YONIS DAHIR

INVESTIGATION OF EXPRESSION LEVELS OF GENES INVOLVED IN APOPTOSIS IN HUMAN OOCYTE SAMPLES OBTAINED FROM PATIENTS WITH POLYCYSTIC OVARIES

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DEPARTMENT OF MEDICAL GENETICS

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Approval

We certify that we have read the thesis submitted by Yonis Dahir's titled, "**Investigation of expression levels of genes involved in apoptosis in human oocyte samples obtained from patients with polycystic ovaries**," 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.

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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.

Yonis Dahir

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Abstract

Investigation of expression levels of genes involved in apoptosis in human oocyte samples obtained from patients with polycystic ovaries

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M.Sc. Program in Medical Biology and Genetics

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Background: The body often goes through a process known as apoptosis, also known as programmed cell death, in order to support the growth and upkeep of healthy tissues. Cells go through structural changes and eventually leave the body during apoptosis. The removal of any damaged or aberrant cells that can one day develop into cancer as well as the maintenance of the body's normal cell balance require this operation. Women of reproductive age are commonly affected by polycystic ovary syndrome (PCOS), a hormonal condition. Numerous tiny ovarian cysts, unbalanced hormone levels, and irregular or nonexistent menstrual periods are the condition hallmarks.

Methods: A total of 13 oocyte samples, seven from the control (non-polycystic ovary, non-PCO) and six from control oocytes were collected. The samples were examined by real-time PCR following nucleic acid extraction and cDNA synthesis.

Result: The real-time PCR settings used to measure the level of gene expression. The amplification conditions for neither of the genes were optimized and primer dimers were obtained following real time PCR analysis.

Conclusions: Further studies including collection of more samples and optimization processes are necessary to establish the *BAX*, and *MAD2L1*gene expression levels. Understanding the connection between variations in their expression levels may have an impact on the underlying mechanisms of PCOS.

Keywords: *BAX*, *MAD2L1*, gene expression, polycystic ovaries, human oocytes

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List of Abbreviations

mRNA:	messenger ribonucleic acid
PCOS:	Polycystic ovary syndrome
FSH:	Follicle-stimulating hormone
cDNA:	complementary deoxyribonucleic acid
GnRH:	Gonadotropin-releasing hormone
HA:	Hyperandrogenism
IR:	Insulin resistance
LDL:	Law-density lipoprotein
LH:	Luteinizing hormone
MII:	Meiosis ll stage
QPCR:	Real-time quantitative polymerase chain reaction

CHAPTER I

Introduction

Polycystic ovary syndrome (PCOS) is the most frequent endocrine condition that affects female reproductive systems all around the world (Dumesic and Lobo 2013). The prevalence ranges from 6% to 17%, depending on the diagnostic guidelines employed. According to widely accepted specialty society recommendations, polycystic ovaries, clinical or biological hyperandrogenism, and persistent anovulation are the three conditions that must be present in order to determine the presence of PCOS. Other conditions with similar clinical indications must be ruled out because PCOS is diagnosed by exclusion. Among these are thyroid illness, hyperprolactinemia, and non-classical congenital adrenal hyperplasia. Some people might require a more thorough workup if their clinical characteristics point to additional factors (Ding et al., 2018).

1.1 Background on apoptosis in polycystic ovarian syndrome

The body often goes through a process known as apoptosis, also known as "programmed cell death", in order to support the growth and upkeep of healthy tissues. Cells go through structural changes and eventually leave the body during apoptosis. The removal of any damaged or aberrant cells that can one day develop into cancer, as well as the maintenance of the body's normal cell balance, require this operation. Women of reproductive age are commonly affected by PCOS, a hormonal condition. Numerous tiny ovarian cysts, unbalanced hormone levels, and irregular or nonexistent menstrual periods are the condition's hallmarks. Insulin resistance is a risk for PCOS patients who have type 2 diabetes and high blood sugar levels. Some studies suggest that ovarian cysts and other symptoms of PCOS may arise because of altered apoptosis in women with the disease. Anti-apoptotic proteins have been found to be more prevalent in PCOS patients, which may prevent planned cell death. Numerous studies have also discovered that women with PCOS have decreased levels of pro-apoptotic proteins, which encourage apoptosis (Ding et al., 2018).

1.2 Etiology

PCOS can be brought on by a number of factors. It has been shown that the etiology of the disease is influenced by numerous genes. These genes take part in a variety of androgenic and steroidogenic processes at various levels. Based on twin studies, PCOS heritability has been estimated to be 70%. The environment has a big impact on both how these genes are expressed and how the sickness shows up (Ying Li et al., 2019).

According to two well-known theories, PCOS symptoms would appear in those who have a genetic vulnerability and are subjected to particular environmental situations. Insulin resistance and obesity are two of the environmental factors that are most frequently present. One explanation is that prenatal androgen exposure occurred (Ying Li et al., 2019).

1.3 Pathophysiology of polycystic ovary syndrome

Androgen excess, overweight, and insulin resistance are the three main symptoms of polycystic ovarian syndrome, an endocrine disorder with a variety of underlying causes. While boosting the maturity of lesser follicles, excessive intraovarian androgen prevents the selection of a dominant follicle. The ovaries appear "polycystic" because of the surplus little follicles that are not able to continue to grow. PCOS was formerly thought to be a syndrome linked to a problem with the production of hypothalamic-pituitary gonadotropins, but it is now known to be largely an ovarian steroidogenesis illness (Tsilchorozidou, et al 2004).

1.4 Diagnostic criteria

The first comprehensive description of PCOS showed varied clinical symptoms, unidentified etiology, complex pathophysiology, and suboptimal diagnosis led to a considerable deal of scientific discussion. The American National Institutes of Health Criteria were established in 1990 in an effort to offer a comprehensive and precise diagnosis for PCOS. The Rotterdam criteria were created in 2003 as a new benchmark for PCOS diagnosis by a lab in Rotterdam. This requirement must be satisfied by unusual ovulation, androgen levels, and/or polycystic ovarian shape (more than 11 follicles for each ovary measuring between 2 and 8.98 mm) (Pundir et al., 2020).

In 2006, diagnostic recommendations were changed by the Society for Androgen Excess (AES) and Polycystic Ovaries. Both oligo- or polycystic ovaries and hyperandrogenism are necessary for the AES. The standardization of diagnosis is not without its challenges. Ovulation that is irregular and frequent is the first indicator of early menarche. The second factor is that transvaginal ultrasonography is rarely performed on young people, which prevents any invasive testing for PCOS and makes it difficult to see the ovaries. The AES raised the bar for diagnostic accuracy. Both oligo- or polycystic ovaries and hyperandrogenism are necessary for the diagnosis by AES. The process of standardizing diagnostics has some challenges. First, frequent, erratic ovulation is a feature of early menarche. Therefore, anovulatory cycles cannot be utilized to establish the syndrome's existence. Second, the limited view of the ovaries due to the scant application of transvaginal ultrasonography in young people prevents any intrusive diagnosis of polycystic ovarian morphology (Legro et al., 2014).

1.5 Androgen excess

LH as well as adrenocorticotropic hormone (ACTH) typically control the ovary and adrenal glands to generate sex hormones. Androgen excess, or the body's high amounts of sex hormones, is a disorder that impacts people with PCOS. Women who have polycystic ovarian syndrome, a prevalent endocrine condition that has an impact on menstruation, fertility, and general health, may experience this (Azziz et al., 2009).

As mentioned previously, multiple ovarian cysts are one of the main indicators of PCOS, and they can cause an imbalance in hormone production, including an excess of androgens. Women with PCOS who have high androgen levels may experience irregular or nonexistent periods, acne, excessive facial and body hair development, and thinning of the scalp hair (Azziz et al., 2009).

1.6 Hyperinsulinemia and obesity

Both obesity and hyperinsulinemia (high amounts of insulin in the blood) have been linked to PCOS. Numerous hormonal imbalances, such as a

propensity for ovarian cysts, irregular menstrual cycles, and high levels of androgens (male hormones), characterize the illness (Ying Li et al., 2019).

The existing findings suggest that overweight and hyperinsulinemia may prevent PCOS by disrupting the body's normal hormonal balance. Particularly higher levels of androgens have been associated with obesity and hyperinsulinemia, which can cause PCOS symptoms like irregular periods, acne, hair growth, and difficulty becoming pregnant. Hyperinsulinemia, obesity, and insulin resistance are all conditions where the body's cells do not react to insulin as they should. More issues could arise as a result of insulin resistance (Ying Li et al., 2019).

1.7 Fundamentals of folliculogenesis and polycystic ovary syndrome

The development of mature oocytes from immature germ cells into ovarian follicles is known as folliculogenesis. Primordial germ cells change into oocytes as a baby grows, where they remain until they are later required to develop into preovulatory follicles. The cumulus cells, one of the two varieties of granulose cells that surround the oocytes at this stage, are located on the inside layer of the follicle and are situated closer to the eggs (Ying Li et al., 2019).

For the purpose of preparing for ovulation, the "cumulus expansion" process occurs within the cumulus cells. This process results in the production of hyaluronic acid, which is subsequently deposited into the extracellular space together with other proteins. The oocyte will nevertheless continue to go through meiosis and finally become a cumulus-oocyte complex (COC). Due to the fact that this complex stalls at the metaphase of the second meiotic division, the egg in this complex is prepared for ovulation and eventual fertilization (meiosis II stage oocyte, MII stage). The interaction of the oocytes with the surrounding granulosa cells as well as the favorable follicular microenvironment play a crucial role in ovulation and follicular development (Ying et al., 2019).

