

THE EXPRESSION PROFILE OF WNT/B-CATENIN SIGNALING PATHWAY GENES IN MISCARRIAGES

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ABSTRACT

Miscarriage, defined as the spontaneous loss of a fetus before viability, is the most common complication of pregnancy. Among its many causes, genetic factors are thought to play a significant role. One of the key signaling pathways involved in embryonic development is the Wnt/ β -catenin pathway, which regulates critical processes such as embryonic cell migration, cell fate determination, proliferation, and differentiation. This pathway is also essential for early developmental events, including preimplantation development and blastocyst implantation. Although numerous animal studies have linked disruptions in Wnt signaling to pregnancy loss, limited data exist on its role in human miscarriage.

In this study, we aimed to investigate the expression profiles of genes involved in the Wnt/ β -catenin signaling pathway in placental tissues from a total of 23 miscarriage cases, including 15 with normal and 8 with abnormal fetal karyotypes.

Our analysis revealed that GSK3B, WNT3A, WNT4, AXIN2, and APC were upregulated in the normal karyotype group, while CTNNB1 (β -catenin) and WNT5A were downregulated. DVL1 expression showed no significant difference between the groups.

These findings suggest that upregulation of GSK3B, AXIN2, and APC, together with downregulation of β -catenin, may lead to inhibition of the Wnt/ β -catenin signaling pathway. Such disruption could impair key cellular processes—including proliferation, migration, and blastocyst implantation—that are essential for early pregnancy maintenance.

Keywords

Wnt/ β -Catenin signal pathway, Spontaneous abortion, Miscarriage, Aborted foetus

INTRODUCTION

Spontaneous abortion, commonly referred to as miscarriage, is defined as the loss of pregnancy before the 20th week of gestation without medical intervention. According to the American College of Obstetricians and Gynaecologists (ACOG), it is the most prevalent form of pregnancy loss, occurring in approximately 15–20% of clinically confirmed pregnancies [1,2]. The vast majority—about 80%—of these losses occur during the first trimester, with risk significantly declining after the 12th gestational week [3,4]. Miscarriages are clinically classified into several subtypes, including complete, missed, inevitable, incomplete, threatened, septic, and recurrent spontaneous abortions, based on the progression and presentation of symptoms [5–7].

The etiology of spontaneous abortion is multifactorial. Known contributing factors include genetic abnormalities, infections, uterine anatomical defects, endocrine disorders, and immunological dysfunctions [8]. However, a significant proportion of miscarriages remain idiopathic. Notably, chromosomal anomalies are identified in more than 90% of conceptuses resulting in early pregnancy loss, underscoring the role of genetic instability in miscarriage pathophysiology [9]. Maternal factors such as advanced age also significantly increase the risk, often correlating

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with chromosomal abnormalities in the embryo [11,12]. The immune system, particularly cytokine profiles, plays a dual role, either promoting or inhibiting the maintenance of pregnancy [10].

The placenta, as a transient yet vital organ facilitating maternal-fetal exchange, is crucial for successful pregnancy outcomes [13]. Abnormalities in placental development or trophoblast function have been linked to adverse pregnancy outcomes, including intrauterine growth restriction and pre-eclampsia [15,16]. Nonetheless, the molecular mechanisms underlying these complications are still not fully understood. Recent research has drawn attention to the Wnt/ β -catenin pathway, particularly the canonical Wnt/ β -catenin pathway, in regulating key physiological processes during early pregnancy and implantation [17,18].

The Wnt/ β -catenin pathway is a highly conserved signalling cascade involved in embryonic patterning, cell proliferation, differentiation, and migration [19–21]. Its components—including ligands such as WNT4, WNT5A, and WNT7A, as well as inhibitors like DKK1—are expressed in both gametes and preimplantation embryos [17]. These molecules contribute to implantation, endometrial decidualization, and trophoblast invasion—processes essential for the establishment of pregnancy [22–24]. Disruption of this signalling axis has been associated with implantation failure and pregnancy loss, as evidenced by studies in murine models and human tissues [17,25–27].

