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EXPRESSION OF GENES INVOLVED IN STEROID PATHWAY IN HUMAN EUPLOID AND ANEUPLOID EMBRYOS

M.Sc. THESIS

2023

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Approval

We certify that we have read the thesis submitted by Joshua Adebola Ayobami Idowu titled **"Expression of Genes Involved in Steroid Pathway in Human Euploid and Aneuploid Embryos"** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Medical Biology and Genetics.

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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.

Joshua Adebola Ayobami IDOWU

09/02/2023

Acknowledgments

Firstly, I would like to thank the almighty God for the strength and grace to complete this project.

I would also like to give gratitude to my supervisor, Prof. Dr. Pinar TULAY for her patience, guidance and assistance all through my studies and particularly in this project.

I am also grateful to Mr. HakanAytacoglu for his immense contribution and guidance in my laboratory work. Also, to the Examining committee for their impact in this study.

Lastly, I am super grateful to my parents for their contributions and impact towards my studies. God bless you so much.

Joshua Adebola Ayobami IDOWU

Abstract

Expression of Genes Involved in Steroid Pathway in Human Euploid and Aneuploid Embryos

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M.Sc. Program in Medical Biology and Genetics

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February 2023, 56 pages

Introduction: Steroid mechanism plays an important role during gametogenesis and preimplantation embryo development.It is well established that aneuploidies rise during gametogenesis due to non-disjunction. However, there has not been studies investigating the molecular pathways that are involved in or affected by the aneuploidies. Thus, the aim of this study was to analyse the expression levels of genes involved in steroid metabolismpathway in euploid and aneuploid human embryos, and to elucidate the role of these genes with aneuploidies.

Materials and Methods:A total of10 euploid and 10 aneuploid human embryo samples were used for this study. RNA was extracted after which the complementary DNA (cDNA) synthesis was performed following the manufacturer's protocol. The real time polymerase chain reaction (PCR) was conducted to evaluate the level of gene expression. The negative control in the absence of cDNA sample was performed for each PCR. The cycle of threshold (CT) values were obtained using the software of the real time PCR equipment.

Results: Five out of the 10 aneuploid samples did not work, and 7 out of the control samples also did not work. The median for the control samples were 2.3, and the median for the aneuploid samples were 1.07. The P-value from the analysis was 0.881 which shows that there was no statistical significance.

Conclusion: This study shows that the levels of expression of *CYP11A1* in euploid and aneuploid human embryo samples is similar and the expression level of this gene did not vary in the presence of aneuploid embryos.

***Keywords**:*gene expression, aneuploidies, *CYP11A1*, embryos, steroid metabolism

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List of Abbreviations

aCGH - Array Comparative genomic hybridization

AKR - Aldo-keto reductases

AKR1C3 - Aldo-Keto Reductase Family 1 Member C3

A4 –Androstenedione

BAK1: BCL2 Antagonist/Killer 1

cDNA - Complementary Deoxyribonucleic acid

CGH - Comparative genomic hybridization

CYP - Cytochrome P450

Cyp17 - Cytochrome P450 Family 17

Cyp11A1 - Cytochrome P450 Family 11 Subfamily A Member 1

CYP19A1 - Cytochrome P450 Family 19 Subfamily A Member 1

DNA - Deoxyribonucleic acid

DHEA - Dehydroepiandrosterone

DHT - Dihydrotestosterone

ESHRE - European Society for Human Reproduction and Embryology

FAD - Flavin adenine dinucleotide

FISH - Fluorescence in situ hybridization

FMN - Flavin mononucleotide

FSH - Follicle Stimulating Hormone

GnRH - Gonadotropin releasing hormone

HFEA - Human Fertilization and Embryology Authority

hCG - Human Chorionic Gonadotrophin

HIV - Human Immunodeficiency Virus

HLA - Human leukocyte antigen

HSD - Hydroxysteroid Dehydrogenase

HSD3B1 - Hydroxysteroid 3-Beta Dehydrogenase 1

HSD3B2 - Hydroxysteroid 3-Beta Dehydrogenase 2

HSD17B1 - Hydroxysteroid 17-Beta Dehydrogenase 1

IVF - In vitro fertilization

KHDC1P1: KH Domain Containing 1 Pseudogene 1

LDL – Low density lipoproteins

LH - Luteinizing Hormone

MPB - Male Pattern Baldness

mRNA – Messenger Ribonucleic acid

NADPH - Nicotinamide Adenine Dinucleotide Phosphate

PAPSS - PAPS synthase

PCOS –Poly-cystic ovarian syndrome

PCR - Polymerase chain reaction

PGT - Preimplantation genetic testing

PGS - Preimplantation genetic screening

PK - Proteinase K

POR - Cytochrome P450 oxidoreductase

RNA - Ribonucleic acid

SR-BI - Scavenger receptor class B type I

SNP - Single nucleotide polymorphism

StAR - Steroidogenic acute regulatory protein

SULT1E1 - Sulfotransferase Family 1E Member 1

tRNA - Transfer Ribonucleic acid

CHAPTER I

Introduction

Aneuploidy is one of the reasons for loss of pregnancy. The risk of having an aneuploid gamete and thus embryo elevates substantially with the age of the mother within the decennium following the end of the menstrual cycle (Hassold& Hunt, 2001). Most of the aneuploidies and monosomies in the autosome chromosomes are mostly fatal with a number of exceptions. A minor number of trisomies are in accordance with complete development mostly with various abnormalities including mental anomalies. At parturition the occurrence of aneuploidy, majorly Down Syndrome and sex chromosomal abnormalities is about 0.3%, while in still births the occurrence elevates to 4% (Spandorfer et al., 2004). However, in miscarriages that happen spontaneously within the gestation period of six and twenty weeks, there is an increase to about 35%(Spandorfer et al., 2004). The possibility of conceiving a child with aneuploidies, such as Down Syndrome, is extremely linked with the age of the mother, elevating from about 0.1% at the age of 30 years, to 1% at the age of 40 years (Spandorfer et al., 2004). Many Down Syndrome pregnancies are lost *via* spontaneousmiscarriages. It is evaluated that 7 to 10% of pregnancies which are acknowledged clinically are affected by aneuploidy, which is not a full estimate of the total aneuploidy considering that it does not include any preclinical loss (Spandorfer et al., 2004).