The ovarian follicle's early, middle, late, and primordial antral developmental stages, the corpus luteum ruptures when an egg eventually ejects after ovulation. Beginning with the initiation of the hyperandrogenic

condition in PCOS, defective folliculogenesis started. The pituitary and hypothalamus will create higher levels of gonadotropin-releasing hormone (GnRH) and gonadotropin hormone more quickly when there is more testosterone present. Additionally, LH and FSH will promote the ovarian theca cells' generation of androgens and the ovarian granulosa cells' conversion of androgens to estrogens. Since the hypothalamic-pituitary-ovarian axis is inconsistent, women with PCOS will have a higher ratio (Qiao and Feng 2011).

Cell survival and cell death are both regulated by Bcl2 family proteins. The cumulus cells of mature, fertilized oocytes exhibit increased expression. These genes' altered expression in oocytes and cumulus cells has an impact on oocyte development in PCOS. The ability of oocytes to complete nuclear maturation and ovulate was shown to be enhanced by Bcl2 expression. One such gene targeted by miR-155 is Bcl2. A higher expression of miR-155 led to a downregulation of Bcl2 (Ghaffari et al., 2020).

MicroRNA miR-155 promotes cleavage, nuclear and cytoplasmic maturation, and cumulus expansion. Granulosa cells in PCOS overexpress miR-155, which is favorable for embryonic development but bad for nuclear and cytoplasmic maturation (Ghaffari et al., 2020).

1.8 Fertilization and gametogenesis in polycystic ovary syndrome

The process by which an organism produces gametes, or reproductive cells (eggs in females and sperm in men), is known as gametogenesis. Gametogenesis occurs in the gonads and is managed by an intricate web of hormones and signaling pathways (ovaries in females and tests in males) (Hatok and Racay 2016).

An egg is generated and released by the ovary during the process of ovulation, and then the egg is fertilized by a sperm. Gametogenesis and fertilization typically take place in females in this manner. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which enhance the development and maturation of eggs in the ovaries, are hormones that control this process (Hatok and Racay 2016).

PCOS, a common endocrine disorder, can prevent healthy gametogenesis and pregnancy in females of reproductive age. Numerous ovarian cysts, high levels of androgens (male hormones), irregular menstrual cycles, and other hormonal irregularities are the hallmarks of PCOS. These hormonal abnormalities could stop eggs from maturing and releasing correctly from the ovaries, which could lead to delayed or missing ovulation, in addition to other issues with fertility (Hatok and Racay, 2016).

PCOS is not only characterized by impairing proper gametogenesis and conception, insulin sensitivity, but also by its influence on next generation and release of hormones including FSH and LH (Dumesic et al., 2015).

1.9 Pregnancy and PCOS

Obese women may have a change in uterine receptivity due to their heightened risk of infertility and lower fecundity. Obesity is typically associated with poor obstetric outcomes throughout the whole pregnancy, regardless of whether it arises before conception or as a result of an uncontrollable weight gain during pregnancy. Because obese women have lower levels of leptin receptor mRNA in the syncytiotrophoblast without higher levels of leptin protein, leptin sensitivity, which is linked to obesity, may occur in these individuals. In spite of the fact that pregnant obese women have higher blood leptin levels than pregnant non-obese women, there is evidence that leptin per unit mass of adipose declines over the duration of pregnancy (Legro et al., 2014).

1.10 The role of apoptosis during preimplantation development

The ability of a preimplantation embryo to adapt to its environment and protect itself from cellular injury caused by either inherent or external sources determines how successfully that embryo develops. Somatic cells interrupt the cell cycle and activate repair mechanisms in response to DNA or organelle damage. The ability of a preimplantation embryo to adapt to its environment and protect itself from cellular injury caused by either inherent or external sources determines how successfully the embryo will develop. Somatic cells

interrupt the cell cycle and activate repair mechanisms in response to DNA or organelle damage (Friedberg, 2014).

If the damage is repaired, the cell cycle resumes; if not, programmed cell death, also known as apoptosis, occurs. The process of apoptosis causes the DNA, cytoplasm, and nuclear structures to fragment. The cell apoptosizes, splinters into membrane-bound fragments, and is either dispersed or phagocytized by neighboring cells (Wyllie et al., 1980).

The ability of a preimplantation embryo to adapt to its environment and protect itself from cellular injury caused by either internal or external influences determines how successfully the embryo will develop. Somatic cells stop their cell cycle when their DNA or organelles are damaged, and they start the repair procedures (Friedberg, 2003). The cell cycle resumes if the damage is repaired; if not, programmed cell death, often known as apoptosis, takes place (Friedberg, 2014).

1.11 Apoptosis Pathways

Two established apoptosis routes are intrinsic and extrinsic apoptotic pathways, often known as mitochondrial apoptosis and receptor-mediated apoptosis, respectively. Both of these occurrences trigger the caspase and DNase enzymes, which then start to work, destroying DNA and cytoplasmic material and causing the classic morphological apoptosis signals of membrane blebbing and apoptotic bodies. The essential difference between the two methods is the presence of a signal that is receptor-mediated as opposed to one that is internally produced by the mitochondria (Voss and Strasser, 2020). Apoptosis is necessary for maintaining tissue homeostasis. It promotes the growth of the embryo, cell regeneration, and the immune system (Voss and Strasser, 2020).

1.11.1 The Extrinsic Pathway

Using specific membrane-bound death receptors from the tumor necrosis factor family, the extrinsic pathway, the cytoplasm of these receptors contains a homologous domain called the death domain (DD), which has

roughly 80 amino acids (Nagata, 1999). A few extracellular signaling molecules that can bind are Fas ligand (FASLG) (Nagata, 1997) and TNFSF10 (formerly TRAIL) (Metcalfe et al., 2004).

The activation of these receptors, Fas-associated protein with death domain, a 20-adaptor molecule, is requested by the receptor and activated (FADD). Following that, Procaspase-8 interacts using FADD. Two death instructions (DED) are located on the N-terminal region of procaspase-8 and make up the death-inducing signaling complex (DISC). The weak proteinase activity of procaspase-8 is activated when a DISC develops, assisting in its self-cleavage and production of active caspase-8 (Metcalfe et al., 2004).

1.11.2 The Intrinsic Pathway

The intrinsic route, which is apoptosis's stress-induced mechanism, is not driven by the extrinsic system, which is dependent on membrane-bound receptors; rather, it is activated by a stress-induced signal, such as the heat shock-induced activity of sphingomyelin phosphodiesterase (SMPD), which causes sphingomyelin to hydrolyze into ceramide. Ceramide then stimulates either mitogen-activated protein kinase 8 (MAPK8; formerly JNK) or mitogen-activated protein kinase 9, which in turn stimulates the BH3-only 21 BCL2 family members. Apoptosis cannot be stopped by BH3-only proteins because they bind to the anti-apoptotic B-cell CLL/lymphoma 2 (BCL2) and BCL2-like 1 (BCL2L1, formerly BCL-xL) proteins. BCL2-associated X protein (BAX) and BCL2-antagonist/killer 1 (BAK) are consequently released, which causes apoptosis (Wyllie et al., 1981).

Some members of the BH3-only family may focus on the mitochondrial outer membrane and create multimers in addition to inhibiting BCL2 and BCL2L1 by binding to BAX and BAK1 and altering their conformation. The holes created by BAX and BAK1 in the mitochondrial outer membrane cause the mitochondria to depolarize. Cytochrome C is consequently delivered to the cytoplasm. Cytochrome C binds to a protein called apoptosis-inducing factor 1 (APAF1), which causes a conformational change that allows the formation of the 7-membered apoptosome. The apoptosome draws in a large number of procaspase-9 zymogen units. When procaspase-9 levels are sufficient,

procaspase-9 undergoes a conformational change that reveals the enzyme's active site. Caspase-9 activates and cleaves group II, or executioner caspases (-3, -6, and -7). The caspase cascade is terminated by DFFA cleavage, which results in its separation from DFFB and the activation of DNase. Then, DFFB splits DNA into fragments with internucleosomal apoptotic signatures (Wyllie et al., 1981).

1.12 The statement of the problem

PCOS is a very common reproductive health issue that affects one in ten females. Unusual periods are among the most common symptoms of PCOS. PCOS is one of the main reasons for infertility since it can disrupt ovulation, an important step in becoming pregnant. Underlying genetic mechanism of PCOS is not very well known.

1.13 Importance of the research

The aim of the present study was to investigate if PCOS affects the level of *BAX, and MAD2L1* gene expression levels in human oocytes obtained from patients with polycystic ovaries**.**

1.14 Limitation of the study

The number of patients and oocyte samples are limited, which can affect the statistical power and generalizability of the study results.

CHAPTER II

Literature Review

2.1 BCL2 gene

The *BCL2* gene, the prominent member of the BCL2 protein family, is essential for regulating apoptosis (Kvansakul et al., 2014). Due to their roles in mitochondrial apoptosis and the existence of shared BH (BCL2 homology) domains, the 25-member BCL2 protein family is divided into three subfamilies (Hatok and Racay, 2016).

