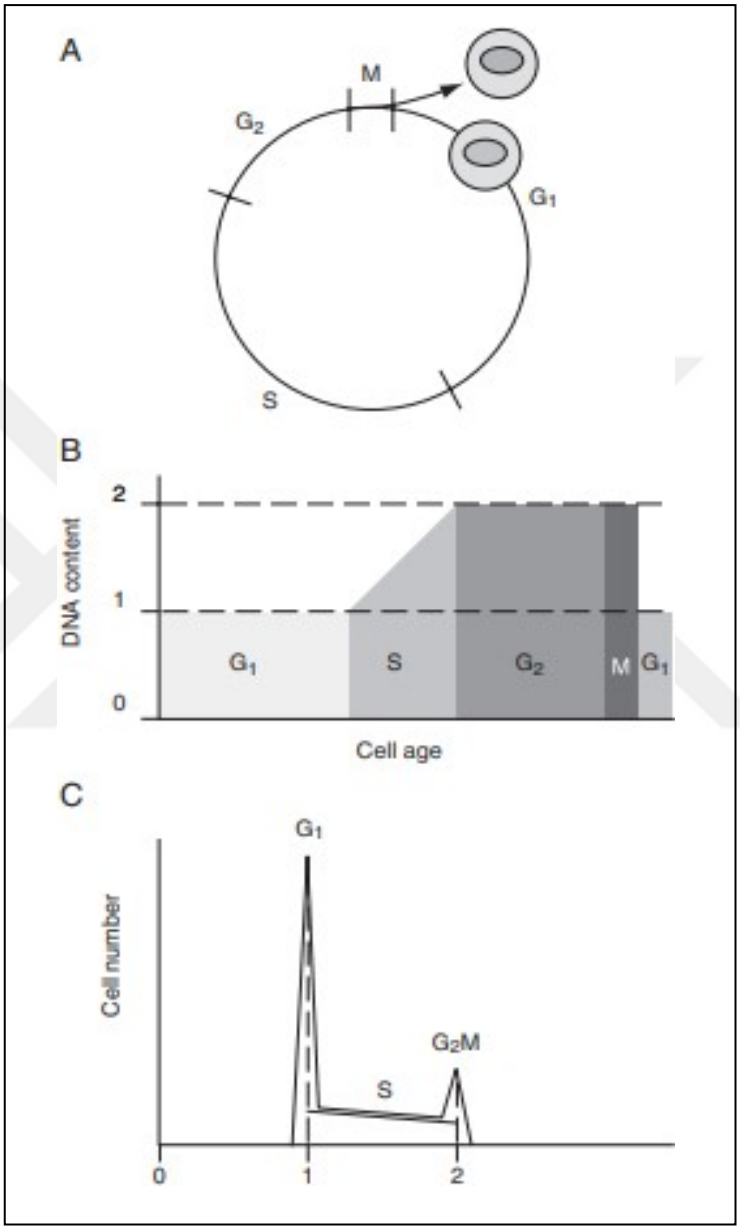

Figure 1. Cell cycle stages demonstrated by flow cytometry analysis

(Darzynkiewicz, Z., Juan, G., & Bedner, E. (2001). Determining Cell Cycl Stages by Flow Cytometry. Current Protocols in Cell Biology. Doi:10.1002/0471143030.cb0804s01)

When haploid cells are exposed to a mating pheromone, they form mating projections, stick together, and eventually unite to generate a dumbbell-shaped zygote.

Mitochondrial segments generated from each parent rapidly merge in the zygote before or simultaneously with nuclear fusion, forming a single and continuous network. Most mitochondrial materials are completely mixed and redistributed across the network as a result of this mitochondrial fusion. Buds formed from any place on the

Zygotes inherit mitochondrial networks with fairly homogeneous membrane and protein content as a result of this mitochondrial fusion and compartment mixing (41).

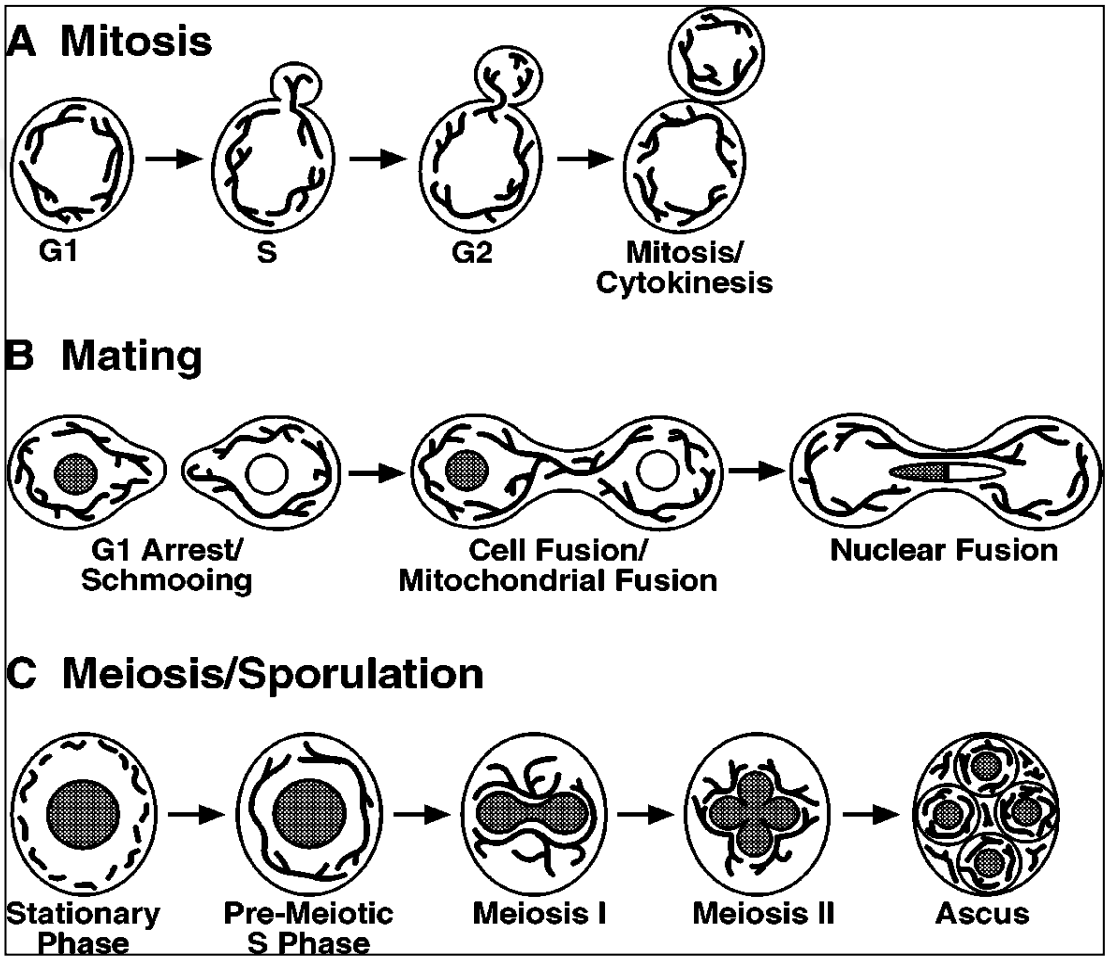

Figure 2. Mitochondrial dynamics during the life cycle of *S. cerevisiae*

(Hermann GJ, Shaw JM. Mitochondrial dynamics in yeast. Annu. Rev. Cell Dev Biol [Internet]. 1998;14(1):265–303. Available from: https://pubmed.ncbi.nlm.nih.gov/9891785)

Mitochondria are a branched tubular network found near the cell's cortex. In between the beginning of the S phase and cytokinesis, they are continually separated from the mother cell into the bud. During mating, mitochondrial fusion occurs. By

cellular and nuclear fusion, haploid yeast cells of various mating types generate a diploid zygote. The paternal mitochondrial networks in the zygote combine to form one linked organelle before nuclear fusion. During meiosis and sporulation, mitochondrial structure and inheritance happen. In diploid yeast, meiosis and sporulation result in four haploid daughter cells encapsulated inside the mother cell.

Specific mitochondrial partitions are distributed at the cell cortex during the pre-meiotic stationary phase. Individual mitochondria fuse to generate a vast branching network during the pre-meiotic S phase. The mitochondrial membrane stays strongly attached to the nucleus during meiotic nuclear divisions. Some portion of the network in mitochondria is incorporated into newly produced spores as a result of this interaction.

