TURKISH REPUBLIC OF NORTHERN CYPRUS

NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDİES

The gene expression profile of WNT/β-catenin pathway genes in spontaneous abortion materials

Elif Gülseren

Master of Science in Molecular Medicine

Thesis Advisors:

Assoc. Prof. Mahmut Çerkez Ergören

Nicosia, 2022

TURKISH REPUBLIC OF NORTHERN CYPRUS

NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDİES

The gene expression profile of WNT/β-catenin pathway genes in spontaneous abortion materials

Elif Gülseren

Master of Science in Molecular Medicine

Thesis Advisors:

Assoc. Prof. Mahmut Çerkez Ergören

Nicosia, 2022

APPROVAL

We certify that we have read the thesis submitted by **'The gene expression profile of WNT/β-catenin pathway genes in spontaneous abortion materials'** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Health Sciences.

Examining Committee	Name-Surname	Signature
Committee Member (Supervisor):	Assoc. Prof. Mahmut Cerkez Ergoren	
Committee Member:	Dr. Gulten Tuncel Dereboylu	
Committee Member:	Asist. Prof. Özel Yuruker	

Approved by the Head of the Department

…../…../20…

……

Prof. Dr. Selma Yılmaz

Head of Department

Approved by the Institute of Graduate Studies

…../…../20…

Prof. Dr. Kemal Hüsnü Can Başer

Head of the Institute

THE ETHICAL APPROVAL FOR MASTER'S PROJECT

YAKIN DOĞU ÜNİVERSİTESİ
BİLİMSEL ARAŞTIRMALAR ETİK KURULU

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi :25.11.2021
Toplantı No : 2021/97
Proje No :1449

Yakın Doğu Üniversitesi Tıp Fakültesi öğretim üyelerinden Doç. Dr. Mahmut Cerkez Ergoren'in sorumlu araştırmacısı olduğu, YDU/2021/97-1449 proje numaralı ve **"The gene expression profile of WNT/ β-catenin pathway genes in spontaneous abortion materials"** 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)*
Prof. Dr. Tamer Yılmaz	✓	✓
Prof. Dr. Şahan Saygı	✓	✓
Prof. Dr. Nurhan Bayraktar	✓	✓
Prof. Dr. Mehmet Özmenoğlu	✓	✓
Prof. Dr. İlker Etikan	✓	✓
Doç. Dr. Mehtap Tınazlı	✓	✓
Doç. Dr. Nilüfer Galip Çelik	✓	✓
Doç. Dr. Emil Mammadov	✓	✓
Doç. Dr. Ali Cenk Özay	✓	✓

DECLERATION

I declare that I have no unethical behaviour in all stages from the time I started the laboratory work of the thesis to my writing of the thesis itself, I have gained all the information in this thesis within the academic and ethical guidelines, and I have involved all the information and details in the version and have added these citations to the references part. I hereby have not violated my patents and copyrights while studying and writing this letter.

Elif Gülseren
Signature

The gene expression profile of WNT/β-catenin pathway genes in spontaneous abortion materials

TABLE OF CONTENTS:

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATION

CTNNB1: B-catenin 1 gene

GSK3B: Glycogen synthase kinase-3 beta gene

DVL1: Dishevelled Segment Polarity Protein 1 gene

µl Microliter

ng/µl: Nano gram/ Microliter

β: Beta

γ: Gamma

DNA: Deoxyribonucleic acid

RNA: Ribonucleic acid

PCR: Polymerase chain reaction

PCOS: polycystic ovaries syndrome

F: forward

R: reverse

LH: Luteinizing Hormone

FSH: Follicle Stimulating Hormone

AMH: Anti Mullerian Hormone

NC: Negative control

Ct: Cycle threshold

NA: Nucleic acid

Wnt: Wingless+Int-1

Fz: Frizzled

Dsh: disheveled

DNA: Deoxyribonucleic acid

RNA: Ribonucleic acid

Cdk3: Cyclin-dependent kinase 3

IVF: Invitro fertilization

ZGA: Zygotic genome activation

B: Beta

ACOG: American College of Obstetrics and Gynaecology

HLA: Human Leukocyte Antigen

APC: Adenomatous Polyposis Coli

GSK3: Glycogen synthase kinase 3

CK1α: Casein Kinase 1 Alpha

LRP5: Low Density Lipoprotein Receptor Related Protein 5

PCP: Polarity Cell Pathway

DAAM1: Dishevelled Associated Activator of Morphogenesis 1

CGM: Cyclic Guanosine Monophosphate

JNK: Jun N-Terminal Kinase

Wnt 3: Wingless 3 family member

Wnt 10B: Wingless 10B family member

Wnt 1: Wingless 1 family member

Wnt 2: Wingless 2 family member

Wnt 4: Wingless 4 family member

Wnt 5A: Wingless 5A family member

Wnt 5B: Wingless 5B family member

Wnt 6: Wingless 6 family member

Wnt 7: Wingless 7 family member

RSA: Recurrent spontaneous abortion

URSA: Unexpected Recurrent Spontaneous Abortion

cDNA: complimentary Deoxyribonucleic acid

bp: Base pair

EDTA: Ethylene Diamine Tetra-acetic Acid

TAE: Tris Acetic Acid EDTA

ml: Milliliter

mg: Milligram

µl: Microliter

PCR: Polymerase Chain Reaction

µm: Micro Molar

qRT: Quantittive Real Time

dH_2O: Deionized Water

Ct: Cycle threshold

DW: distilled water

ACKNOWLEDGMENT

I would like to give my sincere thanks to my mentor Assoc. Professor Mahmut Cerkez Ergören, for his continued support, patience, enthusiasm and great knowledge. His guidance helped me through all stages of working and writing this thesis. I would like to thank my other teacher Dr. Gülten Tuncel. Their guidance helped me through all stages of working and writing this thesis. I never thought that I would have such good advisers who are very good in every way and will be my guide. Thank you for everything.

It is my privilege to thank and thank the support of my family and friends for their generous care and encouragement throughout the study. I would like to especially thank my parents and my siblings have always supported me in achieving my goals and dreams, without them it would have been very difficult for me to continue in this profession that I am passionate about. I would like to thank my very valuable friends from my business and school life, Cyprus and Turkey, who have always been my support and morale throughout my graduate education. I would like to thank all my teachers who have given me this knowledge, experience and confidence throughout my life. And finally, I would like to thank my dear family and the Ministry of National Education of the Turkish Republic of Northern Cyprus for their financial support.

ABSTRACT

The gene expression profile of WNT/β-catenin pathway genes in spontaneous abortion materials

Elif Gülseren

Master of Science in Molecular Medicine

ADVISOR: Assoc. Prof. Mahmut Çerkez Ergören

AIM:

The most important aim of this study was to examine the expression of WNT/β-catenin signaling pathway genes (β-catenin, GSK3B, and DVL1) in spontaneous abortion material, that are suspected of having a foremost impact on embryonic development, early pregnancy, and implantation failure.

BACKGROUND:

One of the most important conventional and important signaling pathway that regulates embryonic migration, cell fate determination, and cell proliferation is the Wnt/β-catenin signaling pathway. It is one of the major signaling pathways in the cell. This WNT/β-catenin signaling play a vital role in regulating many processes during the development along with growth of all organisms and uses autocrine or paracrine communication in cell communication. It is involved in many important and different processes, as well as in the management of embryonic development, cell polarity, cell migration, cell fate and proliferation. It is thought that the etiology of several diseases is caused by disruptions in genes encoding proteins in the signaling pathway and, accordingly, activation problems in the Wnt/β-catenin signaling pathway. Studies have shown that abnormalities in these genes can lead to a variety of diseases, and genes in this signaling pathway may be associated with miscarriage.In previous studies, the Wnt/β-catenin signaling was required for evolutionary enhancement including preimplantation development, blastocyst transplantation, and cell proliferation and differentiation. Although many studies have shown that problems with these genes can cause miscarriage, most studies have been done in mice or dams. This study used placental samples from pregnancies that ended in miscarriage.

MATERIAL AND METHODS:

Aborted fetus samples with 8 abnormal karyotypes and 15 normal karyotypes obtained from the Near East University Hospital Medical Genetics Laboratory were analyzed. RNA was isolated from these 23 tissue samples using the Hybrigen Genomic RNA isolation kit in accordance with the manufacturer's directions. After samples isolated, they have been measured with a Thermo Scientific Nanodrop Microvolume spectrophotometer. cDNA synthesis has been performed according to the producer's directions using the abm cDNA kit. Expression of these three genes was analyzed by RT-PCR using SYBR green qPCR mastermix.

RESULTS:

Three of Wnt/β-Catenin signal pathway genes *DVL1, GSK3B* and *β-catenin* examined in spontaneous abortion samples with normal and abnormal karyotype, has been shown that GSK3B was down-regulated in the abnormal karyotype, DVL1 did not show any significant difference on the abnormal and normal karyotype, CTNNB1' was upregulated in the abnormal karyotype.

CONCLUSION:

This study showed, when samples of spontaneous abortions with normal and abnormal karyotype were compared for Wnt/β-Catenin signal pathway genes *DVL1, GSK3B* and *β-catenin*, it was observed that DVL did not change, but GSK3β was observed to be down-regulated in abnormal karyotype, it may be important information that this gene differs in abnormal and normal karyotype, a more detailed study with more samples is required for more precise results. It is expected that *β-catenin* is upregulated while GSK3β is down-regulated because GSK3β acts as a *β-catenin* degradation complex in the cell. When *β-catenin* is high at cell , GSK3β should not work or work less. So, what does the upregulation of *β-catenin* in the abnormal karyotype indicate, namely, that as described earlier, *β-catenin* shows an crucial role in embryonic development. It is already known that the probability of miscarriage is high in the abnormal karyotype, downregulation of B-catenin in the normal karyotype may result in a high probability of miscarriage in the normal karyotype.

KEYWORDS: Wnt/β-Catenin signal pathway, Spontaneous abortion, B-catenin , Wnt Genes, Aborted fetus

CHAPTER I: INTRODUCTION

1.1 INTRODUCTION

Spontaneous abortion can say miscarriage too, can define as the loss of the baby from natural causes before the twentieth week. As seen in Figure 1.1, causes of miscarriage could be genetic, infections, anatomic, hormonal reasons can be counted. The reason for almost 50% of these reasons is still unexplained. Clarifying this 50% will reduce the problem of miscarriage to very low levels in the future, which is why this research is of importance. Most miscarriages happen spontaneously, but often do not recur. Therefore, it is estimated that more than birth-carrying pregnancies are lost spontaneously (Sirait *et al.*, 2022). It has also been said that 50%–60% spontaneously aborted manufactured from thought have chromosomal anomalies. Spontaneous fetal aneuploidy is the most commonplace cause for spontaneous loss, specially inside the first trimester of being pregnant (Hyde and Schust, 2015).

Wnt/β-catenin pathway and associated genes have role in early pregnancy and implantation failure. In reproductive biology, the early phase of embryogenesis is a complex and important cell-remodeling activity is happened. Gene targeting and β-catenin activation assay in mice demonstrated that not only β-catenin (*Ctnnb1*) and several Wnts, including Wnt4, Wnt6, Dkk1, Wnt5a, and Wnt7a are required for various developmental processes including pre-implantation development, implantation of blastocyst but also required for Decidualization of the endometrium, cell proliferation, and differentiation. In gametes and embryos, all of the main elements of the WNT signaling pathway are expressed (Knöfler and Pollheimer, 2013).

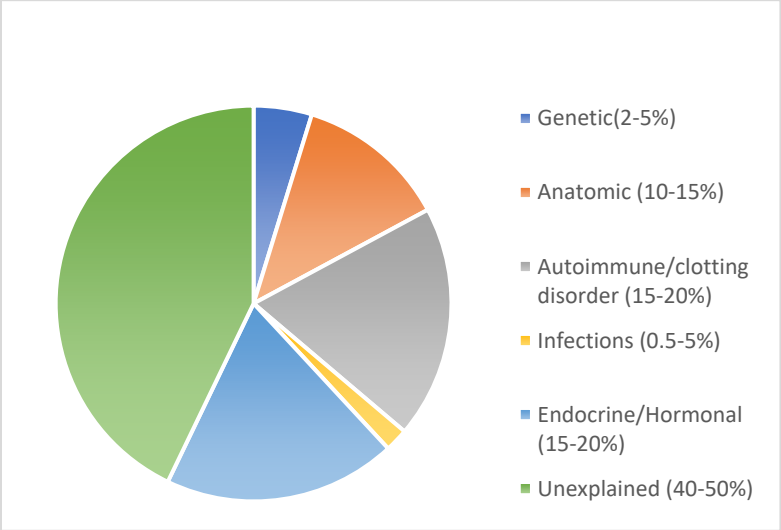

Figure 1.1: Causes of pregnancy lost

1.2 CELL DIVISION

Cells perform cell division as a result of a series of events that take place in order to produce a duplicate cell identical to themselves. As a result of the cell replication and division, two different cells are formed. Every cycle in which duplication and division takes place is called the cell cycle, the mechanism necessary for the reproduction of living organisms (Israels and Israels, 2000). Cell division differs from living-to-living thing. For example, in living creatures such as bacteria and yeast, every cell division creates a brand-new living thing, while in multicellular organisms, cell division is a necessary event for the proliferation of cells and the transfer of genetic information. Cell division is a part of the cell cycle, which is a large and important process in humans, and cell division, which can be of two different types, mitosis and meiosis (I and II), takes role in many important tasks.

1.2.1 MITOSIS

The eukaryotic cell cycle includes 4 phases. Two important events in the cell cycle is nuclear division, called mitosis, and cytokinesis, in which the cell divides into two. These two processes are called the M phase. Before this phase, there are G1, S, G2 phases. The phase between the M phase and the next, which includes G1, S, and G2 phases, in which the cell prepares to divide, is called the interphase. The cell copies the DNA at the S phase (S=Synthesis). Before and after the S phase, two phases have to do, named gap phases called G1 and G2, and the cell continues to grow in these phases. At certain points of G1 and G2, the "control points" decide whether the cell will continue to the next phase or stop to prepare again when it encounters any problems. During interphase, the cell mostly go on to transcribe genes, produce proteins, and increase cell mass. This copying and replication process is important so that the cell does not shrink with each division and continues to exist without shrinking or diminishing.

During prophase, paired chromatids shorten and thicken to become chromosomes. Centrosomes begin to produce spindle fibers. In the prometaphase phase, the nuclear envelope disappears, the chromosomes attach to the spindle fibers and become ready to move because of their kinetochores. In metaphase, the chromosomes fall into line at the equator, and sister chromatids are held by kinetochores by two opposite centrosomes. In anaphase, sister chromatids begin to pull to opposite poles thanks to the spindle fibers, and meanwhile, the

nuclear membrane begins to form again. In telophase, the contractile ring begins to form, the cytogenesis of which will allow the cell to divide into two. In the last stage of cytokinesis, actin and myosin filaments function in the contractile ring and two different cells with nuclei are formed.

1.2.2 MEIOSIS

Meiosis is a type of cell division in which a haploid cell is formed from a diploid cell. It is essential for the production of gametes such as eggs and sperm in sexual reproduction in eukaryotes and haploid cells. The decrease in the number of chromosomes from diploid to haploid in meiosis is important for preventing the doubled chromosome number during fertilization from increasing from generation to generation and keeping it stable. Meiosis contributes to diversity with the events of recombination and chromosome separation (Ohkura, 2015).

