NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF MEDICAL BIOLOGY AND GENETICS

INVESTIGATION OF BAK, BAX AND MAD2L1 GENE EXPRESSION IN HUMAN ANEUPLOID BLASTOCYSTS

M.Sc THESIS

MIHAD SAMI SALAELDIN AHMED

Nicosia

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M.Sc. THESIS

Mihad Sami Salaheldin AHMED

Supervisor

Prof. Pinar TULAY

Nicosia
June, 2022

Approval

We certify that we have read the thesis submitted by Mihad Ahmed titled **"Investigation Of *BAK*, *BAX* and *MAD2L1* Gene Expression In Human Aneuploid Blastocysts"** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Educational Sciences.

Examining Committee	Name-Surname	Signature
Head of the Committee:	Prof. Rasime Kalkan	.....
Committee Member*:	Assist. Prof. Dr. Özel Yürüker	.....
Supervisor:	Prof. Pınar Tulay	.....

Approved by the Head of the Department

27/6/2022

Prof. Pınar Tulay
Head of Department

Approved by the Institute of Graduate Studies

27/6/2022

Prof. Kemal Hüsnü Can Başer
Head of the Institute

Declaration

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Mihad Sami Salaheldin Ahmed

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Abstract

Investigation Of *BAK, BAX* and *MAD2L1* Gene Expression In Human Aneuploid Blastocysts

Ahmed, Mihad

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Background:

Early in embryonic development, a blastocyst is a hollow structure with a thin wall that holds the inner cell mass, from which the embryo develops. The outer layer of cells produces the placenta and other tissues necessary for fetal development in the uterus, whereas the inner cell mass produces the tissues of the body.

Aneuploidy is the presence of one extra or missing chromosome(s), resulting in an imbalanced chromosomal complement, or any abnormality in chromosome number, instead of 46.

Apoptosis is a form of cell death program that eliminates unsuitable cells that disease-causing, immune-system-maintaining, aging and development. It is initiated by a controlled signal cascade that results in caspase activation and initiates apoptosis. Apoptosis mediates the removal of certain cells in the second half of development, primarily during the morula and blastocyst stages, and has a physiological involvement in cell proliferation and death regulation, as well as the transmission of genomically changed defective cells. Apoptosis is a normal feature of human preimplantation development, even *in vivo*, and it can play an active role in embryo development by removing genetically abnormal cells.

The processes of apoptosis are extremely sophisticated and complicated, including a series of energy-dependent chemical reactions. Cell death mechanisms activated caspases have extrinsic and intrinsic pathways. The intrinsic path of signalling of the BCL2 family protein regulates the apoptotic process.

BAX and BAK are two nuclear-encoded proteins that are pro-apoptotic members of the BCL2 family and are found in MOM (mitochondrial outer membrane) where they regulate the intrinsic apoptotic pathway.

The *MAD2L2* gene, which is located on chromosome 1, is a component of the mitotic SAC (spindle assembly checkpoint), which prevents anaphase from occurring until all chromosomes are properly aligned at the metaphase plate.

This study aimed to determine the relationship between expression of genes involved in apoptosis in human aneuploid and euploid blastocysts. Usually, genetic anomalies are corrected by repair mechanisms, and if they cannot be corrected the cell would be removed by apoptosis which does not happen in the aneuploid blastocyst. Therefore, the investigation to know if there is something different in aneuploid embryos compared to euploid embryos.

Materials and Methods:

A total of 47 embryos belonging to 21 different patients, where 18 showed no genetic anomalies (control) and 29 were diagnosed as aneuploid, were used for this study. The human surplus embryos were obtained from patients attending the British IVF Centre. RNA was extracted and followed by an RNA purity measurement using a Nano-drop spectrophotometer. cDNA was synthesized using the extracted RNA. Gene expression level in each sample was evaluated using RT-PCR. Furthermore, a statistical analysis was performed on the RT-PCR data to evaluate the expression of the desired gene using the student's T-tests analysis.

Results:

There is a significant difference between the sample (aneuploid) and the control group in *BAX* gene expression. According to that, there is a relationship between the *BAX* expression in aneuploid and euploid blastocysts. The statistical results for *BAK* and *MAD2L1* genes by student's T-test statistical analysis indicated no significant difference between aneuploidy and the control group.

Conclusion:

Our study aimed to investigate the expression of the *BAK*, *BAX*, and *MAD2L1* genes in the blastocyst embryos and to evaluate the relationship between apoptosis in aneuploid and euploid embryos.

The findings of this study revealed that there was a relationship between apoptosis and *BAX* gene in aneuploid and euploid embryos. On the other hand, there was no statistical difference in the expression levels of *BAK* and *MAD2L1*.

Keywords:

Blastocyst, Aneuploidy, Apoptosis, Gene Expression

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List of Abbreviations

ATP: Adenosine Triphosphate

FSH: Follicular Stimulating Hormone

LH: Luteinizing Hormone

GV: Germinal Vesicle

cAMP: cyclic Adenosine MonoPhosphate

DNA: Deoxyribonucleic Acid

FISH: Fluorescence *In Situ* Hybridization

PB: Polar Body

ICM: Inner Cell Mass

TE: Trophectoderm

IVF: *In Vitro* Fertilization

PGT: Preimplantation Genetic Testing

NGS: Next Generation Sequencing

MOM: Mitochondrial Outer Membrane

IMS: Intermembrane Space

MOMP: Mitochondrial Outer Membrane Permeabilization

ER: Endoplasmic Reticulum

***BCL2*:** B Cell Lymphoma-2

***BAK*:** *BCL2* Antagonist Killer

***BAX*:** *BCL2* Associated X

SAC: Spindle Assembly Checkpoint

***MAD2L1*:** Mitotic Arrest Deficient-Like1

RT-PCR: Real time-Polymerase Chain Reaction

AMA: Advanced Maternal Age

RML: Recurrent Pregnancy Loss

RIF: Recurrent Implantation Failure

k-MT: kinetochore-MicroTubule

CHAPTER I
Introduction

The process of generating gametes or germ cells is known as gametogenesis (Larose, et al., 2019). Technically, the formation of egg cells, or ova, is known as oogenesis and the formation of sperm cells, or spermatozoa, is known as spermatogenesis. The haploid cells are formed by meiosis and gametes are produced following gametogenesis (Vedantu, 2020).

1.1 Gametogenesis

Gametogenesis is the mechanism utilized by an organism to generate the gametes or germ cells within the testes in males and theovaries in females. It is a complex procedure that involves a wide range of metabolic and morphological changes. Gametes are formed by a special cell division, called meiosis (Libretexts, 2018). Meiosis is a process of cell division that transforms a diploid cell (2n) with two sets of chromosomes, into a haploid cell (n) with a single set of chromosomes (Ohkura, 2015). The reason for this is that the total number of chromosomes in a species does not change from one generation to the next (Ohkura, 2015). Meiosis is divided into two phases; meiosis I and II. Each meiotic division has the following stages; prophase, metaphase, anaphase, telophase, and cytokinesis (Ohkura, 2015). After a single replication cycle, chromosomal segregation occurs in two cycles. During the first separation (meiosis I), chromosomes replicate and homologous chromosomes are separated which is similar to mitotic cell division, and during the second division (meiosis II), there is no replication and the sister chromatids are separated (Ohkura, 2015). The haploid cells are the sex cells, the sperm in males, and the oocytes in females. These gametes are all haploid, but they are not genetically identical (Ohkura, 2015). Each gamete has a unique genetic material due to crossing-over (Ohkura, 2015). Germ cells vary from somatic cells in that they undergo a sequence of mitotic and meiotic divisions before differentiating into either sperm or egg. Somatic cells do not go through meiotic divisions. The mature gametes are called spermatogonia in males and oogonium in females, respectively. The gametes following fertilization, generate a diploid cell termed a zygote. It has half of the genetic materials of the parent's cells (Marks, 2021).