Mitochondrial distribution and morphology change significantly through meiosis and sporulation phases. Mitochondria change in a papillary formation scattered across the cell cortex in pre-meiotic cells. These separated components had merged to create a tubular reticulum by the early prophase. The mtDNA nucleoids in this reticulum are packed tightly. Within both meiosis I and II, the mitochondrial reticulum moves to the center of the cell, where it stays attached to the nuclear membrane. After meiosis II, there are four distinct mitochondrial tubules are preserved, each of which is positioned near a different nuclear lobe. When each nuclear lobe is surrounded by the prospered cell membrane, the mitochondrial and nuclear membranes are strongly connected, ensuring that mitochondria are involved (41,42,43).

3 MATERIALS AND METHODS

3.1 PBMC Isolation

Blood sample was collected from 3 volunteers who will participate in the experiment with the help of a nurse. 15 ml Ficoll (Sigma-Aldrich) was transferred into the conical tube. 30 ml of blood sample was carefully layered onto the Ficoll solution and centrifuged at 800 x g for 25 minutes. After centrifugation, the density gradient separation of the blood cells were seen. The cloudy layer on middle position (Mononuclear cells) were collected and transfered to a sterile conical tube. The mononuclear cells were washed twice, as adding of PBS without Ca^{+2} Mg^{+2} (Gibco) and centrifuging at 400 x g for 10 minutes. After washing step, the mononuclear cells suspended with 20 ml ringer lactat solution. 50 µl sample was taken for the cell counting. PBMC was cryopreserved in cryovials with adding DMSO (Wak Chemie) and Dextran 40 (Polifarma). The cryovials were stored in -80 deep freezer until the mitochondria isolation step.

3.2 Mitochondria Isolation from PBMC

Mitochondria isolation was performed using the mitochondria isolation kit (miltenyi Biotec) according to the following steps.

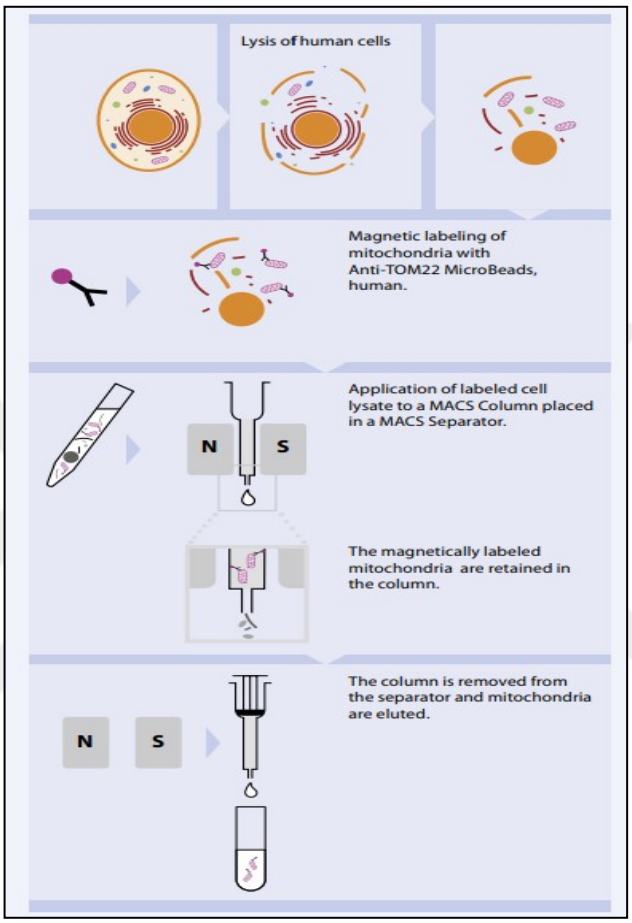

Figure 3. Mitochondria extraction kit manual (Miltenyi Biotec).

3.2.1 Preparation of cell lysate

Cryopreserved PBMC's were thawing in the water bath and transferred into a sterile conical tube containing 10 ml DMEM low glucose (Gibco). PBMC was washed in DMEM low glucose (Gibco), as centrifuging at 400 x g for 10 minutes. Pellet was suspended in 1ml of PBS without Ca^{+2} Mg^{+2} (Gibco). 50µlcells were diluted 1:10 ratio with PBS without Ca^{+2} Mg^{+2} (Gibco) for the cell counting. 100µlsample was taken and mixed with 100µlTrypan Blue (Sigma Aldrich). 10µlsample was loaded in to slide of the automatic cell counter (BioRad). The cell count result was recorded from cell counter screen. After 10^6 cells transferred to the sterile tube, 1 ml of Lysis Buffer (Sigma Aldrich) was added in tube and homogenized for 1 minute with homogenizer.

3.2.2 Magnetic labeling

9 ml of Separation Buffer was added into the tube that contain cell lysate and the obtained suspension mixed well. 50 µl of Anti-TOM22 Beads were added into suspension and mixed. 10 ml of suspension incubated for 1 hour on orbital shaker on 50 rpm, at +4 degrees.

3.2.3 Magnetic separation

LS Column was placed on magnetic separation stand and prepared via washing with 3 ml of Separation Buffer. Firstly 3.3 ml of the cell lysate was added into the column and allowed to flow. This process was repeated 3 times and with this way all of the lysate was passed through the column. The column was washed with 3 ml Separation Buffer for 3 times to get rid of unlabeled components. Column was taken from magnetic stand and 1.5 ml of Separation Buffer was added to the column. Marked mitochondria were pushed from the column into the conical tube with the help of a plunger. The mitochondria suspension was centrifuged at 13,000 x g for 2 minutes. The supernatant was discarded and 100 µl of Storage Buffer was added into the mitochondria pellet.

3.2.4 Stain with MitoTracker

The isolated mitochondria were centrifuged at 13,000 x g for 7 minutes for get rid of Storage Buffer. 100 µl of MitoTracker was added onto pellet and incubated at +4 degree for 15 minutes. PBS with Ca+2 Mg +2 (Gibco) was added on the mitochondria and centrifuged at 13.000 x g for 5 minutes for washing.

3.3 MSC Culture

Umbilical cord tissue of baby boys and baby girls was donated by donors with consent form and collected by the physician during the birth. Umbilical cord tissue was washed three times with isotonic saline (Eczacıbaşı Baxter Pharmaceuticals) and Wharton's gelly was mechanically divided into small pieces. These Wharton's gelly pieces were placed into T-25 flask. The flask was incubated at 37°C 5% CO2 condition for 20 minutes. After adhesion of the tissue pieces on flask layer the 2 ml of complete media (Msc Nutristem XF Basal Medium, MSC Nutristem XF Supplement, %1 penicillin&streptomycin) (Biological İndustries) was added into the flask and incubated at 37°C 5% CO2 condition for 4 days. When cell colonies were visible subculture was performed. The medium and tissue pieces were removed, and the flask was washed with PBS without Ca^{+2} Mg^{+2} (Gibco). 5 ml of Trypsin EDTA (Biological Industries) was added into the flask and incubated at 37°C for 4 minutes. After the detachment of the cells from the flask layer, neutralization was performed with HSA (Biological İndustries), and the cells were transferred into the sterile conical tube. The cells were washed twice, as adding of PBS without Ca^{+2} Mg^{+2} (Gibco) and centrifuging at 400 x g for 10 minutes. After the washing step the cells were cultured at the same media and condition.

3.3.1 Termination process

At the end of the 2nd subculture, mesenchymal stem cells were collected with the help of the Trypsin EDTA as in the subculturing process (Biological Industries). 100µlof the mesenchymal stem cell sample was taken for the cell count analyses on the cell counter (Biorad). Mesenchymal stem cells were cryopreserved in cryovials with adding DMSO (Wak Chemie) and Dextran 40 (Polifarma). The cryovials were stored in -80 deep freezer by the gender of donors.

3.3.2 Thaw of MSC

The MSC's were thawed in water bath by the gender of donors (male and female) and transferred into the sterile conical tube that contain 10 ml DMEM low glucose (Gibco). MSC's were washed in DMEM low glucose, as centrifuging at 400 x g for 10 minutes. Pellet was suspended in 1ml of PBS without Ca^{+2} Mg^{+2} (Gibco). 50µlcells were diluted 1:10 ratio with PBS without Ca^{+2} Mg^{+2} (Gibco) for the cell counting.