Meiosis involves of two stages called Meiosis 1 and Meiosis 2. Meiosis 1 consists of 4 stages while Meiosis 2 consists of 4 stages. Prophase 1 in meiosis 1 begins with the shortening and thickening of the chromosomes. Prophase 1 is divided into 5 stages with uncertain boundaries. These stages are the Leptoten stage where the chromosomes begin to appear, the Zygotene stage where the tetrad (4 chromatids, that is, the structure formed by the combination of two chromosomes) is formed, the Pachytene stage, where the exchange of genetic material between the tetrads (crossing-over), the Diploten stage, where homologous chromosomes begin to separate, the chromosomes take their final form (Bolcun-Filas and Handel, 2018). It consists of the Diakines phase in which the nuclear membrane is destroyed, in which the nucleolus it receives is lost. In Metaphase 1, which takes place after Prophase I, homologous chromosomes are fall into line on the equatorial plane. In next phase, Anaphase I, the chromosomes separate from each other and move towards opposite poles. Diversity is achieved by the random separation of chromosomes that adhere to each other in Prophase I in Anaphase I (Baudat, Imai and De Massy, 2013). In Telophase I, which is the last stage of meiosis I, the chromosomes that divide into two poles begin to elongate and get thinner, a nuclear membrane forms around it, and two separate daughter cells are formed by the cleavage of the cytoplasm by cleavage in the

middle. So far it is called Meiosis I. Meiosis II begins with the onset of Prophase II. In prophase II, the nuclear membrane breaks down and spindle fibers begin to form. While the chromosomes line up at the equator in Metaphase II, sister chromatids separate to opposite poles in Anaphase II, and the irregularity of sister chromatids at this stage ensures genetic diversity. In the last phase, Telophase II, the helix of the chromosomes opens, and they become invisible, the nuclear membranes form and the cytoplasm divides. Thus, as a result, 4 cells are formed from I cell.

1.2.2.1 MEIOTIC RECOMBINATION

Meiotic recombination occurs during meiosis during spermatogenesis and oogenesis, expressed as the swap of DNA between the parent's chromosomes and helps the chromosomes to segregate properly (Kauppi, Jeffreys and Keeney, 2004). In the Anaphase 1 phase of meiosis, a 4-stranded structure known as tetrad occurs, exchange of parts in homologous chromosomes occurs between two separate tetrads (Hunter, 2015). The exchange of parts among these homologous chromosomes occurs by breaking the double strand in the DNA sequence and re-gluing after the change. By this recombination event, genetic material is mixed among homologous chromosomes, providing a large amount of the genetic variation that makes it appear to differ between individuals, even within families. Although double-chain breakage occurs during recombination, recombination sites usually do not cause mutations because the recombination process is a highly efficient one.

The frequency of recombination among two loci on a chromosome depends on the physical distance among them. The genetic closeness of the two loci is evaluated by the percent recombination. Comparing genetic and physical distances and measuring the frequency of recombination between different chromosome regions is possible thanks to the human genome sequence. The recombination ratio is higher in certain regions of the chromosomes, for example the shorter arms of the chromosomes and the distal regions of the arms have been shown to have a higher average recombination rate (Broman *et al.*, 1998). In addition, it has been shown that the recombination rate in women is 1.6 times higher than in men. Females have more recombination at centromeres, while males have higher recombination at telomeres (Broman *et al.*, 1998).

1.2.2.2 ERRORS DURING MEIOSIS

Chromosomal duplication, pairing, and separation are constantly repeated throughout the cellular divisions, which are of great importance and aim at the transfer of genetic material. Errors that occur in these processes can cause errors in fertilization and embryogenesis, causing miscarriage, as well as vital or nonvital diseases (Magli, Gianaroli and Ferraretti, 2001). After meiosis, it is important that the cells have half the amount of DNA, that is, the cells are haploid. Because when the egg and sperm unite, that is, after fertilization, the number of chromosomes must be neither missing nor more so for the living thing to continue its life in a healthy way. Excess or lack of chromosome number causes several diseases. The most widespread cause of spontaneous abortions in the first trimester are genetic defects, particularly chromosomal abnormalities. It has previously been proven that chromosomal abnormalities occur in approximately 60% of miscarriages (Hassold, 1980).

Inherited disorders can occur when chromosomes abnormally behave during meiosis. Chromosomal disorders can be examined in two categories. Abnormalities in chromosome number and abnormalities in chromosome structure. Chromosomal disorders are characteristically dynamic and can often be fatal. Karyotype is a method by which chromosomal abnormalities can be detected from a single cell. Cells (such as white blood cells) collected from a person's blood or other tissue are used to observe a person's karyotype. The isolated cells are first stimulated to begin dividing, and then chemicals are applied to stop cell division during metaphase. Then the chromosomes are made visible by staining and examined using a bright field microscope. Chromosomes are identified by looking at the sizes of the chromosomes, the centromere positions and the bands of the stained parts. While the karyogram basically shows genetic abnormalities with more or less chromosomes per cell, problems in the genetic material such as deletion and translocation can be detected by looking at the bands. Chromosomal abnormalities are divided into two as abnormalities in chromosome number and chromosome structure. Examples of diseases caused by abnormalities in the number of chromosomes are Down syndrome, trisomy 18, Turner syndrome, trisomy 13, Klinefelter syndrome, XXY syndrome and triple X syndrome.

Defined by the third copy of the 21st chromosome, Down syndrome is an abnormality that increases with age. Developmental and mental disabilities are the main problems of individuals with Down syndrome, besides these features, there are also diseases such as slow growth,

upward slanted eyes, and heart diseases. In addition, babies with Down syndrome generally learn basic skills (such as sitting, walking, speaking and learning) later than other babies (Antonarakis *et al.,* 2020). Trisomy 18 syndrome (Edwards syndrome) is the second most frequent chromosomal syndrome caused by the presence of an extra third chromosome (Cereda and Carey, 2012). The syndrome includes psychomotor and cognitive disability as well as increases the risk of miscarriage, neonatal and infant death, as well as other chromosomal disorders. Confirmation of the disease can be made by demonstrating a partial trisomy of the long arm of chromosome 18, which is extra in the Standard G-banded karyotype (Carey, 2010; Cereda and Carey, 2012). On the other hand, Turner syndrome (TS) is an abnormality in which the X chromosome is partially or completely missing (Kesler, 2007) While approximately 50% of girls with Turner syndrome have a 45,X or non-mosaic karyotype, some have various karyotype variations. The physical phenotype caused by TS comprises short stature, webbed open neck, cardiac malformations, triode disease, impaired glucose tolerance, ovarian failure, and hearing loss (Gravholt *et al.*, 2019).

1.3 EMBRYOGENESIS

The initiation of an embryo from a somatic cell or a zygote is called embryonic development or embryogenesis (Mahajan, Ganguly and Pagrut, 2018). Two types of embryogenesis, namely the development of the embryo, consist of stages. As a result of the gradual events that take place in somatic embryogenesis, the result of a non-embryogenic cell with an embryogenic cell (Von Arnold *et al.*, 2002), that is, an embryo, is called, whereas in zygotic embryogenesis, an embryo arises from a zygote. The stages and place of Embrogenesis, which is seen in sexually reproducing organisms and is called the development of the creature during the period between fertilization and birth or exiting the egg or cocoon, differ from organism to living thing (Gartstein., Putnam. and Kliewer., 2016) (Von Arnold *et al.*, 2002). Embryonic development in animals is seen in 4 stages: fertilization, shape, shrinkage, and unchanged.

1.3.1 STAGES OF HUMAN EMBRYO DEVELOPMENT

The four stages of embryonic development are morula, blastula, gastrula and organogenesis. In the first stage of embryogenesis, fertilization, the sperm pass through the outer glycoprotein

layer of the egg, called the zona pellucida, and fuse its genetic material with that of the egg. However, since the zygote's own genes are not active in the first moments, division is under the control of the mother's genome, thanks to the proteins and mRNAs given by the mother through the fertilized egg. (Minami, Suzuki and Tsukamoto, 2007). At this stage, the zygote performs successive mitotic divisions to produce smaller cells that carry the same genome. When the zygote that continues to undergo divisions has a 32-cell structure, it is called a morula (Mahajan, Ganguly and Pagrut, 2018). The cleavage phase of embryogenesis is completed. The cells still inside the zona pellucida stay firmly in the middle of the structure, this stage is called compaction and the cells on the outside begin to differentiate, and this process is called differentiation. The outer cells are called trophoblast and the inner cells are called embryoblast, this process continues and passes to the next stage. The inner cells (embryoblasts) are intertwined even more and as you can see in figure 2, they are collected on one side and a gap is formed on the other side(Gartstein., Putnam. and Kliewer., 2016). This cell mass in the middle is called the i-cell mass and the space on the other side is called the blastocoele. This structure, which contains an inner cell mass and a blastocoele and is surrounded by a trophoblast, is called the blastocoele. And this period is called blastulation and at the same time, the zona pellucida begins to break down. After this stage, the inner cells begin to differentiate and form a cavity, as shown in figure 3. Differentiating cells are called hypoblasts, while cells forming the amniotic cavity are called epiblasts. After the bilaminar disc is formed, the region where the cells in the epiblast layer in the bilaminar disc, called the primitive line, proliferate, collect and migrate, is formed, and this primitive line is the precursor of gasturulation, which is the next stage of early embryogenesis.

The cells migrate on the primitive line and further differentiate, after which a structure with 3 cell layers is formed and these layers are called germ layers. The upper layer is called ectoderm, the middle layer is called mesoderm and the lower layer is called endoderm and the process of formation of these 3 layers is called gastrulation. In the gastrula stage, the zygote now activates its own genes. The final stage of early embrogenesis is called neurolation. The notochord structure, which is involved in neurulation, is formed and a thickened area called the neural plate is formed in the ectoderm. Neural plate cells begin to penetrate the mesoderm, while some cells that break off the ectoderm pass into the mesoderm and the neural crest cells begin to differentiate into these cells that will form their own tissues in the future (Rossant and Tam, 2018). Over time, the cells in the embryo form the cell types in adult living things, and this

stage is called change (differentiation). These cells become layers, forming tissues, tissue organs and systems in organs that are important for the survival of the living thing. After it has occurred in all systems, most animals enter the stage of development where new cells and intercellular fluids are formed (Mahajan, Ganguly and Pagrut, 2018).

1.3.2 MOLECULAR BASIS FOR EMBRYONIC DEVELOPMENT

To fully understand embryonic development, it is necessary to integrate the molecular and morphological facets of embryology. There are very critical families of molecules known to drive embryonic development (Massaro and Haven, 1969). One of the most important insights is that genes that drive development are preserved. In many species, varying from worms to humans, the genes responsible for development have proven to have significantly minor changes in the nucleotide bases relative to other genes (Zeitlinger and Stark, 2010). Thanks to such conservation of genes, it is easier to find equivalents for genes with significant developmental roles in different species.

It has been proven in many previous studies that the same gene can function differently in different times and places. This mechanism of reuse in development and normally in different organs is very important as it significantly reduces the number of molecules required. In the prenatal and postnatal period, certain genes can be expressed in usual and unusual processes. Some transcription factors are significant molecules that handle embryonic development. Transcription factors generally control the transcription of genes by binding to promoter, enhancer, or silencing regions. Other molecules are molecules that play a role in signal communication between cells. When they form a complex by binding to the receptors in the cells from which they are produced or in the plasma membrane of another cell, it initiates a series of events whose molecular signal is transmitted to the responding cell. That signal effects the regulation of genes and the upcoming developmental process of the cell (Çikgöz, 2015) (Massaro and Haven, 1969).

Some transcription factors are particular for certain cell types and developmental stages. Particular transcription factors are so significant that they induce major developmental changes by initiating certain gene expression patterns. Transcription factors are separated into various

major groups according as their formation and how they interrelate with DNA (Tam and Loebel, 2007). For example, one of the most vital transcription factors is homeodomain (homebox) proteins. These proteins are a kind of highly conserved helix-loop-helix region of 60 amino acids. HOX (homeobox) genes play an essential role in many developmental processes (for instance developing organs, intestines, blood cells, limbs, internal and external genitalia, hair follicles, blood cells as well as developing sperm cells (Lufkin, 1997). PAX gene family, which consists of 9 known members, are also genes that perform a role in significant events in mammalian development. PAX genes play an important role in the development of the nervous system and sensory organs, as well as in cellular differentiation during epithelial-mesenchymal transitions (Wang *et al.*, 2008). POU gene family, Lim proteins, Dlx gene family, Msx genes are gene families that play a role in developmental processes. While POU gene family plays an vital role during early division, Lim proteins are involved in one stage of formation of almost all segments, Dlx gene family is involved in appendage development as well as early development of placenta, morphogenesis of jaws and inner ear, another gene family is Msx genes. Msx genes are involved in embryonic development especially limbs and While playing a role in facial epitheliomeneschymal interactions, they are widespread inhibitors of cell differentiation in prenatal development and help preserve the proliferation capacity of cells after birth (Kraus and Lufkin, 2006; Singh, Singh and Modi, 2021). T-Box gene family genes also play an vital role in the developmental process, such as controlling the growth of the leg or arm by inducing the mesoderm layer. The Helix-Loop-Helix configuration, on the other hand, is exhausted in multiple transcription factors that regulate myogenesis. The FOX gene family is also expressed in many developing organs. Sox genes, which are expressed by most constructs at the developmental stage, were identified as the male responsible factor in gender differentiation when they were first identified (Golson and Kaestner, 2016). The WT1 (Wilms tumor suppressor gene) gene plays an vital role in the development of both gonads and kidneys. The transforming growth factor-β (TGF-β) superfamily comprises of many molecules and these play important roles in embryogenesis and postnatal life (Miller-Hodges and Hohenstein, 2012)(Wall and Hogan, 1994).

As resources show, many genes are participating in embryonic development. In addition to the known genes, there are many genes that are related to the development of the embryonic and keep the placenta alive, waiting to be investigated and found.

1.3.3 DEVELOPMENTAL DISORDERS

Errors that occur during embryo development cause developmental abnormalities. These abnormalities cause miscarriage or any disorder or disease in the fetus. Having a baby primary challenge to be overcome in reproduction, the second most important problem is to have a healthy baby without genetic defects (Piyamongkol, 2020). Genetic problems impact, approximately 1% of living births and are in charge for 20% of pediatric admission to hospital for treatment and 20% of newborn deaths. Mutations, especially X-linked recessive mutations, can be carried by a large part of the population and cause diseases. Especially in IVF technology, to avoid such genetic defects, selective treatment is performed and unaffected normal and carrier females are selectively transferred (Rubio and Simón, 2021).

1.4 SPONTANEOUS ABORTION

Spontaneous abortion is identified as the expulsion of pregnancy sooner than 20 weeks of pregnancy without any intervention. The ACOG (American College of Obstetricians and Gynecologists) says it is the most common form of pregnancy (Kanmaz, İnan, *et al.*, 2019). Spontaneous abortion appears in 15-20% of clinically defined pregnancies (George *et al.*, 2002). In addition, about 80% of early pregnancy losses happen in the first trimester, in addition the possibility of miscarriage decreases after the 12th week (Wilcox *et al.*, 1988; Zinaman *et al.*, 1996; Kanmaz, Inan, *et al.*, 2019). Genetic defects, particularly chromosomal abnormalities, are the most frequent cause of spontaneous abortions in the first trimester.