There are significant biological differences between male and female meiosis, particularly in terms of time.

Meiosis in females begins in utero, pauses during prophase I at approximately 20 weeks of gestation, and continues only when oocytes are picked from the pool for ovulation following the onset of puberty. Only one follicle gains primacy and becomes a mature follicle. Therefore, only one mature oocyte is formed at the end of meiosis (Marks, 2021). Meiosis II is only completed upon fertilization. In contrast, males undergo spermatogenesis, approximately 74-day-long process in which spermatogonial stem cells develop into spermatozoa. At the outset of adolescence, spermatogonia experience continuous mitotic proliferation in men, and these primordial germ cells develop, continue with meiosis without arrest, and ultimately create mature spermatozoa (Marks, 2021). The number of haploid gametes produced after meiosis is also a substantial difference between males and females. In males, four haploid gametes are produced at the end of meiotic division, whereas in females, only one haploid gamete and two polar bodies are produced (Marks, 2021). In addition, there is progressive and significant atresia of follicles until menopause in females, whereas there is a constant proliferation of spermatogonial stem cells following puberty in males, resulting in the production of millions of sperm cells per day throughout their lifetime. Given the inherent distinctions between male and female meiosis, it is perhaps not unexpected that female gametes have a higher incidence of chromosomal aneuploidy than male gametes (Ioannou, 2019).

1.1.1 Spermatogenesis

Spermatogenesis is the process by which spermatocytes (also known as sperm) grow and mature. The production of sperm occurs in the seminiferous tubules of male reproductive organ; testes. Gametogenesis in males does not start until puberty, which usually happens between the ages of 10 and 16 (Vedantu, 2020). Every single day, males produce approximately 200 million sperm cells as part of their normal reproductive process. Primordial germ cells (PGCs) are the cells that undergo mitotic divisions during fetal development. In males, the PGCs do not undergo meiotic divisions and this is initiated at puberty. To generate four haploid daughter cells, which are known as spermatids, spermatocytes undergo two meiotic divisions. The spermatids are subsequently differentiated into functional sperm cells called spermatozoa (Vedantu, 2020). There are three stages to this procedure; multiplication, growth, and maturation phases, respectively.

During multiplication phase of reproductive maturity, mitosis occurs several times, resulting in the division of identical primordial germ cells. The primary goal is to increase the number of spermatogonia. Type A and Type B spermatogonia are the two main classifications. Type A spermatogonia serve as a stem cell, which divides to produce more spermatogonia. Type B spermatogonia can be seen in male sex cell prototypes (Crumbie, 2021). During the growth phase, every type B spermatogonia evolve dynamically into a superior primary spermatocyte by feeding on nursing cells. The maturation phase involves the meiotic divisions producing haploid cells. Proteolytic enzymes necessary for acrosome reaction and fertilization are found in the acrosome region of the mature sperm head. The mature sperm cells are composed of the head, mid-piece, and tail (Crumbie, 2021). The DNA is located within the sperm head. The mitochondria, which are responsible for producing adenosine triphosphate (ATP) used for motility, are located within the mid-piece. The tail region of the sperm cell, the flagellum, is responsible for motility (Crumbie, 2021).

1.1.2 Oogenesis

Oogenesis is the process of gamete formation in females. It begins as a germ cell known as an oogonium, which is located within the ovaries (Joseph, 2021). Meiosis I is a process that takes place in the cell that will eventually become the primary oocyte during the development of the fetus. Similar to spermatogenesis, the process of meiotic division starts with PGCs, but it gets arrested at the prophase I stage. Primary oocytes are seen in abundance within the glandular tissue. They are surrounded by structures known as primary follicles, which are made up of epithelial cells that are flattened (Joseph, 2021). Primary oocytes remain in prophase I up to adolescence and puberty. A great number of follicles that grow in the ovary are prompted by hormones released by the anterior pituitary gland during puberty. Due to GnRH regulation of the anterior pituitary gland in producing FSH and LH, the follicle development is stimulated and the oocyte enters meiotic divisions. The four phases of oocyte maturation include pre-antral, antral, pre-ovulatory, and ovulatory stages (Joseph,2021). During the pre-antral stage, the primary oocyte is still in meiotic phase I of the cell cycle. The follicular cells' cuboidal epithelium expands and multiplies as the process continues. Granulosa cells, which are also referred to as granulocytes, are responsible for the secretion of glycoproteins. Proteins from the zone pellucida surround the primary oocyte found in the zona pellucida(Joseph,2021).

The cells of the surrounding connective tissue are also responsible for the formation of theca folliculi, a specialized layer of the surrounding cells that is receptive to LH and can release androgens when it is stimulated. The primary follicles continue on the maturation forming secondary follicles (Joseph, 2021). During the pre-ovulatory stage, which is supported by the LH stimulation, meiosis I is completed and meiosis II is initiated. The process by which fluid-filled spaces between granulosa cells fuse to generate the antrum results in the formation of a central cavity that is filled with fluid and is called the antrum. Only one of the follicles reaches the full maturity that takes place every month. At the end of meiosis I, one cell is smaller in size (first polar body) and it cannot continue with meiotic division. This is called the first polar body, which is formed when one of the two daughter cells receives much less cytoplasm than the other one. Another haploid cell, known as the secondary oocyte, is also produced during this process (Joseph, 2021).

1.1.3 Follicle development and fertilization

The autocrine and paracrine signalling pathways of the ovaries are responsible for regulating follicle formation during the preantral phase, also known as the gonadotropin-independent phase. Granulosa cells rapidly proliferate and secrete their contents during the antral or gonadotropin-dependent phase of follicle development. This phase of follicle development is also known as the antral phase. At this stage, the granulosa cells of the follicle are responsible for producing follicular fluid, which assists in the growth of the antrum of the follicle (Larose, et al., 2019). The gaps in between follicles are lined with stromal cells, while squamous theca cells wrap each growing follicle immediately outside the basal lamina. Cytokines and hormones work together to control the size of follicles as they develop (Larose, et al., 2019). TGF-family members have an impact, particularly in the earlier phases of follicular development, on the process (Larose, et al., 2019). Oocytes are responsible for the secretion of two TGF-superfamily members; BMP-15 and GDF-9. mRNA for GDF-9 can only be generated in oocytes that have reached maturity and moved on from the main follicular phase (Larose, et al., 2019). These two hormones, FSH and LH, are responsible for supporting the growth of follicles and the maturation of oocytes, respectively. At this point in the process, the granulosa cells that make up the primary follicle start to express FSH receptors. FSH is responsible for the stimulation of preantral to primary granulosa cell proliferation (Larose, et al., 2019).

FSH is also responsible for the promotion of estrogen production and follicle expansion. Early-stage follicles contain theca cells, which are responsible for steroidogenesis and have LH receptors, which are required for theca cell steroidogenesis (the production of progesterone and testosterone) (Larose, et al., 2019).

During the process of follicle formation, the oocyte and the granulosa cells can interact with one another through cellular extrusions that are referred to as the trans zona pellucida (Larose, et al., 2019). During the process of follicle formation, these extrusions are linked by gap junctions (connexin 43 and connexin 37), which enable the interchange of small molecules with one another. This stage of the development of the oocyte is distinguished by the presence of a big nucleus that is called the germinal vesicle (GV). Because both the oocyte and the cumulus cells produce cyclic adenosine monophosphate (cAMP), the oocyte continues to be in the prophase stage of the cell cycle (Larose, et al., 2019).