BCL-B proteins that inhibit apoptosis contain a certain number of BH domains. Each anti-apoptotic BCL2 protein is helical in shape and rests on a single hydrophobic helix in the center. The BH1-3 domains are positioned to display a hydrophobic groove in order to function in a pro-survival manner and bind to partners that cause apoptosis (Hatok and Racay 2016). Pro-apoptotic proteins from the BCL2 family's second subclass, BOK, BAX, and BAK, contain BH 1-3 domains (Hatok and Racay 2016). There is a third diverging class, called BH3. The majority of linkages are mediated by the principal structural component of the BCL2 family. Because they are made up of numerous amphipathic alpha helices, they can adhere to mitochondrial membranes and pro-apoptotic subunit domains (Hatok and Racay, 2016). Each method requires a distinct caspase, and they are all dependent on the BCL2 protein family, which makes it easier to distinguish between them. The BCL2 family of proteins tightly controls this intrinsic system, often referred to as the mitochondrial pathway or the BCL2-regulated, which is activated by a range of developmental cues or cytotoxic insults like DNA damage, viral infection, and growth hormone shortage. Phosphokinases and phosphatases are both necessary for the dynamic process of phosphorylating BCL2 (Westphal, Kluck, and Dewson, 2014).

2.2 BAK/BAX

Apoptosis is regulated by the BCL-2 family proteins, which include BAK. The outer membrane of the mitochondria, the organelle in charge of generating the cell's energy, contains a latent monomer called BAK. The self-association of the activated BAK protein allows for the release of important signaling molecules that open pores in the mitochondrial outer membrane to start the apoptotic process. Because BAK activation is one of the important variables influencing apoptosis, understanding the intricate mechanisms underlying BAK regulation and channel creation continues to be a high research focus in cell death. Full-length BAK cannot currently be expressed as a recombinant protein; hence, it has not been feasible to thoroughly analyze full-length BAK's activation process biochemically or structurally (Westphal et al., 2011).

These BCL-2-associated proteins, or BAX and BAK, are younger BCL-2 family members with similar roles. The BCL-2 homology domain 3 (BH3), an essential helix motif seen in each protein, is required for the deadly action based on oligomerization. Anti-apoptotic BCL-2 family proteins have a groove on their surface that conceals the exposed BAK and BAX BH3 domains. It is widely known that cancer cells will modify and increase this normal regulatory system to favor pathologic cell survival due to how well this type of BAK and BAX suppression inhibits apoptosis. To start the cancer death process in people, significant attempts are being made to pharmacologically disrupt these restricting BAK and BAX links (Pea-Blanco et al., 2018).

In NMR experiments, the solution structure of BAX was successfully ascertained using full-length recombinant BAX. BAK is a membrane protein that is challenging to produce and isolate in monomeric form, creating a significant knowledge gap. As far as we are aware, efficient production or purification of filled monomeric BAK has yet to be achieved (O’neill et al., 2016).

Mitochondrial BAX and BAK have been revealed to include a region that can activate monoclonal antibodies. The 1-2 loop is inaccessible to BH3-only proteins, but other BAX/BAK activators may increase MOMP by

activating this particular activation site. Inserting the BAX helix into the membrane, which ensures that the BAX/BAK BH3 domain is accessible and available to form self-assemblies with other BAX/BAK molecules, is one of the steps in the molecular mechanism that characterizes BAX/BAK activation. Most cells include the protein BCL2, which interacts with proteins to either promote or prevent apoptosis and determine whether a cell will survive or not (O'neill et al., 2016).

Understanding the structure, metabolic function, activation patterns, and mechanisms of pro-apoptotic BAX has advanced significantly in recent years, partly as a result of the effective isolation of BAX in its purest monomeric form. Using full-length recombinant BAX in NMR studies, the solution structure of BAX2 was effectively determined. A significant information gap exists because BAK, a membrane protein, is challenging to generate and purify in monomeric form. As far as we know, filled monomeric BAK has not yet been efficiently produced or purified (O'neill et al., 2016).

2.3 *MADL2*

The *MADL2* gene (mitotic arrest defective 2) generates the MADL2 protein, which functions in the cell cycle, the procedure by which cells divide and multiply, which is what causes cells to divide and multiply. The spindle checkpoint, which is regulated by *MADL2*, is a process that ensures that the genetic material of the cell is accurately copied and divided during cell division. (O'neill et al., 2016).

Data point to a potential link between the *MADL2* gene and polycystic ovarian syndrome (O'neill et al., 2016). Women with PCOS showed larger quantities of *MADL2* gene levels in their ovaries than women without PCOS. The study suggests that greater *MADL2* mRNA levels may influence the emergence of PCOS (O'neill et al., 2016). This work suggests that the malignant progression of human cancer may include the overexpression of *MADL2*, which results in a damaged mitotic spindle checkpoint. Numerous clinicopathological traits, including metastasis, prognosis, and histology grade, have been linked in studies to the overexpression of *MADL2* (differentiation). The result of almost all of these investigations is that *MADL2* overexpression

is a risk factor for a worse outcome. Therefore, *MADL2* overexpression promotes both carcinogenesis and the development of cancer (O'neill et al., 2016).

It has been established that h *MAD2L1*, also known as human Mad2p, is required for checkpoint security. *MADL2* has a wide nuclear distribution and is only present in kinetochores without a mitotic spindle connection. These results suggest that the detection and perception of the checkpoint signal are significantly influenced by the kinetochore structure (O'neill et al., 2016).

Chapter lll

Methodology

The Near East University Scientific Research Ethics Committee (YDU/2021/96-1432) gave its clearance following an ethical review. All of the participants gave informed consent.

The goal of the experiments was to find out the level of *BAK*, *BAX*, and *MAD2L1*genes in human oocytes.

3.1 Sample Size

A total of 13 oocyte samples, 1–7 from the control (non-PCOS) and 8–13 oocytes from PCOS group were included in the study.

3.2 Nucleic acid extraction, cDNA synthesis and real-time PCR

In Nicosia, North Cyprus, at the Near East University DESAM Research Institute, this experiment was carried out. Nucleic acid extraction was done using the Hibrigen total nucleic acid isolation kit, by the manufacturer's instructions (Hibrigen, Turkey, cat. no. MG-TNA-01-10).

The samples were first treated with cell lysis buffer before the RNA was extracted. After that, the RNA was preserved from deteriorating by first being incubated on ice and then being added to chloroform. The DNA and chloroform were separated using ethanol after the samples were vortexed and centrifuged twice. The solution was then centrifuged and rinsed. Following the manufacturer's instructions, using the Nano-drop Spectrophotometer, the extracted RNA's purity and concentration were determined (Thermo-Scientific, Pittsburgh, USA). Hibrigen's cDNA synthesis kit (Hibrigen, Turkey, cat. no. MD-CDNA-01-100) was used.

For the real-time PCR, the LightCycler® 480 SYBR Green I Master kit (Roche, Germany, ref no. 04707516001) was used in accordance with the manufacturer's instructions without any changes. According to the optimization process described in Table 1. PCR conditions are shown in Table 2. Table 3 shows the final concentrations of the reagents. Melting curve

analysis was used during the PCR to distinguish between the primer-dimer and the product.

3.3 Statistical analysis

The statistical analysis was completed using the program GraphPad Prism v8.

Table 1

Sequences of primers

BAK	
Forward primer	TACATGTCTACCAGCACGGC
Reverse primer	CCTTGTTGCAGCATGAAGACC
BAX	
Forward primer	GTGGTTGGGTGAGACTCCTC
Reverse primer	GCAGGGTAGATGAATCGGGG
MAD2L1	
Forward primer	TTTGGCATGGTGCTCCACTA
Reverse primer	CGGTTCTCAAGCTCAAGCAAA

Table 2

Conditions for Real-Time PCR

	PCR procedures	**Temperature ^{0}C/ Time**	**Cycles**
Steps	Initial Denaturation	95 ^{0}C/ 10 minutes	1
	Denaturation	95 ^{0}C/ 10 seconds	40-45
	Annealing	62-64 ^{0}C/ 10-20 seconds	
	Extension	72 ^{0}C/ 30 seconds	

Table 3

Final concentrations of the reagents in PCR

Sample	BAX	*MAD2L1*
Primer Forward	0.5 µM	0.3 µM
Reverse primer	0.5 µM	0.3 µM
	1 µl cDNA	1µl cDNA +10% glycerol + 1.25 µl $MgCl_2$
	63 °C 20 seconds	62 °C 20 sec

CHAPTER lV

Results

The experiment's findings are presented in this chapter. The following numerical and graphical data are provided in this chapter as a consequence of further examination of the real-time PCR analysis results using the student's T-test statistical method.

Table 4 shows the details of the oocytes used in this project. RNA was successfully extracted from each sample and cDNA was synthesized. The concentration of cDNA samples is shown in table 5.

Table 4

Details about the oocyte donors

Patient number	PCO
1	Yes
2	Yes
3	Yes
4	Yes
5	Yes
6	Yes
7	Yes
8	No
9	No
10	No
11	No
12	No
13	No

Table 5

Measurements of the cDNA samples' concentration and absorbance.