Furthermore, the Wnt/ β -catenin pathway includes both canonical (β -catenin-dependent) and non-canonical branches (such as planar cell polarity and Wnt/ Ca^{2+} pathways), each eliciting distinct cellular responses based on temporal and spatial context [25]. Mutations in genes encoding Wnt pathway components, such as dishevelled (DVL) proteins, have been implicated in congenital anomalies, highlighting the developmental significance of this pathway [26]. Experimental inhibition of Wnt/ β -catenin pathway in animal models has led to impaired implantation and aberrant embryo-endometrium interactions [17,27–29].

The evolutionary conservation and functional diversity of Wnt genes, observed in a range of species including *Drosophila*, *Xenopus*, zebrafish, and mammals, further underscore their essential role in developmental biology [30–32]. In light of these findings, investigating the involvement of Wnt/ β -catenin signalling and related genes in spontaneous abortion may offer valuable insights into the molecular basis of implantation failure and open new avenues for therapeutic intervention, particularly in the context of assisted reproductive technologies. Thus, in this study, we planned to compare the gene expressions of Wnt/ β -catenin pathway genes in fetuses with abnormal karyotype and fetuses with normal karyotype after spontaneous abortion.

MATERIALS AND METHODS

Sample collection: In this study a total of 23 spontaneous abortion materials were obtained from Near East University Hospital (NEUH) Medical Genetics Laboratory. 15 of these samples were categorized as having a normal karyotype (46, XX or 46, XY) and eight were detected to have abnormal karyotypes (Table 1). The study was approved by the Near East University Scientific Review Board (registration number YDU/2021/97-1449).

RNA extraction, cDNA synthesis and gene expression- The RNA was extracted from samples (abortion materials) using MG-RNA-01 Hibrigen General RNA extraction kit (Hibrigen, Biotechnology, Gebze, Turkey) following manufacturer's guidelines (Hibrigen Biotechnology, Gebze, Turkey). The cDNA synthesis was carried out using the OneScript Plus cDNA synthesis kit according to the manufacturer's guidelines (ABMGood, Canada).

The expression levels of *WNT3A*, *WNT4*, *WNT5A*, *APC*, *AXIN2*, *GSK3 β* , *DVL1* and β -catenin genes were analysed using gene-specific primers and 2x SYBR Green qPCR Mix (Hibrigen, Turkey) according to the manufac-

Table 1. Samples with normal and abnormal karyotypes.

Sample No	Karyotype	Year
1	45, X	2019
2	46, XX	2017
3	46, XX	2021
4	46, XX	2021
5	46,XY,inv(9)(p11q13)	2018
6	45, X	2019
7	46, XX	2019
8	46, XX	2020
9	92, XX	2020
10	46, XX	2020
11	46, XX	2021
12	46, XX	2019
13	47, XX	2022
14	46, XX	2022
15	46, XX	2020
16	46, XX	2021
17	69, XXY	2018
18	46, XX	2018
19	46, XX	2021
20	46, XX	2019
21	46, XY+21	2017
22	46, XX	2021
23	46, XX	2019

Table 2. The sequence of primers forward and reverse primers obtained from oligomer stock for Wnt signalling genes.

Gene	Forward primers	Reverse primers
<i>B-Catenin</i>	AGACGGAGGAAGGTCTGAGG	TTCAAATACCCTCAGGGGAACA
<i>APC</i>	ACGCGCTTACTGTGAAACCT	GCCTGTAGTCCCCCTAGTTC
<i>AXIN 2</i>	CCCAGAGCCGGGAAATAAA	CTCCTCTCTTTACAGCAGGGC
<i>WNT3A</i>	GAGCAGGACTCCACCTAAAC	AGACACTAGCTCCAGGGAGGA
<i>WNT4</i>	CATGAGTCCCCGCTCGTG	CCAGGTACAGCCAGTTGCTC
<i>WNT5A</i>	TCGCTGATGGACGTTGGAAA	CCAATGGACTTCTTCATGGCG
<i>GSK3β</i>	ACAGCAGCGTCAGATGCTAA	TGACCAGTGTGCTGAGTGA
<i>DVL1</i>	CAGCAGAGTGAAGGGAGCAA	GGTTCCCCATAGCCTTCTGG

Table 3. statistical analysis of *WNT3A*, *WNT4*, *WNT5A*, *B-Catenin*, *APC*, *AXIN2*, *DVL1*, and *GSK3 β*