Spontaneous abortion is subdivided into complete abortion, missed abortion, inevitable abortion, incomplete abortion, threatened abortion, septic abortion, and recurrent spontaneous abortion (Griebel *et al.*, 2005). Many pregnancies are lost on their own before the woman even realizes she is pregnant. During the miscarriage event, heavy or late menstruation is among the clinical symptoms. The threat of miscarriage is the presence of vaginal bleeding in early pregnancy, the fetus is alive but there is a threat of miscarriage. Inevitable abortion is the type of abortion in which the fetus with vaginal bleeding and the products of conception are expected

to be expelled soon (Maconochie *et al.*, 2007). A complete abortion is the initial vaginal bleeding and expulsion of the fetus and pregnancy products from the cervix (Birch, Gulati and Mandalia, 2017).

1.4.1 ETIOLOGY OF SPONTANEOUS ABORTION

As seen in Figure 1.1, causes of miscarriage could be genetic, infections, anatomic, hormonal reasons can be counted. If counted in detail, some viruses, chromosomal disorders, luteal phase defects, immunological abnormalities, major trauma, and uterine abnormalities can be counted as causes of spontaneous abortions (George *et al.*, 2006). The majority of the causes of spontaneous abortions are idiopathic, that is, the reasons that have not yet been explained. A previous study found that more than 90% of fetal lives that result in miscarriage have a chromosomal abnormality (Philipp *et al.*, 2003). Cellular immune effectors and cytokines, some cytokines have recently been shown to be beneficial for pregnancy while others are actually anti-pregnancy (Raghupathy, 2003). Maternal age is the biggest risk factor for spontaneous abortion (Friebe and Arck, 2008). As age progresses, the probability of miscarriage increases significantly with the possibility of having a chromosomal abnormality (Magnus *et al.*, 2019).

Abnormal genetic sequence from the mother and father can cause miscarriages (Wilcox *et al.*, 1988). Translocations, in which genetic material changes between chromosomes, can be counted among these causes. If any genetic material is lost, the parents may not be affected because they have a healthy copy, but because the baby has only one copy and is likely not to receive a full set of chromosomes, this can result in miscarriage or serious birth defects (Puscheck and Jeyendran, 2007). Chromosome inversion, that is, reattachment of the chromosome with a segment that breaks and turns in the wrong direction, can again lead to miscarriage or serious birth defects when half a chromosome set is passed to the fetus and these wrong and missing parts (Suzumori and Sugiura-Ogasawara, 2012). Single gene mutations are a common cause of genetic diseases as in many diseases, and mutations in many unknown genes that play a significant roles in the developmental process are likely to cause miscarriages (Dawood, Farquharson and Stephenson, 2010). It is aimed to clarify the functions of these genes and to know the diseases they cause.

Anatomical abnormalities in the uterus cause spontaneous abortions. It is estimated to cause 10-20% of spontaneous abortions. An example of a uterine abnormality is the septa uterus, if an implantation occurs on or near the septum, blood flow is poor there, increasing the risk of miscarriage (Passos and Britto, 2020). Another uterine abnormality is fibroids, which increase the chances of miscarriage if fibroids grow in or near the uterine cavity (El Hachem *et al.*, 2017). Hormonal and metabolic problems can increase the risk of miscarriage. Endocrine factors include hyperprolactinemia, thyroid dysfunction and diabetes. Women aged 35 and over are defined as advanced reproductive age. Aging eggs are more possible to be abnormal, resulting in an increase in genetically abnormal embryos with advancing female age (Suzumori and Sugiura-Ogasawara, 2012; Magnus *et al.*, 2019). Women who experience accelerated reproductive aging have higher rates of unexplained infertility and spontaneous abortions. Estradiol, FSH (follicle stimulating hormone) and AMH (anti-mullerian hormone) amounts, which reflect the number of healthy eggs growing for the menstrual cycle, are important for fertility, that is, for the prevention of spontaneous abortions (Passos and Britto, 2020). One of the important factors that increase the risk of miscarriage is Polycystic ovary syndrome (PCOS) (Chakraborty *et al.*, 2013). When we look at the immunological causes of miscarriage, forexample, Antiphospholipid antibody syndrome (APS), when antiphospholipid (aPL) antibodies bind to phospholipids, which are an important part of cell wall development and help the formation of blood vessels, they adversely affect blood vessel development and cause pregnancy loss (Girirajan, Campbell and Eichler, 2011). Low uterine reaction to progesterone or low progesterone manufacture by the ovary is one of the leading causes of miscarriage, and studies have shown that the rate of live birth increased in women who received progesterone supplementation in the first 3 months of pregnancy (Rubio and Simón, 2021).

1.4.2 MOLECULAR BASIS OF SPONTANEOUS ABORTION

From all pregnancies 10-15% of them end in spontaneous abortion, most spontaneous abortions (SM) occur in the first trimester (Warren and Silver, 2008; Zhang *et al.*, 2009). It is well known

that more than 50% of genetic abnormalities that cause spontaneous abortions are caused by chromosomal abnormalities (Nagaishi *et al.*, 2004) (Table 1.1).

Chromosomal Abnormalities	% of cases
Structural anomalies	10%
Numerical abnormalities	90%
monosomy X	20%
Polyploidy	20%
Triploidy	6%
autosomal trisomies	60%
trisomy 16	20%-30%
trisomy 21	20%
trisomy 22	20%
Parental balanced translocation	3%-6%
Confined placental mosaicism (CPM)	20%
Single gene disorders	10%
Thrombophilia	10%
X-linked conditions	10%

Table 1.1 Genetic abnormalities of spontaneous abortions

As described above, spontaneous abortions, familial genetic disorders, anatomical disorders in the uterus, hormonal problems, metabolic problems, advanced reproductive age, PCOS, immunological causes, luteal phase defects can be counted as well as exposure to toxins, recurrent embryonic aneuploidy, excessive blood coagulation disorder, male reproductive disorders, anomalies, presence of contagious infection, stress and psychological aspects are also counted among the causes of miscarriage (Quach *et al.*, 2015)

Another reason for spontaneous abortions is the level of hCG, hCG is a glycoprotein consisting of two subunits, and it is secreted in an increasing amount from the syncytiotrophoblast with

the increase in pregnancy in the first trimester and prevents the decrease of LH (Luteinizing Hormone) hormone by binding to its receptors in the corpus luteum. He says that early miscarriages may be caused by a decrease in hCG levels, with previous studies (Barnhart *et al.*, 2004).

Methylenetetrahydrofolate reductase (MTHFR) defect is another genetic abnormality that causes complications in pregnancy. This gene mutation has been associated with high k-homocysteine (Gaboon, 2013).

Mutations in processes such as angiogenesis, coagulation, cell adhesion (eg, trophoblast/endometrium interaction), immunological occupation response/modulation, metabolism (eg receptor activation in addition specific cell function regulation such as proliferation, apoptosis, migration, and differentiation) are likely to affect spontaneous abortions (Fukuda and Sugihara, 2012)(Maruyama, Bell and Majerus, 1985). For example, TRO, CDH11, CDHA genes affect cell adhesion, trophoblast/endometrium interaction, THBD, F5, FGA genes are involved in coagulation, FGA, MMP10, MMP9, COL6A3, ADAMTS1 and TNC genes are responsible for extracellular matrix remodeling, while FLT1, EPAS1 genes are responsible for the vascularization process, LIFR, FGFR2, BMP7 genes are involved in proliferation, differentiation, migration and apoptosis processes, while AMN gene affects metabolism, IDO2, CR1, TLR3, TRAF3IP1 is involved in modulation of immunological function and mutations in these genes can cause spontaneous abortions (Fukuda and Sugihara, 2008)(Puscheck and Jeyendran, 2007)(Zhao *et al.*, 2010; Nakamura and Saji, 2014) (Quintero-Ronderos *et al.*, 2017)

1.5 WNT/β-CATENIN SIGNALING PATHWAYS

The classical signaling pathway that regulates embryonic migration, cell fate determination, and cell proliferation, and also the WNT/B-catenin signaling pathway uses paracrine or autocrine cell communication to regulate a range of biological processes during the growth and development of all organisms (Clevers, 2006; Clevers and Nusse, 2012). The WNT signaling pathway plays an critical role in many vital events. For example, it is one of the most important biological components in charge for the regulation of cell fate, cell polarity, cell proliferation, cell migration, and adult tissue homeostasis and more importantly in human embryonic development (MacDonald, Tamai and He, 2009).

Wnt (Growth Stimulatory Factors) is a ligand in the WNT/B-catenin signalling pathway, there are 19 WNT genes in mammals. B-catenin is an intracellular protein controlled by degradation; it acts as a transcription regulatory factor depending on the presence of the signal. The WNT/β-catenin signalling pathway is a highly conserved signaling pathway . Mutation in this signaling pathway can cause problems in the embryo as it is responsible for embryonic development, and mutation in this signaling pathway shows an important role in carcinoma since it is responsible for the directive of cell migration, cell polarity, cell proliferation, and cell fate. WNT/B-catenin signaling pathways diversifies into at least three branches:, these are the, Non-Canonical Wnt signaling pathway/Calcium pathway, Non-Canonical Wnt-signaling pathway (β-catenin Independent pathway) and Canonical Wnt-signaling pathway (β-catenin dependent pathway) (Huelsken and Behrens, 2002).

Canonical Pathway

As the name implies the canonical pathway falls in the category of canonical pathways which has a link with the beta-catenin (β-catenin) (Freese, Pino and Pleasure, 2010). As seen in Figure 1.2, when the Wnt ligand does not bind to the receptor and the WNT/β-catenin signaling pathway is inactive, a small amount of β-catenin in the cell is degraded by the degradation complex consisting of Dvl, Axin, CKI, GSK3, APC, β TrCP, taken to the proteosome and degraded. and thus this small amount of B-catenin cannot enter the nucleus, the transcription factor (TCF) is inhibited by Grucho and transcription does not occur. Once the Wnt ligand binds to the Frizzled receptor, it causes phosphorylation of the LRP receptor in the membrane. When LRP is phosphorylated, it goes to the canine membrane and the degradation process is stopped. When the degradation process is stopped, B-catenin begins to accumulate in the cytoplasm, when there is a large amount of β-catenin in cytoplasm, β-catenin begins to enter the nucleus, B-catenin entering the nucleus causes the Grucho to separate from TCF, and B-catenin itself binds to TCF and this TCF-B-catenin complex causes Wnt target genes to be transcribed and causes events such as growth and differentiation to occur.

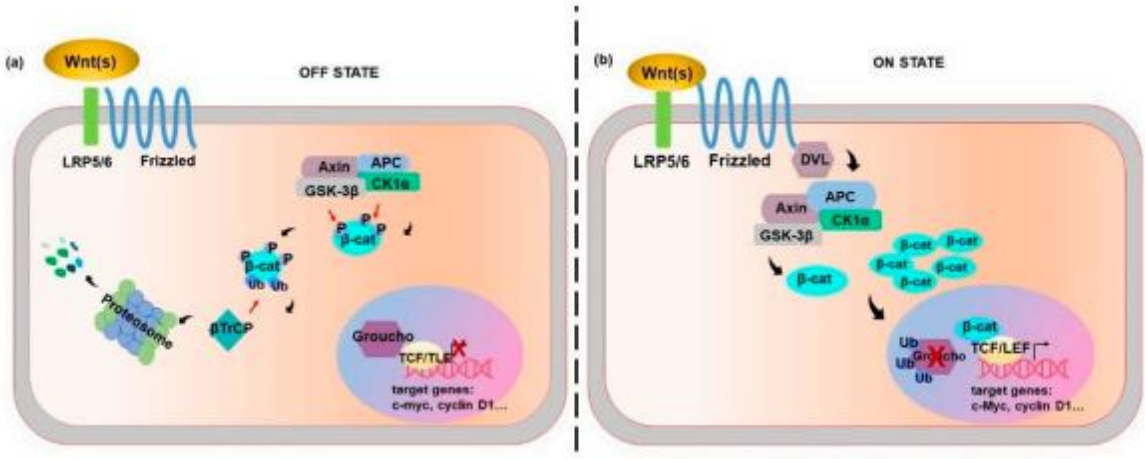

Fig 1.2 The Canonical Wnt signalling pathway. (Adapted from (Koni, Pinnarò and Brizzi, 2020))

Noncanonical Pathway

The non-canonical Wnt signaling pathway (PCP signaling) is a β-catenin-free pathway, but this pathway doesn't use LRP-5/6 as a coreceptor. This pathway is triggered by binding of the WNT ligand to the Fz receptor and its co-receptor. DAAM1, which is then affected by Dsh, activates the guanine exchange factor Rho to activate one of the main regulators of the cytoskeleton, Rho-associated kinase (ROCK) (Fig 1.3b). Dsh also activates Rac1 to activate JNK, which also enables actin polymerization. This can lead to the binding of the profile to actin and reorganization of the cytoskeleton, which is also important in the gastrulation stage.

Another non-Canonical signaling pathway is the Non-Canonical Wnt/calcium pathway, which does not contain B-catenin . Its main role is help control calcium release. As in other non-canonical signaling pathways, the Wnt ligand connects to the Fz receptor, activating the PDZ and Dep regions of Dsh that interact directly with Fz, which are involved in Wnt/calcium signaling. The Fz receptor can also stimulate the G-protein, providing activation of both (Dsh and G-protein). Activated DSH can activate two separate paths by activating PLC and PDE. If PLC is activated, it activates IP3. When IP3 binds to the ER receptor, calcium is released, increasing calcium and PKC resulting in activation of CDC42, a significant regulator of ventral patterning. Increasing calcium activates Calcineurin and CamKII. CamKII stimulates the transcription factor NFAT and consequently regulates cell adhesion, migration and tissue separation. Calcineurin, which is also activated by calcium increase, activates TAK1, while Tak1 activates NKL, a kinase that can affect with TCF/β-Catenin signaling in the canonical Wnt signalling pathway. However, if PDE is triggered, it causes inhibition of calcium release via PKG (Fig 1.3a)

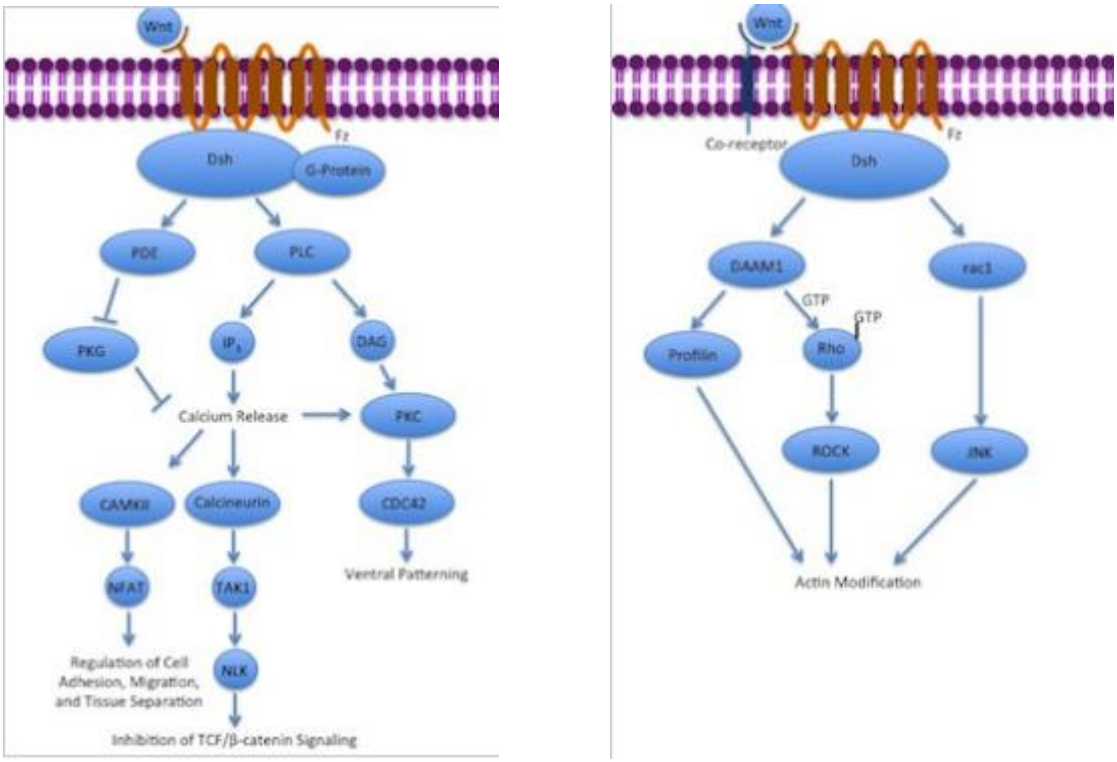

Fig 1.3. The NonCanonical Wnt signalling pathways. Figure a, Non-Canonical Wnt/calcium pathway, Figure b, Wnt signaling pathway (PCP signaling). Adapted from (Deng *et al.*, 2021)