It has been ascertained that most pregnancies which are abnormal as well as live births originate from anomalies during female oogenesis (Hassold et al., 1993; Lamb et al., 2005; Sherman et al., 2006; Spandorfer et al., 2004). Moreover, most trisomies emerge due to mis-segregation mistakes in meiosis I (Spandorfer et al., 2004). It has been confirmed that trisomy is related with decrease in crossover numbers. This is mostly with an unusual dispersal either proximal to the telomeresor contrarily proximal to the centromeres. Furthermore, in some situations, there is no identification of any crossover specifying that the divalent chromosome that takes part in the aneuploidy may have been achiasmate (Spandorfer et al., 2004). So, as crossovers play an important function in sustaining the bond between homologues till, they are sorted at first stage of anaphase, this might make the chromosomes susceptible to segregation mistake. A comparable aneuploidy pattern takes place in pregnancies after *in vitro* fertilization (IVF) (Spandorfer et al., 2004). Just a small

percentage of human embryos that are cultured for up to a week and then transferred to the uterus progress to a live birth. When implantation takes place as proven by the existence of elevated levels of human chorionic gonadotrophin (hCG), fourteen days after fertilization, 10 to 20% of the pregnancies do not advance to the level where a heartbeat can be observed. Also, 10 to 20% of clinical pregnancies end due to miscarriage (Spandorfer et al., 2004). Previously published studies showed that 11% of pregnancies following IVF were lost which was extremely linked with the age of the mother (Spandorfer et al., 2004). Additionally, the occurrence of aneuploidy in young women was lower, in such approximately 20%(Kazemi&Taketo, 2022).

1.1. Gene Expression

Gene expression has an effect on the phenotype and the end products are often proteins. Gene expression occurs in prokaryotes, eukaryotes and even viruses. In DNA, the genotype is the genetic information that is stored and the phenotype represents the interpretation of the information (Chen, 2020). Gene expression is very important because it has a great effect on the control of the structure and function of cells. Abnormal gene expression process can result in different kinds of genetic disorders. Furthermore, due to an abnormality, such as aneuploidy, changes of the gene expression pattern may be observed. During gametogenesis, steroidogenesis plays an important role. Therefore, during meiotic division, if there is non-disjunction, this may have a direct impact on the expression of the steroidogenesis related genes. On the contrary, it is a possibility that if there is an abnormal level of gene expression, it may have an impact on the segregation of chromosomes. *CYP11A1*, *CYP17* and *CYP19* genes are involved in steroid metabolism, and steroid hormones have functions in regulating water, salt uniformity, stress response, metabolism, and the initiation and sustaining sexual differentiation and reproduction (Schiffer et al., 2019).

CYP11A1 codes for an enzyme that activates the initial and challenging step of steroid production. The gonads and adrenals are the sites of expression. The SF-1 transcription factor which joins to the promoter of *CYP11A1* takes part in hormonally and tissue-precise controlled expression.In cases where there is a partial *CYP11A1* abnormality, there are genotype and phenotype discrepancies, in such patients with 46,XY genotypes may have female phenotypes(Meng-Chun et al., 2004).

The monooxygenases that make up the *CYP17* subunit of the P450 enzymes play a crucial part in the generation of steroids, cholesterol, and other lipids as well as several drug metabolism-related events. The steroidogenic pathway, which produces progestins, glucocorticoids, mineralocorticoids, estrogen, and androgens, is largely dependent on this enzyme. Adrenal hyperplasia and pseudo-hermaphroditism are associated with *CYP17* mutations (Heather et al., 1998).

The aromatase enzyme, which is a key player in the manufacture of estrogen is coded by *CYP19*. While aromatase is primarily expressed in the gonads and brain of most mammals, it is also expressed in additional extragonadal locations in primates. As the evolutionary tree progresses and reaches its peak in humans, aromatase expression and estrogen production keep rising. This is made possible by adding additional new tissue-specific promoters to the fat, placenta, skin, and bone, as well as more effectively using existing promoters (Serdar et al., 2003). In particular regions of the brain, aromatase cytochrome stimulates the conversion of androgens to estrogens(Birgit et al., 1999).

1.2. Statement of the Problem

There have not been any studies investigating the expression of the steroidogenesis related genes in human aneuploid embryos to date. This study is very unique in understanding the variation in the expression levels between euploid and aneuploid human embryo samples, and also to understand whether the genes involved in the steroidogenesis are involved in the development of aneuploidies or the expression of these genes are affected by the level of aneuploidies. The result of this study can also give an insight to understand if genes involved in the steroidogenesis are involved in the development of other syndromes.

1.3. Purpose of the Study

This research aimed to analyse the patterns of gene expression that are related with steroid metabolism in euploid and aneuploid human embryos, and to elucidate the role of thesegenes in aneuploidies.

1.4. Hypothesis

It was predicted that there would be a notable difference in the expression levels of genes involved in the steroidogenesis between the euploid and aneuploid human embryos.

CHAPTER II

Literature Review

2.1. Gametogenesis and Preimplantation Embryo Development

2.1.1. *Oogenesis*

Oogenesis starts during prenatal development. The ova are formed from oogonia, which are cells in the ovarian cortex that have come from the primordial germ cells by a sequence of about 20 mitoses. Every oogonium is the central cell in a forming follicle. Around the third month of prenatal development, the oogonia of the embryo have started to form primary oocytes, most of which have earlier reached the prophase of meiosis I. The process of oogenesis is not in synchrony, and both the beginning and late stages coexist in the fetal ovary (Nussbaumet al., 2007). There are million oocytes at the period of birth, but most of these oocytes are degenerated. About 400 finally mature and are ovulated (Nussbaum et al., 2007). The primary oocytes are at prophase I stage of meiosisby the period of birth, and the ones that are not degenerated remain arrested for years in that stage till ovulation as part of the woman's menstrual cycle. When a woman has attained sexual maturity, a single follicle starts to develop and mature, and ovulation occurs. Just prior to ovulation, the oocyte swiftly finishes meiosis I, dividing in a way that one cell becomes the secondary oocyte (egg or ovum), consisting most of the cytoplasm with its organelles and the other becomes the first polar body. Meiosis II starts quickly and moves to the metaphase II stage in the course of ovulation, where it stops only to be finalized if fertilization takes place (Nussbaum et al., 2007).