A follicle that has reached its full development and maturity is referred to as a Graafian follicle. Increased levels of LH cause an increase in the amount of collagenase activity in the body. This enzyme is responsible for the breakdown of collagen. As a direct result of this, the follicular wall starts to deteriorate. This, together with the muscle spasms that occur in the ovarian wall, is what ultimately results in the ovum being expelled from the ovary. Following this, the ovum will be moved into the fallopian tube using the fimbriae with the process called ovulation (Joseph, 2021). Meiosis II is completed only at fertilization. If the mature egg is fertilized, the cell completes meiosis and generates a fertilized egg with 46 chromosomes necessary to form a zygote, half of which is derived from the sperm and half from the oocyte, respectively. In the absence of fertilization within twenty-four hours following ovulation, the egg degenerates (Joseph, 2021).

1.2 Preimplantation Embryo Development

The stages between the zygote and the implantation are known as preimplantation embryo development. After fertilization, the zygote starts the cleavage divisions. With the cleavage divisions, the one-cell zygote divides the 2-cell stage by mitosis and the mitotic division continues forming 4-cell, 8-cell, etc stages of preimplantation embryo development (Larose, et al., 2019).

Each embryonic cell is called a blastomere (Larose, et al., 2019). Individual blastomeres are no longer visible at the 32-cell stage of preimplantation embryo development.

At approximately day-5 or day-6 post-fertilization when embryos reach the blastocyst stage, the blastomeres differentiate into two types, the ICM (inner cell mass) which forms the embryo, and the TE (trophectoderm) which forms the extraembryonic tissues that surround the embryo. The fluid-filled blastocoel, which is bordered by a thin layer of TE cells, forms during this time due to cavitation as well. Embryos generally implant in the uterus at the blastocyst stage, which occurs on days 5 or 6 of preimplantation development. Enzymes that penetrate and open up the zona pellucida are released at this point to allow it to penetrate and implant into the mother's endometrium (Larose, et al., 2019).

Any abnormalities during meiotic or mitotic divisions lead to the formation of abnormal gametes and embryos. Anaphase lag or nondisjunction could be reasons for the formation of gametes with an abnormal number of chromosomes. These abnormalities, especially nondisjunction in meiosis I or II, are observed more frequently in females of advanced maternal age. Any abnormal chromosome segregation in gametes leads to the formation of an abnormal embryo. The chromosome segregation errors in mitotic divisions post-fertilization lead to the formation of a mosaic embryo and fetus (Larose, et al., 2019).

1.3 Chromosomal abnormalities in gametes and embryos: Aneuploidy

When a cell contains one or two additional or fewer chromosomes than the normal pair, this condition is known as aneuploidy. In aneuploidy, "an" refers to an incorrect number of chromosomes, and "ploidy" refers to the number of chromosomes; thus, an abnormal number of chromosomes leads to this condition. The addition or deletion of a chromosome (resulting from the loss or gain of an entire chromosome) or aneuploidy is one of the leading causes of miscarriage and infertility. Up to thirty percent of human zygotes may be aneuploid. Approximately one-third of miscarriages are due to chromosomally flawed chromosomes (MacLennan, et al., 2015).

Genome integrity is based on the homogenous distribution of replicated chromosomes to daughter cells during cell division. There can be aneuploidy when there are errors in chromosomal segregation (MacLennan, et al., 2015).

Aneuploidy results from missegregation (nondisjunction) of chromosomes during meiosis. Nondisjunction of meiosis results in eggs or sperm that have additional or missing chromosomes (MacLennan, et al., 2015).

The unequal chromosomal division between daughter cells can occur during either paternal or maternal gamete formation. It results in daughter cells with abnormal number of chromosomes due to the failure of the chromosomes to segregate (MacLennan, et al., 2015).

Anaphase is a phase in which the cell's microtubules assist in the separation of homologous chromosomes in meiosis I or sister chromatids in meiosis II to opposite poles. Rather than segregating, two homologous chromosomes or sister chromatids migrate to the same pole of the cell in nondisjunction. Two aneuploid daughter cells will be formed, one with 45 chromosomes (2n-1) and the other with 47 chromosomes (2n+1). Nondisjunction in anaphase I of meiosis I occurs when the tetrads do not segregate after crossing-over. After meiosis I, two haploid daughter cells, one with n+1 and the other with n-1, are formed. In meiosis II, the failure of sister chromatids to segregate in anaphase II results in nondisjunction as well. Since meiosis I was completed normally, two of the four daughter cells have a normal complement of 23 chromosomes. The other two daughter cells will each have an abnormal number of copies, with one having n+1 and the other having n-1 (MacLennan, et al., 2015).

In human somatic cells, there are 23 chromosomal pairs, making the total number of chromosomes 46 as "diploid", which has two sets of chromosomes, while the "haploid" number is 23, which has just one set of chromosomes. An individual with monosomy (n-1) has 45 chromosomes rather than the usual 46 chromosomes. This type of aneuploidy is monosomy, characterized by the lack of one chromosome (MacLennan, et al., 2015).

A further type of aneuploidy known as trisomy (n+1) is defined by the presence of 47 chromosomes in the cell. Despite the extra chromosome, it is a possibility to have a viable pregnancy and live birth. The most common trisomies that lead to live birth and syndromes are Down Syndrome, Edwards Syndrome and Patau Syndrome. The most common live births resulting from chromosomal trisomies including sex chromosomes are Klinefelter syndrome and Triple X syndrome. Sex chromosome monosomies can also result in a live birth, such as Turner syndrome. It is the only kind of monosomy of chromosomes that is compatible with life (Gottlieb, et al., 2021).

1.4 *In vitro* Fertilization

In vitro fertilization (IVF) is a procedure to help conceive a child in infertile couples. IVF can be used as the primary treatment for infertility in females with advanced maternal age (+40) (Barad &D. H., 2004). IVF is also an option for those with specific health issues, such as polycystic ovary syndrome. IVF may be an option for females with damaged or obstructed fallopian tubes, females with ovulation problems, or females with endometriosis (Barad &D. H., 2004). Furthermore, patients with cancer can also opt for IVF for fertility preservation. Before the radiation or chemotherapy treatments, which could compromise the fertility in both males and females, the gametes can be obtained and stored. Couples with repeated IVF failures, implantation failures, or miscarriages can try preimplantation genetic testing (PGT) since one of the reasons for these failures could be due to the formation of aneuploid embryos (Shahine, et al., 2005).

Controlled ovarian stimulation is applied to the female partner to develop approximately 10-15 mature oocytes. The oocytes are collected upon hCG stimulation under sedation. The semen sample is obtained from the male partner. After the preparation of both gametes, the fertilization is performed in the embryology laboratory and the preimplantation embryo development occurs in the embryology laboratory. Mostly, the embryos are cultured until the blastocyst stage and the embryo transfer takes place on day 5 or day 6 post-fertilization. Morphological parameters are used to determine which blastocysts should be transferred, examining blastocyst development as well as the quality of TE and ICM. Even though morphology can give a good idea of embryo potential, it does not offer much about chromosomal status. Morphology was impacted primarily by embryos with several chromosome abnormalities, monosomies, or aneuploidy that affect the chromosomes. In mammals, female embryos grow at a different rate than male embryos, which may be due to gene dosage compensation resulting from X inactivation or the presence of growth factor genes on the Y chromosome (Fragouli & Wells, 2011). Unusual high rate of chromosomal aberrations has been discovered in studies of human cleavage-stage embryos, on the 3rd post-fertilization day. Furthermore, these embryos present high levels of mosaicism. Human embryos and gametes are frequently affected by aneuploidy (Fragouli & Wells, 2011).