Group Statistics					
Genes	Karyotype	Sample Numbers	Mean	p-Value	Std. Error Mean
<i>WNT3A</i>	Abnormal	8	0.7922	0.170	0.3205
	Normal	15	1.9469		0.7413
<i>WNT4</i>	Abnormal	8	1.4901	0.176	0.5198
	Normal	15	2.8678		0.8356
<i>WNT5A</i>	Abnormal	8	0.5011	0.592	0.1243
	Normal	15	0.4101		0.1108
<i>B-Catenin</i>	Abnormal	8	1,20	0,255	0,101
	Normal	15	1,05		0,09
<i>APC</i>	Abnormal	8	2.3400	0.485	1.2200
	Normal	15	4.1420		1.7310
<i>AXIN2</i>	Abnormal	8	0.0690	0.501	0.4620
	Normal	15	0.1400		0.0710
<i>DVL1</i>	Abnormal	8	0,93	0,350	0,024
	Normal	15	1,01		0,061
<i>GSK3β</i>	Abnormal	8	0,62	0,033	0,06
	Normal	15	1,01		0,09

turer's guidelines using rotor gene. β -actin was used as the housekeeping gene for normalization for all each gene expression analysis Gene-specific primers were designed at the exon-exon junction region and in-silico cross-reaction tests were performed using NCBI PrimerBlast tool (Table 2).

Statistical analysis- The statistical analysis was carried out using the analysis variance (*t-test*) (IBM SPSS Statistics) and correlation analysis between each gene in the normal and abnormal karyotype spontaneous abortion materials. The *P*-values were used to compare the different results between the genes. The *P*-values <0.05 were considered as significant, as those with >0.05 were considered non-significant.

RESULTS

To investigate the expression profile of WNT- β -catenin signalling pathway genes (*WNT3A*, *WNT4*,

WNT5A, *APC*, *AXIN2*, *DVL1*, β -catenin and, *GSK3 β*) in spontaneous abortion materials a total of 23 abortion samples with normal or abnormal karyotypes were analysed (Table 3).

Differences in expression levels of *WNT3A*, *WNT4*, *WNT5A*, *APC*, *AXIN2*, *GSK3 β* , *DVL1* and *B-catenin* genes were observed between normal and abnormal fetus samples (Figure 1).

Gene expression levels of *GSK3 β* between samples with normal and abnormal karyotypes was found to be significant ($p=0.033$, $p<0.05$) (Figure 1a). No statistically significant difference was detected in gene expression levels of *WNT3A*, *WNT4*, *WNT5A*, *APC*, *AXIN2*, *DVL1*, β -catenin genes between samples with normal and abnormal karyotypes.

However, although not significant, gene expression levels were found to be different between samples with normal and abnormal karyotypes for *WNT3A*, *WNT4*,