1.5.1 THE ROLE OF WNT/β-CATENIN SIGNALING IN EMBRIYONIC DEVELOPMENT

Wnt proteins are factors that regulate the differentiation and proliferation of cells (stem and pregenitor) during both embryonic development and adult tissue homeostasis (Logan and Nusse, 2004) Wnt signaling is involved in cell-cell communication in vertebrate early embryonic development, during embryonic development in multicellular organisms, in self-renewal of stem cells, in deciding cell fate, in regulating cell migration and organization of cells in tissues. Wnt ligands can be autocrine, that is, self-acting, as well as pracrine and affect other cells (Sanz-Ezquerro, Münsterberg and Stricker, 2017)

The part of the Wnt/β-catenin signaling pathway, at protection of stem cells is important. While stem cells produce special cells, they are called cells that have the capacity to renew themselves, and these choices are usually determined by external signals (Losick *et al.*, 2011). These incoming signals include tasks such as how much and how long the cells will produce. Although

signaling pathways such as BMP, Hedgehog and Delta/Notch are also important in determining these tasks, the Wnt/β-catenin signaling pathway stands out due to its widespread activity (Clevers and Nusse, 2012). Embryo development is critical for the healthy development of the embryo, as well as the implantation of the embryo. If there is a problem with the implantation and it fails, the event will generally result in a miscarriage. Studies have shown that the Wnt/β-catenin signaling pathway is important for preimplantation embryo development, blastocyte activation for implantation, and uterine decidualization (Chen *et al.*, 2009) (Kemp *et al.*, 2005) (Li *et al.*, 2015)

It is argued that the Wnt/β-catenin signaling pathway regulates the body axis pattern and contributes to both the relative constancy and diversity of body forms (Niehrs, 2010). Another example is the inoculation of Wnt mRNA or factors that initiate B-catenin signaling into the blastomere, resulting in duplication of the dorsal axis resulting in a two-headed frog (Moon and Kimelman, 1998). A study in mice supports the crucial part of Wnt/β-catenin signaling in uterine growth and development. Inhibition of Wnt signaling has been shown to inhibit estrogen-dependent activation and cell proliferation of β-catenin , thereby inhibiting steroid hormone-induced uterine cell growth (Hou *et al.*, 2004).

It is identified that the canonical Wnt/β-catenin signalling pathway shows important roles in development, Wnt ligands can bind to multiple receptor molecules (WNT/Ca2+ pathways, non-canonical WNT/planar cell polarity (PCP) and canonical WNT/βcatenin-dependent signaling pathway), the same signal cell may produce different cellular responses liable on the type, context, and time of development (Sanz-Ezquerro, Münsterberg and Stricker, 2017). Has been examined the task of the canonical WNT/β-catenin-dependent signaling pathway in eye development and showed that it plays important roles in the modeling of ocular tissue, differentiation of the retinal pigment epithelium, and morphogenesis of the optic lid (Fujimura, 2016). Again, it has been shown that mutations in DVL genes, one of the important genes in the Wnt/β-catenin signaling pathway, may cause human congenital diseases, emphasizing their important role in development (Gentzel and Schambony, 2017). Yet another study showed that implantation was significantly reduced in mice with inhibited Wnt signaling pathway. That show us Wnt/β-catenin signaling pathway performs a crucial role in coordinating uterus–embryo interactions necessary for implantation (Ciarke *et al.*, 2005).

1.5.2 WNT/β-CATENİN SIGNALING IN EARLY PREGNANCY

The majority of our knowledge about the importance of Wnt signaling in implantation emerges from research on rodents. The function and the physical organization of cadherins are maintained by the β-catenin molecule as it binds strongly to the cytoplasmic area of cadherins to link the cadherins through α-catenin to the actin cytoskeleton, indeed, β-catenin functions in two distinct ways: As a mediator of interactions between cells besides the co-factor of the canonical Wnt/β-catenin signaling pathway. As human setting illustrates, the risk of ectopic pregnancy increases with increased β-catenin expression in the tubular epithelium (Bellati *et al.*, 2019). Gene expression analysis demonstrated the downregulation of Canonical Wnt signaling in porcine parthenogenetic embryos, and the suppression of Wnt signaling with a small molecule drug had little influence on the development of these embryos (IWP-2), moreover, trophectoderm development was halted. Excessive Wnt activation caused apoptosis, as well as blastocyst hatching was hampered (Huang *et al.*, 2019) ; In zygotes with B-catenin activation, development beyond the two-cell stage was inhibited. Additional investigation revealed that in culture embryos, the triggered form of βcatenin protein was enhanced while the phosphorylated form of β-catenin protein was decreased (Li *et al.*, 2017). In contrast another research on bovine embryo indicated, which during preimplantation in the bovine embryo, a functional Wnt signaling system exists. These researchers discovered that activating canonical signaling reduces both the early embryonic cell numbers and embryonic development (Denicol *et al.*, 2013). Inhibiting Wnt/β-catenin signaling with Dkk1 did not appear to be advantageous. RIF (Repeated implantation failure) is a significant barrier in the treatment of human assisted reproduction. A global gene profiling study was conducted on RIF patients to observe the expression levels of SFRP1 (secreted frizzled-related protein 1) and LEF1 in endometria checked with fertile women , which SFRP1 was downregulated whereas Dkk1 expression was discovered to be upregulated (Huang *et al.*, 2019)(Koler *et al.*, 2009). Numerous differentially expressed microRNAs (miRNAs) regulate the Wnt signaling pathways in line with the secretory endometrium expression patterns (Revel *et al.*, 2011) According to a genomic sequence miRNA profiling research in women that she have recurrent spontaneous abortion , Wnt signaling is one of the foremost targets of the expressed miRNAs, hence Wnt signaling is influenced by abnormal miRNA expression in cell adhesion (Dong *et al.*, 2014).

1.6 THESIS OVERVIEW

The Wnt/β-catenin signalling pathway is a classical and important signalling pathway that regulates embryonic migration, cell fate determination, and cell proliferation. It is one of the main signaling pathways in the cell This Wnt/β-catenin signalling pathway plays an important role in regulating many processes during the development and growth of all organisms and uses autocrine or paracrine communication in cell communication. It is involved in many important and different processes, as well as in the regulation of embryonic development, proliferation, cell migration, cell polarity, and cell fate. It is thought that the etiology of many diseases is caused by disruptions in genes encoding proteins in the signaling pathway and, accordingly, activation problems in the Wnt/β-catenin signalling pathway. Studies have shown that disruptions in these genes can cause many different diseases, and genes in this signaling pathway may be related to miscarriages. In previous studies, WNT/β-catenin signalling pathway genes in charges were required for evolutionary enhancements including pre-implantation development, blastocyst implantation, cell proliferation and differentiation Many studies have shown that problems in these genes can cause miscarriages, but most of the studies have been done on either mice or mothers. In this study, the placenta sample from pregnancies that resulted in miscarriage was used.

CHAPTER II: MATERIALS & METHODS

2.1 Materials

2.1.1 Suppliers

- abm OneScript plus cDNA-synthesis kit (Applied Biological Materials Inc. (abm), Richmond, Canada),
- Thermo-scientific marker (Pittsburg, USA),
- Eppendorf Scientific (Hamburg, Germany),
- Nano-drop (Thermo-scientific, Pittsburg, USA),
- Bio-Rad Electrophoresis instrument (Hemel Hemstead,UK),
- Trans-Illuminator (DNR Bio-Imaging System, Neve Yamin, Israel).
- Applied bio-systems thermal cycler PCR, (Waltham, Massachusetts, USA),

- RotarGene Real-Time PCR (Qiagen,Hilden, Germany)

2.1.2 Chemical Reagents

2.1.2.1 Oligonucleotides

Primers that used in this project were from Oligomer Company (Turkey)

2.1.2.2 Molecular Wight Markers

In this project Thermo-scientific 50 bp – 1000 bp (Pittsburg, USA) DNA ladder was used to be molecular weight marker.

2.1.2.3 Human aborted fetuses

Aborted fetus samples were obtained from Near East University Hospital (NEUH) the genetic laboratory when the approval of Near East University Scientific Review Board. 23 miscarriage samples were collected from women who had abortion. 8 of these samples (1,2,5,6,9,13,17,21) had abnormal karyotype, while 15 of them (3,4,7,8,10,11,12,14,15,16,18, 19,20,22,23) had a normal karyotype. This project was performed in the Near East University DESAM Institute Molecular Medicine Laboratory. All the reagents, pipettes and tubes used in the research were UV treated to avert any risk of contamination problem.

2.1.2.4 Standard Solutions

10X electrophoresis buffer (Tris-acetate/EDTA (TAE) was used in this study) was made by adding 100 ml of 10x TAE and 900 ml of distilled water to 1X. Since 10X TAE is too dense, it causes problems in the movement of the bands.

Other solution was Hibrigen 2X SYBR green qPCR mix (İstanbul, Turkey). This Hibrigen 2X SYBR green qPCR mix has antibody mediated hot star, MgCl2, ultrapure dNTPs, Taq DNA polymerase , SYBR green I with enhancers and stabilizers.

2.1.2.5 Other chemical agents

Glycerol, %70 Ethanol, Ethidium Bromide (Serva, Heidelberg, Germany), Agarose biomax 100mg

2.1.3 Computers

Softwarethat used in this project to store data and precede imaging.

2.2 Methods

2.2.1 Karyotyping

Karyotype analysis was previously performed in the Near East University Hospital Medical Genetics Laboratory.

2.2.2 RNA Extraction from aborted fetuses

The RNA had been extracted using with Hibrigen general RNA isolation kit (MG-RNA-01) (Istanbul, Turkey)

2.2.3 Measuring RNA concentration

260/ 280 nm wavelength shows purity and concentration of RNA, through measuring optical density at by Nano-drop and best purified density of the RNA is about 2.1ng/µl.

2.2.4 Complementary DNA (cDNA) synthesis

cDNA synthesis was carried out by using abm OneScript plus cDNA-synthesis kit (abm company, Richmond, Canada). This kit contained OneScript Plus reverse transcriptase, OneScript Plus RT reaction buffer, Oligo(dT) primer, dNTPs mix, and anchored oligo dt primer, stored in -15, - 25℃.

For each sample, 12 µl of the mix formed by these components and 8 µl of RNA were brought together.

Component of the kit	For 1X
Rxn buffer	4 µl
Oligo dT primer	1 µl
dNTPs	1µl
RTase	1 µl
RNAse free water	5 µl
Total	12 µl

Table 2.1 the table shows the necessary calculations done for cDNA synthesis

2.2.5 Primer Optimization for Gradient PCR

It comes in stock from the primary company, and a working solution must be prepared before use. A separate working solution was prepared for the four genes (3 investigated genes and 1 hausekeeping gene) used in the research. 10 ml of stock solution and 90 ml of distilled water were combined and used as working solution.

GENE NAME	FORWARD PRIMER	REVERSE PRIMER
Beta Catenin (CTNNB1) - 112 bp	AGACGGAGGAAGGTCTGAGG	TTCAAATACCCTCAGGGGAACA
GSK3B- 151 bp	ACAGCAGCGTCAGATGCTAA	TGACCAGTGTTGCTGAGTGA
DVL1 – 358bp	CAGCAGAGTGAAGGGAGCAA	GGTTCCCCATAGCCTTCTGG

Table 2.2 Shows designed primer for *DVL1*, *GSK3B*, and *β -catenin* genes.

Gradient PCR was performed to distinguish the optimum annealing temperature for qRT-PCR. The selected temperature range ranged from 56℃ to 62℃ and this step was repeated for all three genes. Table 2.4 lists the conditions used for this gradient PCR. 8 µl of gradient PCR mix, 2 µl of cDNA was added to each tube. To avoid contamination, all reactions were performed in a category II laminar flow hood and all tools and equipment were used in a sterile manner. Additionally, 0.6 µl of glycerol was added to the reaction to avoid the primer dimer.

Component	1X	8X
SYBR green	5 µl	40 µl
Forward primer	0.5 µl	4 µl
Reverse Primer	0.5 µl	4 µl
Glycerol	0.6 µl	4.8 µl
DH_20	1.4 µl	11.2 µl

Table 2.3 Gradient PCR Master

8 µl + 2 µl cDNA (total reaction volume of 10) from the final gradient PCR mastermix mixture was placed in Eppendorf Scientific PCR tubes for analysis. These calculations were for all 6 samples + 1 Negative control (ntc) and + 1 pipetting error. Measurements were performed for 3 different genes with a different primer set each time.

Stage	Temperature	Time	Cycles
Initial denaturation	95 ℃	5 minutes	1 cycle
Denaturation	95 ℃	15 seconds	35 cycles
Annealing	56℃ - 62 ℃	30 seconds	
Extension	72 ℃	45 seconds	
Termination	72 ℃	5 minutes	1 cycle

Table 2.4 Shows condition utilized for gradient PCR.

2.2.6 Real-Time quantitative reverse transcription polymerase chain reaction (qPCR)

Real-time quantitative reverse transcription-polymerase chain reaction (RT-qPCR) was performed using the RotarGene Real Time PCR machine. This device provides reliable results by changing the temperatures according to the process, allowing reactions to take place and measuring data. Primer optimizations were made, such as finding the optimum operating temperatures of genes, such as gradient PCR. 8 µl + 2 µl cDNA (total reaction volume of 10) from the final gradient PCR mastermix mixture was placed in Eppendorf Scientific PCR tubes for analysis. Processing time took about 1 hour and 30 minutes with the preparation, the machine working time was 1 hour and 15 minutes of this time. Master mix preparation calculations are listed in Table 2.5 and conditions are listed in 2.6.

Component	1X	26X
SYBR Mix	10 µl	260 µl
Forward Primer	2 µl	52 µl
Reverse Primer	2 µl	52 µl
dH2O	5 µl	130 µl

Table 2.5 RT-qPCR Master Mixture calculations for each gene

Stage	Temperature	Time	Cycles
Initial denaturation	95 °C	2 minutes	1 cycle
Denaturation	95 °C	0.30 seconds	30 cycles
Annealing	59 °C	0.30 seconds	
Extension	72 °C	0.45 seconds	
Termination	72 °C	10 minutes	1 cycle

Table 2.6 Quantitative real time PCR conditions.