Especially oocytes, and their effects on early development have long been described. For instance, it has been found that chromosomal abnormalities are responsible for roughly two-thirds of spontaneous miscarriages (Barad, 2004).

1.5 Preimplantation Genetic Testing (PGT)

PGT-A refers to preimplantation genetic testing for aneuploidies. Several methods are being used to identify euploid embryos in IVF treatments. It is a standard method in embryo selection based on euploidy (Simopoulou, et al., 2021). PGT approaches include testing embryos for hereditary diseases and screening for chromosomal abnormalities. It is mostly advised for a subset of patients who have RIF (recurrent implantation failure), AMA (advanced maternal age), RM (recurrent pregnancy loss), elective single ET or severe male infertility. Embryo biopsy, diagnosis, and selective embryo transfer are all involved in the PGT process, which aims to limit the risk of genetically faulty embryos being implanted. PGT-A is intended to reduce miscarriage and live birth of trisomic children, as well as to increase IVF success. FISH (fluorescence *in situ* hybridization), PCR (quantitative polymerase chain reaction), NGS (next-generation sequencing), aCGH (array comparative genomic hybridization), and karyomapping are/had been used in PGT-A (Griffin & Ogur, 2018). PGT-A is regarded as a potential approach for selecting chromosomally normal embryos for transfer to improve IVF treatment outcomes (Eggenhuizen et al., 2021). This is accomplished with a blastocyst trophectoderm biopsy of 5–10 cells, followed by an extensive molecular cytogenetic examination of the biopsied cells. The TE cells are applied to determine the remaining embryo's chromosomal arrangement (Eggenhuizen, et al., 2021).

1.5.1 Next Generation Sequencing (NGS)

Next generation sequencing is the newest method for PGT-A. Following genome amplification, a bar-coding method is carried out in which various samples are labelled with distinct sequences. This technology allows for the sequencing of 96 biopsies (depending on the sequencing platform) in a single run, resulting in a lower cost per sequenced embryo. Once all sequences are compared to a reference human genome, the software is used to identify substantial deletion and duplication as well as copy number differences (Greco, et al., 2020). Both chromosomal and monogenic abnormalities can be detected at the same time using NGS and karyomapping. Women using NGS had considerably higher rates of implantation and sustained pregnancy live birth (Greco, et al., 2020).

1.5.2 Fluorescence *in situ* hybridization (FISH)

The detection of chromosomal abnormalities in terms of both number and structure is possible with molecular cytogenetics using a technique called FISH. Genomic abnormalities polyploidy and aneuploidy such as deletions, inversions, duplications, or translocations can be screened for and diagnosed using fluorescence-labeled probes that bind to the homologous areas on the target genome. Repetitive sequence, site-specific, or whole chromosome painting DNA probes can be used to classify FISH techniques, which can be used to identify the nuclear position of genomic regions, genes, and chromosomes (Shete, et al., 2014). It can be used to detect chromosomal imbalance caused by Robertsonian translocations and other chromosome rearrangements, such as inversions, by measuring the number of copies of certain loci in the genome (Scriven & Ogilvie, 2010). Selecting female embryos in patients with X-linked diseases where mutation-specific screening is not available can also be done using FISH (Scriven & Ogilvie, 2010). The efficiency of assisted reproduction has also been improved by screening embryos for rare cases of chromosome aneuploidy (Scriven & Ogilvie, 2010).

1.6 Apoptosis

The evolution of multicellular organisms is directed not only by cell division and differentiation but also by the efficient removal of undesirable cells. This cell suicide or programmed cell death is referred to as apoptosis. It is a type of controlled cell that has a major impact on development, illness, and aging (Elmore, 2007). Apoptosis is initiated by a tightly regulated signal cascade that results in caspase activation. Apoptosis is characterized by cell contraction, membrane breakdown, chromosomal condensation, core fragmentation, DNA resorts, and phagosome trapping (Renehan, et al., 2001).

There is no evidence of programmed cell death in embryos that have been halted. Instead, defective *in vitro* embryos will undergo irreversible cell cycle cessation, which is consistent with cellular aging, which is characterized by high reactive oxygen species levels and active metabolism (Green & Llambi, 2015).

It has a physiological role in balancing cell growth and death, as well as the transmission of genomically altered, damaged cells, during the second stage of development, specifically during the morula and blastocyst stages. It serves both as a preventive and a pathogenic purpose (Pucci, et al.,2000).

Apoptosis is a natural part of human preimplantation development, even *in vivo*, it may be crucial in embryo development by eliminating genetically faulty cells (Giritharan, et al., 2007). The mechanisms of apoptosis are exceedingly deep and complex, involving a cascade of energy-dependent chemical events. Caspase (Cysteine Aspartate Protease) accelerates cell death by proteolyzing over 400 proteins. Both intrinsic and extrinsic apoptotic pathways involve activated caspases. The intrinsic signaling pathway that causes apoptosis is made up of several arrays of non-receiving stimuli that act directly on the target and generate an intracellular signal with a mitochondrial initiation event. The BCL2 family protein, which affects mitochondria's commitment to cell death, modifies it. Permeability of the MOM (mitochondrial outer membrane) is an important stage in the intrinsic cell death pathway and subsequent cells are required for cell death to occur (Levy, et al.,2001). Protein release from the mitochondrial membrane skeleton during permeation treatment increases caspase activation and death. Proteins from the BCL2 family regulate and alter these apoptotic dexmitochondrial processes. The BCL2 protein family affects the permeability of mitochondrial membranes, which can be pro- or anti-apoptotic. Approximately 25 genes from the *BCL2* family have been discovered. Anti-apoptotic proteins include *BCL2*, *BCLX*, *BCLXL*, *BCLXS*, *BCLW*, and *BAG*, whereas pro-apoptotic proteins include *BCL10*, *BAX*, *BAK*, *BAD*, *BIM*, *BIK*, and *BLK*. It is critical to determine whether the cell is going through apoptosis or has stopped the process. The major mode of action of the *BCL2* family protein, which regulates cytochrome c release from mitochondria, is thought to be altering the permeability of mitochondrial membranes (Levy, et al.,2001). Mitochondria play a major part in apoptosis. To begin, the large majority of protein-protein interactions of the *BCL2* family occur in mitochondria. Second, *BAK* and *BAX* generate toroidal holes consisting of lipids and proteins at the MOM, demonstrating that mitochondrial structure plays a part in pore formation. Third, the release of IMS proteins such as cytochrome c into the cytosol is a crucial step in the execution of apoptosis (Metcalfe, et al., 2004).

The former requires proteolytic maturation at the IMS by the protease PARL to achieve its pro-apoptotic action, while the latter is locked in the mitochondrial cristae, cytochrome c cannot be released into the cytosol without a mitochondrial rearrangement (Metcalfe, et al., 2004).

Without caspases, MOMP induced by *BAX/BAK* increases mitochondrial DNA release, activating the cGAS/STING DNA-sensing system leading to the generation of type I interferon and a pro-inflammatory form of cell death. As a result, mitochondrial architecture, protein composition, and lipid content are likely essential components of apoptosis regulation (Metcalfe, et al., 2004). When *BAX* and *BAK* (pro-apoptotic *BCL-2* family members) disrupt the outer mitochondrial membrane, the mitochondrial apoptosis cascade is initiated by OMM (outer mitochondrial membrane). After activated *BAX* and *BAK* produce proteolipid pores in the OMM, the inner membrane space proteins such as cytochrome c are released and produce MOMP which causes caspase activation (Metcalfe, et al., 2004).