3.4 Serial Dilution of MSC

Before single cell experiment, different doses of mitochondria sample were applied to multiple cells (10 µl, 20 µl, 40 µl, and 100 µl of mitochondria were added on MSC). In this single cell experiment, many cells initially present were transformed into single cells in a 96 well plate. Therefore, the MSC was serially diluted with DMEM low glucose (Gibco). The dilution process was terminated when it reached the amount that can be taken with a pipette. The amount that could be taken from the diluted liquid was taken. The volume taken was completed to 10 ml. Prepared 10 ml MSC was dispensed into the wells as 100 µl. The single cell wells were marked. On the first day of the experiment, 100 µl of mitochondria was added to the other wells containing MSC, except for the control group. At the end of the 48th hour, a microscope image was taken. Afterwards, the cells adhered to the 96 well plate were separated from the flask surface with 0.05 ml of Trypsin EDTA (Biological İndustries). For this, the sample with trypsin was incubated at 37°C for 5 minutes. For the

neutralization process, 0.05 ml HSA was added to trypsin EDTA (Biological İndustries) and transferred to eppendorf. It was centrifuged at 400 x g for 10 minutes. The supernatant was discarded and 100 µl of PBS without Ca^{+2} Mg^{+2} (Gibco) was added to the pellet for flow cytometry. The sample was prepared for flow analysis. For PGT analysis, 10 µl of PBS without Ca^{+2} Mg^{+2} (Gibco) was added to the pellet and was prepared for analysis.

3.5 Flow Analysis

Flow cytometry is a method used to measure cell diameter, internal structure, and enzyme activities, as well as a successful method for determining DNA content. A fluorescent dye that binds to DNA is cultured with a single cell suspension of adhesive or fixed cells during this process. The quantity of fluorescent sensors is directly approximately equal to the amount of DNA because the dye attaches to DNA stoichiometrically. Because of the changes that happen during the cell cycle, DNA content can be used to distinguish between the G1, S, G2, and M phases. Figure 2 shows a straightforward cellular analysis protocol. To summarize, cells are corrected and resuspended to allow dye(s) to join the cell while preventing them from migration (44).

Flow cytometry cell cycle analysis is primarily based on the quantification of DNA content using a propidium iodide stain (PI). The stoichiometric essence of PI allows for precise DNA content quantification and reveals the spread of cells in G1, S, and G2 cell cycle stages, as well as sub-G1 cell death, which is described by DNA fragmentation. The majority of commonly used PI-based DNA quantification methods necessitate alcohol or aldehyde fixation, which is time-consuming and labor-intensive. Furthermore, these methods are incompatible with the use of fluorescent-labeled antibodies to stain mitotic markers. As a result, a previously established hypotonic buffer is optimized to permeabilize cells, permitting synchronized dye of DNA with PI (MACS Miltenyi Biotec) and mitotic marker, phospho Histone H3 (pH3), with pH3 specific antibody. This method allows for a quick (less than 20 minutes) and thorough analysis of the cell cycle database (phase of G1, S, G2, M) (45).

In the flow cytometry section, 100 µl of PBS without Ca^{+2} Mg^{+2} (Gibco) samples prepared in the previous section were measured. Furthermore, PI staining was used for cell cycle analysis. In that experiment, a large population was used, not a single MSC cell. PI staining was performed for meiosis. 100µlcells in a sterile tube with dye were incubated for 10 min. Then it was viewed in the flow cytometry. When evaluating the ploidy of a new sample, it was useful to use a sample with known numerical value, like Chicken Erythrocyte Nuclei, in a flow cytometer.

Flow Cytometry Analysis was conducted by Acıbadem LabMed Laboratories.

3.6 Preimplantation Genetic Testing

For PGT-A (aneuploidy), and PGT-M (monogenetic/single-gene defects), advances in next-generation sequencing (NGS) techniques have enabled simple, modular, and creative sample-to-answer workflow solutions. PGT by NGS is now available to any lab, despite expertise, thanks to integrated analysis tools.

Infertility affects an approximate 48.5 million couples worldwide, driving up the use of infertility treatment technologies around the world. As a consequence, PGT during IVF methods has become commonplace in research. These cutting-edge solutions provide quick results from valuable samples without the challenges that come with traditional genetic analysis tools (46).

Preimplantation Genetic Testing was conducted by Acıbadem LabGen Laboratories.

4 RESULTS

4.1 Mitochondria Transfer into MSC

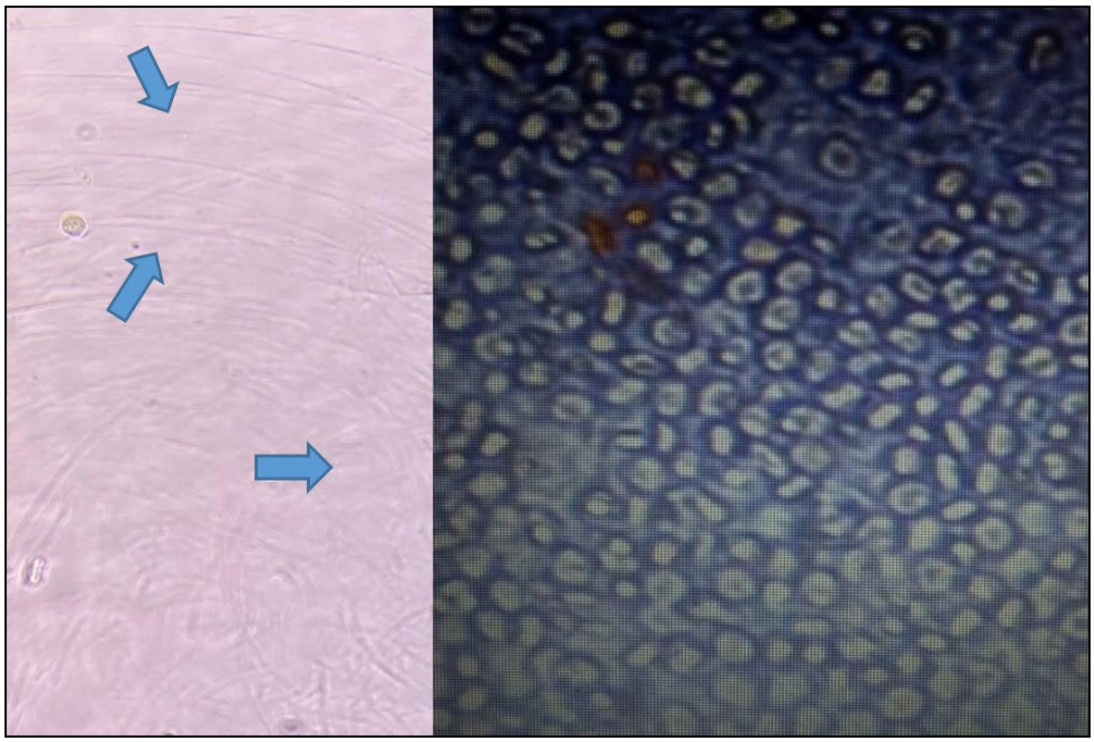

Figure 4. MSC and Mitochondria in the same environment on the first day (left). Mitochondria passed into the MSC after 48 hours (right).