Sample ID	Conc. (ng/µl)	260/280
1	982.9	1.74
2	1080.6	1.74
3	1038.5	1.75
4	1055.4	1.73
5	1045.0	1.73
6	1030.6	1.74
7	1063.6	1.74
8	1072.0	1.74
9	1042.5	1.74
10	1036.4	1.74
11	1046.2	1.74
12	1039.4	1.74

Real-time PCR was performed to analyze the expression levels of *MADL2* and *BAX* genes in oocyte samples. To investigate the level of gene expression, real time PCR conditions were tested using different primer concentrations ranging from 2.5 μM to 0.1 μM with different annealing temperatures and annealing time in the presence of glycerol and magnesium chloride. For each PCR amplification, a negative control in the absence of any DNA was included to evaluate contamination and formation of primer dimer. Melting curve analysis was performed at the end of each real time PCR to identify the primer dimer and the actual product. The PCR conditions were not being able to be optimized. The final PCR conditions are shown in table 5. A total of two samples showed an amplification for the *BAX2* and six samples showed an amplification for *MADL2* genes, respectively. Two samples from the PCO group showed an amplification with a CT value of 28.1 and 29.4 for *BAX2* and three samples from the control group with a CT value of 30, 29.8 and 29.9 and three samples from the PCO group with CT values of 29.8, 29.9 and 29.4 for *MADL2* genes, respectively. Figure 1 shows the melting curve analysis result following *BAX* gene amplification. Figure 2 shows the melting curve analysis of primer dimers following *MADL2* amplification. Figure 3 shows the *BAX* gene amplification plot analysis following real-time PCR.

Table 5

Real time PCR conditions

Reagents	**Volume/ concentration**
SYBR- Green	5 µl
Forward primer	0.3 µM
Reverse primer	0.3 µM
$MgCl_2$	1.25 µl
Glycerol	2 µl
Water	-
cDNA	1 µl

Figure 1

Melting curve analysis of an optimization attempt for the *BAX* gene from real-time PCR.

Negative control sample shows a peak coinciding with the samples and as a result, real-time PCR melting curve analysis showed primer dimer.

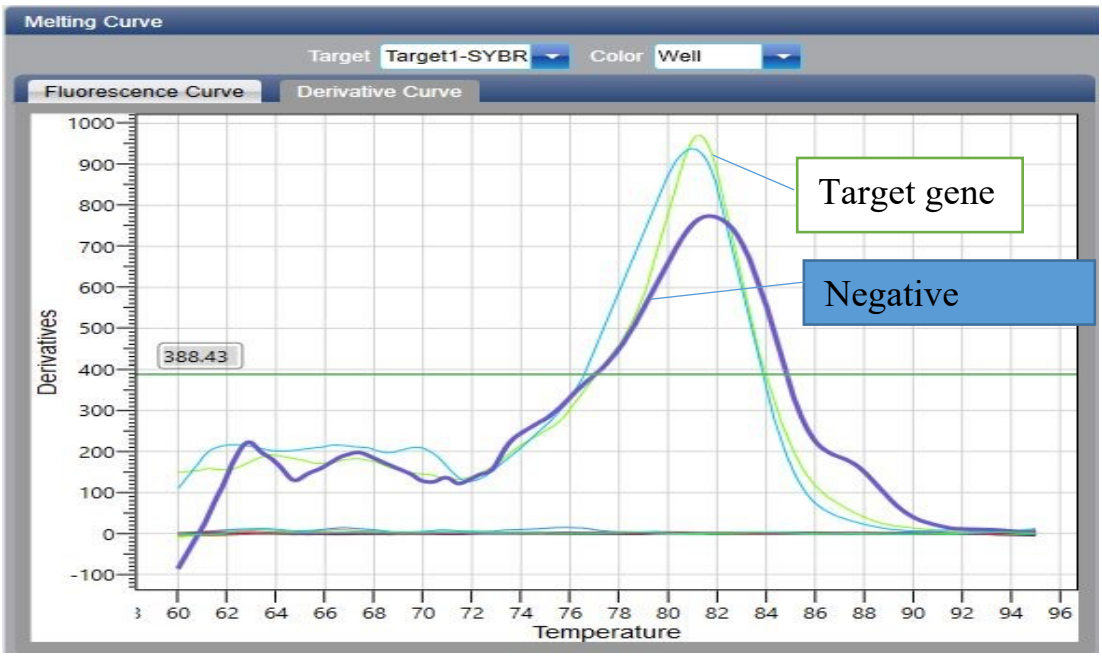


Figure 2

Melting curve analysis of a PCR optimization try for the *MADL2* target gene.

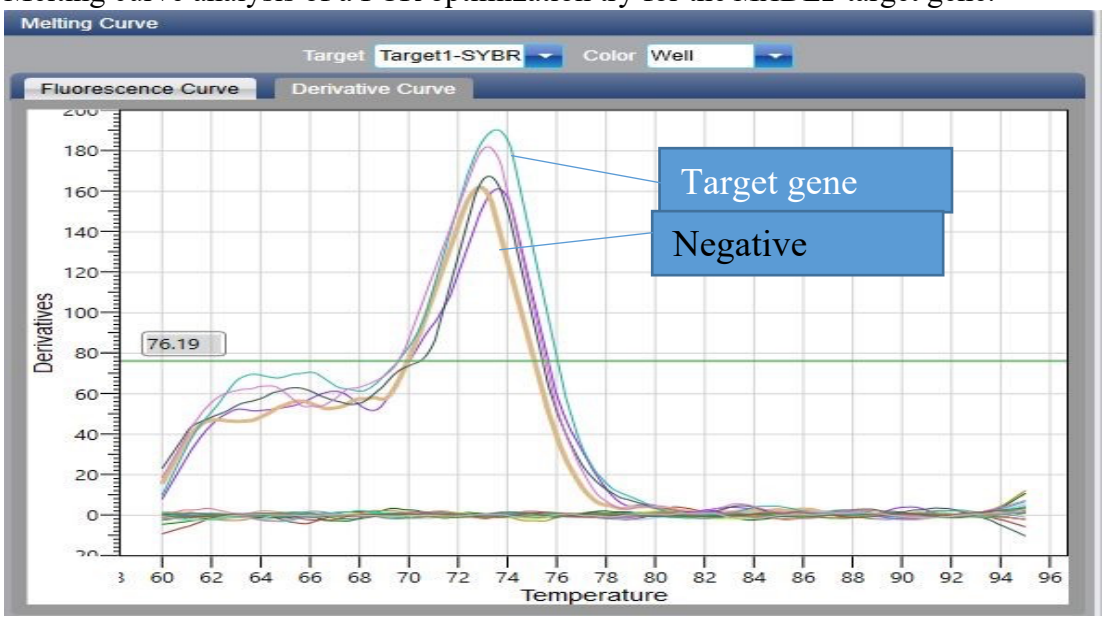
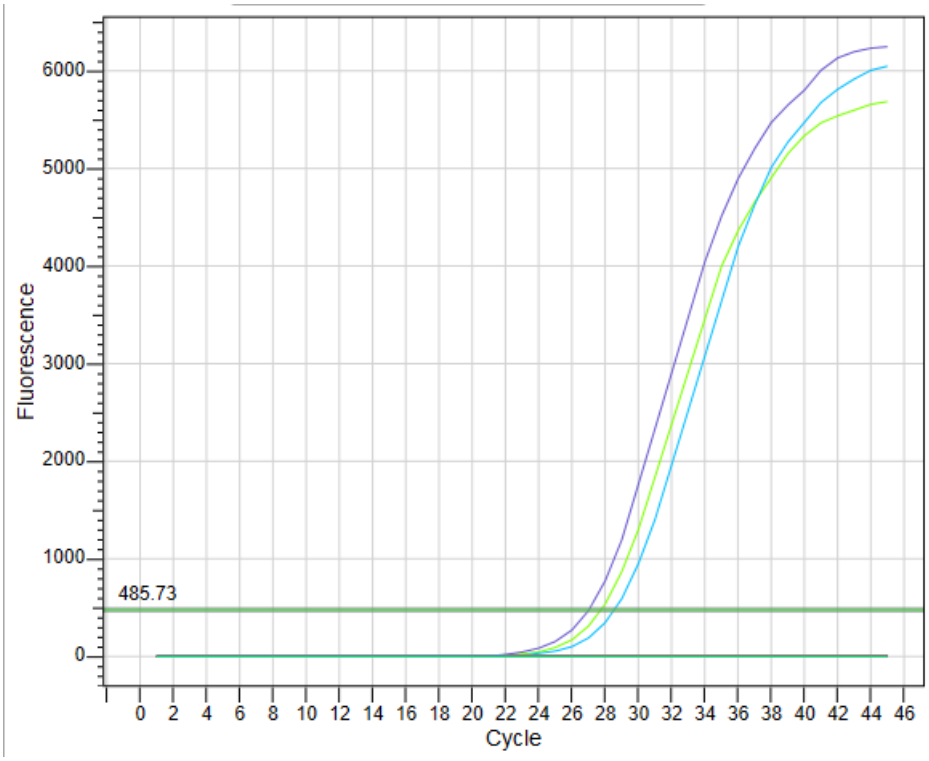


Figure 3

BAX gene amplification plot analysis following real-time PCR.



CHAPTER V

Discussion

By examining the gene expression levels of *BAX, and MAD2L1*, this study attempted to ascertain the link between the apoptosis-related genes and PCOS in the oocytes. Important mechanisms governing genomic stability during early human development, mechanisms like cell arrest, cell cycle checkpoints, and apoptosis may be ineffective (Mantikou et al., 2012). Programmed cell death, also known as apoptosis, is essential for the human body to operate normally. The development and regulation of the immune system, cell turnover, embryonic development, and gametogenesis are all included in this (Yan Li et al., 2022).