1.7.1 *BCL2* Gene

The most important member of the BCL2 protein family is the *BCL2* gene, which has a major role of apoptosis regulation (Kvansakul, et al., 2014). The *BCL2* gene is located on chromosome 18 and it has been detected in many B-cell leukemias and lymphomas (Thomadaki & Scorilas, 2006). The *BCL2* protein family consists of 25 members, which are classified into 3 subfamilies based on the presence of conserved BH (*BCL2* homology) domains and functions in mitochondrial apoptosis (Hatok & Racay, 2016). Anti-apoptotic proteins containing four BH domains, indicated as 1–4, include *BCL2*, *BCL-XL*, *Mcl-1*, *BLF1*, *BCL-W*, and *BCL-B*. Every anti-apoptotic *BCL2* protein has a helical shape that is localized on a core hydrophobic helix, with the BH1-3 domains positioned to reveal a hydrophobic groove required for both pro-survival activity and binding to pro-apoptotic partners (Hatok & Racay, 2016). *BOK*, *BAX*, and *BAK* are pro-apoptotic proteins having BH 1–3 domains that is the second subclass of the *BCL2* family (Hatok & Racay, 2016). A third divergent class is BH3. All *BCL2* families are commonly found in the cytoplasm and the OMM. The *BCL2* core structural unit mediates the majority of *BCL2* family interactions. It is composed of numerous amphipathic alpha (αa) helices that form a hydrophobic groove that enables them to bind to mitochondrial membranes as well as binds pro-apoptotic BH3 domains (Westphal, et al., 2011).

Each pathway can be separated by whether they require BCL2 family proteins and which caspases are required for their execution. The intrinsic pathway, also known as mitochondrial pathway or the *BCL2*-regulated, is activated by a variety of developmental signals or cytotoxic insults such as DNA damage, viral infection and

growth-factor shortage, and is tightly regulated by the *BCL2* family of proteins (Youle & Strasser, 2008). *BCL2* phosphorylation is a dynamic process involving both kinases and phosphatases. There is a way to quickly and reversibly modify *BCL2* activity and hence influence cell survival (Boumela, et al., 2009).

1.7.2 *BAX/BAK* Genes

Pro-apoptotic *BCL2* family members *BAX* (*BCL2* Associated *X*) and *BAK* (*BCL2* Antagonist Killer) are encoded in the nucleus and present in the MOM to govern the intrinsic apoptotic pathway, where they oligomerize and mediate MOMP, resulting in the release of proapoptotic chemicals such as cytochrome c, which is a key component of apoptosis. In healthy cells, *BAK* is inserted into the MOM, whereas *BAX* is mostly cytosolic with a minor population that is weakly connected to the MOM (Westphal, et al., 2011). *BAX* moves from the cytosol to the outer membrane OM during apoptosis, and BAX /BAK undergoes an inactive-to-active conformational change to promote cell death (Westphal, et al., 2011). Interactions with BH3-only direct activator proteins generated by cell stress, as well as the physicochemical impacts of heat, high pH, and hydrophobics, all enhance BAK and BAX activation (Pea-Blanco & Garca-Sáez, 2018). BAX and BAK must undergo multiple structural changes during apoptosis to permeate the MOM (Pea-Blanco & Garca-Sáez, 2018). BAX accumulates in the mitochondria in response to cytotoxic stress and is activated *via* interactions with BH3-only proteins (Pea-Blanco & Garca-Sáez, 2018). The traditional hydrophobic groove and the rear activation site (helices 1–6) on the hydrophobic groove's opposing side are both binding sites for BAX (Pea-Blanco & Garca-Sáez, 2018). When BH3 interacts with the activation site, the loop between 1 and 2 helices moves, allowing BAX's transmembrane domain to leave the hydrophobic groove and enter the membrane (Pea-Blanco & Garca-Sáez, 2018).

Activator BH3 can attach to the normal hydrophobic groove, causing the N-terminus anterior to helix 1 to be pushed into the cytosol and rearranging the BH3 domain (Pea-Blanco & Garca-Sáez, 2018).

The BH3 domain is momentarily exposed, which is essential for *BAX* dimerization. BH3-only activator proteins generate bimodal activation by first inserting the *BAX* domain and then making the BH3 domain accessible (Pea-Blanco & Garca-Sáez, 2018). In the case of BAK, the normal hydrophobic groove was considered to be the only activation site for activator BH3-only proteins.

In BAK and mitochondrial BAX, the 1–2 loop has been identified as a monoclonal antibody activation site. Although BH3-only proteins do not bind 1–2 loop, additional BAX/BAK activators may use this unique activation site to promote MOMP. One of the steps in the molecular mechanism that determines BAX/BAK activation is the insertion of helix9 BAX into the membrane sure that BAX/BAK BH3's domain is exposed and available to form self-assemblies with other BAX/BAK molecules (Renault, et al., 2013). The majority of cells have both anti- and pro-apoptotic BCL2 proteins, and their interactions determine whether a cell lives or dies (Westphal, et al., 2011). Direct and indirect models of BAX/BAK activation have been developed based on the BCL2 binding of various members. The BH3-only proteins BID, BIM, and PUMA (activators) bind directly to BAX/BAK in the direct approach. When prosurvival proteins are replaced by BH3-only proteins, BAX/BAK are activated in the indirect approach. Additionally, components from both the direct and indirect models may be required (Renault, et al., 2013). The point of no return in mitochondrial apoptosis occurs when BAX or BAK produces the apoptotic pore in the OMM (Renault, et al., 2013).

1.7.3 *MAD2L1-DT* Gene

MAD2L1 is an RNA gene belonging to the lncRNA family known as *MAD2L1-DT* (mitotic arrest deficient 2 like 1 divergent transcript). A member of the *MAD2* family of genes. Anaphase cannot begin until all chromosomes are paired correctly at the metaphase plate, and this is controlled by a part of the spindle assembly checkpoint. Requirement for the execution of mitotic checkpoint, which checks spindle attachment and sequestering CDC20 until all chromosomes are paired at the metaphase plate, and blocks anaphase-promoting complex by sequestering CDC20 as a proofreading network during meiosis. The SAC (spindle assembly checkpoint) works to prevent aneuploidy by delaying the commencement of anaphase as long as necessary to ensure proper k-MT (kinetochore-microtubule) attachments and tensions.

SAC is a multicomponent route in which a k-MT attachment error of at least one kinetochore is required for a temporary metaphase arrest. The molecular mechanism of SAC activation involves the kinetochores of both mitotic and meiotic chromosomes being enriched with BUB and MAD proteins (Maciejewska, et al., 2009).

Once mismatched chromosomes have been corrected, a mitotic cell-cycle inhibitory SAC signal is activated to ensure equal chromosomal allocation to daughters. Mitotic

arrest deficient 2 *MAD2* is a key player in the SAC signal transduction cascade. Aneuploid metaphase II oocytes are formed when the *MAD2* gene is silenced during meiosis I, resulting in chromosome missegregation (Shi, et al., 2011).