4.2 Flow Cytometry for Cell Cycle Analyzation

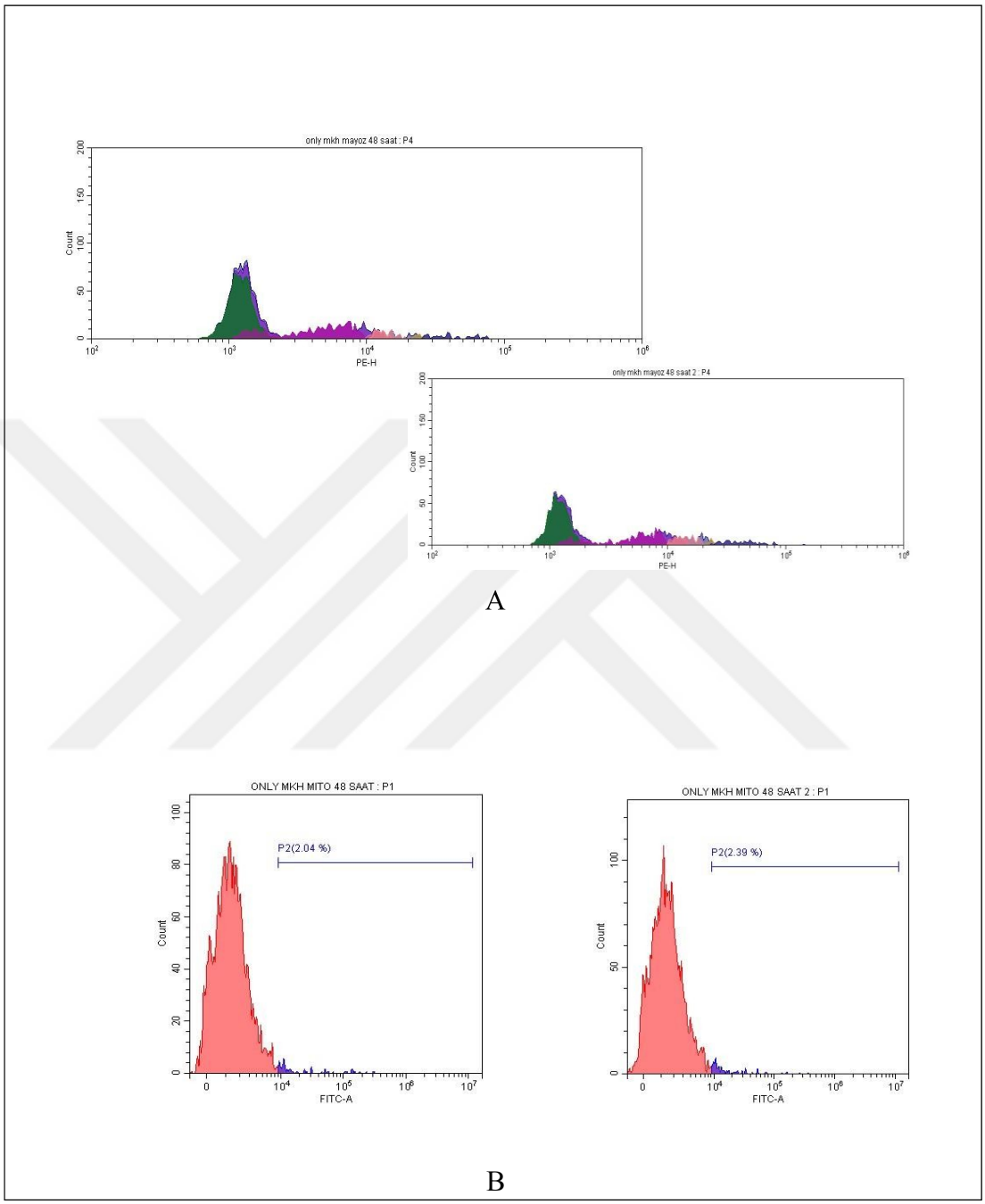

Figure 5. Flow cytometry DNA content distribution in a cell cycle analysis assay. The figures represent the control group containing only MSC at 48 hours. A) DNA Ploidy Assay. Diploid and aneuploidy are examined. B) Cell Count on flow cytometry. The amount of MSC entering the mitochondria is measured (MitoTracker determination analysis).

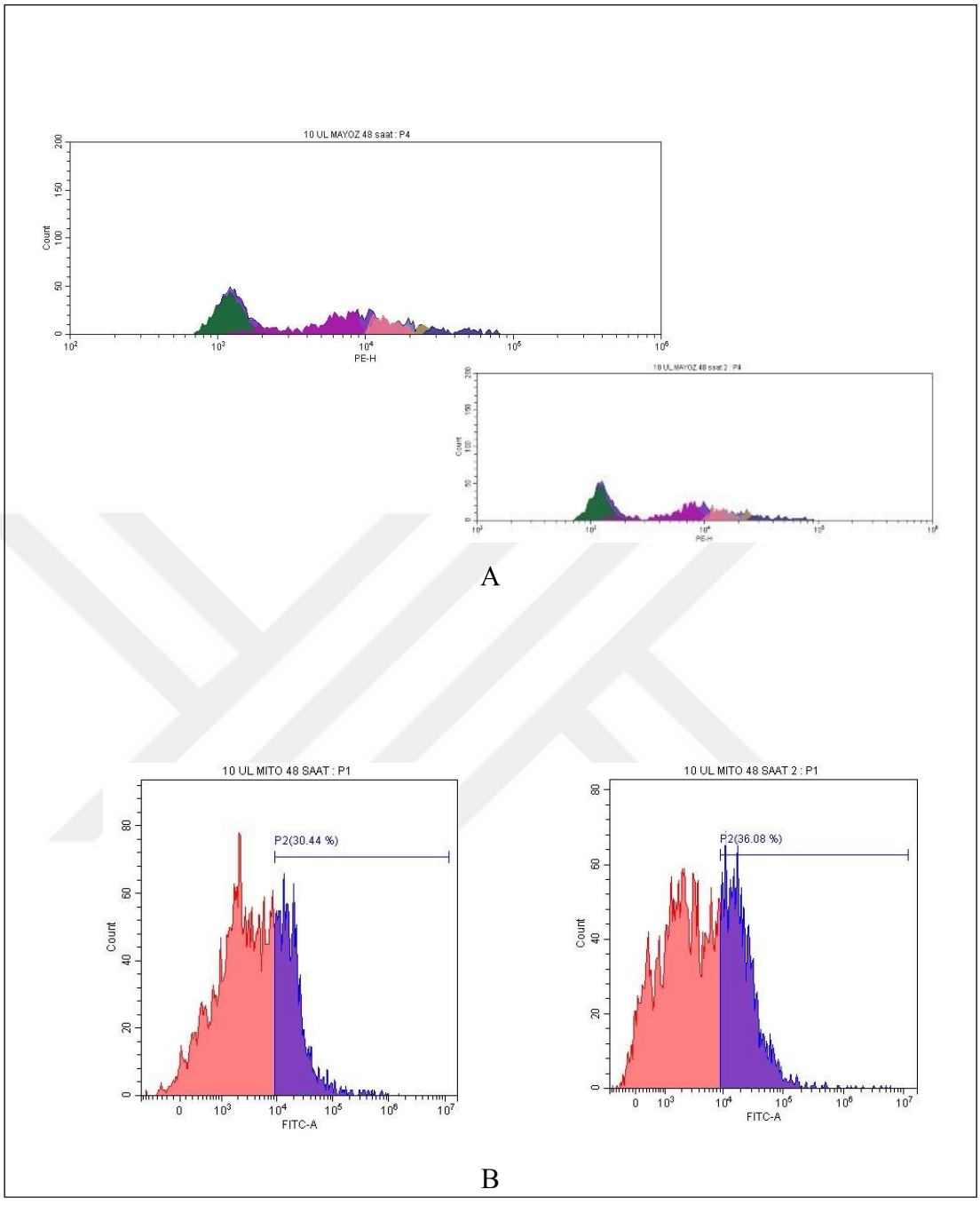

Figure 6. Flow cytometry DNA content distribution in a cell cycle analysis assay. The figures represent a sample group containing 10 µl Mitochondria/MSC at 48 hours. A) DNA Ploidy Assay. Diploid and aneuploidy are examined. B) Cell Count on flow cytometry. The amount of MSC entering the mitochondria is measured (MitoTracker determination analysis).