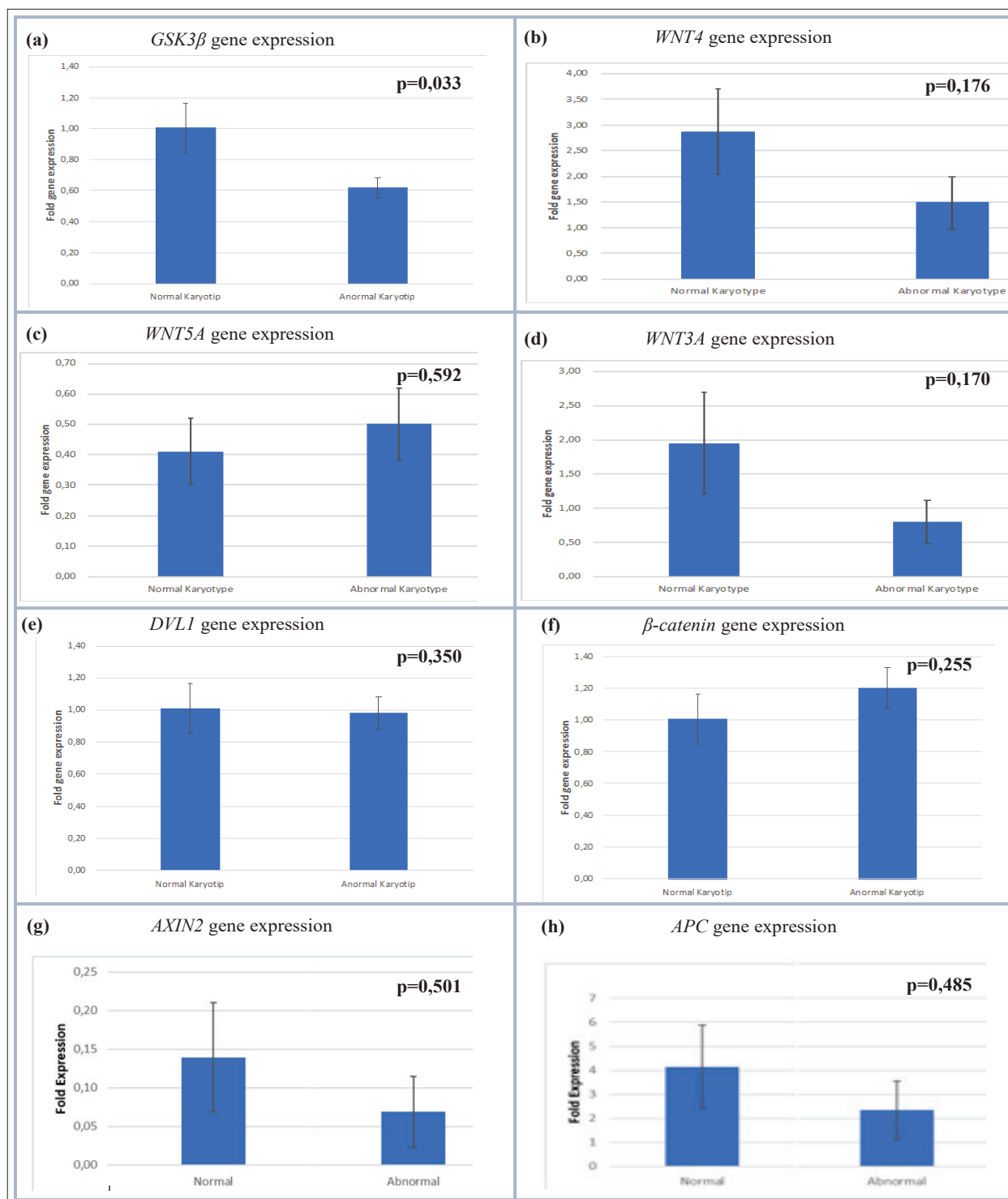


Figure1. Expression levels of *GSK3β* (a), *WNT4* (b), *WNT5A* (c), *WNT3A* (d), *DVL1* (e), *β-catenin* (f), *AXIN2* (g), *APC* (h) genes in normal karyotype and abnormal karyotype in spontaneous abortion sample groups.

WNT5A, *APC*, *AXIN2*, *DVL1*, *β-catenin* genes (Figure 1b-h). A decreased expression in *WNT3A*, *WNT4*, *GSK3β*, *DVL1*, *AXIN2*, and *APC* and an increased expression in

WNT5A and *β-catenin* were observed in abnormal karyotype when compared to normal karyotype according to the gene expression analysis.

DISCUSSION

Miscarriage is the most common form of complication during pregnancy, and it is characterized by the involuntary loss of a foetus before maturation [33]. The Wnt proteins are there to regulate biological processes which include the development of an embryo. The signal pathways can be carried out through the transmission of signals, promoting the transfer of molecules from one cell to another cell surface receptors [34]. From research Wnt signals can regulate different types of activities such as pluripotency and proliferation of an embryo from a mouse model stem cells and as well somatic cells adjustment [35]

It has been reported that there is a relationship between the uterus and hatched blastocyst, significantly promoting implantation and greater adaptation to the uterine tissue [36]. The role of Wnt/ β -catenin have being studied expressly using the mouse model on its effect on the embryo. The Wnt/ β -catenin can regulate certain mechanisms in the embryo such as the formation of the luminal epithelial evagination. [28]. Wnt signalling has been identified as vital in developing blood vessels, as several studies have shown [37]. The Wnt signal pathways have more specific and vital roles to play in the physiology of cells [38]. The Wnt signal was first identified in *Drosophila*, but later found to be present in a variety of other species such as humans, zebrafish, and frogs comprising 19 essential Wnt genes with varying functions [39]. It was after this discovery that researchers did a thorough study and came up with a conclusion that these genes can contribute to the development of the embryo in *Drosophila* [40]. As a result, it has been thought that Wnt signalling may aid in the regulation and proliferation of cells and the development of organs and organ systems [41].