2.2.7 Agarose gel Electrophoresis

The product from gradient PCR was later taken for gel electrophoresis using agarose. The gel concentration was prepared at 2 %, in which 4grams of agarose were weighed and dispensed into a transparent glass holding 200ml TAE buffer. The solution was then taken into the microwave at high voltage to ensure there was clarity seen in the glass every 30 seconds. The glass was taken to a cool dry place to ensure the cooling down of the mixture. The tray (20 cm x 20cm) was wiped to remove debris, Ethidium Bromide 5 µl was added to the solution before it was poured into the tray for it to get solidified. Both loading dye and sample were mixed (2 µl to 8µl respectively) and were loaded into the wells. The ladder wells were later loaded with 2 µl and the tank was covered. The samples ran for 1hr 30mins at 100volts. The bands were later viewed using an ultraviolet trans-illuminator (DNR Bioimaging system, Neve Yamin, Israel).

CHAPTER III: RESULTS

3.1 Results of Karyotyping

As shown Table 3.1, 15 of samples have normal karyotype, 8 of samples have abnormal karyotype. Among of his abnormal samples have Mosaic Down syndrome, Tetraploidy, Turner syndrome, invasion 9 and translocation 13:14.

Sample Names	Karyotype
1	Abnormal (45,X),
2	Abnormal (45,XX)(t13:14)
3	Normal (46,XX)
4	Normal (46,XX)
5	Abnormal (46,XY) inv.9
6	Abnormal (45,X),
7	Normal (46,XX)
8	Normal (46,XX)
9	Abnormal (92,XXXX)
10	Normal (46,XX)
11	Normal (46,XX)
12	Normal (46,XX)
13	Abnormal (47,XY)+21
14	Normal (46,XX)
15	Normal (46,XX)
16	Normal (46,XX)
17	Abnormal (69,XXY)
18	Normal (46,XX)
19	Normal (46,XX)
20	Normal (46,XX)
21	Abnormal (46,XY) +21
22	Normal (46,XX)
23	Normal (46,XX)

Table 3.1 Shows the results of karyotyping (15 of sampes have Normal karyotype (46,XX), 8 of samples have Abnormal karyotype)(these 8 abnormal karyotype kinds are; 45,X Turner Syndrome; 45,XX(t13:14) chromosome 14 translocated to chromosome 13; 46,XY (inv 9)invasion on chromosome 9; 92,XXXX tetraploidy syndrome ; 47,XY (+21) Down syndrome ; 69,XXY Triploidy syndrome)

3.2 Extracted RNA Measurement

RNA Purification extracted aborted fetuses that were investigated by Nano drop. Table 3.2 shows 260/280 ratio and nucleic acid concentration

Sample Names	Karyotype	Nucleic Acid Concraction (ng/µl)	A260/280
1	Abnormal	6.4	2.22
2	Abnormal	6.45	1.62
3	Normal	17.25	1.86
4	Normal	19.15	1.63
5	Abnormal	16.24	1.87
6	Abnormal	3	1.94
7	Normal	26.25	1.66
8	Normal	7.2	1.75
9	Abnormal	19.5	1.72
10	Normal	1.6	2.40
11	Normal	47.75	2.04
12	Normal	6.7	1.92
13	Abnormal	111.65	2.04
14	Normal	314.65	2.07
15	Normal	56	1.95
16	Normal	28.8	1.92
17	Abnormal	27.65	1.47
18	Normal	21.5	1.59
19	Normal	17.3	1.70
20	Normal	2.65	1.67
21	Abnormal	4.8	1.71
22	Normal	24.15	1.63
23	Normal	37.15	1.55

Table 3.2 Shows the results of the RNA isolation. (samle 1,2,5,6,9,13,17,21 has abnormal karyotype, sample 3,4,7,8,10,11,12,14,15,16,18,19,20,22,23 has normal karyotype. Samples nuclic acid concentration and A260/238 results that show purity of RNA in sample , has been shown in this table)

3.3 Gradient PCR and Agarose Gel Electrophoresis Results

Gradient PCR has been carried out to get the optimum annealing temperature for the genes (*DVL1, GSK3B,* and *β-catenin* genes). The bands displayed were recorded at varying base pairs.. For 3 genes (GSK3B,DVL1 and B-catenin), Gradient PCR was performed with the temperature varying between 56 ℃-61℃, and it was decided that the anealling temperature would be 59℃ from this PCR result.

3.4 Gene expression analysis of *DVL1, GSK3B, β -catenin* and *ACTB*(β-Actin) genes

Expression profiles of these genes were performed using cDNA synthesized samples for all three Wnt signaling genes (DVL1, GSK3B, β-catenin and B-actin genes). The double-stranded nucleic acid chain that separates at each loop begins to be synthesized from where the primer is attached and again to form the double-stranded strand. SYBR green dye binds to these double-chain structures, this dye causes radiation and the device that detects the amount of radiation shows them as a result. The loop threshold, or Ct, is where the fluorescent radiation exceeds the threshold amount. The lower the Ct amount, the earlier the sample started to glow, which means that there is more product in it. In other words, the amount of nucleic acid is always inversely proportional to the Ct number. A sample with a high nucleic acid number will have a low Ct number. RT-q PCR analysis was performed for all 23 abortion fetus samples, and the status of the genes in the sample was shown with the help of the Ct of the examined gene and the amount of Housekeeping genes. The results of Ct values are shown in Table 3.3.

	Karyotype	**GSK3B Ct**	**DVL1 Ct**	**CTNNB1 Ct**	**B-Actin (Housekeeping gene) Ct**
3	Normal	29,97	28.69	19.41	17,72
4	Normal	16,95	29.78	19.41	20,02
7	Normal	13,59		17.76	16,54
8	Normal	13,61	29.04	20.12	22,65
10	Normal	12,43	29.20	17.72	19,68
11	Normal	16,54		19.38	18,85
12	Normal	13,19	28.50	17.32	18,39
14	Normal	25,1		18.95	18,05
15	Normal	24,23			13,86
16	Normal	18,55		18.60	18,03
18	Normal	16,44	29.75	19.91	23,08
19	Normal	27,96	29.02	19.54	17,8
20	Normal		29.27	19.43	24,33
22	Normal		29.18	19.52	22,15
23	Normal	29,04	29.31	19.37	19,23
5	Abnormal	25,9	29.56	18.62	18.03
6	Abnormal	26,68	29.40	20.04	20.37
1	Abnormal	20,96	29.29	18.50	17.90
2	Abnormal	14,86	28.40	17.34	19.09
9	Abnormal	15,67	29.31	18.38	23.59
13	Abnormal	17,08		19.40	17.31
17	Abnormal	23,45	29.81	19.65	20.07
21	Abnormal	20,24	29.47	19.70	18.54

Table 3.3 Expression levels of four genes (*DVL1*, *GSK3B*, *β -catenin* and *β-Actin* (Housekeeping gene) genes) in all 23 samples.

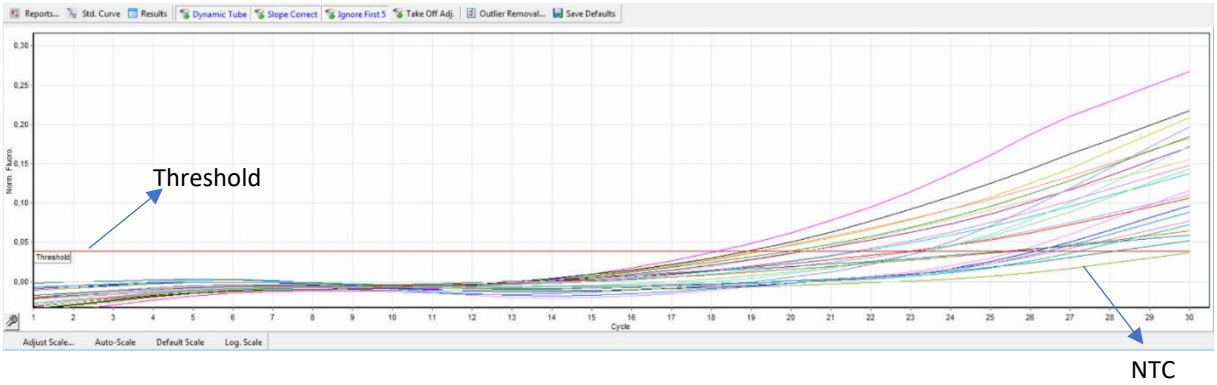

Figure 3.1 RT-qPCR reaction curves for *GSK3B* for all 23 samples and 1 negative control. (NTC: Negative Control, Threshold has been showed with arrows, each sample *GSK3B* gene expression curve has been shown with different colour)

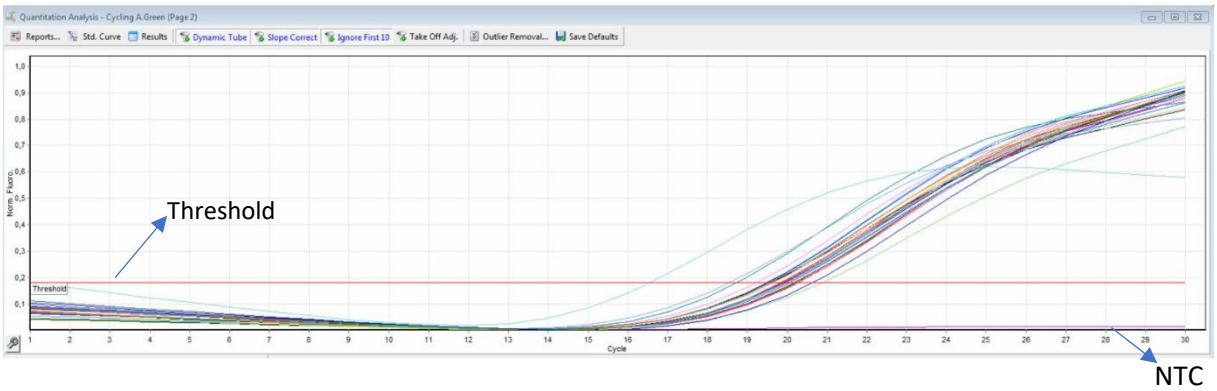

Figure 3.2 RT-qPCR reaction curves for *DVL1* for all 23 samples and 1 negative control. (NTC: Negative Control, Threshold has been showed with arrows, each sample *DVL1* gene expression curve has been shown with different colour)

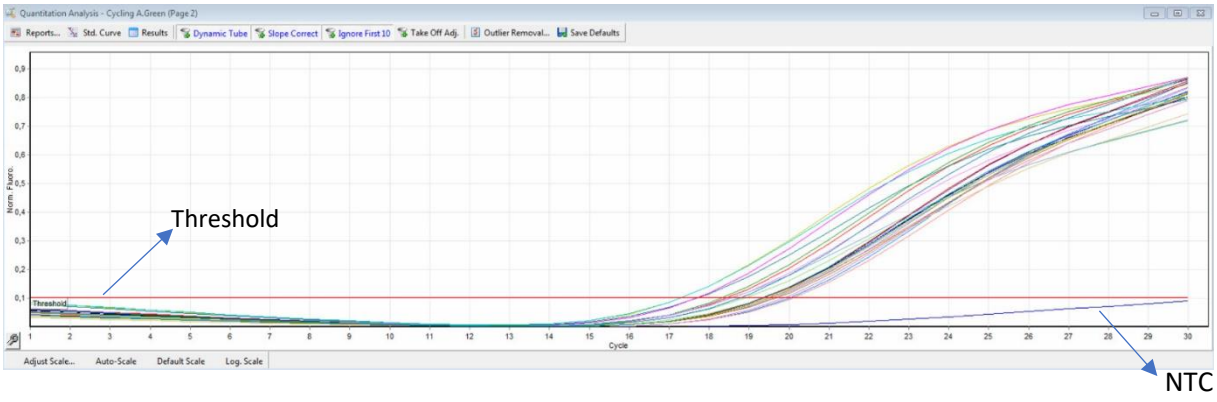

Figure3.3 RT-qPCR reaction curves for *β -catenin* for all 23 samples and 1 negative control. (NTC: Negative Control, Threshold has been showed with arrows, each sample *β -catenin* gene expression curve has been shown with different colour)

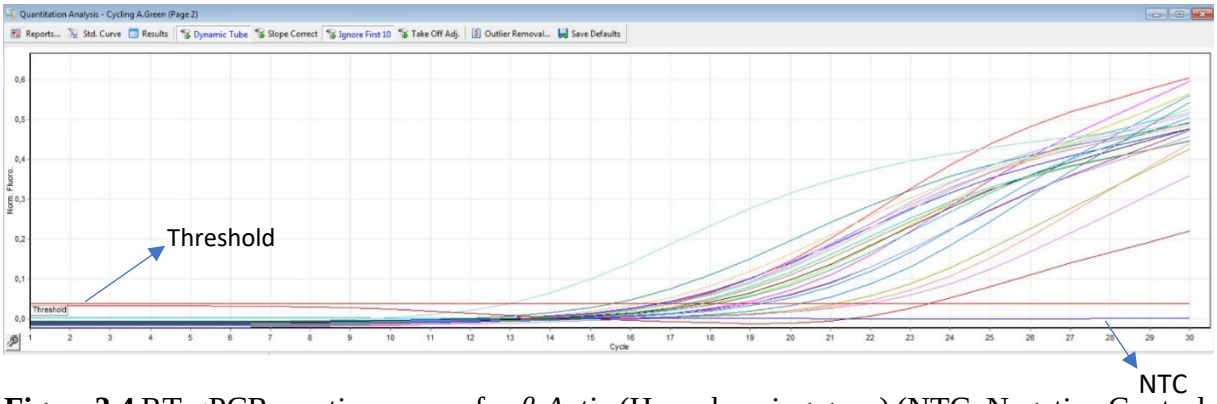

Figure3.4 RT-qPCR reaction curves for *β-Actin* (Housekeeping gene) (NTC: Negative Control, Threshold has been showed with arrows, each sample *β-Actin* gene expression curve has been shown with different colour)

Samples of spontaneous abortions with normal and abnormal karyotype were compared. As seen in Table 3.1, Table 3.2, Table 3.3 and Table 3.4, the threshold was drawn above the negative control curve, and samples passing the threshold have an expression curve. There is no expression in samples that do not exceed the threshold.

As seen Table 3.5, Table 3.6 and Table 3.7, *DVL* gene expression level did not change normal and abnormal karyotype (Table 3.6) , but *GSK3β* was less expressed in the abnormal karyotype than in the normal karyotype that means its down regulated (Table 3.5), On the other hand, the β-catenin gene was more expressed in the abnormal karyotype than in the normal karyotype, that is, it was upregulated (Table 3.7).

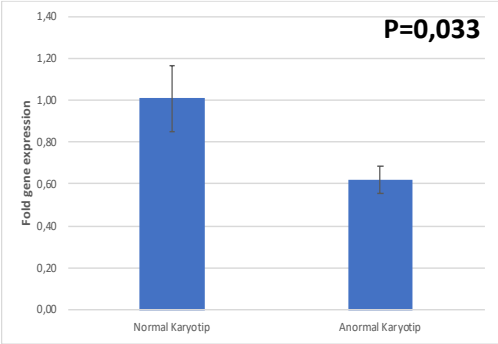

Figure3.5 Comparison of the *GSK3B* gene in samples of spontaneous abortions with normal and abnormal karyotypes. (*GSK3B* gene expression was less expressed (downregulated) in the abnormal karyotype than in the normal karyotype.)