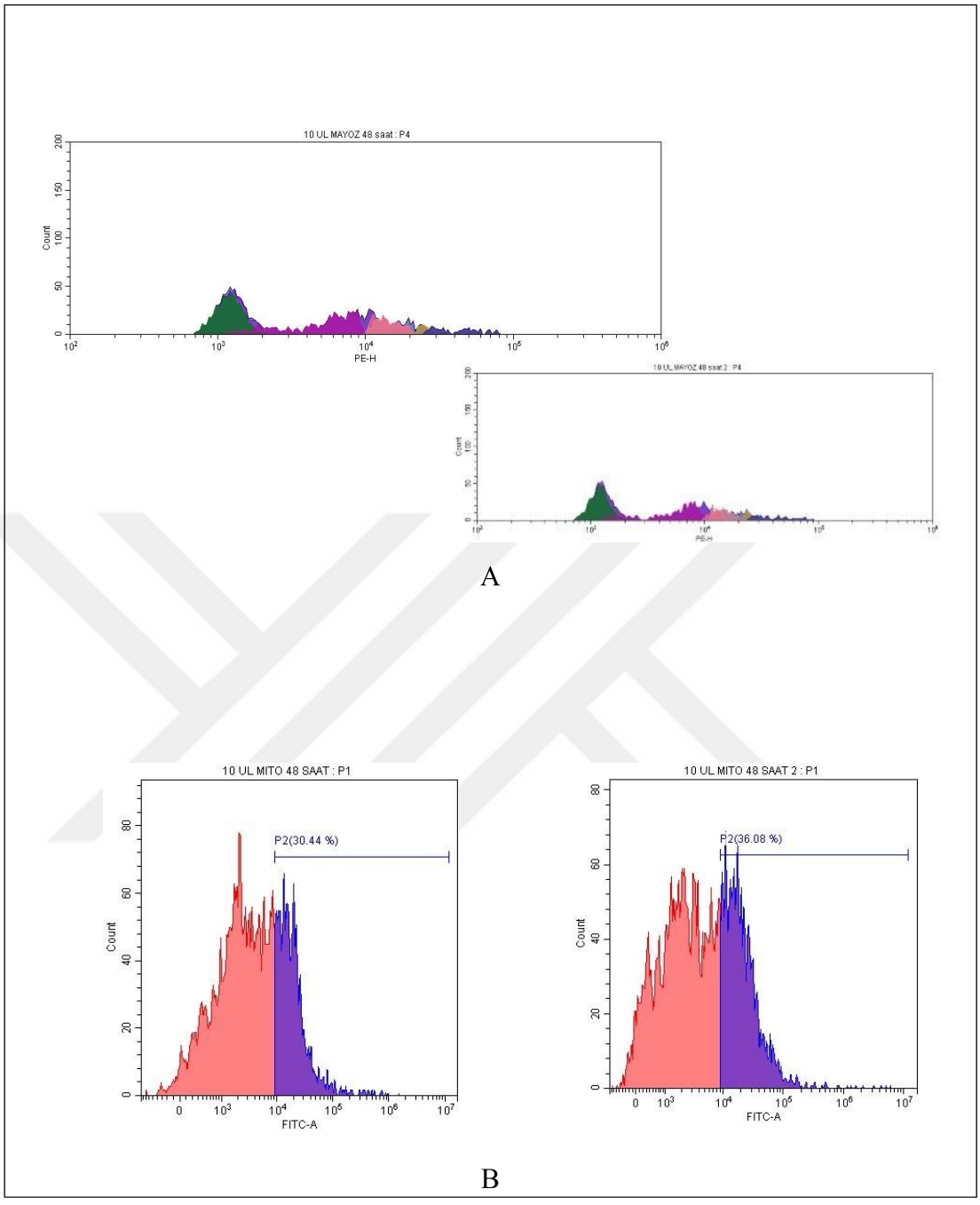

Figure 7. Flow cytometry DNA content distribution in a cell cycle analysis assay. The figures represent a sample group containing 20 µl Mitochondria/MSC at 48 hours. A) DNA Ploidy Assay. Diploid and aneuploidy are examined. B) Cell Count on flow cytometry. The amount of MSC entering the mitochondria is measured (MitoTracker determination analysis).

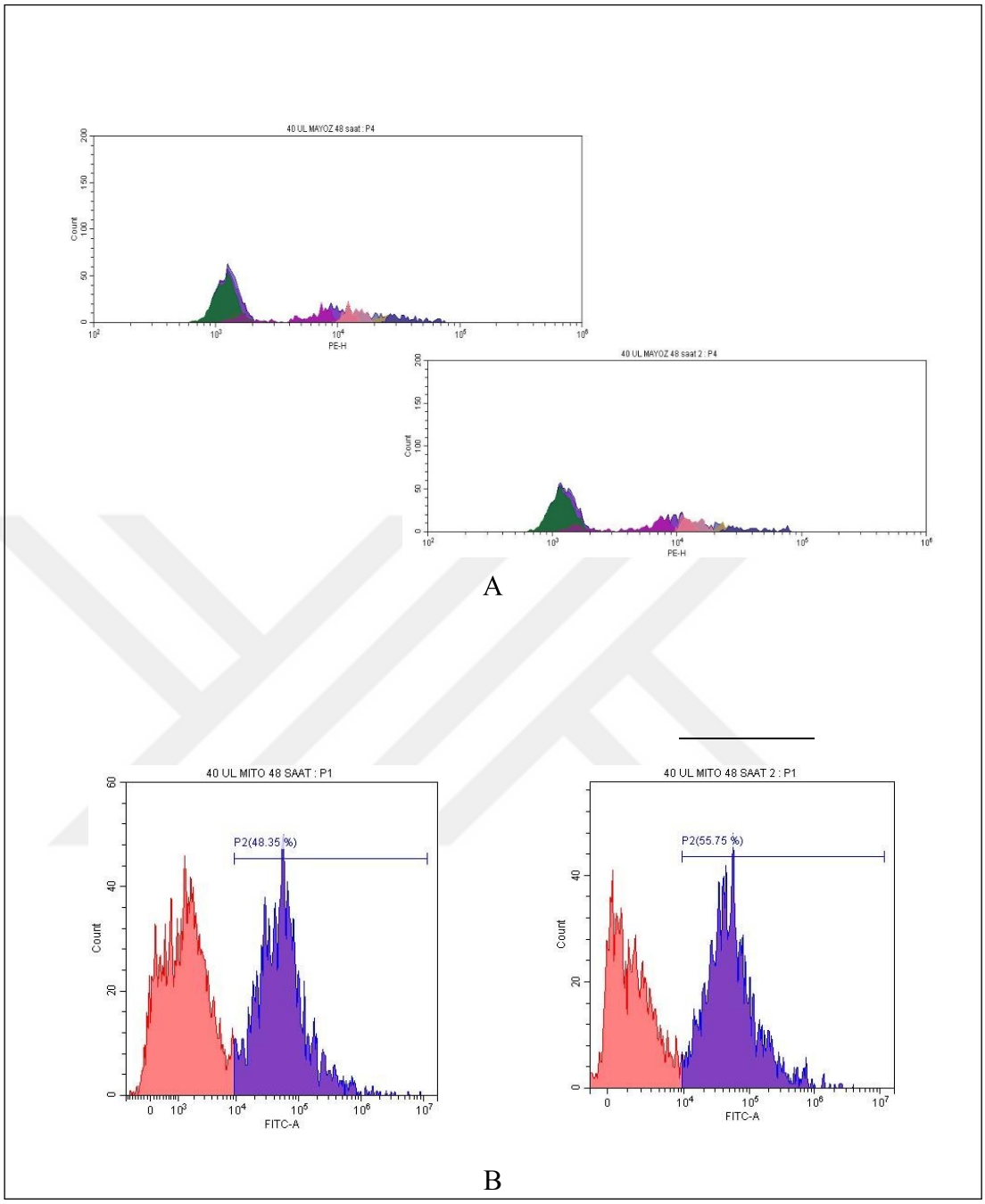

Figure 8. Flow cytometry DNA content distribution in a cell cycle analysis assay. The figures represent a sample group containing 40 µl Mitochondria/MSC at 48 hours. A) DNA Ploidy Assay. Diploid and aneuploidy are examined. B) Cell Count on flow cytometry. The amount of MSC entering the mitochondria is measured (MitoTracker determination analysis).