Loss of beta-catenin function can result in a reduction in trophoblast cells that can affect blastulation and endanger the human embryo [42]. As studies have shown, the WNT4 and Beta-catenin from animal model studies have shown to play a role in implantation and placental development [43]. Also, the downregulation of DKK (Dickkopf WNT Signalling Pathway Inhibitor 1) can affect the invasion of trophoblast negatively [44]. Therefore, an increase in DKK1 and SFRP4 (Secreted Frizzled-Related Protein 4) expression may result in a decrease in WNT4 and CTNNB1(β -catenin) expression, which might either decrease or increase Wnt5A expression [45]. E. Chronopoulou et al, aimed to study the expression of shown to play a role in implantation and placental WNT4, WNT6, and β -catenin using human placenta tissue obtained from first-trimester miscarriage, [47]. In their conclusion, their finding shows the significance of balanced Wnt signalling in an event surrounding early pregnancy. A study dem-

onstrated that a total of fourteen Wnt ligands and eight Fzd receptors were expressed in the placenta of a human sample, indicating the role of the Wnt signalling pathway in placental development [18]. The β -catenin expression may lead to hyperplasia and subfertility, and the destruction of β -catenin can lead to infertility [46]. In animals, a balanced state of equilibrium of Wnt-signalling is important for placentation in humans. In two different ways such as the hyper-activation and under-activation of Wnt signalling are associated with the pathology of the placenta and trophoblast abnormalities [44].

According to the gene expression analysis, an increase in WNT3A, WNT4, GSK3 β , AXIN2 and APC expression and a decrease in WNT5A and β -catenin expression were observed in normal karyotype compared to abnormal karyotype as shown in Figure 1a-h. In our findings, while the expression of GSK3 β , APC and AXIN increases in normal karyotype, the decrease in β -catenin is a logical finding, but it may be important. Increased expression of GSK3 β , APC and AXIN and decreased expression of β -catenin inhibits the functioning of the Wnt/ β -catenin signal pathway, so many cellular events involving the Wnt/ β -catenin signal pathway do not occur. No significant change was observed in DVL1. There has been evidence, which was gathered using animal models, suggesting the key role of Wnt signalling in placental development and also impedes the regulation of trophoblast proliferation and invasion [47]. The Wnt beta-catenin has been shown to inactivate the function of blastocyst implantation [48]. According to H. Bao et al., the over-expression of Wnt-Beta signalling, when the Wnt inhibitors are silent can hinder trophoblast differentiation [49, 50].

The WNT3A is a canonical ligand pathway that helps in promoting embryonic development, which includes regulating pluripotency, migration of cells during neurulation and gastrulation, and the formation of body axis [51]. WNT3A has been shown to promote both β -catenin dependent and β -catenin independent YAP/TAZ responses [52]. The WNT3A plays an integral role in the maintenance of bovine trophoblast by regulating and activating CDX2 expression levels using the Wnt-YAP/TZ signal pathways. However, the WNT3A from a study has a function of promoting β -catenin-dependent and independent responses.

A total of fourteen Wnt ligands and eight FZ receptors were identified in the placenta tissue during the first trimester [53]. The Wnt4 is mostly expressed in Wnt signal genes [54]. WNT4 remains to be the most expressed Wnt signalling gene [54]. So far there has been a lot of research has been done in the adult uterus about WNT4, which is crucial for implantation and forms the shaped portions of the decidua [55]. WNT4 is involved in the canonical and

non-canonical Wnt/ β -catenin signalling pathway [56]. An increase in Dkk-1 and sFRP4 expression decreased the expression of WNT4 and β -catenin, that cause an increase or decrease in WNT5A expression, as a result, they showed that these associated with pre-eclampsia development [57]. The relationship between the decidua and trophoblast is important later in pregnancy, influencing the regulation of trophoblast proliferation, differentiation and migration [58]. Defects that affect survival in the placenta such as vascularization and trophoblast invasion cause complications such as premature birth and miscarriage [59-61]. Activation of the WNT/ β -catenin signalling pathway activates the transcription of genes that promote cell cycle progression and differentiation [62]. WNT5A is claimed to suppress the canonical WNT signal. It is also known that WNT5A is necessary for the uterus and female reproductive system and has an important role in implantation [63, 64]. The fact that WNT5A is expressed 0.08 times lower may support this information.