2.1.2. *Spermatogenesis*

The process by which mature sperm develop from primordial germ cellsis known as spermatogenesis. Similar to the oogenesis, the primordial germ cell travel to the gonadal ridge early on during the embryo's development and mature into spermatogonia. In the testicular seminiferous tubules, these spermatogonia are located. During embryo development, spermatogonia are formed. Spermatogonia begin to go through mitotic split, expansion,differentiation through multiple developmental phases around adolescence in order to produce sperm (Guyton & Hall, 2008; Praveen et al., 2020). Spermatogenesis begins on average at the age of thirteen and lasts for the majority of the other years of life. It occurs during active

sexual life and decreases noticeably as people age(Guyton & Hall, 2008; Praveen et al., 2020).

After the initiation of adolescence in males, the maturation of spermatocytes begins. The next process, known as spermiogenesis, occurs in adults and entails the production of mature sperm (Guyton & Hall, 2008; Praveen et al., 2020). The cells that develop into sperm are called spermatogonia.Spermatogonia, which are the progenitors to sperm cells, are produced from early childhood until adolescence in males.

2.1.3.*Preimplantation Embryo Development*

Preimplantation embryo development starts with zygote formation, continue through the cleavage division, and end with implantation of the embryo. (Tulay et al., 2017; Jaroudi&SenGupta, 2007). Fertilization takes place when the sperm cell penetrates the oocyte leading to finalization of meiosis, and the oocyte which is fertilized is referred to as the zygote. Fertilization happens when the oocyte and the sperm are joined together. Then, there is a succession of splitting in the cleavage that results in different stages. Parental genes are functioning at the earlier stages of cleavage phase and later on the activation of the genome of the embryo occurs. Due to the embryo's inability to perform cellular tasks, the development will not proceed if the activation of the genome of the embryo fails (Tulay et al., 2017). The breakdown of maternal genes, some RNAs retained in oocytes, proteins, and other molecules induces this activation. The preimplantation embryo experiences remarkable transformation of expression following activation of the genome of the embryo (Tulay et al., 2017).

2.2. Chromosome Segregation in Mitotic Division in Preimplantation Embryos and Meiotic Division in Gametes

When replication takes place in the cell cycle at the S stage as well as the G2 stage, every chromosome now comprises of sets of sister chromatids. On the spindle of the metaphase, they position equally. A bipolar amphitelic extension is formed from the microtubules across the spindle pillars to specialized shapes of proteins gathered on the centromeres of both sister chromatids. There is a reduction in the microtubules at anaphase that detach thesister chromatids, and they move to various spindle pillars where the chromosomes decondense at telophase. There is an adjustment in the nuclear partition, and cytokinesis finishes the division into two

daughter cells comprising each of them with twochromosome pair (Miller & Therman, 2001).

During gametogenesis, meiosis goes through one stage of replication accompanied by two cytokinesis. Meiosis I occurs after the replication of DNA and the premeiotic S phase. Chromosomes that have been replicated which consist of sets of sister chromatids unite with their pair. They form a synaptonemal complex which produces a haploid pair of divalent chromosomes. Then, recombination takes place between unidentical pair of chromatids. After the loss of the synaptonemal complex, the chromosomes, which are divalent is reduced but stay together towards the arms of the chromosomes, positions at metaphase I on the spindle. In contrast to mitosis, the two sets of sister kinetochores which are divalent function as one. They develop extensions that are amphitelic to microtubules from opposing spindle pillars. There is a reduction in microtubules at anaphase I, and the chiasmata move along the ending of each divalent. As the crossovers are rectified and the homologues are divided into several pillars, they are eliminated. Meiosis II moves with no replication, close to mitosis. The sets of sister chromatids position on the anaphase II and metaphase II spindle. They eventually divide into different pillars and produces daughter cells with a haploid chromosome pair(Miller & Therman, 2001).

2.2.1. *Mis-segregation and Nondisjunction in Meiosis*

Mis-segregation is exceptionally recurrent during meiosis, and in consequence aneuploid embryos are being produced. Nondisjunction is specially known to be the inability of homolog chromosomes or sister chromatids to segregate (Miller & Therman, 2001).

The process involved in mis-segregation is reported in accordance to two models. The meiotic nondisjunction mechanism shows that if the set of homologs made up of a divalent at meiosis I is unable to divide, and therefore a single daughter cell will possess two chromosomes while the other will possess none. Nondisjunction may also take place in meiosis II. Sister chromatids are unable to divide in meiosis II after these errors in nondisjunction, the conceptus at fertilization then results as monosomic or trisomic, supposing the other gamete to be normal (Gardner et al., 2018).

Pre-division is referred to the “precocious” division of chromatids in the course of meiosis I, as first presented by Angell (1997), and it involves three

chronological events. Firstly, the homolog chromosomes are unable to pair in the course of meiosis I. However, if they pair, division takes place prior to the finalization of meiosis I. So, in place of the both chromosomes showing up as a united divalent, they show up as two different monovalents. Secondly, these monovalents are susceptible to pre-division which means that the division of both chromatids that should (on the long-established plan) occur at meiosis II, rather occurs while they are yet in the initial cycle of meiosis. Thirdly, during meiosis I in the anaphase stage, there is a separation in the single or both chromatid chromosomes alone to the polar body and oocyte, or spermatocytes that have developed(Gardner et al., 2018).

Achiasmate nondisjunction takes place when the homologs had never attached, and then segregate jointly to the same daughter cell. The final outcome is the same as if the long-established nondisjunction had taken place, but without any recombination (Uroz&Templado, 2012).

The majority of human mis-segregation of chromosomes takes place in oogenesis (Uroz&Templado, 2012). The chromosomes 21, X and Y are very susceptible to nondisjunction in males during meiosis I or II. The most common aneuploidies that result in live births are trisomy 13, 18, 21 and the sex chromosomes X and Y (Hassold, 1998; Robinson et al., 1999).

2.2.2. *Nondisjunction in Mitosis*

Nondisjunction in mitosis also result in abnormalities. Nondisjunction following fertilization, either in the growing embryo or in extraembryonic tissues like the placenta, can result in chromosomal mosaicism that can underlie some medical conditions, such as the measure of patients with Down syndrome. Also, unusual chromosome segregation in tissues that divide rapidly such as in cells of the colon, is normally a step in the growth of chromosomally abnormal tumours (Nussbaum et al., 2007).