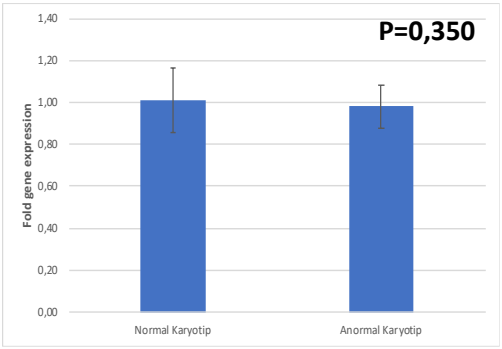

Figure3.6 Comparison of the *DVL1* gene in samples of spontaneous abortions with normal and abnormal karyotypes. (DVL1 gene expression has the same expression level in normal and abnormal karyotype)

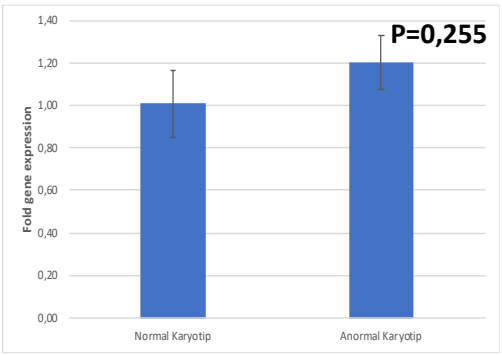

Figure 3.7 Comparison of the *β -catenin* gene in samples of spontaneous abortions with normal and abnormal karyotypes. (*β -catenin* gene expression was higher (upregulated) in abnormal karyotype than in normal karyotype)

3.6 Statistical Analysis

Group Statistics					
Genes	Karyotype	Sample Numbers	Mean	p-Value	Std. Error Mean
GSK3B	Abnormal	8	0.62	0.033	0.06
	Normal	15	1.01		0.09
DVL1	Abnormal	8	0.93	0.350	0.024

	Normal	15	1.01		0.061
β -catenin	Abnormal	8	1.20	0.255	0.09
	Normal	15	1.05		0.101

Table 3.5 shows a statistical analysis of *GSK3B, DVL, β -catenin*

(P values <0.05)= Significant (P values >0.05)= Not significant

3.5 Conclusion

In this study Wnt/β-Catenin signal pathway genes *DVL1, GSK3B* and *β-catenin* examined in spontaneous abortion samples with normal and abnormal karyotype and has been shown that *GSK3B* is significantly down-regulated in abnormal karyotype, *DVL1* does not differ significantly in abnormal and normal karyotype, and *CTNNB1* changes insignificantly but is upregulated (not significant) in abnormal karyotype.

CHAPTER IV: DISCUSSION

4.1 DISCUSSION

The aim of this study is to show the connection between Wnt/β-catenin pathway and associated genes in early pregnancy and implantation failure. In reproductive biology, the early phase of embryogenesis is a complex and important cell-remodeling activity. Maternal-to-zygotic transition (MZT) is critical for both the destruction of maternal products and zygotic genome activation (ZGA) (Minami, Suzuki and Tsukamoto, 2007) , which gene targeting and β-catenin activation assay in mice demonstrated that not only β-catenin (*Ctnnb1*) and several Wnts, including Wnt4, Wnt6, Dkk1, Wnt5a, and Wnt7a are required for various developmental processes including pre-implantation development, implantation of blastocyst but also required for Decidualization of the endometrium, cell proliferation, and differentiation (Deng *et al.*, 2021). In gametes and embryos, all of the most important elements of the WNT signaling pathway are expressed (Knöfler and Pollheimer, 2013). The WNT pathway is mediated by βcatenin, which is phosphorylated at Ser/Thr residues that are highly conserved as a result of its association with the Adenomatous Polyposis Coli (APC) complex, in the absence of WNT ligands. The E3 ubiquitin ligase complex identifies the modified form of βcatenin, which is

then destroyed by the proteasome. βcatenin can no longer be targeted by the APC complex in the presence of WNT ligands, resulting in catenin's nuclear localization and subsequent stability (Clevers and Nusse, 2012). The interaction of βcatenin with a variety of factors in the nucleus, particularly components of the TCF/LEF family, stimulate the transcription of specific genes (Cadigan and Waterman, 2012). It is suggested that Wnt4 has a pleiotropic signal that supports the follicle formation, particularly maturation and coordination of the polarized structure, as well as follicular cell survival, the inactivation of the Wnt4 gene lead to compromised ovarian folliculogenesis and diminished female fertility.Wnt4 knockout mice resulted in ovarian failure (Prunskaite-Hyyryläinen *et al.,* 2014). However, gain-of-function (GOF) causes mesenchymal tumors and infertility due to oviducts malformation, the 4.5th day of a simulated pregnancy, or in treated mice with oestrogen and progesterone to simulate early pregnancy, the progesterone-IHH and oestrogen-LIF-ERK pathways in GOF β-catenin mice are mostly intact; however, JAK/STAT signaling is affected. All in all, dysregulation of β-catenin in connective tissue influences both the epithelial cell STAT3 signaling and uterine conditions (Patterson *et al.*, 2017).

The developmental stages are critical for survival of an embryo as well as proper formations of the tissues. *CTNNB1* is indeed a component of a protein complex, which it helps endothelial and epithelial tissues generate cell–cell junctions. Furthermore, β-catenin 1 increases neurogenesis by retaining sympathetic neuroblasts in the cell cycle thus *CTNNB1* is clearly involved in early developmental process as well as cellular recognition (Komiya and Habas, 2008). *CTNNB1* is a significant factor of the canonical Wnt signaling pathway's downstream components, This gene was discovered to be elevated in this research, making it a useful marker for disease research (Guha *et al.,* 2021). The implications of unregulated β-catenin activation at the developing Mullerian duct mesenchyme were also investigated. β-catenin stabilization in the mesenchyme resulted in epithelial changes such as decreased proliferation, delayed uterine gland development, and the activation of an EMT (epithelial–mesenchymal transition). EMT occurrence occurs prior to birth and ends about 5 days of birth (Patterson *et al.,* 2017). Use of Matrigel invasion assay, illustrated that *WNT3A* induced both trophoblast migration and invasion, which could have been inhibited by *Dkk1*, implying that Wnt/β-catenin signaling is aberrant. *WNT3A* enhanced trophoblast outgrowth in villous explant cultures while also activating the canonical pathway and phosphorylating AKT (Pollheimer *et al.,* 2006). In another study, *WNT7A* (rs104893832) polymorphism was found as a significant association in recurrent

spontaneous abortion ,which comparing to control group the frequency of G allele was significantly different (Mazdapour, Ashkezari and Seifati, 2018). *GSK3β* has a pleiotropic involvement, and knocking it out during the periconceptional stage does not affect fertility but triggers cardiovascular abnormalities, cardiac hyperplasia, and a considerably higher neonatal death rate (Monteiro da Rocha *et al.*, 2015). Antibody array technology demonstrated the downregulation of Dickkopf-related protein (Dkk) in Recurrent spontaneous abortion (RSA) , therefore it is a potential biomarker for the diagnosis and prediction of RSA (Wu *et al.*, 2017). The *DVL2* p. R633W variants adversely affected the protein and increased the activity of all three Wnt signaling pathways in mammals, *DVL1* variants (*DVL1* p. R558H) and (*DVL1* p. R606C) conversely, reduced canonical Wnt/ β-catenin signaling however elevated Wnt/Ca2+ signaling activity. In zebrafish embryos, *DVL2* p. R633W variation leads to fetal abnormalities. Uncommon DVL mutations, particularly *DVL2* p. R633W, by blocking Wnt signaling pathways, possibly play a role in human neural diseases (Liu *et al.*, 2020). As a result, Wnt signaling is particularly important in placental vascularization. Formation of migratory extra villous trophoblasts (EVTs) play an important role in embryonic development. Different trophoblast cell models were used to indicate that TCF4 is linked with stimulation of canonical Wnt signaling in EVTs regulating cell motility and gene expression in EMT and cell migration (Meinhardt *et al.*, 2014).

Has been founded that many genes cause spontaneous abortion. For example, *TRO, CDH11, CDHA* genes affect cell adhesion, trophoblast/endometrium interaction, *THBD, F5, FGA* genes are involved in coagulation, *FGA, MMP10, MMP9, COL6A3, ADAMTS1* and *TNC* genes are responsible for extracellular matrix remodelling, while *FLT1, EPAS1* genes are responsible for the vascularization process, *LIFR, FGFR2, BMP7* genes are involved in proliferation, differentiation, migration and apoptosis processes, while *AMN* gene affects metabolism, *IDO2, CR1, TLR3, TRAF3IP1* is involved in modulation of immunological function and mutations in these genes can cause spontaneous abortions (Fukuda and Sugihara, 2008)(Puscheck and Jeyendran, 2007)(Zhao *et al.*, 2010; Nakamura and Saji, 2014) (Quintero-Ronderos *et al.*, 2017)

A total of 23 samples were obtained from the IVF clinic at Near East Hospital, wherein they were placed into two categories namely Normal and Abnormal karyotype. The study was actualized to observe the expression level of Wnt-signalling pathway genes in (*DVL1, GSK3B* and *β-catenin*) in all 23 abortion materials. The RNAs were isolated from these samples and further processed for cDNA synthesis. The gradient PCR was completed to obtain the optimum

temperature ranging from 56℃ to 61℃ of the three genes along with the housekeeping gene (β-actin). The genes were further processed using Agarose gel electrophoresis, but each gene displayed no bands rather primer dimers were visualized.

Gradient PCR has been carried out to get the optimum annealing temperature for the genes (*DVL1, GSK3B,* and *β-catenin* genes). Gradient PCR showed a band around 150 bp as a result of *GSK3B* agarose gel, no specific bands were seen in *DVL1* and *CTNNB1*. As a result of the analysis, the bonding temperatures were set as 59℃.

In this study, samples of spontaneous abortions with normal and abnormal karyotype were compared. As seen Table 3.5, Table 3.6 and Table 3.7, *GSK3B* is significantly down-regulated in abnormal karyotype (Table 3.5), *DVL1* does not differ significantly in abnormal and normal karyotype (Table 3.6). On the other hand, *CTNNB1* changes insignificantly but is upregulated (not significant) in abnormal karyotype (Table 3.7). It may be important information that this gene differs in abnormal and normal karyotype, a more detailed study with more samples is required for more precise results. It is expected that β-catenin is upregulated while *GSK3β* is down-regulated. because *GSK3β* acts as a β-catenin degradation complex in the cell. When β-catenin is high, *GSK3β* should not work or work less. So, what does the upregulation of β-catenin in the abnormal karyotype indicate, namely, that as described earlier, β-catenin have a significant role in embryonic development. It is already known that the probability of miscarriage is high in the abnormal karyotype, downregulation of β-catenin in the normal karyotype may result in a high probability of miscarriage in the normal karyotype. However, more samples are needed for conclusive results, monitoring of protein levels by different techniques such as immunochemistry, and fetal unrealized fetal samples for better comparison. When these conditions are met, more precise information will be obtained, and this information is important. Finding out this signaling pathway and which genes cause spontaneous abortions is of great importance in learning and treating the cause of spontaneous abortions of unknown cause, which is at the level of 50%.

4.2 CONCLUSION

This study showed, when samples of spontaneous abortions with normal and abnormal karyotype were compared for Wnt/β-Catenin signal pathway genes *DVL1, GSK3B* and *β-*

catenin, It was observed that *DVL1* did not change, but *GSK3β* was observed to be down-regulated in abnormal karyotype, it may be important information that this gene differs in abnormal and normal karyotype, a more detailed study with more samples is required for more precise results. It is expected that β-catenin is upregulated while GSK3B is down-regulated. because *GSK3B* acts as a β-catenin degradation complex in the cell. When β-catenin is high, *GSK3B* should not work or work less. So, what does the upregulation of β-catenin in the abnormal karyotype indicate, namely, that as described earlier, β-catenin have a significant role in embryonic development. It is already known that the probability of miscarriage is high in the abnormal karyotype, recent studies showed abnormal karyotypes like invasion 9 associated pregnancy failure, idiopathic miscarriage (Xie *et al.*, 2020), another example has been known that tetraploidy fetuses end in miscarriage (Bothur-Nowacka *et al.*, 2013), other research showed tetraploidy leads to early miscarriage (Filges *et al.*, 2014), etc. so many research improved that the probability of miscarriage is high in the abnormal karyotype, its possible that downregulation of β-catenin in the normal karyotype may result in a high probability of miscarriage in the normal karyotype.

REFERENCES

Antonarakis, S. E. *et al.* (2020) 'Down syndrome', *Nature Reviews Disease Primers*. Springer US, 6(1), pp. 1–20. doi: 10.1038/s41572-019-0143-7.

Von Arnold, S. *et al.* (2002) 'Developmental pathways of somatic embryogenesis', *Plant Cell, Tissue and Organ Culture*, 69(3), pp. 233–249. doi: 10.1023/A:1015673200621.

Barnhart, K. *et al.* (2004) 'Decline of serum human chorionic gonadotropin and spontaneous complete abortion: defining the normal curve.', *Obstetrics and gynecology*. United States, 104(5 Pt 1), pp. 975–981. doi: 10.1097/01.AOG.0000142712.80407.fd.

Baudat, F., Imai, Y. and De Massy, B. (2013) 'Meiotic recombination in mammals: Localization and regulation', *Nature Reviews Genetics*. Nature Publishing Group, 14(11), pp.

794–806. doi: 10.1038/nrg3573.

Bellati, F. *et al.* (2019) ‘Low endometrial beta-catenin and cadherins expression patterns are predictive for primary infertility and recurrent pregnancy loss’, *Gynecological Endocrinology*. Taylor & Francis, 35(8), pp. 727–731. doi: 10.1080/09513590.2019.1579790.

Birch, J. D., Gulati, D. and Mandalia, S. (2017) ‘Cervical shock: A complication of incomplete abortion’, *BMJ Case Reports*, 2017. doi: 10.1136/bcr-2017-220452.

Bolcun-Filas, E. and Handel, M. A. (2018) ‘Meiosis: The chromosomal foundation of reproduction’, *Biology of Reproduction*, 99(1), pp. 112–126. doi: 10.1093/biolre/ioy021.

Bothur-Nowacka, J. *et al.* (2013) ‘Tetraploidy in the era of molecular karyotyping - What we need to remember’, *Pediatria Polska*, 88(5), pp. 467–471. doi: 10.1016/j.pepo.2013.06.002.

Broman, K. W. *et al.* (1998) ‘Comprehensive human genetic maps: Individual and sex-specific variation in recombination’, *American Journal of Human Genetics*, 63(3), pp. 861–869. doi: 10.1086/302011.

Cadigan, K. M. and Waterman, M. L. (2012) ‘TCF/LEFs and Wnt signaling in the nucleus.’, *Cold Spring Harbor perspectives in biology*, 4(11). doi: 10.1101/cshperspect.a007906.

Carey, J. C. (2010) ‘Trisomy 18 and Trisomy 13 Syndromes’, *Management of Genetic Syndromes: Third Edition*, pp. 807–823. doi: 10.1002/9780470893159.ch54.

Cereda, A. and Carey, J. C. (2012) ‘The trisomy 18 syndrome’, *Orphanet Journal of Rare Diseases*, 7(1), pp. 1–14. doi: 10.1186/1750-1172-7-81.

Chakraborty, P. *et al.* (2013) ‘Recurrent Pregnancy Loss in Polycystic Ovary Syndrome: Role of Hyperhomocysteinemia and Insulin Resistance’, *PLoS ONE*, 8(5), pp. 1–6. doi:

10.1371/journal.pone.0064446.

Chen, Q. *et al.* (2009) 'Embryo – uterine cross-talk during implantation : the role of Wnt signaling †', 15(4), pp. 215–221. doi: 10.1093/molehr/gap009.

Ciarke, H. *et al.* (2005) 'Uterine Wnt/ß-catenin signaling is required for implantation', *Proceedings of the National Academy of Sciences of the United States of America*, (24), pp. 8579–8584.