1.7.4 *ACTB;* Housekeeping Gene

The application of RT-qPCR (Real-time quantitative polymerase chain reaction) to quantify mRNA transcription has changed the understanding of the cellular responses induced by individual developmental progression or experimental therapy (Chapman, et al., 2015). Because qPCR is an extremely sensitive method that detects tiny dynamic changes in gene expression between samples, every step of sample preparation and processing must be done carefully. To assure data reliability, this technique necessitates a normalization strategy (Panina, et al., 2018). One popular approach is to compare the target gene to an endogenous reference gene in the same sample (Chapman, et al., 2015), also known as housekeeping genes. The experimental treatment(s) and/or developmental phases under consideration must not affect the reference gene expression pattern (Chapman, et al., 2015).

ACTB (*Actin Beta*) encodes 1 of 6 actin proteins. Actins are highly conserved proteins that play an important role in cellular movement, structure, and signaling. The protein encoded is an important component of the contractile apparatus and one of the two globally expressed non-muscle cytoskeletal actins (Chapman, et al., 2015).

CHAPTER II
Materials and Methods

Ethical approval was granted by the Near East University Scientific Research Ethics Committee (YDU/2021/96-1432). Informed consent was given by all the participants. The information provided in this chapter highlights the materials and processes involved in the experiment. The experiments aimed to evaluate the expression of *BAK*, *BAX*, and *MAD2L1-DT* genes in human embryos to identify gene expression differences between aneuploid and euploid embryos.

2.1 Sample Collection and Sample Size

The samples were human surplus embryos from patients attending the British IVF Centre. A total of 47 samples belonging to 21 different patients,18 of them with no genetic anomalies (control) and 29 with aneuploidy, were involved in this study. Next generation sequencing (NGS) was used for detection of aneuploidy.

2.2 Analysis of the Samples: Nucleic Acid Extraction, cDNA Synthesis, and Real-Time PCR

This experiment was conducted at NEU DESAM Research Institute laboratory, Nicosia, North Cyprus. Nucleic acid extraction was performed using the Hibrigen total nucleic acid isolation kit (Hibrigen, Turkey, cat. No. MG-TNA-01-10) following the manufacturer protocol.

The first step in RNA extraction was adding cell lysis buffer to the samples. After that, the RNA was kept from degrading by incubating it in ice and then adding chloroform. After vortexing the samples and centrifuging them again, the DNA was separated from the chloroform using ethanol. Finally, the solution was placed into spin columns and centrifuged, and washed. The purity and concentration of the extracted RNA were estimated using the Nano-drop Spectrophotometer following manufacturer's protocol (Thermo-scientific, Pittsburg, USA).

For cDNA synthesis, Hibrigen cDNA synthesis kit was used (Hibrigen, Turkey, cat. No. MD-CDNA-01-100). The general protocol of cDNA synthesis includes adding the reaction mix, reverse transcriptase, and RNA sample.

All kits were used to reverse the transcription from RNA to synthesize cDNA following the protocol of the manufacturers with no modifications.

The LightCycler® 480 SYBR Green I Master kit (Roche, Germany, ref no. 04707516001) was utilized for the real-time PCR following the manufacturer protocol without any modifications. The final concentration of 0.3 µM of the primers (Table 1) was used for forward and reverse primers as determined by the optimization procedure explained in Table 2. During the PCR, melting curve analysis was performed to diffrentiate between the primer-dimer and the product. The primers were designed before the experiment by research assistant Hakan Aytaçoğlu. Primer sequences are listed in Table 1. *ACTB* gene was used as a housekeeping gene for normalization.

2.3 Statistical analysis

The tool GraphPad Prism v8 was used in the process of carrying out the statistical analysis.

Table 1

List of Primer Sequences

Genes	Forward Primer	Reverse Primer
MAD2L1-DT	TTTGGCATGGTGCTCCACTA	CGGTTCTCAAGCTCAAGCAAA
BAK	TACATGTCTACCAGCACGGC	CCTTGTTGCAGCATGAAGACC
BAX	GTGGTTGGGTGAGACTCCTC	GCAGGGTAGATGAATCGGGG

Table 2

Real-Time PCR Conditions

	PCR Steps	Temperature ^{0}C/ Time	Cycles
Steps	Initial Denaturation	95 ^{0}C/ 10 minutes	1
	Denaturation	95 ^{0}C/ 10 seconds	40
	Annealing	64 ^{0}C/ 10 seconds	
	Elongation	72 ^{0}C/ 30 seconds	

CHAPTER III

Results

This chapter presents the results of the conducted experiment. The results were obtained from real-time PCR analysis and were further examined using the student's T-test statistical method to create the following numerical and graphical results that are provided in this chapter.

A total of 32 human embryos and one sample from each couple were used. 19 embryos were determined as aneuploid and 13 as the control group. The nanodrop results that display the concentration and purity of RNA for each sample are listed in table 3.

Table 3

The Concentration and Purity of RNA Samples.

Sample	Group	Concentration (**ng/µl**)	260/280
1	Aneuploid	11.2	1.71
2	Aneuploid	6.2	1.75
3	Aneuploid	27	1.63
4	Aneuploid	33.5	1.67
5	Aneuploid	6.2	1.67
6	Aneuploid	4.2	1.81
7	Aneuploid	5.4	1.73
8	Aneuploid	7.6	1.75
9	Aneuploid	19.2	1.56
10	Aneuploid	15.8	1.60
11	Aneuploid	8.6	1.61
12	Aneuploid	7.1	1.67
13	Aneuploid	7.1	1.69

14	Aneuploid	7.8	1.66
15	Aneuploid	13.7	1.54
16	Aneuploid	23.1	1.61
17	Aneuploid	31.3	1.54
18	Aneuploid	8.5	1.62
19	Aneuploid	14.1	1.52
20	Control	26.3	1.52
21	Control	13.8	1.55
22	Control	12.9	1.60
23	Control	10.1	1.63
24	Control	7.9	1.67
25	Control	18.2	1.56
26	Control	24.0	1.54
27	Control	14.3	1.27
28	Control	78.4	1.32
29	Control	12.6	1.60
30	Control	22.3	1.59
31	Control	37.4	1.57
32	Control	18.4	1.25

Table 4

Ct Value that shows the expression level of the genes

	ACTB		***MAD2L1***		***BAK***			***BAX***		
Sample	**Ct**	**Ct**	**ΔΔCt**	**2^-(ΔΔCt)**	**Ct**	**ΔΔCt**	**2^-(ΔΔCt)**	**Ct**	**ΔΔCt**	**2^-(ΔΔCt)**
1	22.1	19.0	0.67	0.63	28.2	2.36	0.2	26.5	5.01	0.03
2	22.7	19.5	0.57	0.68	27.8	1.36	0.39	27.0	4.91	0.03
3	22.7	21.4	2.47	0.18	28.1	1.66	0.32	27.3	5.21	0.03
4	24.5	21.4	0.67	0.63	26.8	-	2.72	27.8	3.91	0.07
5	22.2	20.9	2.47	0.18	27.4	1.46	0.36	25.8	4.21	0.05
6	23.4	20.6	0.97	0.51	27.2	0.06	0.96	26.5	3.71	0.08