Genetic studies have focused on the association between the *BAX* genes with polycystic ovary syndrome (PCOS). Apoptosis is aided by the pro-apoptotic gene *BAK* as well. Researchers have looked at how the *BAK* gene may impact PCOS-related apoptotic processes in the ovaries and how it may contribute to the onset of the condition (Yan Li et al., 2022). Studies suggest that variations in the *BAX* gene may increase the likelihood of developing PCOS. This is thought to occur as modifications to the *BAX* gene may affect ovarian function and cause cyst development (Yan Li et al., 2022). It is crucial to remember that not all studies have discovered a statistically significant connection between the *BAX* gene and PCOS and that additional investigation is still needed in this field. To fully comprehend how the *BAX* gene influences the development of PCOS, more studies are required (Ying Li et al., 2019).

Apoptosis may result from internal or extrinsic processes. Extrinsic pathways are engaged when a cell detects DNA damage and receives a signal from one of its genes or proteins to start apoptosis, as opposed to intrinsic pathways, which are activated when a cell detects DNA damage and receives a signal from other cells in the body. When a cell is useful or presents a problem that the organism cannot address, the extrinsic cascade is set off (Wang et al., 2021).

The *BCL2* gene and its critical role as an apoptosis regulator make up the BCL2 protein family's most important members. Found that proteins from

the BCL2 family are important in regulating intrinsic apoptotic pathways. They also participate in intracellular processes related to cell survival (Yan Li et al., 2022). Some non-canonical behaviors of BCL2 family members differ greatly based on the cell type or cell structure in endogenous or exogenous conditions. (Hatok & Racay, 2016). The BCL2 family of proteins is shown to be essential for regulating oocyte and early embryonic survival. Certain family members present themselves differently throughout oocyte differentiation and the first few weeks of embryonic development. One of the main rivals of these members is the pro-apoptotic factor *BAX*. Because *BAX* is constitutively generated, oocytes and early embryos might always be in danger of dying, thus it's probable that their capacity to control proapoptotic activity is crucial to guaranteeing their (Yan Li et al., 2022).

Studies have shown that mutations in the *BAK* gene may increase a person's risk of developing PCOS. It is anticipated that this will occur since cyst formation, a characteristic of PCOS, may be brought on by mutations in the *BAK* gene (Ying Li et al., 2019). It is important to keep in mind that not all studies have discovered a conclusive link between the *BAK* gene and PCOS, and that this field of study is currently ongoing. To fully comprehend how the *BAK* gene contributes to the emergence of polycystic ovarian syndrome, more investigation is required (Ying Li et al. 2019).

On the regulation of cell division and apoptosis, the *MAD2L1* gene plays a significant role. Variations in *MAD2L1* expression and the emergence of ovarian cysts in the context of PCOS have been studied as potential associations (Ying Li et al., 2019). Studies have suggested that changed *MAD2L1* expression may have a role in the pathophysiology of PCOS and the development of ovarian cysts, even though additional research is necessary to completely understand the relationship between apoptosis, altered *MAD2L1* expression, and PCOS (Boumela et al., 2011).

This investigation looked at the connection between oocyte apoptosis and *MAD2L1* expression using oocyte samples from women with PCOS. Unfortunately, the PCR conditions were not able to be optimized for these genes and primer dimers were obtained. Thus, further samples must be

collected for optimizing the PCR conditions. When analyzing the findings of studies investigating the association between *MAD2L1* expression and PCOS, it is crucial to take into account the potential impact of sample populations. It is necessary to conduct additional research on the association between both *BAX* and *MAD2L1* expression and PCOS utilizing bigger and more varied sample sets.

CHAPTER VI

Conclusion

Further studies are necessary to establish how the *BAX*, and *MAD2L1* genes manifest themselves in relation to polycystic ovarian syndrome. The onset of PCOS has been linked to these genes. They are known to contribute to apoptosis as well. Although several studies have shown a connection, more research is needed to completely grasp the connections and underlying mechanisms. As potential new targets for PCOS treatment and prevention, these genes show promise. Understanding the connection between variations in their expression and a higher likelihood of getting PCOS will require more study.

REFERENCES

Li, Y., Liu, Y., Zhou, X., Chen, S., Chen, X., Zhe, J., Zhang, J., Zhang, Q., & Chen, Y. (2019b). MiR-29a regulates the proliferation, aromatase expression, and estradiol biosynthesis of human granulosa cells in polycystic ovary syndrome. *Molecular and Cellular Endocrinology*, *498*, 110540. https://doi.org/10.1016/j.mce.2019.110540

Dumesic, D. A., Oberfield, S. E., Stener-Victorin, E., Marshall, J., Laven, J. S., & Legro, R. S. (2015). Scientific Statement on the Diagnostic Criteria, Epidemiology, Pathophysiology, and Molecular Genetics of Polycystic Ovary Syndrome. *Endocrine Reviews*, *36*(5), 487–525. https://doi.org/10.1210/er.2015-1018

Tsilchorozidou, T., Overton, C., & Conway, G. S. (2004). The pathophysiology of polycystic ovary syndrome. *Clinical Endocrinology*, *60*(1), 1–17. https://doi.org/10.1046/j.1365-2265.2003.01842.x

Andreas, E., Pandey, H. M., Hoelker, M., Salilew-Wondim, D., Gebremedhn, S., Schellander, K., & Tesfaye, D. (2021). The regulatory role of miR-20a in bovine cumulus cells and its contribution to oocyte maturation. *Zygote*, *29*(6), 435–444. https://doi.org/10.1017/s0967199420000933

Cheng, J., Huang, J., Yuan, S., Zhou, S., Yan, W., Shen, W., Chen, Y., Xia, X., Luo, A., Zhu, D., & Wang, S. (2017). Circular RNA expression profiling of human granulosa cells during maternal aging reveals novel transcripts associated with assisted reproductive technology outcomes. *PLOS ONE*, *12*(6), e0177888. https://doi.org/10.1371/journal.pone.0177888

Dang, Y., Qiao, J., Hu, B., Fan, X., Ren, Y., Li, R., Lian, Y., Yan, J., Li, Q., Zhang, Y., Minteer, S. D., Ren, X., Huang, J., Wu, Y., Liu, P., Wen, L., Zhang, C., Huang, Y., & Tang, F. (2016). Tracing the expression of circular RNAs in human pre-implantation embryos. *Genome Biology*, *17*(1). https://doi.org/10.1186/s13059-016-0991-3

Azziz, R., Carmina, E., Dewailly, D., Diamanti-Kandarakis, E., Escobar-Morreale, H. F., Futterweit, W., Janssen, O. E., Legro, R. S., Norman, R. J., Taylor, A. G., & Witchel, S. F. (2009). The Androgen Excess and PCOS Society criteria

for the polycystic ovary syndrome: the complete task force report. *Fertility and Sterility*, *91*(2), 456–488. https://doi.org/10.1016/j.fertnstert.2008.06.035

Ghaffari, M., Dehghan, G., Baradaran, B., Zarebkohan, A., Rasmi, Y., Soleymani, J., Dolatabadi, J. E. N., & Hamblin, M. R. (2020). Co-delivery of curcumin and Bcl-2 siRNA by PAMAM dendrimers for enhancement of the therapeutic efficacy in HeLa cancer cells. *Colloids and Surfaces B: Biointerfaces*, *188*, 110762. https://doi.org/10.1016/j.colsurfb.2019.110762

Wyllie, A. H., Kerr, J. F. R., & Currie, A. R. (1980). Cell Death: The Significance of Apoptosis. *International Review of Cytology*, 251–306. https://doi.org/10.1016/s0074-7696(08)62312-8

Legro, R. S., Kunselman, A. R., Dodson, W. C., & Dunaif, A. (1999c). Prevalence and Predictors of Risk for Type 2 Diabetes Mellitus and Impaired Glucose Tolerance in Polycystic Ovary Syndrome: A Prospective, Controlled Study in 254 Affected Women[1]. *The Journal of Clinical Endocrinology and Metabolism*, *84*(1), 165–169. https://doi.org/10.1210/jcem.84.1.5393

Ekart, J., McNatty, K. P., Hutton, J. C., & Pitman, J. K. (2013a). Ranking and selection of MII oocytes in human ICSI cycles using gene expression levels from associated cumulus cells. *Human Reproduction*, *28*(11), 2930–2942. https://doi.org/10.1093/humrep/det357

Assidi, M., Dufort, I., Ali, A. A., Hamel, M. A., Algriany, O., Dielemann, S., & Sirard, M. (2008b). Identification of Potential Markers of Oocyte Competence Expressed in Bovine Cumulus Cells Matured with Follicle-Stimulating Hormone and/or Phorbol Myristate Acetate In Vitro. *Biology of Reproduction*, *79*(2), 209–222. https://doi.org/10.1095/biolreprod.108.067686

Dumesic, D. A., & Lobo, R. A. (2013). Cancer risk and PCOS. *Steroids*, *78*(8), 782–785. https://doi.org/10.1016/j.steroids.2013.04.004

McKenzie, L. J., Pangas, S. A., Carson, S. A., Kovanci, E., Cisneros, P., Buster, J. E., Amato, P., & Matzuk, M. M. (2004b). Human cumulus granulosa cell gene expression: a predictor of fertilization and embryo selection in women undergoing IVF. *Human Reproduction*, *19*(12), 2869–2874. https://doi.org/10.1093/humrep/deh535

Mukherjee, S., Shinde, G., & Hinduja, I. (2021). Compromised Cumulus-Oocyte Complex Matrix Organization and Expansion in Women with PCOS.