2.3. Preimplantation Genetic Testing (PGT)

PGT is a term which describes the testing of the embryo before implantation, formed with the aid of *in vitro*fertilization (IVF) technology. This is used for defects that are inheritable in the chromosomes (structural chromosome abnormalities) or deficiencies in single genes after which the embryos which are not affected are

transferred to the uterus to create a pregnancy (Kakourou et al., 2009; Mykitiuk et al., 2006; Delhanty et al., 1994; Delhanty& Wells, 2002). In addition to this PGT is also applied to patients with recurrent miscarriages, implantation failure or recurrent IVF failures to screen for aneuploidies. Clinical geneticists and medical doctorsgive a first-hand counselling to couples that wish to undergo this process. Then, embryologists and doctors provide counselling on IVF treatment. PGT is not a simple conceptive choice and different challenges may be faced in the course of the procedure which includes the reasonable cost for every cycle, the possibility of wrong diagnosis as well as the low chance of success. Also, starting treatment does not give an assurance that the embryos needed for implantation would be available. It could be the outcome of poor feedback from the ovary, unsuccessful fertilization, embryos with low quality, affected embryos as a result of diagnosis or due to indeterminate results. In spite of these possible challenges, PGT is a very pleasant choice to a lot of patients, specifically to those with high moral standards or those who do not wish to undergo pregnancy termination and to people that have been through pregnancy terminations (Kakourou et al., 2009). In fact, one study reported the perspective of the patient's on PGT, where eighty six percent of women revealed that avoiding pregnancy termination was the major benefit they noticed in PGT (Pergament et al., 1991). Furthermore, patients who already had a PGT experience are fully satisfied with PGT (Lavery et al., 2002).

The initial confirmed pregnancies after PGT were disclosed in 1990 with X-linked mental retardation (Handyside et al., 1990). The initial implementation of PGT in 1992 was for cystic fibrosis, and this was for single gene deficiency (Handyside et al., 1992). The application of PGT has changed reasonably, not just from the technical perspective but also concerning its application generally(Kuliev&Verlinsky, 2005; Kuliev&Verlinsky, 2008).

PGT has also been utilized in the detection of late-onset disorders with genetic susceptibility. PGT can be done to spot embryos that match for a sibling who is affected with the need for transplantation (Fiorentino et al., 2004; Rechitsky et al., 2004; Delhanty et al., 2006; Mantzouratou et al., 2007; Renwick et al., 2007). Laws for PGT application is different amongst countries (Soini et al., 2007). PGT is controlled by HFEA in the United Kingdom. The PGT information, such as number of cycles performed for the monogenic diseases or aneuploidies with the outcomes, is gathered by the ESHRE. Many children have been conceived and delivered

globally following different types of PGT applications (Banerjee et al., 2008; Goossens et al., 2008; Nekkebroecket al., 2008; Sanders et al., 2021; Conley et al., 2020).

2.3.1. *Biopsy and Diagnosis of the Embryo*

Three phases can be used to perform the biopsy; the polar body, cleavage and blastocyst stages, respectively. Biopsy of the polar body is labour demanding since it is recommended to perform PGT using the first and the second polar bodies (Sermon et al., 2004). One disadvantage of biopsy of the polar body is the fact that the defects that are inherited paternally or other defects starting after fertilization cannot be identified (Swanson et al., 2007).

The cleavage phase embryo biopsy requires obtaining the blastomere on third day of growth after fertilization. The cleavage stage biopsy is performed at the six to eight cell and the cells in this phase are interpreted as totipotent (Vos &Steirteghem, 2001). The major issue concerning this approach has been with the use of one or two blastomeres. Therefore, evaluation has been carried out by researchers on this issue regarding the effect on how it will affect diagnosis and the growth and implant of the embryo. Currently, because there is no particular protocol to this issue, every PGTcenter makes use of their guiding rules when carrying out cleavage phase biopsy (Lewis et al., 2001; Goossens et al., 2008a; Dreesen et al., 2008). One of the disadvantages of cleavage stage biopsies is thehigher rates of mosaicism in aneuploidies.Mosaicism might particularly affect PGT in testing aneuploidies (Kokkali et al., 2007). Ultimately, blastocyst stage biopsy can be performed on the fifth- or sixth-day following fertilizationby obtaininga few cells from the layer of the trophectoderm which can give rise to the membranes of the placenta. The higher number of cells makes the diagnosis easier. The human blastocysts have an elevated chance of implantation compared to those that would be implanted after the third day, i.e., the cleavage stage (Gardner et al., 1998a; Gardner et al., 1998b; Blake et al., 2007). About forty to fifty percent of embryos mature to the phase of blastocyst by the fifth day following fertilization (Kokkali et al., 2007; McArthur et al., 2008).

Polymerase chain reaction (PCR) is utilized for the testing of disorders that are classified as monogenic disorders. The major method for the detection of abnormalities present in the chromosomes has been array comparative genomic hybridisation (aCGH) and next generation sequencing (NGS). Fluorescence *in situ*

hybridisation (FISH)was the first technique developed in PGT for aneuploidies (Handyside et al., 1992). FISH wasutilized as a screening test in embryos from individuals of increased maternal age,miscarriages, individuals with constant IVF failure as its associated with aneuploidy (Fragouli et al., 2007). FISH has a limit of low number of probes that can be utilized in the same test (Baart et al., 2007a; Baart et al., 2007b; Mantzouratou et al., 2007). Following FISH, CGH technique was developed and has brought a solution to the limit of FISH test by allowing the concurrent study of every chromosome (Wells et al., 1999). aCGH has been utilized on embryos from the third day to the blastocyst phase embryos, and also polar bodies (Wells &Delhanty, 2000; Wilton et al., 2001; Wells et al., 2002; Fragouli et al., 2006a; Fragouli et al., 2006b; Fragouli et al., 2008). aCGHhas sorted out the limit from CGH by producing fast results that can be ascertained in twenty-four hours (Hu et al., 2004; Le et al., 2006). The advantages of using NGS in PGT are the excellent precision, improved throughput, and lower costs in comparison to aCGH. It is possible to test many DNA samples at once, with results coming back in thirteen to sixteen hours. The Personal Genome Machine from Thermo-Fischer Scientific and the MiSeq from Illumina are examples of the two platforms used for PGT most frequently (Sachdev et al., 2017).