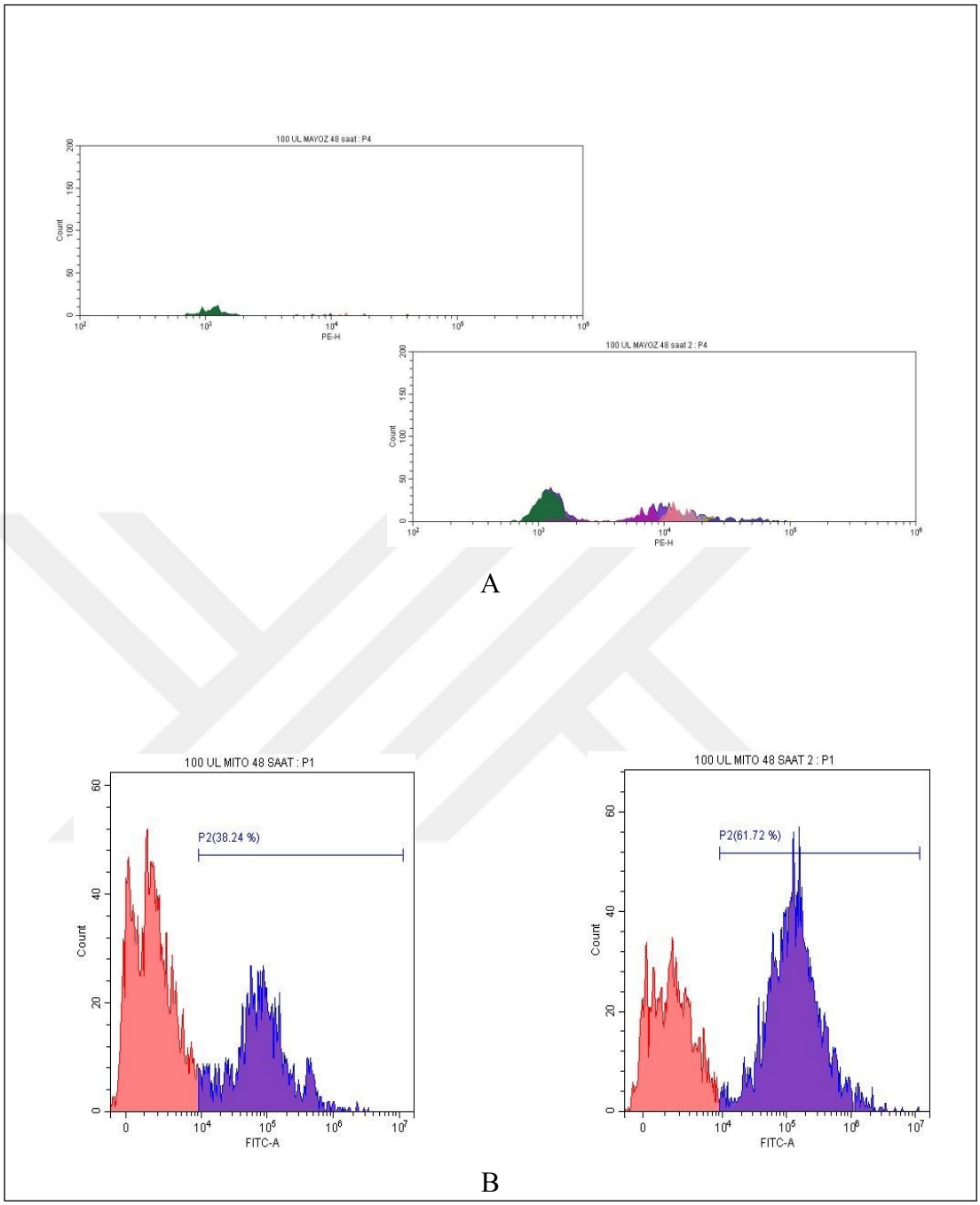

Figure 9. Flow cytometry DNA content distribution in a cell cycle analysis assay. The figures represent a sample group containing 100 µl Mitochondria/MSC at 48 hours. A) DNA Ploidy Assay. Diploid and aneuploidy are examined. B) Cell Count on flow cytometry. The amount of MSC entering the mitochondria is measured (MitoTracker determination analysis).

4.3 Single Cell Experiment

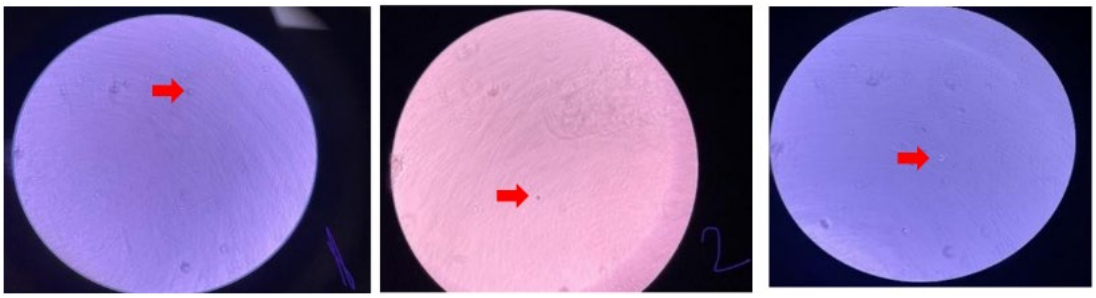

Figure 10. The samples shown in this figure were examined under the light microscope with a magnification of 10x. In this figure, single male MSC are shown.

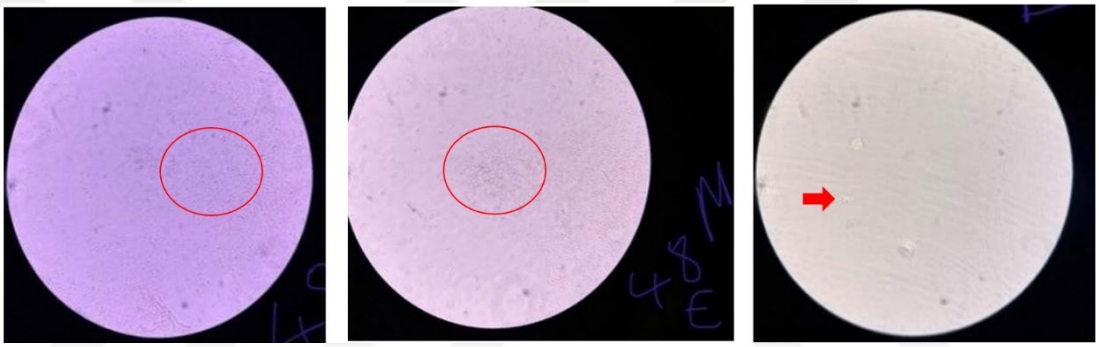

Figure 11. The samples shown in this figure were examined under the light microscope with a magnification of 10x. The left and middle pictures show the male MSC and mitochondria associations at 48 hours. The picture on the right represents control group containing only male MSC.

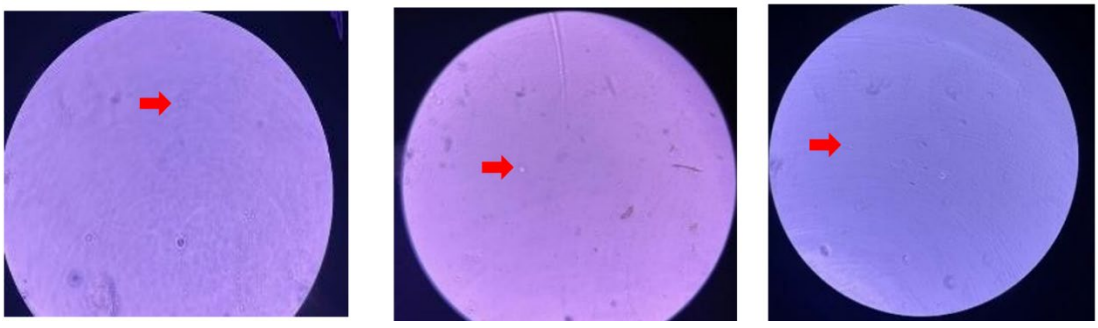

Figure 12. The samples shown in this figure were examined under the light microscope with a magnification of 10x. In this figure, single female MSC are shown.

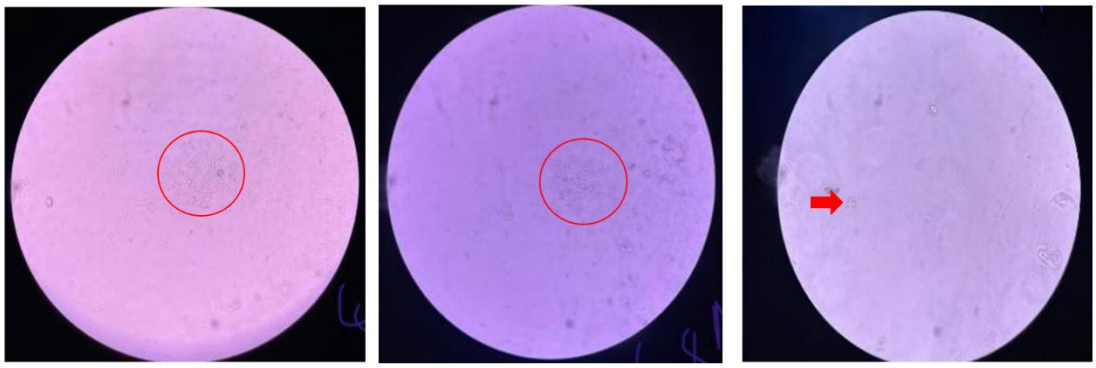

Figure 13. The samples shown in this figure were examined under the light microscope with a magnification of 10x. In this figure, the left and middle pictures show the female MSC and mitochondria associations at 48 hours. The picture on the right represents control group containing only female MSC.

4.4 Preimplantation genetic testing (PGT)

Table 1. Preimplantation genetic testing (PGT) report. The table represents chromosomal abnormality of control group containing 'only male MSC' at 48 hours.

EMBRIYO NO	BİYOPSİ TİPİ	KROMOZOM TARAMA SONUCU	SONUÇ
5224573535	Trofektoderm	NORMAL	-

EMBRIYO NO	DETAYLI SONUÇ TABLOSU																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	XY
5224573535	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N: NORMAL
G: GAIN(Kromozom sayısında artış)
L: LOSS (Kromozom sayısında azalma)
X: PARSİYEL ANÖPLOİDİ

Table 2. Preimplantation genetic testing (PGT) report. The table represents chromosomal abnormality of 'male MSC/Mitochondria' at 48 hours.