Reproductive Sciences, *29*(3), 836–848. https://doi.org/10.1007/s43032-021-00775-0

Papler, T. B., Bokal, E. V., Maver, A., & Lovrečić, L. (2015). Specific gene expression differences in cumulus cells as potential biomarkers of pregnancy. *Reproductive Biomedicine Online*, *30*(4), 426–433. https://doi.org/10.1016/j.rbmo.2014.12.011

Hassani, F., Oryan, S., Eftekhari-Yazdi, P., Bazrgar, M., Moini, A., Nasiri, N., & Sharifi-Zarchi, A. (2019). Downregulation of Extracellular Matrix and Cell Adhesion Molecules in Cumulus Cells of Infertile Polycystic Ovary Syndrome Women with and without Insulin Resistance. *Cell*, *21*(1), 35–42. https://doi.org/10.22074/cellj.2019.5576

Feuerstein, P., Puard, V., Chevalier, C., Teusan, R., Cadoret, V., Guerif, F., Houlgatte, R., & Royere, D. (2012). Genomic Assessment of Human Cumulus Cell Marker Genes as Predictors of Oocyte Developmental Competence: Impact of Various Experimental Factors. *PLOS ONE*, *7*(7), e40449. https://doi.org/10.1371/journal.pone.0040449

Zhang, X., Jafari, N., Barnes, R. B., Confino, E., Milad, M. P., & Kazer, R. R. (2005). Studies of gene expression in human cumulus cells indicate pentraxin 3 as a possible marker for oocyte quality. *Fertility and Sterility*, *83*(4), 1169–1179. https://doi.org/10.1016/j.fertnstert.2004.11.030

Pan, J., Zhou, C., Zhou, Z., Yang, Z., Dai, T., Huang, H., & Jin, L. (2021). Elevated ovarian pentraxin 3 in polycystic ovary syndrome. *Journal of Assisted Reproduction and Genetics*, *38*(5), 1231–1237. https://doi.org/10.1007/s10815-021-02105-4

Pan, J., Zhou, C., Zhou, Z., Yang, Z., Dai, T., Huang, H., & Jin, L. (2021b). Elevated ovarian pentraxin 3 in polycystic ovary syndrome. *Journal of Assisted Reproduction and Genetics*, *38*(5), 1231–1237. https://doi.org/10.1007/s10815-021-02105-4

Brentnall, M., Rodriguez-Menocal, L., De Guevara, R. L., Cepero, E., & Boise, L. H. (2013). Caspase-9, caspase-3 and caspase-7 have distinct roles during intrinsic apoptosis. *BMC Cell Biology*, *14*(1). https://doi.org/10.1186/1471-2121-14-32

Maliqueo, M., Sir-Petermann, T., Pérez, V. F., Echiburú, B., De Guevara, A. L., Gálvez, C. A., Crisosto, N., & Azziz, R. (2009). Adrenal Function during

Childhood and Puberty in Daughters of Women with Polycystic Ovary Syndrome. *The Journal of Clinical Endocrinology and Metabolism*, *94*(9), 3282–3288. https://doi.org/10.1210/jc.2009-0427

Legro, R. S., Kunselman, A. R., Dodson, W. C., & Dunaif, A. (1999b). Prevalence and Predictors of Risk for Type 2 Diabetes Mellitus and Impaired Glucose Tolerance in Polycystic Ovary Syndrome: A Prospective, Controlled Study in 254 Affected Women[1]. *The Journal of Clinical Endocrinology and Metabolism*, *84*(1), 165–169. https://doi.org/10.1210/jcem.84.1.5393

Glueck, C. J., Papanna, R., Wang, P., Goldenberg, N., & Sieve-Smith, L. (2003). Incidence and treatment of metabolic syndrome in newly referred women with confirmed polycystic ovarian syndrome. *Metabolism-Clinical and Experimental*, *52*(7), 908–915. https://doi.org/10.1016/s0026-0495(03)00104-5

Chen, L., Li, S., Zhang, Y., Chen, Z., Li, Y., Li, H.,..., & Lu, L. 2015). Androgens and Insulin – Two Key Players in Polycystic Ovary Syndrome. *Gynakologisch-Geburtshilfliche Rundschau*, *48*(1), 9–15. https://doi.org/10.1159/000111465

Palomba, S., De Wilde, M. A., Falbo, A., Koster, M. P., La Sala, G. B., & Fauser, B. C. (2015). Pregnancy complications in women with polycystic ovary syndrome. *Human Reproduction Update*, *21*(5), 575–592. https://doi.org/10.1093/humupd/dmv029

Boomsma, C. M., Eijkemans, M., Hughes, E. G., Visser, G. H. A., Fauser, B. C., & Macklon, N. S. (2006). A meta-analysis of pregnancy outcomes in women with polycystic ovary syndrome. *Human Reproduction Update*, *12*(6), 673–683. https://doi.org/10.1093/humupd/dml036

Brezina, P. R., Ke, R. W., & Kutteh, W. H. (2013b). Preimplantation Genetic Screening: A Practical Guide. *Clinical Medicine Insights*, *7*, CMRH.S10852. https://doi.org/10.4137/cmrh.s10852

Qin, J., Pang, L., Li, M., Fan, X., Huang, R., & Chen, H. (2013). Obstetric complications in women with polycystic ovary syndrome: a systematic review and meta-analysis. *Reproductive Biology and Endocrinology*, *11*(1). https://doi.org/10.1186/1477-7827-11-56

Roos, N., Kieler, H., Sahlin, L., Ekman-Ordeberg, G., Falconer, H., & Stephansson, O. (2011). Risk of adverse pregnancy outcomes in women with polycystic

ovary syndrome: population based cohort study. *BMJ*, *343*(oct13 1), d6309. https://doi.org/10.1136/bmj.d6309

Schvartzman, J., Duijf, P. H., Sotillo, R., Coker, C., & Benezra, R. (2011). Mad2 Is a Critical Mediator of the Chromosome Instability Observed upon Rb and p53 Pathway Inhibition. *Cancer Cell*, *19*(6), 701–714. https://doi.org/10.1016/j.ccr.2011.04.017

Comparison of the metabolic parameters and androgen level of umbilical cord blood in newborns of mothers with polycystic ovary syndrome and controls. (2012b, March 1). PubMed. https://pubmed.ncbi.nlm.nih.gov/23267369/

Qiao, J., & Feng, H. L. (2011). Extra- and intra-ovarian factors in polycystic ovary syndrome: impact on oocyte maturation and embryo developmental competence. *Human Reproduction Update*, *17*(1), 17–33. https://doi.org/10.1093/humupd/dmq032

Yasmin, A., Roychoudhury, S., Choudhury, A. P., Ahmed, A. B. F., Dutta, S., Mottola, F., Verma, V., Kalita, J. C., Kumar, D., Sengupta, P., & Kolesarova, A. (2022). Polycystic Ovary Syndrome: An Updated Overview Foregrounding Impacts of Ethnicities and Geographic Variations. *Life*, *12*(12), 1974. https://doi.org/10.3390/life12121974

Asunción, M., Calvo, R. M., Millán, J. L. S., Sancho, J. M. L., Avila, S., & Escobar-Morreale, H. F. (2000). A Prospective Study of the Prevalence of the Polycystic Ovary Syndrome in Unselected Caucasian Women from Spain1. *The Journal of Clinical Endocrinology and Metabolism*, *85*(7), 2434–2438. https://doi.org/10.1210/jcem.85.7.6682

Gleicher, N., Weghofer, A., & Barad, D. H. (2010). Anti-Müllerian hormone (AMH) defines, independent of age, low versus good live-birth chances in women with severely diminished ovarian reserve. *Fertility and Sterility*, *94*(7), 2824–2827. https://doi.org/10.1016/j.fertnstert.2010.04.067

Gleicher, N., Weghofer, A., & Barad, D. H. (2010). Anti-Müllerian hormone (AMH) defines, independent of age, low versus good live-birth chances in women with severely diminished ovarian reserve. *Fertility and Sterility*, *94*(7), 2824–2827. https://doi.org/10.1016/j.fertnstert.2010.04.067

Google Scholar. (n.d.). https://scholar.google.com/scholar_lookup?title=International%20evidence-based%20guidelines%20for%20the%20assessment%20and%20management

%20of%20polycystic%20ovary%20syndrome&author=H.L.J.%20Teede&publication_year=2018

Teede, H. J., Misso, M., Costello, M. F., Dokras, A., Laven, J. S., Moran, L. J., Piltonen, T., Norman, R. J., Andersen, M., Azziz, R., Balen, A. H., Baye, E., Boyle, J., Brennan, L., Broekmans, F. J., Dabadghao, P., Devoto, L., Dewailly, D., Downes, L., . . . Yildiz, B. O. (2018). Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome†‡. *Human Reproduction*, *33*(9), 1602–1618. https://doi.org/10.1093/humrep/dey256