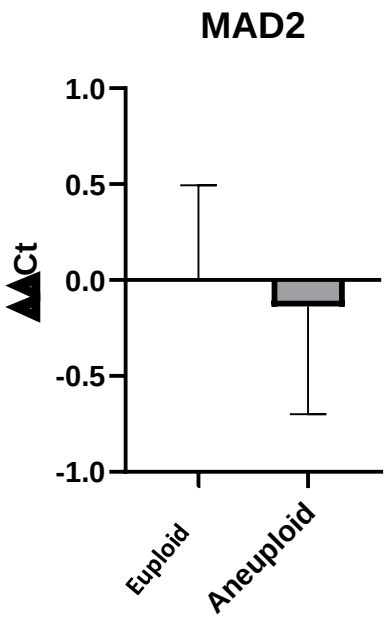
7	24.1	17.5	-	7.13	26.5	-	2.53	25.7	2.21	0.22
8	23.9	20.2	0.07	0.95	-	-	-	26.6	3.31	0.10
9	22.9	20.1	0.97	0.51	27.4	0.76	0.59	27.0	4.71	0.04
10	24.1	19.0	-	2.52	27.7	-	1.10	26.6	3.11	0.12
11	24.9	18.2	-	7.64	27.6	-	2.06	28.7	4.41	0.05
12	24.9	19.9	-	2.35	27.6	-	3.12	29.7	5.41	0.02
13	22.9	19.2	0.07	0.95	27.1	0.46	0.73	27.1	4.81	0.04
14	23.3	20.7	1.17	0.45	26.6	-	1.27	26.5	3.81	0.07
15	24.4	25.9	5.27	0.03	26.8	-	2.53	26.5	2.71	0.15
16	26.4	24.0	1.37	0.39	25.6	-	23.29	28.2	2.41	0.19
17	22.4	18.4	-	1.18	26.7	0.56	0.68	27.0	5.21	0.03
18	22.8	19.6	0.57	0.68	24.5	-	4.12	27.8	5.61	0.02
19	23.4	19.6	-	1.02	25.9	-	2.36	29.3	6.51	0.01
20	23.6	19.0	-	1.78	26.9	-	1.36	21.7	-	2.45
21	22.1	18.8	0.47	0.72	26.7	0.86	0.55	23.2	1.71	0.31
22	21.7	17.6	-	1.26	26.7	1.26	0.42	22.9	1.81	0.29
23	23.2	19.5	0.07	0.95	27.4	0.46	0.73	22.1	-0.49	1.41
24	23.4	20.4	0.77	0.59	26.8	-	1.27	22.7	-	1.07
25	23.6	21.2	1.37	0.39	-	-	-	21.9	-	2.13
26	22.0	21.8	3.57	0.08	26.8	1.06	0.48	21.6	0.21	0.87
27	23.1	18.6	-	1.66	25.8	-	2.06	23.8	1.31	0.40
28	22.0	17.7	-	1.45	26.9	1.16	0.45	22.4	1.01	0.50
29	22.7	18.6	-	1.26	26.8	0.36	0.78	22.9	0.81	0.57
30	25.8	18.5	-	11.58	27.0	-	5.82	22.5	-	6.46
31	21.6	17.9	0.07	0.95	28.6	3.26	0.10	22.8	1.81	0.29
32	25.20	-	-	-	24.9	-	16.47	21.6	-	7.96

3.1 *MAD2L1-DT* Gene Expression Analysis

In this study, the levels of gene expression in human embryo samples were evaluated using student's T-tests analysis. In the *MAD2L1* gene, as shown in figure 1, there is a slight downregulation in the aneuploid group. The T-test results showed that there was no statistical significance ($p>0.05$, figure 1).

Figure 1

The statistical analysis of the ΔΔCT values of MAD2L1-DT in euploid and aneuploid embryos

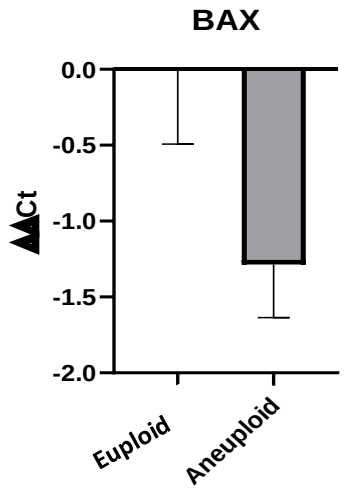


3.2 *BAX* Gene Expression Analysis

According to figure 2, a student's T-test was also used to determine the level of gene expression for the *BAX* gene. There is downregulation in an aneuploid group. The student's T-test showed that there is a significant difference in the level of expression between the euploid and aneuploid samples ($p<0.05$).

Figure 2

The statistical analysis of the ΔΔCT values of BAX in euploid and aneuploid embryos

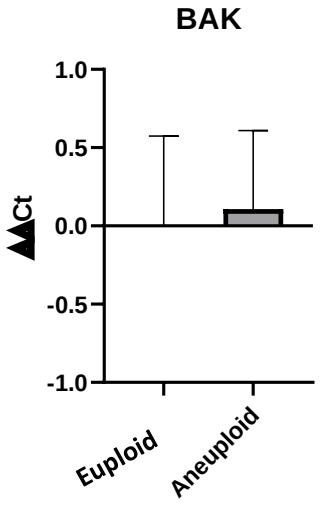


3.3 *BAK* Gene Expression Analysis

The results of gene expression in human embryo samples were evaluated using student's T-tests analysis. In the *BAK* gene, as shown in figure 3, there is slightly upregulation in an aneuploid group. The student's T-test results showed that there is no significant difference in the level of this gene expression pattern in the two groups analyzed ($p>0.05$).

Figure 3

The statistical analysis of the ΔΔCT values of BAK in euploid and aneuploid embryos



CHAPTER IV
Discussion

This study aimed to determine the relationship between the gene expression levels that are involved in apoptosis in relation to aneuploidy in blastocysts by investigating *BAX, BAK,* and *MAD2L1* gene expression levels. The samples used in the experiment were euploid and aneuploid human embryos in the blastocyst stage. In this section, the experiment is explained in detail, how the experiment is relevant, how it connects with other previous experiments, and the results are found.

Important systems for regulating genomic stability, such as cell arrest, cell cycle checkpoints, and apoptosis, are slack or lacking throughout early human development, resulting in an increased rate of aneuploidies (Mantikou, et al., 2012). Apoptosis, or programmed cell death, is required for the human body to operate properly, including cell turnover, immune system development and regulation, embryonic development, and gametogenesis (Vartak, et al., 2017).

DNA damage has the potential to affect a wide range of biological activities, including cell cycle control, DNA repair, and apoptosis (Bazrgar, et al., 2014). The researchers looked at the expression of genes involved in DNA repair. They discovered that it was increased in low-quality preimplantation human embryos with complicated aneuploidy. As a result, DNA repair pathways are more activated in these embryos than cell cycle control and apoptotic pathways. This suggests that DNA repair, rather than cell proliferation or apoptosis, is the main mechanism of DNA damage in poor-quality embryos with complicated aneuploidy (Bazrgar, et al., 2014).

Apoptosis has two types of pathways; intrinsic and extrinsic. Intrinsic pathways are involved in which a cell receives a signal to destroy itself from one of its genes or proteins in response to DNA damage detection; and extrinsic pathways function in which a cell receives a signal to initiate apoptosis from other cells in the organism (Straszewski-Chavez, et al., 2005). When the organism detects that a cell has usefulness or abnormality that is no longer for the organism to support, the extrinsic cascade is activated (Straszewski-Chavez, et al., 2005).

The most important members of the BCL2 protein family are the *BCL2* gene and it has the major role of apoptosis regulators. In a study by Hatok and Racay (2016), they found that BCL2 family proteins are engaged in the regulation of intrinsic apoptotic pathways. In addition, they are involved in the intracellular pathways related to cell survival (Hatok and Racay, 2016),

Some non-canonical effects of BCL2 family members vary significantly depending on the cell type or cell architecture under endogenous or external conditions (Hatok & Racay, 2016). The key role for *BCL2* family members in regulating oocyte and early embryo survival is described in the review by Boumela and colleagues (2011). Several members of the family exhibit differential expression throughout oocyte differentiation and early embryonic development. Among these members, the pro-apoptotic factor *BAX* has emerged as a leading contender. *BAX* is expressed constitutively, implying that both oocytes and early embryos are under constant threat of death and that their survival is dependent on their ability to regulate their proapoptotic activity. The antiapoptotic component *BCL2L10* is expressed at high levels in oocytes and early cleaving embryos, making it a promising candidate to inhibit *BAX* function (Boumela, et al., 2011).