EMBRIYO NO	BİYOPSİ TİPİ	KROMOZOM TARAMA SONUCU	SONUÇ
5224573536	Trofektoderm	Mozaik 45 X0 (%25) / 46 XY (%75)	-

EMBRIYO NO	DETAYLI SONUÇ TABLOSU																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	XY
5224573536	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	L

N: NORMAL
G: GAIN(Kromozom sayısında artış)
L: LOSS (Kromozom sayısında azalma)
X: PARSİYEL ANÖPLOİDİ

Table 3. Preimplantation genetic testing (PGT) report. The table represents chromosomal abnormality of ‘male MSC and mitochondria’ associations at 48 hours.

EMBRIYO NO	BİYOPSİ TİPİ	KROMOZOM TARAMA SONUCU	SONUÇ
5224573537	Trofektoderm	Mozaik 45 X0 (%25) / 46 XY (%75)	-

EMBRIYO NO	DETAYLI SONUÇ TABLOSU																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	XY
522457353 7	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	L

N: NORMAL
G: GAIN(Kromozom sayısında artış)
L: LOSS (Kromozom sayısında azalma)
X: PARSİYEL ANÖPLOİDİ

Table 4. Preimplantation genetic testing (PGT) report. The table represents chromosomal abnormality of ‘female MSC and mitochondria’ associations at 48 hours.

EMBRIYO NO	BİYOPSİ TİPİ	KROMOZOM TARAMA SONUCU	SONUÇ
5224829301	Trofektoderm	Mozaik monozomi 13 (%30), Mozaik trizomi 19 (%30), Mozaik monozomi 22 (%30)	-

EMBRIYO NO	DETAYLI SONUÇ TABLOSU																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	XY
522482930 1	N	N	N	N	N	N	N	N	N	N	N	N	L	N	N	N	N	N	G	N	N	L	N

N: NORMAL
G: GAIN(Kromozom sayısında artış)
L: LOSS (Kromozom sayısında azalma)
X: PARSİYEL ANÖPLOİDİ

Table 5. Preimplantation genetic testing (PGT) report. The table represents chromosomal abnormality of ‘female MSC and mitochondria’ associations at 48 hours.

EMBRIYO NO	BİYOPSİ TİPİ	KROMOZOM TARAMA SONUCU	SONUÇ
5224829302	Trofektoderm	Mozaik trizomi 6 (%60), Mozaik trizomi 8 (%25), Mozaik trizomi 10 (%25)	-

EMBRIYO NO	DETAYLI SONUÇ TABLOSU																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	XY
522482930 2	N	N	N	N	N	G	N	G	N	G	N	N	N	N	N	N	N	N	N	N	N	N	N

N: NORMAL
G: GAIN(Kromozom sayısında artış)
L: LOSS (Kromozom sayısında azalma)
X: PARSİYEL ANÖPLOİDİ

Table 6. Preimplantation genetic testing (PGT) report. The table represents chromosomal abnormality of control group containing ‘only female MSC’ at 48 hours.

EMBRİYO NO	BİYOPSİ TİPİ	KROMOZOM TARAMA SONUCU	SONUÇ
5224829303	Trofektoderm	Yeterli veri yok	-

EMBRİYO NO	DETAYLI SONUÇ TABLOSU																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	XY
5224829303	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N: NORMAL
G: GAIN(Kromozom sayısında artış)
L: LOSS (Kromozom sayısında azalma)
X: PARSİYEL ANÖPLOİDİ

Table 7. Preimplantation genetic testing (PGT) report. The table represents chromosomal abnormality of ‘male MSC and mitochondria’ associations at 48 hours.

EMBRİYO NO	BİYOPSİ TİPİ	KROMOZOM TARAMA SONUCU	SONUÇ
5224829304	Trofektoderm	Mozaik trizomi 10 (%25)	-

EMBRİYO NO	DETAYLI SONUÇ TABLOSU																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	XY
5224829304	N	N	N	N	N	N	N	N	N	G	N	N	N	N	N	N	N	N	N	N	N	N	N

N: NORMAL
G: GAIN(Kromozom sayısında artış)
L: LOSS (Kromozom sayısında azalma)
X: PARSİYEL ANÖPLOİDİ

Table 8. Preimplantation genetic testing (PGT) report. The table represents chromosomal abnormality of ‘male MSC and mitochondria’ associations at 48 hours.

EMBRİYO NO	BİYOPSİ TİPİ	KROMOZOM TARAMA SONUCU	SONUÇ
5224829305	Trofektoderm	NORMAL	-

EMBRİYO NO	DETAYLI SONUÇ TABLOSU																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	XY
5224829305	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N: NORMAL
G: GAIN(Kromozom sayısında artış)
L: LOSS (Kromozom sayısında azalma)
X: PARSİYEL ANÖPLOİDİ

Table 9. Preimplantation genetic testing (PGT) report. The table represents chromosomal abnormality of control group containing ‘only male MSC’ at 48 hours.

EMBRİYO NO	BİYOPSİ TİPİ	KROMOZOM TARAMA SONUCU	SONUÇ
5224829306	Trofektoderm	NORMAL	-

EMBRİYO NO	DETAYLI SONUÇ TABLOSU																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	XY
5224829306	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N: NORMAL
G: GAIN(Kromozom sayısında artış)
L: LOSS (Kromozom sayısında azalma)
X: PARSİYEL ANÖPLOİDİ

4.5 Preimplantation Genetic Testing Result Comparison on Chart

Table 10. This table shows how many of the nine examples have gain and loss in total

	Female 48h	Male 48h	Total
loss	2	2	4
gain	4	1	5

Table 11. This table shows how many of the nine examples have gain and loss separately

	loss	gain
Experiment 1 & only MSC from male	-	-
Experiment 1 & male MSC / mitochondria	1	-
Experiment 1 & male MSC / mitochondria	1	-
Experiment 2 & female MSC / mitochondria	2	1
Experiment 2 & female MSC / mitochondria	-	3
Experiment 2 & only MSC from female	-	-
Experiment 2 & male MSC / mitochondria	-	1
Experiment 2 & male MSC / mitochondria	-	-
Experiment 2 & only MSC from male	-	-

Table 12. This table shows loss and gain in different kinds of chromosomals

Chromosomal numbers (type)	loss	gain
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	1
7	-	-
8	-	1
9	-	-
10	-	2
11	-	-
12	-	-
13	1	-
14	-	-
15	-	-
16	-	-
17	-	-
18	-	-
19	-	1
20	-	-
21	-	-
22	1	-
XY	2	-

4.6 Flow Analytics on Graphics

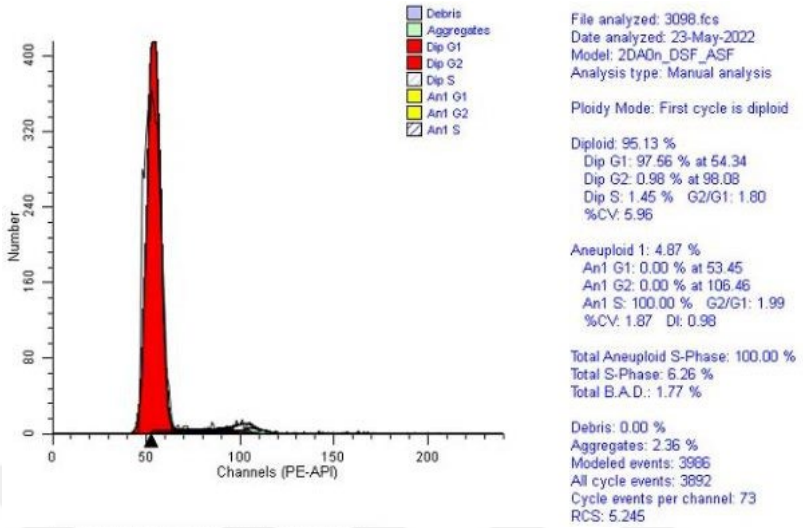

Figure 14. Flow cytometry DNA content evaluation in a cell cycle analysis assay. The figure represents diploid and aneuploidy of control group containing only MSC at 48 hours. The red peak indicates diploid G1.