Skiba, M. A., Islam, R. M., Bell, R. J., & Davis, S. R. (2018). Understanding variation in prevalence estimates of polycystic ovary syndrome: a systematic review and meta-analysis. *Human Reproduction Update*, *24*(6), 694–709. https://doi.org/10.1093/humupd/dmy022

March, W., Moore, V. M., Willson, K., Phillips, D., Norman, R. J., & Davies, M. J. (2010). The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Human Reproduction*, *25*(2), 544–551. https://doi.org/10.1093/humrep/dep399

MAD2L1 Protein - ACROBiosystems. (n.d.). https://www.acrobiosystems.com/L-370MAD2L1.html?gclid=CjwKCAjwj42UBhAAEiwACIhADoQl5Ee8cwlCLmGFDNuAx7jpbZ0mczBHG8M8G8epFLyPYTpK4DXp6RoCdHUQAvD_BwE

Mantikou, E., Wong, K., & Repping, S. (2012). Molecular origin of mitotic aneuploidies in preimplantation embryos. *Biochimica Et Biophysica Acta: Molecular Basis of Disease*, *1822*(12), 1921–1930. https://doi.org/10.1016/j.bbadis.2012.06.013

Scriven, P. N., & Ogilvie, C. M. (2010). FISH for Pre-implantation Genetic Diagnosis. *Methods in Molecular Biology*. https://doi.org/10.1007/978-1-60761-789-1_20

De Ziegler, D., Pirtea, P., Fanchin, R., & Ayoubi, J. M. (2018). Ovarian reserve in polycystic ovary syndrome: more, but for how long? *Fertility and Sterility*, *109*(3), 448–449. https://doi.org/10.1016/j.fertnstert.2017.11.027

Singla, S., Iwamoto-Stohl, L. K., Zhu, M., & Zernicka-Goetz, M. (2020). Autophagy-mediated apoptosis eliminates aneuploid cells in a mouse model

of chromosome mosaicism. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-020-16796-3

Bálint, É., & Vousden, K. H. (2001). Activation and activities of the p53 tumour suppressor protein. *British Journal of Cancer*, *85*(12), 1813–1823. https://doi.org/10.1054/bjoc.2001.2128

Barnes, R. B., Rosenfield, R. L., Namnoum, A. B., & Layman, L. C. (2000). Effect of Follicle-Stimulating Hormone on Ovarian Androgen Production in a Woman with Isolated Follicle-Stimulating Hormone Deficiency. *The New England Journal of Medicine*, *343*(16), 1197–1198. https://doi.org/10.1056/nejm200010193431614

Bergeron, L., Perez, G. I., MacDonald, G. M., Shi, L., Sun, Y., Jurisicova, A., Varmuza, S., Latham, K. E., Flaws, J. A., Salter, J. C., Hara, H., Moskowitz, M. A., Li, E., Greenberg, A. H., Tilly, J. L., & Yuan, J. (1998). Defects in regulation of apoptosis in caspase-2-deficient mice. *Genes & Development*, *12*(9), 1304–1314. https://doi.org/10.1101/gad.12.9.1304

Thomadaki, H., & Scorilas, A. (2006). *BCL2*Family of Apoptosis-Related Genes: Functions and Clinical Implications in Cancer. *Critical Reviews in Clinical Laboratory Sciences*, *43*(1), 1–67. https://doi.org/10.1080/10408360500295626

Youle, R. J., & Strasser, A. (2008). The BCL-2 protein family: opposing activities that mediate cell death. *Nature Reviews Molecular Cell Biology*, *9*(1), 47–59. https://doi.org/10.1038/nrm2308

Dong, B., Shi, M., Capelo, J. L., & Zhang, H. (2019). Insight into long noncoding competing endogenous RNA networks in hepatic fibrosis: The potential implications for mechanism and therapy. *Gene*, *687*, 255–260. https://doi.org/10.1016/j.gene.2018.11.063

Wang, X., Lu, Z., Gomez, A. M., Hon, G. C., Yue, Y., Han, D., Fu, Y., Parisien, M., Dai, Q., Jia, G., Ren, B., Pan, T., & He, C. (2014). N6-methyladenosine-dependent regulation of messenger RNA stability. *Nature*, *505*(7481), 117–120. https://doi.org/10.1038/nature12730

Borruel, S., Fernández-Durán, E., Alpañés, M., Martí, D., Álvarez-Blasco, F., Luque-Ramírez, M., & Escobar-Morreale, H. F. (2013). Global Adiposity and Thickness of Intraperitoneal and Mesenteric Adipose Tissue Depots Are Increased in Women With Polycystic Ovary Syndrome (PCOS). *The*

Journal of Clinical Endocrinology and Metabolism, *98*(3), 1254–1263. https://doi.org/10.1210/jc.2012-3698

Comim, F. V., Hardy, K., & Franks, S. (2013). Adiponectin and Its Receptors in the Ovary: Further Evidence for a Link between Obesity and Hyperandrogenism in Polycystic Ovary Syndrome. *PLOS ONE*, *8*(11), e80416. https://doi.org/10.1371/journal.pone.0080416

Ching, H., Burke, V., & Stuckey, B. G. A. (2007). Quality of life and psychological morbidity in women with polycystic ovary syndrome: body mass index, age and the provision of patient information are significant modifiers. *Clinical Endocrinology*, *66*(3), 373–379. https://doi.org/10.1111/j.1365-2265.2007.02742.x

Rosenfield, R. L., & Caprio, S. (2016). The Pathogenesis of Polycystic Ovary Syndrome (PCOS): The Hypothesis of PCOS as Functional Ovarian Hyperandrogenism Revisited. *Endocrine Reviews*, *37*(5), 467–520. https://doi.org/10.1210/er.2015-1104

Glueck, C. J., Dharashivkar, S., Wang, P., Zhu, B., Gartside, P. S., Tracy, T., & Sieve, L. (2005). Obesity and extreme obesity, manifest by ages 20–24 years, continuing through 32–41 years in women, should alert physicians to the diagnostic likelihood of polycystic ovary syndrome as a reversible underlying endocrinopathy. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *122*(2), 206–212. https://doi.org/10.1016/j.ejogrb.2005.03.010

Recabarren, S. E., Smith, R., Rios, R., Maliqueo, M., Echiburú, B., Codner, E., Cassorla, F., Rojas, P., & Sir-Petermann, T. (2008). Metabolic Profile in Sons of Women with Polycystic Ovary Syndrome. *The Journal of Clinical Endocrinology and Metabolism*, *93*(5), 1820–1826. https://doi.org/10.1210/jc.2007-2256

Azziz, R., & Adashi, E. Y. (2016). Stein and Leventhal: 80 years on. *American Journal of Obstetrics and Gynecology*, *214*(2), 247.e1-247.e11. https://doi.org/10.1016/j.ajog.2015.12.013

Azziz, R. (2006). Diagnosis of Polycystic Ovarian Syndrome: The Rotterdam Criteria Are Premature. *The Journal of Clinical Endocrinology and Metabolism*, *91*(3), 781–785. https://doi.org/10.1210/jc.2005-2153

Huang, H., Weng, H., Sun, W., Qin, X., Shi, H., Wu, H., Zhao, B. S., Mesquita, A., Liu, C., Yuan, C. L., Hu, Y.-C., Hüttelmaier, S., Skibbe, J. R., Su, R., Deng, X., Dong, L., Sun, M., Li, C., Nachtergaele, S., … Chen, J. (2018). Recognition of RNA N6-methyladenosine by IGF2BP proteins enhances mrna stability and translation. Nature Cell Biology, 20(3), 285–295. https://doi.org/10.1038/s41556-018-0045-z

Li, Y., Liu, Y., Zhou, X., Chen, S., Chen, X., Zhe, J., Zhang, J., Zhang, Q., & Chen, Y. (2019). MiR-29a regulates the proliferation, aromatase expression, and estradiol biosynthesis of human granulosa cells in polycystic ovary syndrome. *Molecular and Cellular Endocrinology*, *498*, 110540. https://doi.org/10.1016/j.mce.2019.110540

Hatok, J., & Racay, P. (2016). Bcl-2 family proteins: master regulators of cell survival. *Biomolecular Concepts*, *7*(4), 259–270. https://doi.org/10.1515/bmc-2016-0015

Ding, D., Chen, W., Wang, J. R., & Lin, S. Z. (2018). Association between PCOSand endometrial, ovarian, and breast cancer. *Medicine*, *97*(39), e12608. https://doi.org/10.1097/md.0000000000012608

O'Neill, K. L., Huang, K., Zhang, J., Chen, Y., & Luo, X. (2016). Inactivation of prosurvival Bcl-2 proteins activates Bax/Bak through the outer mitochondrial membrane. *Genes & Development*, *30*(8), 973–988. https://doi.org/10.1101/gad.276725.115

Friedberg, E. C. (2014). Master Molecule, Heal Thyself. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.x114.572115

Metcalfe, A. D., Hunter, H. R., Bloor, D. J., Lieberman, B. P., Picton, H. M., Leese, H. J., Kimber, S. J., & Brison, D. R. (2004). Expression of 11 members of the BCL-2 family of apoptosis regulatory molecules during human preimplantation embryo development and fragmentation. *Molecular Reproduction and Development*, *68*(1), 35–50. https://doi.org/10.1002/mrd.20055