Çikgöz, E. A. (2015) 'Embriyoner gelişimde transkripsiyonel ağlar ve hücre sinyalleri Transcriptional networks and cell signals in embryonic development', 54(2), pp. 101–105.

Clevers, H. (2006) 'Wnt/β-Catenin Signaling in Development and Disease', *Cell*, 127(3), pp. 469–480. doi: 10.1016/j.cell.2006.10.018.

Clevers, H. and Nusse, R. (2012) 'Wnt/β-catenin signaling and disease', *Cell*, 149(6), pp. 1192–1205. doi: 10.1016/j.cell.2012.05.012.

Dawood, F., Farquharson, R. G. and Stephenson, M. D. (2010) 'Investigation of recurrent miscarriage', *Early Pregnancy*, 331, pp. 59–66. doi: 10.1017/CBO9780511777851.008.

Deng, D. *et al.* (2021) 'Canonical Wnt Signaling Pathway on Polarity Formation of Utricle Hair Cells', *Neural Plasticity*, 2021. doi: 10.1155/2021/9950533.

Denicol, A. C. *et al.* (2013) 'Canonical WNT signaling regulates development of bovine embryos to the blastocyst stage', *Scientific Reports*, 3, pp. 1–7. doi: 10.1038/srep01266.

Dong, F. *et al.* (2014) 'Genome-wide miRNA profiling of villus and decidua of recurrent spontaneous abortion patients', *Reproduction*, 148(1), pp. 33–41. doi: 10.1530/REP-14-0095.

Filges, I. *et al.* (2014) 'Recurrent triploidy due to a failure to complete maternal meiosis II:

Wholeexome sequencing reveals candidate variants', *Molecular Human Reproduction*, 21(4), pp. 339–346. doi: 10.1093/molehr/gau112.

Freese, J. L., Pino, D. and Pleasure, S. J. (2010) 'Wnt signaling in development and disease', *Neurobiology of Disease*, 38(2), pp. 148–153. doi: 10.1016/j.nbd.2009.09.003.

Friebe, A. and Arck, P. (2008) 'Causes for spontaneous abortion: What the bugs "gut" to do with it?', *International Journal of Biochemistry and Cell Biology*, 40(11), pp. 2348–2352. doi: 10.1016/j.biocel.2008.04.019.

Fujimura, N. (2016) 'WNT/β-catenin signaling in vertebrate eye development', *Frontiers in Cell and Developmental Biology*, 4(NOV), pp. 1–7. doi: 10.3389/fcell.2016.00138.

Fukuda, M. N. and Sugihara, K. (2008) 'An integrated view of L-selectin and trophinin function in human embryo implantation.', *The journal of obstetrics and gynaecology research*, 34(2), pp. 129–136. doi: 10.1111/j.1447-0756.2008.00776.x.

Fukuda, M. N. and Sugihara, K. (2012) 'Cell adhesion molecules in human embryo implantation.', *Sheng li xue bao : [Acta physiologica Sinica]*. China, 64(3), pp. 247–258.

Gaboon, N. E. A. (2013) 'Recurrent spontaneous abortion: An overview of genetic backgrounds and impact of male factors: A review', *International Journal of Human Genetics*, 13(2), pp. 79–83. doi: 10.1080/09723757.2013.11886200.

Gartstein., M. A., Putnam., S. and Kliewer., R. (2016) '乳鼠心肌提取 HHS Public Access', *Physiology & behavior*, 176(3), pp. 139–148. doi: 10.1016/j.cell.2020.11.003.Principles.

Gentzel, M. and Schambony, A. (2017) 'Dishevelled paralogs in vertebrate development: Redundant or distinct?', *Frontiers in Cell and Developmental Biology*, 5(MAY), pp. 1–8. doi: 10.3389/fcell.2017.00059.

George, L. *et al.* (2002) ‘Plasma folate levels and risk of spontaneous abortion.’, *JAMA*. United States, 288(15), pp. 1867–1873. doi: 10.1001/jama.288.15.1867.

George, L. *et al.* (2006) ‘Risks of repeated miscarriage.’, *Paediatric and perinatal epidemiology*. England, 20(2), pp. 119–126. doi: 10.1111/j.1365-3016.2006.00703.x.

Girirajan, S., Campbell, C. and Eichler, E. (2011) ‘乳鼠心肌提取 HHS Public Access’, *Physiology & behavior*, 176(5), pp. 139–148. doi: 10.1146/annurev-pathmechdis-012418-012743.Immunological.

Golson, M. L. and Kaestner, K. H. (2016) ‘Fox transcription factors: from development to disease.’, *Development (Cambridge, England)*, 143(24), pp. 4558–4570. doi: 10.1242/dev.112672.

Gravholt, C. H. *et al.* (2019) ‘Turner syndrome: mechanisms and management’, *Nature Reviews Endocrinology*. Springer US, 15(10), pp. 601–614. doi: 10.1038/s41574-019-0224-4.

Griebel, C. P. *et al.* (2005) ‘Management of spontaneous abortion’, *American Family Physician*, 72(7), pp. 1243–1250.

Guha, P. *et al.* (2021) ‘A comparative cross-platform meta-analysis to identify potential biomarker genes common to endometriosis and recurrent pregnancy loss’, *Applied Sciences (Switzerland)*, 11(8), pp. 1–17. doi: 10.3390/app11083349.

El Hachem, H. *et al.* (2017) ‘Recurrent pregnancy loss: Current perspectives’, *International Journal of Women’s Health*, 9, pp. 331–345. doi: 10.2147/IJWH.S100817.

Hassold, T. J. (1980) ‘A cytogenetic study of repeated spontaneous abortions’, *American Journal of Human Genetics*, 32(5), pp. 723–730.

Hou, X. *et al.* (2004) 'Canonical Wnt signaling is critical to estrogen-mediated uterine growth.', *Molecular endocrinology (Baltimore, Md.)*, 18(12), pp. 3035–3049. doi: 10.1210/me.2004-0259.

Huang, L. *et al.* (2019) 'Down-regulation of DKK1 and Wnt1/β-catenin pathway by increased homeobox B7 resulted in cell differentiation suppression of intrauterine fetal growth retardation in human placenta', *Placenta*. Elsevier, 80(March), pp. 27–35. doi: 10.1016/j.placenta.2019.03.001.

Huelsken, J. and Behrens, J. (2002) 'The Wnt signalling pathway', *Journal of Cell Science*, 115(21), pp. 3977–3978. doi: 10.1242/jcs.00089.

Hunter, N. (2015) 'Meiotic recombination: The essence of heredity', *Cold Spring Harbor Perspectives in Biology*, 7(12), pp. 1–35. doi: 10.1101/cshperspect.a016618.

Hyde, K. J. and Schust, D. J. (2015) 'Pregnancy Loss', pp. 1–18.

Israels, E. D. and Israels, L. G. (2000) 'The Cell Cycle', *The Oncologist*, 5(6), pp. 510–513. doi: 10.1634/theoncologist.5-6-510.

Kanmaz, A. G., İnan, A. H., *et al.* (2019) 'The effects of threatened abortions on pregnancy outcomes.', *Ginekologia polska*. Poland, 90(4), pp. 195–200. doi: 10.5603/GP.a2019.0035.

Kanmaz, A. G., Inan, A. H., *et al.* (2019) 'The effects of threatened abortions on pregnancy outcomes', *Ginekologia Polska*, 90(4), pp. 195–200. doi: 10.5603/GP.a2019.0035.

Kauppi, L., Jeffreys, A. J. and Keeney, S. (2004) 'Where the crossovers are: Recombination distributions in mammals', *Nature Reviews Genetics*, 5(6), pp. 413–424. doi: 10.1038/nrg1346.

Kemp, C. *et al.* (2005) 'Expression of all Wnt genes and their secreted antagonists during mouse blastocyst and postimplantation development', *Developmental Dynamics*, 233(3), pp. 1064–1075. doi: 10.1002/dvdy.20408.

Kesler, S. R. (2007) 'Turner Syndrome', *Child and Adolescent Psychiatric Clinics of North America*, 16(3), pp. 709–722. doi: 10.1016/j.chc.2007.02.004.

Knöfler, M. and Pollheimer, J. (2013) 'Human placental trophoblast invasion and differentiation: A particular focus on Wnt signaling', *Frontiers in Genetics*, 4(SEP), pp. 1–14. doi: 10.3389/fgene.2013.00190.

Koler, M. *et al.* (2009) 'Disrupted gene pattern in patients with repeated in vitro fertilization (IVF) failure.', *Human reproduction (Oxford, England)*. England, 24(10), pp. 2541–2548. doi: 10.1093/humrep/dep193.

Komiya, Y. and Habas, R. (2008) 'Wnt Secretion and Extra-Cellular Regulators', 4(2), pp. 68–75. Available at: www.landesbioscience.com.

Koni, M., Pinnarò, V. and Brizzi, M. F. (2020) 'The wnt signalling pathway: A tailored target in cancer', *International Journal of Molecular Sciences*, 21(20), pp. 1–26. doi: 10.3390/ijms21207697.

Kraus, P. and Lufkin, T. (2006) 'Dlx homeobox gene control of mammalian limb and craniofacial development', *American Journal of Medical Genetics Part A*, 140A(13), pp. 1366–1374. doi: https://doi.org/10.1002/ajmg.a.31252.

Li, N. *et al.* (2017) 'Decreased secretion of WNT3 by deciduas of URSA patients leads to trophoblast cell dysfunction', *International Journal of Clinical and Experimental Pathology*, 10(6), pp. 6827–6835.

Li, S. *et al.* (2015) ‘Decreased β-catenin expression in first-trimester villi and decidua of patients with recurrent spontaneous abortion’, *Journal of Obstetrics and Gynaecology Research*, 41(6), pp. 904–911. doi: 10.1111/jog.12647.

Liu, L. *et al.* (2020) ‘DVL mutations identified from human neural tube defects and Dandy-Walker malformation obstruct the Wnt signaling pathway’, *Journal of Genetics and Genomics*. Institute of Genetics and Developmental Biology, 47(6), pp. 301–310. doi: 10.1016/j.jgg.2020.06.003.

Logan, C. Y. and Nusse, R. (2004) ‘The Wnt signaling pathway in development and disease’, *Annual Review of Cell and Developmental Biology*, 20, pp. 781–810. doi: 10.1146/annurev.cellbio.20.010403.113126.

Losick, V. P. *et al.* (2011) ‘Drosophila Stem Cell Niches: A Decade of Discovery Suggests a Unified View of Stem Cell Regulation’, *Developmental Cell*, 21(1), pp. 159–171. doi: 10.1016/j.devcel.2011.06.018.

Lufkin, T. (1997) ‘Transcriptional regulation of vertebrate Hox genes during embryogenesis.’, *Critical reviews in eukaryotic gene expression*. United States, 7(3), pp. 195–213. doi: 10.1615/critreveukargeneexpr.v7.i3.10.

MacDonald, B. T., Tamai, K. and He, X. (2009) ‘Wnt/β-Catenin Signaling: Components, Mechanisms, and Diseases’, *Developmental Cell*, 17(1), pp. 9–26. doi: 10.1016/j.devcel.2009.06.016.

Maconochie, N. *et al.* (2007) ‘Risk factors for first trimester miscarriage—results from a UK-population-based case–control study’, *BJOG: An International Journal of Obstetrics & Gynaecology*, 114(2), pp. 170–186. doi: https://doi.org/10.1111/j.1471-0528.2006.01193.x.

Magli, M. C., Gianaroli, L. and Ferraretti, A. P. (2001) 'Chromosomal abnormalities in embryos', *Molecular and Cellular Endocrinology*, 183(SUPPL. 1), pp. 29–34. doi: 10.1016/S0303-7207(01)00574-3.

Magnus, M. C. *et al.* (2019) 'Role of maternal age and pregnancy history in risk of miscarriage: Prospective register based study', *BMJ (Online)*, 364, pp. 1–8. doi: 10.1136/bmj.l869.

Mahajan, T., Ganguly, S. and Pagrut, N. (2018) 'Embryogenesis : a comprehensive review Embryogenesis : A comprehensive review', (February). doi: 10.22271/j.ento.6.1.267.1.

Maruyama, I., Bell, C. E. and Majerus, P. W. (1985) 'Thrombomodulin is found on endothelium of arteries, veins, capillaries, and lymphatics, and on syncytiotrophoblast of human placenta.', *The Journal of cell biology*, 101(2), pp. 363–371. doi: 10.1083/jcb.101.2.363.

Massaro, E. J. and Haven, N. (no date) 'development'.

Mazdapour, M., Ashkezari, M. D. and Seifati, S. M. (2018) 'WNT7A (rs104893832) polymorphism increases the risk of recurrent spontaneous abortion in Iranian women', *Universa Medicina*, 37(3), pp. 167–172. doi: 10.18051/univmed.2018.v37.167-172.

Meinhardt, G. *et al.* (2014) 'Wnt-Dependent T-Cell Factor-4 controls human etravillous trophoblast motility', *Endocrinology*, 155(5), pp. 1908–1920. doi: 10.1210/en.2013-2042.

Miller-Hodges, E. and Hohenstein, P. (2012) 'WT1 in disease: Shifting the epithelial-mesenchymal balance', *Journal of Pathology*, 226(2), pp. 229–240. doi: 10.1002/path.2977.

Minami, N., Suzuki, T. and Tsukamoto, S. (2007) 'Zygotic gene activation and maternal factors in mammals', *Journal of Reproduction and Development*, 53(4), pp. 707–715. doi:

10.1262/jrd.19029.

Monteiro da Rocha, A. *et al.* (2015) ‘Loss of glycogen synthase kinase 3 isoforms during murine oocyte growth induces offspring cardiac dysfunction.’, *Biology of reproduction*, 92(5), p. 127. doi: 10.1095/biolreprod.115.128181.

Moon, R. T. and Kimelman, D. (1998) ‘From cortical rotation to organizer gene expression: toward a molecular explanation of axis specification in Xenopus’, *BioEssays*, 20(7), pp. 536–546. doi: https://doi.org/10.1002/(SICI)1521-1878(199807)20:7<536::AID-BIES4>3.0.CO;2-I.

Nagaishi, M. *et al.* (2004) ‘Chromosome abnormalities identified in 347 spontaneous abortions collected in Japan’, *Journal of Obstetrics and Gynaecology Research*, 30(3), pp. 237–241. doi: https://doi.org/10.1111/j.1447-0756.2004.00191.x.

Nakamura, H. and Saji, H. (2014) ‘A worldwide trend of increasing primary adenocarcinoma of the lung’, *Surgery Today*, 44(6), pp. 1004–1012. doi: 10.1007/s00595-013-0636-z.

Niehrs, C. (2010) ‘On growth and form : a Cartesian coordinate system of Wnt and BMP signaling specifies bilaterian body axes’, 857, pp. 845–857. doi: 10.1242/dev.039651.

Ohkura, H. (2015) ‘Meiosis: An overview of key differences from mitosis’, *Cold Spring Harbor Perspectives in Biology*, 7(5), pp. 1–15. doi: 10.1101/cshperspect.a015859.

Passos, I. de M. P. e. and Britto, R. L. (2020) ‘Diagnosis and treatment of müllerian malformations’, *Taiwanese Journal of Obstetrics and Gynecology*, 59(2), pp. 183–188. doi: 10.1016/j.tjog.2020.01.003.

Patterson, A. L. *et al.* (2017) ‘Gain-of-function β-catenin in the uterine mesenchyme leads to impaired implantation and decidualization.’, *The Journal of endocrinology*, 233(1), pp. 119–

130. doi: 10.1530/JOE-16-0502.