2.4. Steroidogenesis During Gametogenesis

2.4.1. *De novo Steroidogenesis*

Steroid hormones areproduced*via* new steroidogenesis in the adrenal cortex, placenta and gonads. Their tissues have the ability to utilize cholesterol to be the initiating substance for the mitochondrial production of pregnenolone. Different origins can produce cholesterol as well as *de novo* production in the endoplasmic reticulum from acetate (Rittenberg & Bloch, 1945; Bloch & Rittenberg, 1942; Little & Bloch, 1950). Esters of cholesteryl preserved in lipid drops are hydrolyzed by hydrolases. Esters of cholesterol from the LDL receptor that are received exogenously by lipoproteins and transmitted by cellular uptake or SR-BI transmitted uptake routes, as well as cholesterol present in the plasma partition(Gwynne & Strauss, 1982; Azhar&Reaven, 2002; Azhar et al., 2003; Kraemer et al., 2007).

2.4.2. *Steroidogenic Enzymes*

There are two main enzymes categories that function in the synthesis of all steroid hormones. The hydroxysteroid dehydrogenase (HSD) enzymes and cytochrome P450 (CYP). CYP group, which includesheme, activates molecular oxygen by using NADPH as asource of electron(Guengerich et al., 2001; Guengerich& Yoshimoto, 2018).

2.4.3. *Gonadal Steroidogenesis*

The synthesis ofestrogensand androgensis the focus of gonadal steroidogenesis, with progesteroneplaying a significant role in this process. The cell-specific expression order of steroid enzymes in every cell type, which is located close to the zone of the adrenal, regulates steroid outcome. The development of the hypothalamicpituitarygonadal locus during adolescence is the first step in the beginning of gonadal steroidogenesis. Pulsatile production and release of GnRH by the hypothalamus triggers the pituitary's production and release of LH. Androgens and estrogens cause the hypothalamus and pituitary to respond negatively, inhibiting LH (Handa&Weiser, 2014).

2.5. Importance of Gene Expression

The gene expression is important in controlling the level of protein production as well as the timing of the protein production. The control of gene expression is important to control the proteins to be expressed when required by cells which in turn, gives the flexibility for cells to adapt to a different environment and respond to external signals or stimuli. Gene expression also controls the structures and functions of cells. A trait that is phenotypic is an observable way to know how genes are expressed in an obvious and measurable trait. Phenotypes can be different depending on the makeup of an organism. The environment that is exposed to an organism can also influence the phenotype (Chen, 2020). There is a strong link between aneuploidies and the expression of different genes. The presence of an aneuploidy can result in different mutations such as deletions, duplications. These mutations can be located on any chromosome and it affects the level at which genes can be expressed, in such either reduced expression or over-expression (Dürrbaum&Storchová, 2016).

CHAPTER III

Materials and Methods

3.1. Study Approach and Participants

This project was carried out at Near East University DESAM Research Institute laboratory which is located in the Veterinary Medicine Faculty on the fifth floor. The ethical approval was granted by the Institutional Review Board of Near East University (YDU/2021/96-1432). The specimens used in this project was received from the British Cyprus IVF Clinic in Nicosia, Northern Cyprus which consists of 10 euploid (control groups) and 10 aneuploid (affected) human embryos. The aneuploidies were detected by an external laboratory that performed NGS on day 5 using the trophectoderm samples. In this study, the housekeeping gene (*ACTB*) which is located on chromosome 7 was utilized.

3.2. RNA Extraction

RNA samples were extracted from each embryo using the guidelines of the Hibrigen total nucleic acid isolation kit (Istanbul, Turkey).

3.3. Complementary DNA (cDNA) synthesis

The RNA samples were converted into cDNAs using the Hibrigen cDNA synthesis kit (Istanbul, Turkey) following manufacturer's guidelines. Then, ThermofisherScientific NanoDrop® ND-1000 UV-Vis Spectrophotometerwas used to measure the concentration of the cDNA samples. The quality of the cDNA samples was checked by using the pipette to take 1µl of cDNA samples and placing them on the nanodrop machine. Water was used for normalization. The machine displays the cDNA quality and concentrations on a system connected to it.

3.4. Real-time PCR reaction

Hibrigen SYBR-Green mix (Istanbul, Turkey) (5 µl), *CYP11A1* primers (0.5 µM final concentration) were used for the preparation of the master mix. The primer sequences and the melting temperatures are listed in table 1. Then, 6 µl of the mix was transferred into the tubes, and 2 µl of cDNA was transferred into each tube. The final volume of 10 µl was achieved by addition of nuclease free water. The negative control for the reaction was carried out in the absence of any cDNA sample.

Different annealing temperatures and primer concentrations were used in this study (Table 2). The finalized real-time PCR conditions are listed in table 3.

Table 1.

Information for the Primers

Gene	**Forward sequence**	**Reverse sequence**	**Tm(°C)**
CYP11A1	AAGTGTTCACCACGATTACCG	CAGCATATCCTGCACCTTCA	52

Table 2.

Summary Table forOptimization

Genes	**Forward and reverse primer final concentration (µM)**	**cDNA (µl)**	**Annealing temperature°C/time**
CYP11A1	0.2	1	52°C/20 seconds
CYP11A1	0.1	1	57°C/20 seconds

Table 3.

Optimized PCR Conditions

PCR steps	**Temperature°C/time**	**Cycles**
Initial denaturation	95°C/ 10 minutes	1
Denaturation	95°C/ 10 seconds	40
Annealing	58°C/20 seconds	
Elongation	72°C/30 seconds	
High resolution melting analysis		1

3.5. Data Analysis

Cycle of threshold (CT) values were obtained from the software system of the real time PCR equipment Insta Q96™ Real Time Machine. The delta-delta

CTanalysis was utilized for the statistical analysis. PASW Statistics 18 software was used to derive the p-values.

CHAPTER IV

Results

4.1. The Size of the Samples

In this study, 10 control and 10 aneuploid human embryo samples were utilized to analyse the levels of *CYP11A1 gene* expression. The blastocyst qualities and the NGS result following trophectoderm biopsy on day 5 or 6 post fertilization are shown in table 4.The RNA was successfully obtained from all the samples and cDNA synthesis was performed successfully (Table 5).