Renehan, A. G., Booth, C., & Potten, C. S. (2001). What is apoptosis, and why is it important? *BMJ*, *322*(7301), 1536–1538. https://doi.org/10.1136/bmj.322.7301.1536

Westphal, D. L., Kluck, R. M., & Dewson, G. (2014). Building blocks of the apoptotic pore: how Bax and Bak are activated and oligomerize during apoptosis. *Cell Death & Differentiation*, *21*(2), 196–205. https://doi.org/10.1038/cdd.2013.139

Li, Y., Xu, J., Li, L., Bai, L., Wang, Y., Zhang, J., & Wang, H. (2022). Inhibition of Nicotinamide adenine dinucleotide phosphate oxidase 4 attenuates cell apoptosis and oxidative stress in a rat model of polycystic ovary syndrome through the activation of Nrf-2/HO-1 signaling pathway. *Molecular and Cellular Endocrinology*, *550*, 111645. https://doi.org/10.1016/j.mce.2022.111645

ElInati, E., Zielinska, A. P., McCarthy, A., Kubikova, N., Maciulyte, V., Mahadevaiah, S. K., Sangrithi, M. N., Ojarikre, O. A., Wells, D., Niakan, K. K., Schuh, M., & Turner, J. M. A. (2020b). The BCL-2 pathway preserves mammalian genome integrity by eliminating recombination-defective oocytes. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-020-16441-z

Wu, G., Yang, Z., Chen, Y., Li, X., Yang, J., & Yin, T. (2020). Downregulation of LNC-OC1 attenuates the pathogenesis of polycystic ovary syndrome. Molecular and Cellular Endocrinology, 506, 110760. https://doi.org/10.1016/j.mce.2020.110760

Martinez-Fierro, M. L., Carrillo-Arriaga, J. G., Luevano, M., Lugo-Trampe, Á., Delgado-Enciso, I., & Rodriguez-Sanchez, I. P. (2019). Serum levels of miR-628-3p and miR-628-5p during the early pregnancy are increased in women who subsequently develop preeclampsia. *Pregnancy Hypertension*, *16*, 120–125. https://doi.org/10.1016/j.preghy.2019.03.012

Liu, G., Liu, S., Xing, G., & Wang, F. (2020). RETRACTED: lncRNA PVT1/MicroRNA-17-5p/PTEN Axis Regulates Secretion of E2 and P4, Proliferation, and Apoptosis of Ovarian Granulosa Cells in PCOS. *Molecular Therapy. Nucleic Acids*, *20*, 205–216. https://doi.org/10.1016/j.omtn.2020.02.007

Zhang, X., Hong, R., Chen, W., Xu, M., & Wang, L. (2019). The role of long noncoding RNA in major human disease. *Bioorganic Chemistry*, *92*, 103214. https://doi.org/10.1016/j.bioorg.2019.103214

Liu, G., Liu, S., Xing, G., & Wang, F. (2020b). RETRACTED: lncRNA PVT1/MicroRNA-17-5p/PTEN Axis Regulates Secretion of E2 and P4, Proliferation, and Apoptosis of Ovarian Granulosa Cells in PCOS. *Molecular Therapy. Nucleic Acids*, *20*, 205–216. https://doi.org/10.1016/j.omtn.2020.02.007

Quinn, J. J., & Chang, H. Y. (2015). Unique features of long non-coding RNA biogenesis and function. Nature Reviews Genetics, 17(1), 47–62. https://doi.org/10.1038/nrg.2015.10

Artika, I. M., Dewi, Y. P., Nainggolan, I. M., Siregar, J. E., & Antonjaya, U. (2022). Real-Time Polymerase Chain Reaction: Current Techniques, Applications, and Role in COVID-19 Diagnosis. *Genes*, *13*(12), 2387. https://doi.org/10.3390/genes13122387

Appendix

Turnition Similarity Report

Yoonis Dahir Thesis

ORİJİNALLİK RAPORU

%15	%13	%7	%
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

1	docs.neu.edu.tr İnternet Kaynağı	%5
2	www.researchgate.net İnternet Kaynağı	<%1
3	hdl.handle.net İnternet Kaynağı	<%1
4	joe.bioscientifica.com İnternet Kaynağı	<%1
5	estudogeral.sib.uc.pt İnternet Kaynağı	<%1
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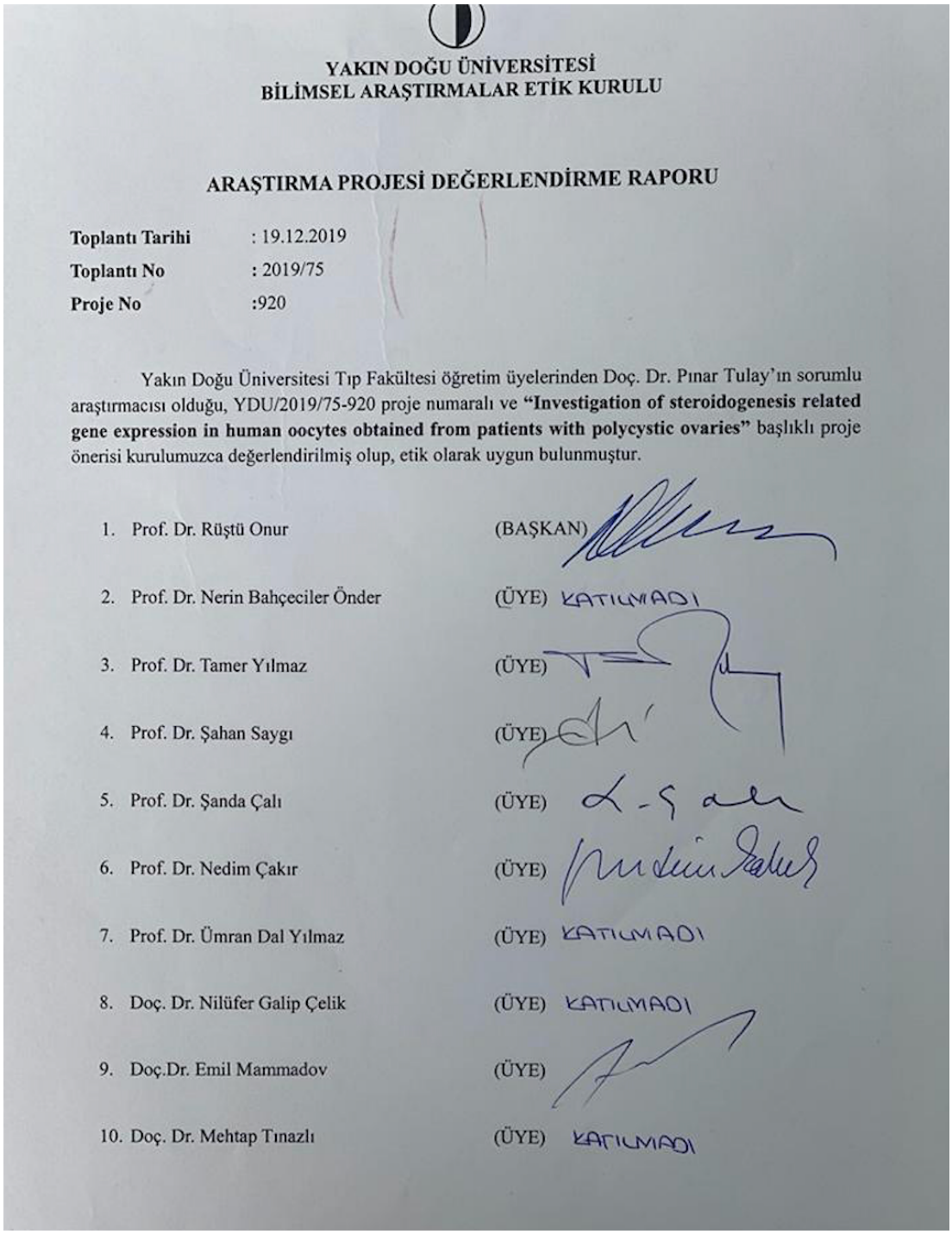
7	paduaresearch.cab.unipd.it İnternet Kaynağı	<%1
8	944fee2a-009a-4e87-9af6-fd7f295bdd78.filesusr.com İnternet Kaynağı	<%1
9	Practical Manual of In Vitro Fertilization, 2012. Yayın	<%1

ETHICAL APPROVAL



YAKIN DOĞU ÜNİVERSİTESİ
BİLİMSEL ARAŞTIRMALAR ETİK KURULU

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi : 19.12.2019
Toplantı No : 2019/75
Proje No :920

Yakın Doğu Üniversitesi Tıp Fakültesi öğretim üyelerinden Doç. Dr. Pınar Tulay'ın sorumlu araştırmacısı olduğu, YDU/2019/75-920 proje numaralı ve **"Investigation of steroidogenesis related gene expression in human oocytes obtained from patients with polycystic ovaries"** başlıklı proje önerisi kurulumuzca değerlendirilmiş olup, etik olarak uygun bulunmuştur.

1. Prof. Dr. Rüştü Onur (BAŞKAN)
2. Prof. Dr. Nerin Bahçeciler Önder (ÜYE) KATILMADI
3. Prof. Dr. Tamer Yılmaz (ÜYE)
4. Prof. Dr. Şahan Saygı (ÜYE)
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ACADEMIC BACKGROUND

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Ladnan hospital	2019-2020