Philipp, T. *et al.* (2003) 'Embryoscopic and cytogenetic analysis of 233 missed abortions: Factors involved in the pathogenesis of developmental defects of early failed pregnancies', *Human Reproduction*, 18(8), pp. 1724–1732. doi: 10.1093/humrep/deg309.

Piyamongkol, W. (2020) 'Pre-implantation genetic testing for aneuploidy (PGT-A)', *Thai Journal of Obstetrics and Gynaecology*, 28(3), pp. 130–135.

Pollheimer, J. *et al.* (2006) 'Activation of the canonical wingless/T-cell factor signaling pathway promotes invasive differentiation of human trophoblast', *American Journal of Pathology*, 168(4), pp. 1134–1147. doi: 10.2353/ajpath.2006.050686.

Prunskaite-Hyyryläinen, R. *et al.* (2014) 'Wnt4, a pleiotropic signal for controlling cell polarity, basement membrane integrity, and antimüllerian hormone expression during oocyte maturation in the female follicle', *FASEB Journal*, 28(4), pp. 1568–1581. doi: 10.1096/fj.13-233247.

Puscheck, E. E. and Jeyendran, R. S. (2007) 'The impact of male factor on recurrent pregnancy loss.', *Current opinion in obstetrics & gynecology*. England, 19(3), pp. 222–228. doi: 10.1097/GCO.0b013e32813e3ff0.

Quach, T. *et al.* (2015) 'Adverse birth outcomes and maternal complications in licensed cosmetologists and manicurists in California.', *International archives of occupational and environmental health*. Germany, 88(7), pp. 823–833. doi: 10.1007/s00420-014-1011-0.

Quintero-Ronderos, P. *et al.* (2017) 'OC-2a: Novel genes and mutations in patients with recurrent spontaneous abortion', *Thrombosis Research*, 151, p. S104. doi: 10.1016/s0049-3848(17)30082-8.

Raghupathy, R. (2003) 'The immunology of unexplained recurrent spontaneous abortion: cytokines as key mediators', *Bull. Kuwait Inst. Med. Spec*, (March), pp. 32–38. Available at: http://kims.org.kw/bulletin/Issues/issue3/Immunology.pdf.

Revel, A. *et al.* (2011) 'MicroRNAs are associated with human embryo implantation defects', *Human Reproduction*, 26(10), pp. 2830–2840. doi: 10.1093/humrep/der255.

Rossant, J. and Tam, P. P. L. (2018) 'Exploring early human embryo development', *Science*, 360(6393), pp. 1075–1076. doi: 10.1126/science.aas9302.

Rubio, C. and Simón, C. (2021) 'Embryo genetics', *Genes*, 12(1), pp. 1–3. doi: 10.3390/genes12010118.

Sanz-Ezquerro, J. J., Münsterberg, A. E. and Stricker, S. (2017) 'Editorial: Signaling pathways in embryonic development', *Frontiers in Cell and Developmental Biology*, 5(AUG), pp. 1–3. doi: 10.3389/fcell.2017.00076.

Singh, N., Singh, D. and Modi, D. (2021) 'LIM Homeodomain (LIM-HD) Genes and Their Co-Regulators in Developing Reproductive System and Disorders of Sex Development', *Sexual Development*, pp. 1–15. doi: 10.1159/000518323.

Sirait, B. *et al.* (2022) 'Potential Use of Immature Oocyte to Improve Fertility Preservation Outcome: A Narrative Review', *Journal of Human Reproductive Sciences*, In-press, pp. 247–253. doi: 10.4103/jhrs.jhrs.

Suzumori, N. and Sugiura-Ogasawara, M. (2012) 'Genetic Factors as a Cause of Miscarriage', *Current Medicinal Chemistry*, 17(29), pp. 3431–3437. doi: 10.2174/092986710793176302.

Tam, P. P. L. and Loebel, D. A. F. (2007) 'Gene function in mouse embryogenesis: Get set for gastrulation', *Nature Reviews Genetics*, 8(5), pp. 368–381. doi: 10.1038/nrg2084.

Wall, N. A. and Hogan, B. L. M. (1994) 'TGF-β related genes in development', *Current Opinion in Genetics & Development*, 4(4), pp. 517–522. doi: https://doi.org/10.1016/0959-437X(94)90066-C.

Wang, Q. *et al.* (2008) 'Pax genes in embryogenesis and oncogenesis: Genes...', *Journal of Cellular and Molecular Medicine*, 12(6A), pp. 2281–2294. doi: 10.1111/j.1582-4934.2008.00427.x.

Warren, J. E. and Silver, R. M. (2008) 'Genetics of pregnancy loss.', *Clinical obstetrics and gynecology*. United States, 51(1), pp. 84–95. doi: 10.1097/GRF.0b013e318161719c.

Wilcox, A. J. *et al.* (1988) 'Incidence of Early Loss of Pregnancy', *New England Journal of Medicine*. Massachusetts Medical Society, 319(4), pp. 189–194. doi: 10.1056/NEJM198807283190401.

Wu, Y. *et al.* (2017) 'Serum biomarker analysis in patients with recurrent spontaneous abortion', *Molecular Medicine Reports*, 16(3), pp. 2367–2378. doi: 10.3892/mmr.2017.6890.

Xie, X. *et al.* (2020) 'Analysis of the clinical features of pericentric inversion of chromosome 9', *Journal of International Medical Research*, 48(9). doi: 10.1177/0300060520957820.

Zeitlinger, J. and Stark, A. (2010) 'Developmental gene regulation in the era of genomics', *Developmental Biology*. Elsevier Inc., 339(2), pp. 230–239. doi: 10.1016/j.ydbio.2009.12.039.

Zhang, Y.-X. *et al.* (2009) 'Genetic analysis of first-trimester miscarriages with a combination of cytogenetic karyotyping, microsatellite genotyping and arrayCGH', *Clinical Genetics*, 75(2), pp. 133–140. doi: https://doi.org/10.1111/j.1399-0004.2008.01131.x.

Zhao, Y. *et al.* (2010) 'The impact of luteal phase support on gene expression of extracellular matrix protein and adhesion molecules in the human endometrium during the window of

implantation following controlled ovarian stimulation with a GnRH antagonist protocol.', *Fertility and sterility*. United States, 94(6), pp. 2264–2271. doi: 10.1016/j.fertnstert.2010.01.068.

Zinaman, M. J. *et al.* (1996) 'Estimates of human fertility and pregnancy loss*†*Supported by grant CR-820787 from the United States Environmental Protection Agency, Washington, D.C.†The views expressed in this paper are those of the authors and do not necessarily reflect the views or ', *Fertility and Sterility*, 65(3), pp. 503–509. doi: https://doi.org/10.1016/S0015-0282(16)58144-8.

thesis 2

ORIGINALITY REPORT

13%	10%	5%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	docs.neu.edu.tr Internet Source	7%
2	Aya Badeea Ismail, Marwan 'Mohammad Saeed' Naji, İnci Nebih, Gulten Tuncel et al. "The expression profile of WNT/β-catanin signalling genes in human oocytes obtained from polycystic ovarian syndrome (PCOS) patients", Zygote, 2022 Publication	1%
3	en.wikipedia.org Internet Source	<1%
4	Bruce M. Carlson. "Molecular Basis for Embryonic Development", Elsevier BV, 2014 Publication	<1%
5	Filippo Bellati, Flavia Costanzi, Maria Paola De Marco, Claudia Cippitelli et al. "Low endometrial beta-catenin and cadherins expression patterns are predictive for primary infertility and recurrent pregnancy loss", Gynecological Endocrinology, 2019 Publication	<1%

6	lra.le.ac.uk Internet Source	<1 %
7	docksci.com Internet Source	<1 %
8	www.tandfonline.com Internet Source	<1 %
9	academic.oup.com Internet Source	<1 %
10	J. Wang. "Increased Expression of -catenin, Phospho-Glycogen Synthase Kinase 3 , cyclinD1, and c-myc in Laterally Spreading Colorectal Tumors", Journal of Histochemistry and Cytochemistry, 12/08/2008 Publication	<1 %
11	Hayden J. Selvadurai. "Wnt/β-catenin Signalling Is Active in a Highly Dynamic Pattern during Development of the Mouse Cerebellum", PLoS ONE, 08/08/2011 Publication	<1 %
12	infectagentscancer.biomedcentral.com Internet Source	<1 %
13	textarchive.ru Internet Source	<1 %
14	www.spandidos-publications.com Internet Source	<1 %

15	Tao Tong, Nahyun Kim, Taesun Park. "Topical Application of Oleuropein Induces Anagen Hair Growth in Telogen Mouse Skin", PLOS ONE, 2015 Publication	<1 %
16	Craig, Nancy. "Molecular Biology- Principles of Genome Function", Oxford University Press, 2020 Publication	<1 %
17	Hiroyasu Okazaki, Seidai Sato, Kazuya Koyama, Shun Morizumi et al. "The novel inhibitor PRI-724 for Wnt/β-catenin/CBP signaling ameliorates bleomycin-induced pulmonary fibrosis in mice", Experimental Lung Research, 2019 Publication	<1 %
18	Straimer, Judith. "Studies on evolution of drug resistance in P. falciparum malaria in vivo and in vitro", 'American Association for Cancer Research (AACR)', 2010 Internet Source	<1 %
19	docplayer.net Internet Source	<1 %
20	research-repository.st-andrews.ac.uk Internet Source	<1 %
21	Anil Sigdel, Rafael S. Bisinotto, Francisco Peñagaricano. "Genes and pathways	<1 %

	associated with pregnancy loss in dairy cattle", Scientific Reports, 2021 Publication	
22	Young Sun Hwang, Minseok Seo, Sohyun Bang, Heebal Kim, Jae Yong Han. "Transcriptional and translational dynamics during maternal - to - zygotic transition in early chicken development", The FASEB Journal, 2018 Publication	<1 %
23	eprints.nottingham.ac.uk Internet Source	<1 %
24	mafiadoc.com Internet Source	<1 %
25	purehost.bath.ac.uk Internet Source	<1 %
26	repository.upenn.edu Internet Source	<1 %
27	researchspace.ukzn.ac.za Internet Source	<1 %
28	www.who.int Internet Source	<1 %
29	Jiashuo wang, Sai Wang, Xiaoxu Ding, Yue Hu, Aihui Yan. "Knockdown of GOLM1 inhibits growth of laryngeal cancer cells via the Wnt/	<1 %

β-catenin pathway", Research Square Platform LLC, 2022
Publication

30	Zainab Jagani, Roya Khosravi-Far. "Chapter 15 Cancer Stem Cells and Impaired Apoptosis", Springer Science and Business Media LLC, 2008 Publication	<1 %
31	epdf.pub Internet Source	<1 %
32	www.assignmenthelp.net Internet Source	<1 %
33	www.mdpi.com Internet Source	<1 %
34	www.omicsonline.org Internet Source	<1 %
35	www.science.gov Internet Source	<1 %
36	Anthony Veltri, Christopher Lang, Wen-Hui Lien. "Wnt Signalling Pathways in Skin Development and Epidermal Stem Cells", STEM CELLS, 2017 Publication	<1 %
37	Fagen Xie, Cheryl Mercado, Sunhea S. Kim, Wei Yu, Chengyi Zheng, Darios Getahun, Steven J. Jacobsen, Wansu Chen. "Identifying	<1 %

	Spontaneous Abortion from Clinical Notes within a Large Integrated Healthcare System", SN Computer Science, 2022 Publication	
38	Roman Anton, Hans A. Kestler, Michael Kühl. "β-Catenin signaling contributes to stemness and regulates early differentiation in murine embryonic stem cells", FEBS Letters, 2007 Publication	<1 %
39	dev.biologists.org Internet Source	<1 %
40	hrcak.srce.hr Internet Source	<1 %
41	studentsrepo.um.edu.my Internet Source	<1 %
42	www.wjgnet.com Internet Source	<1 %
43	Kenyi Saito-Diaz, Tony W. Chen, Xiaoxi Wang, Curtis A. Thorne, Heather A. Wallace, Andrea Page-McCaw, Ethan Lee. "The way Wnt works: Components and mechanism", Growth Factors, 2012 Publication	<1 %
44	Li Zhang, Xiuwei Wang, Rui Cao, Dandan Li, Yufei Wang, Ajab Khan, Keke Wang, Zhizhen Liu, Bo Niu, Jun Xu, Jun Xie. "Reduced H3K27me3 Suppresses Wnt/β-catenin	<1 %

Signaling by S-adenosylmethionine Deficiency in Neural Tube Development", Research Square Platform LLC, 2021
Publication

45	Ksenia Kulikova, Alexey Kibardin, Nikolay Gnuchev, Georgii Georgiev, Sergey Lari. "Chapter 12 Dual Function of Wnts in Human Cutaneous Melanoma", IntechOpen, 2011 Publication	<1 %

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		

ELİF GÜLSEREN(MSc)

Molecular Biologist and Geneticist

PROFILE

I finished Mugla Sıtkı Kocman University, Molecular Biology and Genetics department with a grade of 3.46. I completed master's degree in Molecular Medicine in Near East University. I have the teorical knowledge and laboratory experience in my field thanks to my internships and the workplace I work at.

Techniques Learned in the Lab:

- DNA/RNA Isolation
- PCR (Polymerase Chain Reaction) (PCR)
- SDS-PAGE Electrophoresis
- Agarose Gel Electrophoresis
- Real Time PCR
- Gradient PCR
- Bacteria cultivation in liquid and solid culture
- Gene Cloning to Bacteria
- Microsoft Office programs

AREAS OF INTEREST

Embryology, Developmental Genetics, Genetics, Genetic diseases, Gene Therapy

CONTACT

Address: Famagusta/ TRNC

Tel: 05338610112

Email: elifgulseren@outlook.com

Date of Birth: 07 October 1998

Marital Status: Single

Citizenship: TRNC/ TR

LANGUAGES

Turkish – Native Language

English -- Advanced

Arabic – Beginner

Education

2012-2016 **HIGH SCHOOL** - Namık Kemal High School, Department of Science, TRNC (DiplOma grade 8.65)

2016-2020 **UNDERGRADUATE-** Muğla Sıtkı Koçman University, Molecular Biology and Genetics, TURKEY (Diploma grade3,46)

2021- 2022 **MASTER-** Near East University, Molecular Medicine English), TRNC (Diploma grade: 3,79)

Experience

Ege University, Department of Bioengineering**, Medical Biotechnology Laboratory**, Internship, Izmir
June 2019 – July 2019
During the internship, bacterial semi, bacterial cultivation, research of bacteria, DNA purification were carried out.

Near East University, Department of Medical Genetics, **Medical Genetics Laboratory**, Internship, Nicosia
January 2020 - February 2020
During the internship, the gene region was searched by PCR, and analysed

Near East University, Near East Hospital, **COVID 19 and Genetics Laboratory**, Internship, Nicosia
July 2021-September 2021 Real time PCR, FISH, Karyotyping

Cyprus Central Hospital, Full time work**, Genetics Laboratory**, Famagusta November 2021-2022 Real Time PCR Covid 19 test

Studies

Investigation of gene expression profile of WNT/β-catenin pathway genes in spontaneous abortion materials
(Within the scope of the project, RNA isolation, cDNA synthesis, Gradient PCR, RT-PCR, Agarose gel Electrophoresis applications were made by me.)

REFERENCES

- Assist. Prof. Aysegul Yildiz
  Faculty of Science / Molecular Biology and Genetics
- Assoc. Prof. Mahmut Çerkez Ergören
  Faculty of Medicine / Medical Genetics
- Dr. Gülten Tuncel
  Faculty of Medicine / Medical Genetics