Despite evidence of increased placental apoptosis during pregnancy, Halperin et al. (2000) discovered no difference in the incidence of apoptosis in placental tissue when comparing normal pregnancies with early and late missed abortions, or with ongoing abnormal chromosomally pregnancies (Halperin, et al., 2000).

Singla et al. (2020) discovered that aneuploid mouse cells generated at the 4–8 cell stage are progressively reduced *via* apoptosis from the early blastocyst stage to early post-implantation from the mosaic embryo's epiblast. In normal (diploid) cells, cellular protein quality control systems such as the proteasome machinery and autophagy remove misfolded or unfolded proteins to reduce cytotoxicity and enhance healthy cell survival. They found an aneuploid cell in the epiblast that converts gene mutations into protein mutations. After several mitotic divisions, chronic protein misfolding upregulates autophagy to the point where it mediates cell death rather than protects the cell. This prevents the aneuploid cell from advancing further in the formation of the epiblast (Singla, et al., 2020).

Furthermore, a study on mouse apoptosis of aneuploid cells during blastocyst maturation discovered that apoptosis actively removes aberrant cells from the ICM of chimeric mosaic embryos during blastocyst formation (Bolton, et al., 2016).

Research by Orvieto et al. (2020) uses mouse models to study apoptosis and survival in preimplantation embryos. The concordance between numerous trophectoderm biopsies is low in mosaic embryos. The results indicated that mosaic embryos had higher cell growth and cell death than euploid embryos, indicating that embryos have strong self-correction skills (Orvieto, et al., 2020). This correlates with anomalies commonly linked with spindle assembly checkpoint failures, such as chromosomes being absent or the occurrence of micronuclei. These anomalies appear to be closely associated with poor preimplantation embryo development and contribute to the reduced reproductive success in the mice (Maciejewska, et al., 2009). According to Shi et al. (2011), lower expression of MAD2 and BUB1 proteins is related to spontaneous miscarriages in human embryos with chromosomal abnormalities.

Chromosome segregation failures in female meiosis result in aneuploidy in the developing egg and embryo, making them one of the primary genetic causes of spontaneous abortions and developmental disorders in humans. It is well acknowledged that aneuploidy of meiotic origin increases considerably as women age, and current research suggests that the majority of abnormalities originate in meiosis I. Several studies about the mechanism of maternal age-related aneuploidy have been presented, including a failed spindle assembly checkpoint in meiosis I, failures in early meiosis and low sister chromatid cohesion with age (Chiang, et al., 2016). In a study that was used in mice by Chiang and his researchers (2016), the major factor in maternal age-related aneuploidy was shown to be the gradual loss of cohesin proteins in the chromosomes with age and the chances of segregation errors in chromosomes increase (Chiang, et al., 2016).

A mitotic spindle checkpoint controls the appropriate attachment of microtubules to chromosomes before mitosis to ensure the generation of euploid daughter cells (Percy, et al., 2000). The spindle assembly checkpoint monitors chromosomal segregation during mitosis and meiosis. SAC becomes active when there is an aberrant strain across the bipolar spindle arrangement of chromosomes at the metaphase plate or when there is a deficiency in the attachment of the spindle to the kinetochore (Nath, et al.,

2012). SAC prevents cells from entering metaphase until all abnormalities are repaired. The *MAD* (mitotic arrest deficient) gene family plays an important role in SAC.

In animal model cell investigations, deregulated *Mad2* expression was revealed to be related to faulty SAC-mediated aberrant meiotic progression (Nath, et al., 2012). According to early studies, human cancers may be linked to mutations in these genes. A human breast cancer cell line with a weak mitotic arrest response was previously found to have a reduced expression of the human *MAD2L1* gene. Studies have shown that cells have a weak mitotic arrest response because of reduced expression of the human *MAD2L1* gene (Percy, et al., 2000).

MAD2L1 is related to the kinetochore of detached chromosomes and suppresses *APC*, whereas it is missing when chromosomes are aligned appropriately on the metaphase plate. Abnormalities in the *BRCA2* gene and the mitotic spindle checkpoint gene may cooperate to disrupt checkpoint regulations in the cell cycle, thereby boosting cell division, resulting in aneuploidy and causing cancer (Percy, et al., 2000).

In this study, the relationship between apoptosis in aneuploid and euploid human embryos in the blastocysts stage were investigated. For the *BAX* gene, the Ct value was lower in euploid than aneuploid blastocysts, which means the expression for the gene was higher in the euploid blastocysts. According to statistical analysis by student's T-test, there is a significant difference and a relationship between apoptosis and *BAX* gene in aneuploid blastocysts. The results for the Ct value of the *BAK* gene were similar for aneuploid and euploid blastocysts samples. Furthermore, there was no significant different between aneuploid and euploid blastocysts samples in *BAK* gene expression according to the statistical analysis by student's T-test. For *MAD2L1* gene the Ct value for aneuploid was higher than euploid blastocysts, this indicates that the gene expression on euploid blastocysts were higher from aneuploid blastocysts. According to the statistical analysis, there is no significant different between aneuploid and euploid blastocyst samples.

CHAPTER V
Conclusion

Aneuploidy, mutations, or low/high expression of human genes active during early development can disrupt crucial embryonic functions at specific stages and ultimately cause cleavage arrest or widespread apoptosis. Investigation of such genes and their function will surely be useful in gaining a greater understanding of the genetic circuitry of early human development. It will also provide an opportunity for the development of novel screening methods for evaluating genetic risk in reproduction.

This study on the expression of the *BAK*, *BAX*, and *MAD2L1* genes in the blastocyst samples was used to evaluate the relationship between the expression of genes involved in apoptosis in aneuploid and euploid embryos. The findings of this investigation revealed of the *BAX* gene was expressed significantly different between aneuploid and euploid embryos. On the other hand, there was no statistical difference in the expression levels of *BAK* and *MAD2L1*. There is no study investigating the apoptosis related gene expression levels in the aneuploid blastocyst samples. This study will help future studies by being a starting point for further research.

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YAKIN DOĞU ÜNİVERSİTESİ
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ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

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Toplantı No : 2021/96

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Yakın Doğu Üniversitesi Tıp Fakültesi öğretim üyelerinden Doç. Dr. Pınar Tulay'ın sorumlu araştırmacısı olduğu, YDU/2021/96-1432 proje numaralı ve **"The Molecular Regulation of Oocyte Formation and Preimplantation Embryo Development"** başlıklı proje önerisi kurulumuzca değerlendirilmiş olup, etik olarak uygun bulunmuştur.

Prof. Dr. Şanda Çalı

Yakın Doğu Üniversitesi

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Kurul Üyesi	**Toplantıya Katılım**	**Karar**
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Bibliyografyayı Çıkart	üzerinde		

CURRICULUM VITAE

Personal Information

Name / Surname: Mihad Sami Salaheldin Ahmed.

Date and Place of Birth: 01.01.1990 / Saudi Arabia.

Education:

Degree	Institution	Graduation Year
Bachelor	JUST, Biotechnology and Genetic Engineering	2017
Master	NEU, Medical Biology and Genetics	2022