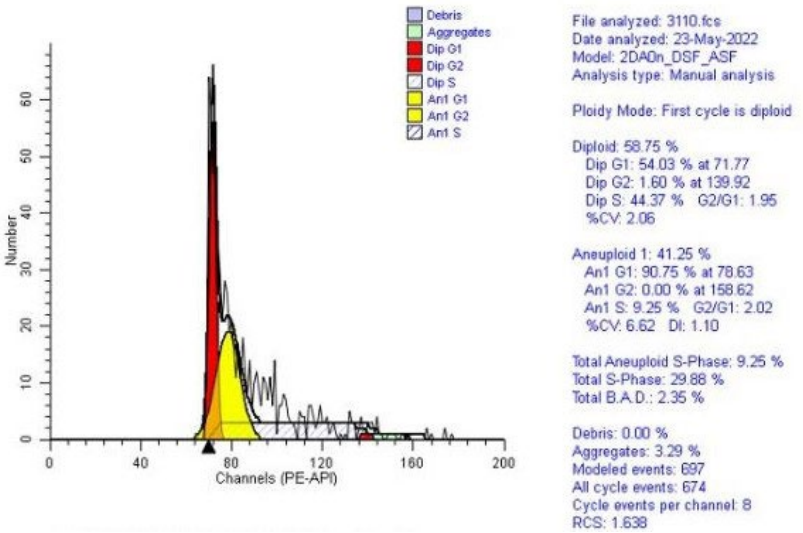

Figure 15. Flow cytometry DNA content evaluation in a cell cycle analysis assay. The figure represents diploid and aneuploidy of MSC and 50 µl mitochondria associations at 48 hours. The red peak indicates diploid G1. The yellow peak indicates aneuploid G1.

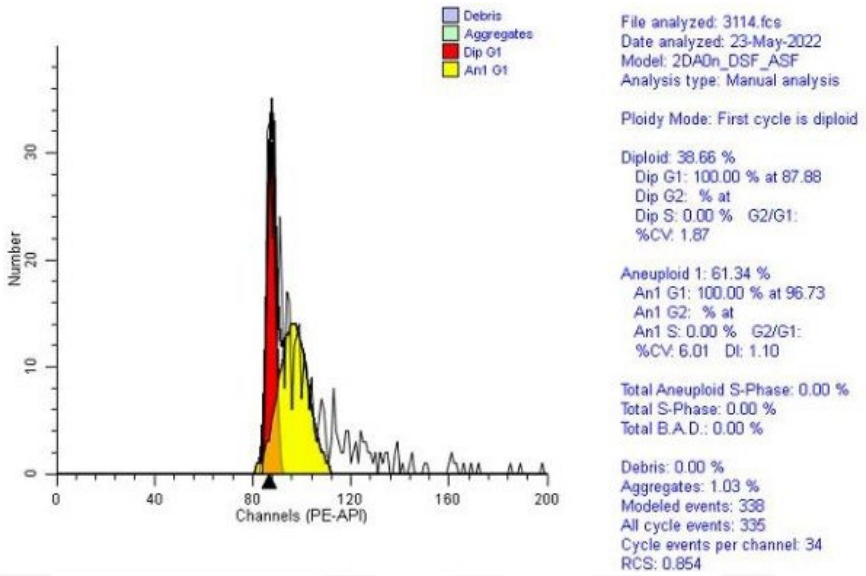

Figure 16. Flow cytometry DNA content evaluation in a cell cycle analysis assay. The figure represents diploid and aneuploidy of MSC and 100 µl mitochondria associations at 48 hours. The red peak indicates diploid G1. The yellow peak indicates aneuploid G1.

5 DISCUSSION

Mitochondria line up around the membrane, allowing cells to divide into two. Mitochondria can envelop DNA particles and take them out. If the amount of DNA in the cell does not increase, it divides the membrane itself into parts and takes the DNA inside, causing loss. Mitochondria transplantation has been performed in infertile patients. It is suggested that they can also provide maturation. This includes expelling the excess DNA inside the egg. The reason is that GV oocytes contain a large amount of DNA. When mitochondria remove a large amounts of DNA, the GV oocyte matures. So, normally there should be 23 chromosomes, but there are still 23+x chromosomes in it. That's why the GV oocyte is not mature. Egg unable to remove germinal vesicle, GV is also arrested. But the moment the germinal vesicle sheds, most of it seems to have matured. There is a human study in which GV oocytes are converted to MII oocytes (47). But the underlying cause has not been investigated. It was tried to understand how this happened in MSC. Due to ethical problems, it could not be worked on oocytes, it was worked in MSC. In this study, it was seen that mitochondria cause changes in the number of chromosomes. In stem cells other than normal germ cells, this can be gain or loss. But it is not known what determines the gain or loss. Looking at the general population, more earnings are seen. It causes few losses. But it obviously results in maturation in cells that have entered the meiotic process. Loss is more prominent in this case. But this could not be studied due to ethical problems.

When it is looked at Figure 4 in the Result section, it is confirmed that mitochondria entered into MSC. In the B sections of figures 6 to 9, different doses of mitochondria were added to multiple MSC cells and MitoTracker determination analysis was analyzed. In B section of figure 5, there was a control group ‘only MSC’. It was observed that as the number of mitochondria increased (10 µl, 20 µl, 40 µl, 100 µl), cell proliferation in the medium increased in direct proportion. For DNA ploidy assay in A section of figures 5 to 9, MSC and chicken erythrocytes were read together. Chicken erythrocyte provides a comparison opportunity because it contains many ploidies. The G1 phase decreases and the cells there pass to the S phase and then to the

G2/M phase. In other words, hypodiploidic peaks can be seen and it can be said that mitochondria trigger cell proliferation. There is a blockade of mitosis in the S phase.

Since it does not receive a mitosis signal, its DNA remains stable up to a certain point and cannot progress. It can be said that mitochondria cause hypodiploidy as the reason for this.

Microscopic analyses of only MSC and mitochondria transfer are represented in figures 10 to 13. Some of the wells marked as single cells remained in the control group (only MSC from male and female separately), while 100 µl of mitochondria was added to some of them. As a result of waiting for 48 hours, it was observed that the number of cells did not change much in the wells containing only female MSCs or male MSCs, classical spindle MSC colonies were observed in those with more than one cell, and a visible proliferation was observed in the wells with mitochondria. Round small cells were present.

After observing the cells in each well of the 96 well plates under the microscope, they were sent for PGT testing to see if there were any abnormalities in their chromosomes. And it saw seen that while all 23 chromosomes were "Normal" in the cell group given only MSC, loss and increase were observed in chromosomes in the female and male MSC group, to which is added mitochondria (table 1 to 9). In tables 1 to 3, PGT analyzes showed that these cells lost one of their sex chromosomes. While pure MSC cells had Normal Karyotype, there was a 25% loss of Y chromosome in both samples that underwent MT. In tables 4 to 9, it is seen gain, loss, or gain plus loss in several different chromosomes in a single sample.

When the PGT test is put on the table and looked at it, it is seen that loss and gain amounts were not that different. But the amount of gain was ahead. In other words, this makes you think that mitochondria stimulate chromosome synthesis. Since the cells with loss are few, they cannot be seen clearly. Playing with cellular mitochondria content can stimulate DNA production or loss, resulting in mitotic and even meiotic

processes. But in order to be able to speak more precisely, research on this subject should be continued (see Table 10,11,12).

Normally, the cells are in the G1 phase, the peak is high there. But if there is proliferation, peaks are seen in the G2 or S phase as evidence of proliferation as mentioned in the introduction section earlier. In our study, while there was no change in DNA cell cycle analysis in only MSCs, it is looked that there was an increase in DNA production in the group in which mitochondria are put. Supports the PGT results, that is, a hyperdiploidic peak that supports the gain formation.

Populations are present to the right and left of the normal peak. This suggests hypo- and hyperdiploidy. Depending on the number of mitochondria at different doses, an increase in hyperdiploidy is observed. The graph has shifted to the right. While 'only MSC' was arrested in the G1 stage, the groups given mitochondria were able to pass to the S and G2 stages. Looking at the percentages in figures 14 to 16, it can be seen that the rate of aneuploidy has increased.

No other mechanism determining this has been analyzed, but what is clear is that the addition of mitochondria results in a change in chromosome number. When looking at the general population (examination of 10 million cells), it can be said that it causes gain, but a small amount of hypodiploidy (loss) can also be seen. This shows that it can push a group of cells within it to meiotic processes. If mitochondria are repeatedly transferred to the cell, the lost ones can go to meiosis. However, there is not enough data on this subject yet. This study should be done on oocytes.

6 CONCLUSION

As a result of the study, the addition of mitochondria can stimulate the gain or loss of DNA in the mitochondrial process, which may determine the mitochondrial content in both mitosis and meiosis. One may speculate that the underlying mechanism is that the mitochondria can attach to the membrane and break up the nucleus. If this breakdown of mitochondria occurs before the cell DNA synthesis takes place, it results in loss which means hypodiploidy. If DNA synthesis is taking place, hyperdiploidic fragments are formed.

In conclusion, it can be said that hypothesis of our study depends on the idea that transferring isolated mitochondria from mononuclear blood cells to mesenchymal stem cells has considerable effects on cellular content and DNA content.

If studies on oocytes are carried out by obtaining ethical permissions, the results can be more clear and more forward-looking.

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