Table 4.

Embryo Details

Sample ID	**Quality of Embryos**	**Embryo Day**	**Detected Abnormalities for the Embryos**
1	4BB	5	+7, +8p, +14, +18, +22
2	3AA	5	-10
3	4BB	5	-11q
4	4AA	5	-9q
5	4AA	6	-19
6	4CC	6	-20
7	3CC	6	-4, -14
8	5AA	5	-17
9	5BB	5	X
10	6CB	6	X
11	6BB	6	EUPLOID
12	5AA	5	EUPLOID
13	5BB	5	EUPLOID
14	4AB	5	EUPLOID
15	5BC	6	EUPLOID
16	5AB	6	EUPLOID
17	5BB	5	EUPLOID
18	5AB	5	EUPLOID
19	4AA	5	EUPLOID
20	5BA	5	EUPLOID

Table 5.

Concentration and Absorbance Information of cDNA Samples Used

Sample ID	**260/280**	**Concentration (ng/ µl)**
1	1.43	189.1
2	1.40	202.9
3	1.43	191.1
4	1.39	178.9
5	1.43	180.5
6	1.44	192.2
7	1.43	221.7
8	1.43	208.9
9	1.43	197.3
10	1.44	190.2
11	1.43	230.0
12	1.42	193.1
13	1.43	181.6
14	1.41	191.0
15	1.43	201.8
16	1.45	191.6
17	1.42	194.7
18	1.42	189.7
19	1.45	197.0
20	1.43	212.0

4.2. Analysis

Five out of the ten aneuploid samples did not show any amplification, and seven of the control samples also did not show any amplification. The CT values for the rest of the samples are listed in table 6. The ΔCt, ΔΔCt and 2^-(ΔΔCt) were calculatedfor each sample following normalization with the housekeeping gene *ACTB*. The data from 2^-(ΔΔCt) was used for the statistical analysis. The non-parametric test (Mann-Whitney U test) was used to calculate the p-value. There was no significant change in the level of expression of *CYP11A1* gene in the euploid and aneuploid samples (0.881).

Table 6.

Delta-Delta CT Analysis of the CYP11A1 Gene Normalized Against ACTBin theAneuploid and EuploidHuman Embryos.

Samples	***CYP11* CT**	***ACTB* CT**	**ΔCt**	**ΔΔCt**	**2^-(ΔΔCt)**
Control 1	29.9	23.60	6.30	-3.30	9.85
Control 2	35	22.00	13.00	3.40	0.09
Control 3	34.7	25.20	9.50	-0.10	1.07
Aneuploid 1	30.5	22.10	8.40	-1.20	2.30
Aneuploid 2	36.2	22.90	13.30	3.70	0.08
Aneuploid 3	33.1	24.90	8.20	-1.40	2.64
Aneuploid 4	32	23.30	8.70	-0.90	1.87
Aneuploid 5	33.9	26.40	7.50	-2.10	4.29

CHAPTER V

Discussion

This study was performed to analyse the levels of expression of genes that take part in the steroidogenesis pathway in euploid as well as aneuploid human embryos, and to elucidate the function of genes involved in the steroidogenesis in aneuploidies. Various studies have been conducted on mouse, oocytes, zebra fish and many more but this is the first study to be performed on human embryo samples, which was performed to analyse the gene expression levels of genes involved in the steroidogenesis.

The results of this study showed that *CYP11A1* gene is expressed in both euploid and aneuploid human embryos.The level of *CYP11A1* gene expression was investigated following normalization with the housekeeping gene, *ACTB*. The p-value (0.881) showed that there is no statistical significance between the expression levels of *CYP11A1* in euploid and aneuploid embryos, though there was a slight downregulation of *CYP11A1* in the aneuploid group which is still statistically insignificant. Due to the low sample size coupled with the fact that not all the samples showed amplification successfully, there was a high standard deviation.

This study is important to understand the correlation between gene expression levels in human embryo samples of *CYP11A1* gene. There is a possibility that the genes involved in the steroid metabolism may also have roles in nondisjunction, thus causing the formation of aneuploidies during gametogenesis. On the contrary, it is a possibility that the level of expression of these genes may be affected due to nondisjunction. These genes are located on chromosomes, and they can be translocated, deleted or duplicated which can lead to some form of aneuploidies and even other types of genetic diseases. *CYP11A1* is positioned on a chromosome which is associated with neurodevelopmental disorders such as autism, dyslexia, trisomy 15 syndrome, Angelman syndrome and Prader-Willi syndrome (Sieg& Karl, 1990). Several studies have been carried out on genes involved in the steroidogenesis and its effects till date. One of such study was carried out on *CYP11* link with hyperandrogenism and polycystic ovary syndrome (PCOS). The information demonstrated that the *CYP19* might be disregarded as an important risk factor for the expression of male pattern baldness and PCOS. On the other hand, both of these are associated with anomalies with *CYP11A1*. The findings show that*CYP11A1*allelic

variations facilitate the emergence of hyper-androgenemia, which is connected to hirsutism and PCOS (Neda et. al., 1997). Another study that was carried out using placenta samples showed that the level of *CYP11A1* gene expression is linked with the risk of preeclampsia (Daniel et al., 2008).In mouse samples, the promoter region of *CYP11A1* was analysed and found to be overexpressed in Leydig cells (Payne et al., 1992).Genes involved in the steroidogenesis can also be linked with cancers. This can happen when there is a downregulation or upregulation of any of the genes involved in steroidogenesis. Studies have associated genes involved in the steroidogenesis to certain types of cancers(Kathryn et al., 2010; Kumazawa et al., 2004).In order to estimate the blastocyst implantation competency, a study on the gene expression in human trophectoderm biopsies was conducted. The level of gene expression and the clinical pregnancy outcome were associated. After RNA sequencing, it was discovered that the cells from the blastocysts implanted in comparison to blastocysts with failed implantation expressed a total of forty-seven transcripts with notable difference. Thirty-six out of the forty-seven transcripts of the blastocysts with implantation failure had considerably lower levels including *CYP11A1* and *HSD17B1*. The other transcripts, including *KHDC1P1* and *BAK1* which were always present in the blastocysts with implantation failure but lacking in all the blastocysts that resulted in clinical pregnancy were all highly elevated. Pathways related in steroidogenic events were discovered through the analysis of differentially regulated RNAs (Ntostis et al., 2019).Thus, it is a possibility that if the number of samples used in this study is increased, there will be a significant difference in the level of *CYP11A1* expression in aneuploid and euploid samples.

CHAPTER VI

Conclusion

Aneuploidies can occur due to errors during recombination, replication and even due to non-disjunctions which can result in Down syndrome, Patau syndrome, Edward's syndrome and many more.

Genes involved in the steroidogenesis can be crucial in relation to aneuploidies due to the fact that they can be deleted or duplicated. Inversion, insertion or translocation in genes involved in the steroidogenesis can lead to birth of children with aneuploidies even if the parents have normal phenotypes.

The analysis for this study has indicated that there is no statistical significance in the expression levels of *CYP11A1* in euploidhuman embryos compared to the aneuploid human embryo samples (p-value=0.881). Therefore, the outcome of this study shows that the gene expression levels of *CYP11A1* is similar in euploid and aneuploid human embryos.

Future studies will include analysis of *CYP11A1* gene expression in more samples, both euploid and aneuploid. Furthermore, other genes involved in the steroidogenesis pathway will be investigated.

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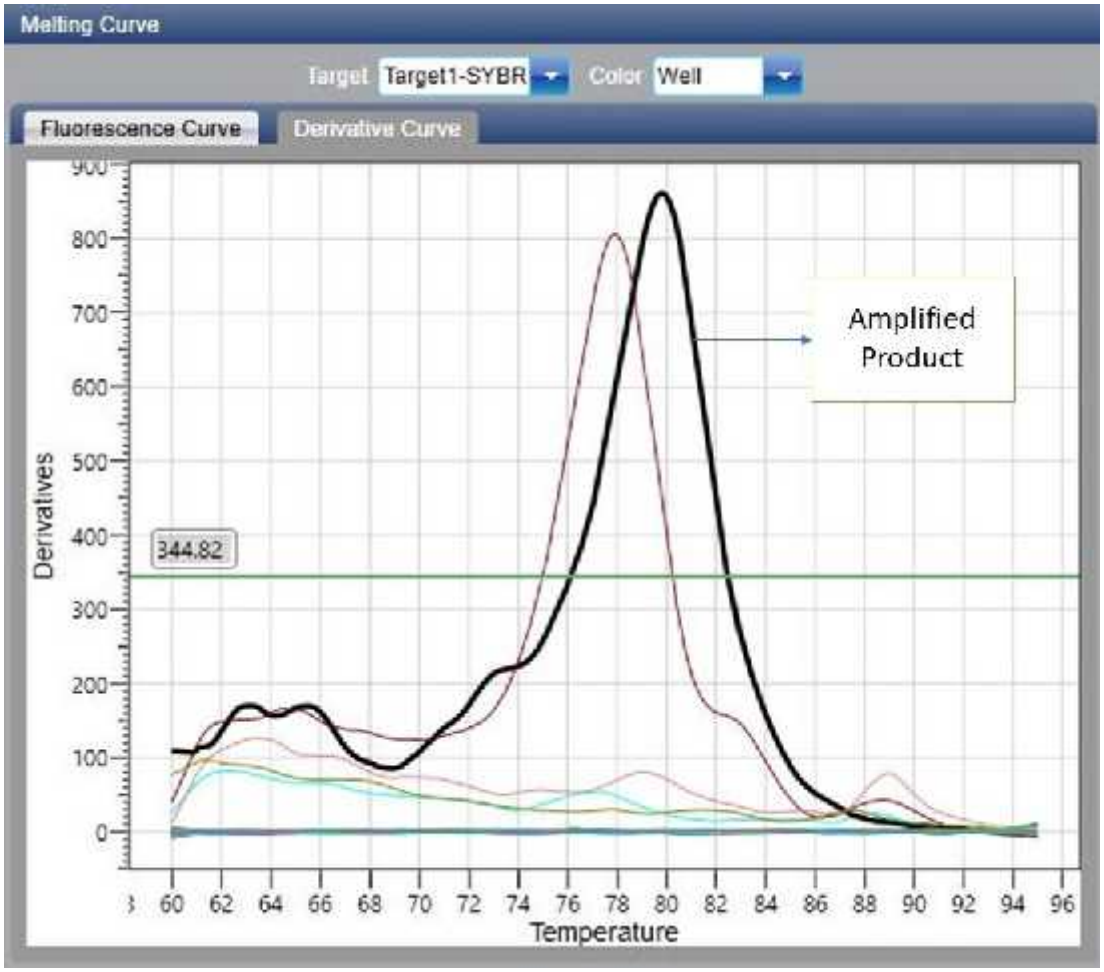
APPENDICES

Appendix A

The figure shown below is from the melting curve analysis taken from the real-time PCR system with primer dimer formation.

Appendix A Figure 1. Real time PCR result showing the melting curve analysis for *CYP11A1* gene expression.



Appendix X

Turnitin Similarity Report

Thesis

ORİJİNALLİK RAPORU

%12	%10	%9	%
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

1	discovery.ucl.ac.uk İnternet Kaynağı	%2
2	scholar.sun.ac.za İnternet Kaynağı	%1
3	Alan H. Handyside. "Molecular origin of female meiotic aneuploidies", Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 2012 Yayın	%1
4	www.ncbi.nlm.nih.gov İnternet Kaynağı	%1
5	hdl.handle.net İnternet Kaynağı	<%1
6	s3.amazonaws.com İnternet Kaynağı	<%1
7	www.frontiersin.org İnternet Kaynağı	<%1
8	iraqijms.com İnternet Kaynağı	<%1

9	coek.info İnternet Kaynağı	<%1
10	www.ijdr.in İnternet Kaynağı	<%1
11	J. Ling. "Evaluation of genome coverage and fidelity of multiple displacement amplification from single cells by SNP Array", Molecular Human Reproduction, 08/11/2009 Yayın	<%1
12	ddd.uab.cat İnternet Kaynağı	<%1
13	research.birmingham.ac.uk İnternet Kaynağı	<%1
14	acronyms.thefreedictionary.com İnternet Kaynağı	<%1
15	rep.bioscientifica.com İnternet Kaynağı	<%1
16	era.ed.ac.uk İnternet Kaynağı	<%1
17	Kirstine Kirkegaard, Thomas F. Dyrlund, Hans Jakob Ingerslev. " Clinical Application of Methods to Select Fertilized Embryos ", Wiley, 2016 Yayın	<%1

18	Crystal Chan, Michelle Ryu, Rhonda Zwingerman. "Preimplantation Genetic Testing for Aneuploidy: A Canadian Fertility and Andrology Society Guideline", Reproductive BioMedicine Online, 2020 Yayın	<%1
19	H. Kang, P. M. Lieberman. "Cell Cycle Control of Kaposi's Sarcoma-Associated Herpesvirus Latency Transcription by CTCF-Cohesin Interactions", Journal of Virology, 2009 Yayın	<%1
20	Zhihong Yang, John Zhang, Shala A Salem, Xiaohong Liu, Yanping Kuang, Rifaat D Salem, Jiaen Liu. "Selection of competent blastocysts for transfer by combining time-lapse monitoring and array CGH testing for patients undergoing preimplantation genetic screening: a prospective study with sibling oocytes", BMC Medical Genomics, 2014 Yayın	<%1
21	epdf.pub İnternet Kaynağı	<%1
22	kar.kent.ac.uk İnternet Kaynağı	<%1
23	scholarscompass.vcu.edu İnternet Kaynağı	<%1

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24	İnternet Kaynağı	<%1
25	Brozic, P.. "Phytoestrogens as inhibitors of the human progesterone metabolizing enzyme AKR1C1", Molecular and Cellular Endocrinology, 20061019 Yayın	<%1
26	Dicka Ar Rahim, Wei Fang, Gaojun Zhu, Haryo Wibowo, Dwi Hantoko, Qing Hu, Herri Susanto, Zengliang Gao, Mi Yan. "Microwave-assisted synthesis of Zn-Fe adsorbent supported on alumina: Effect of Zn to Fe ratio on syngas desulfurization performance", Chemical Engineering and Processing - Process Intensification, 2021 Yayın	<%1
27	academic.oup.com İnternet Kaynağı	<%1
28	bioone.org İnternet Kaynağı	<%1
29	edepot.wur.nl İnternet Kaynağı	<%1
30	theses.hal.science İnternet Kaynağı	<%1
31	www.hindawi.com İnternet Kaynağı	<%1

32	"Abstract book of the 30 th ESHRE Annual Meeting, Munich, Germany, 29 June – 2 July 2014", Human Reproduction, 2014. Yayın	<%1
33	"Invited abstracts", Chromosome Research, 2007 Yayın	<%1
34	Anita H. Payne, Dale B. Hales. "Overview of Steroidogenic Enzymes in the Pathway from Cholesterol to Active Steroid Hormones", Endocrine Reviews, 2004 Yayın	<%1
35	D. Masson. "Inhibition of progesterone production in human luteinized granulosa cells treated with LXR agonists", Molecular Human Reproduction, 04/12/2007 Yayın	<%1
36	F. Peter Guengerich, Francis K. Yoshimoto. "Formation and Cleavage of C–C Bonds by Enzymatic Oxidation–Reduction Reactions", Chemical Reviews, 2018 Yayın	<%1
37	Naoki Watanabe, Shinji Mii, Naoya Asai, Masato Asai et al. "The REV7 Subunit of DNA Polymerase ζ Is Essential for Primordial Germ Cell Maintenance in the Mouse", Journal of Biological Chemistry, 2013 Yayın	<%1

38	Nidhee M. Sachdev, Susan M. Maxwell, Andria G. Besser, James A. Grifo. "Diagnosis and clinical management of embryonic mosaicism", Fertility and Sterility, 2017 Yayın	<%1
39	Webster, Alexandre, and Melina Schuh. "Mechanisms of Aneuploidy in Human Eggs", Trends in Cell Biology, 2016. Yayın	<%1
40	acikbilim.yok.gov.tr İnternet Kaynağı	<%1
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42	medicinescience.org İnternet Kaynağı	<%1
43	ndl.ethernet.edu.et İnternet Kaynağı	<%1
44	patents.google.com İnternet Kaynağı	<%1
45	pt.scribd.com İnternet Kaynağı	<%1
46	qmro.qmul.ac.uk İnternet Kaynağı	<%1
47	www.nature.com İnternet Kaynağı	<%1

48	www.pubfacts.com İnternet Kaynağı	<%1
49	"Preimplantation Genetic Diagnosis", Wiley, 2001 Yayın	<%1
50	&NA;. "Current World Literature", Current Opinion in Obstetrics & Gynecology, 04/2005 Yayın	<%1
51	Chalida Sri-in, Aunyaratana Thontiravong, Lyric C. Bartholomay, Sonthaya Tiawsirisup. "Effects of Aedes aegypti salivary protein on duck Tembusu virus replication and transmission in salivary glands", Acta Tropica, 2022 Yayın	<%1
52	Lina Schiffer, Lise Barnard, Elizabeth S. Baranowski, Lorna C. Gilligan et al. "Human steroid biosynthesis, metabolism and excretion are differentially reflected by serum and urine steroid metabolomes: A comprehensive review", The Journal of Steroid Biochemistry and Molecular Biology, 2019 Yayın	<%1

YAKIN DOĞU ÜNİVERSİTESİ
BİLİMSEL ARAŞTIRMALAR ETİK KURULU

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi :28.10.2021

Toplantı No : 2021/96

Proje No :1432

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
Bilimsel Araştırmalar Etik Kurulu Başkanı

Kurul Üyesi	Toplantıya Katılım	Karar
	Katıldı(✓)/ Katılmadı(X)	*Onay(✓)/ Ret(X)*
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Prof. Dr. Şahan Saygı	✓	✓
Prof. Dr. Nurhan Bayraktar	✓	✓
Prof. Dr. Mehmet Özmenoğlu	X	—
Prof. Dr. İlker Etikan	X	—
Doç. Dr. Mehtap Tınazlı	✓	✓
Doç. Dr. Nilüfer Galip Çelik	✓	✓
Doç. Dr. Emil Mammadov	✓	✓
Doç. Dr. Ali Cenk Özay	✓	✓

CV

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