Dishevelled family protein 1 (DVL1) is a protein in the Wnt/ β -catenin signalling pathway involved in embryonic development [65]. During the development of the placenta, like trophoblast cells, it is the Wnt/ β -catenin signalling pathway that keeps a number of mechanisms under control [66]. DVL1 is also a protein involved in this mechanism. The epithelial-mesenchymal transition (EMT), which allows invasion of trophoblast cells during placentation, is an important event. Timely and uneventful continuation of these processes is essential for successful termination of pregnancy [59,67- 69]. In these results, since it is known that placental implantation is important in pregnancy and the Wnt signalling pathway, which is involved in the event, regulates cell motility and invasion, it can be thought that the proteins and genes in this pathway may be important for the continuation of pregnancy [70]. In the Wnt/ β -catenin signalling pathway, when the wnt ligand binds to the receptor, it deactivates the degradation complex, allowing β -catenin to enter the nucleus after accumulating in the cytoplasm and initiate the transcription of a specific gene [71]. In such a case, it is thought that DVL1 should also be decreased when the expression of β -catenin decreases. This may be due to their lack of linkage at the expression level or the lack of difference in expression of this gene in miscarriage samples with normal and abnormal karyotypes. For a more precise result, the study can be repeated by adding more samples and non-miscarriage placenta samples.

GSK3 is a serine-tyrosine protein kinase encoded by GSK3A and GSK3 β [72]. It is a key enzyme involved in the WNT/ β -catenin signalling pathway, as well as in cellular migration, proliferation, apoptosis, and glucose regulation [43]. WNT/ β -catenin signalling pathway is

involved in embryogenesis and various reproductive tissues [43,53, 73]. When the WNT ligand does not bind to the receptor and combines with other members of the GSK3 β degradation complex, it sends intracellular β -catenin to the proteasome and degrades it. Thus, when the ligand does not bind to the receptor, β -catenin cannot go to the nucleus and initiate transcription. This affects many events such as cellular proliferation, migration, blastocyte implantation, in which the WNT/ β -catenin signalling pathway functions. In this study, it was observed that GSK3 β was more expressed in miscarriage samples with normal karyotype compared to those with abnormal karyotype. Overexpression of GSK3 β means that the WNT/ β -catenin signalling pathway is blocked, and it is inevitable that the inhibition of this signalling pathway will affect many events such as cellular proliferation, migration, blastocyte implantation.

The Wnt/ β -catenin has been shown to contribute immensely toward the development of organ systems, which include the digestive system, respiratory system, skeletal system, nervous system, cardiovascular, hematopoietic, and reproductive systems [74]. Furthermore, the Wnt/ β -catenin signalling plays a pivotal role in tissue homeostasis, and most importantly development and differentiation of trophoblast [75]. An alteration in β -catenin can hinder the process of blastulation [42]. Progesterone excess is known to cause most pregnancies to result in miscarriage [76-78]. In a study conducted with endometriomas of patients with high progesterone, it was shown that β -catenin expression is less than the expression of β -catenin in the endometrium of patients with normal progesterone levels [79].

Axin functions as a negative regulator of the Wnt/ β -catenin signalling pathway and can induce apoptosis. Loss of function of the Wnt/ β -catenin signalling pathway can result in abnormal development of stromal cells, trophoblast, and blood vessels, resulting in failed pregnancies [80]. In our study, AXIN2 gene expression was higher in placentas with normal karyotype compared to abnormal karyotype. According to the information discussed above, excess of Axin protein may cause miscarriage through Wnt/ β -catenin signalling pathway, while the overexpression of AXIN2 gene supports this information. Acquired APC resistance in pregnant women is associated with an increased prevalence of second trimester fetal discharge in early and late miscarriages [81-83]. The anticoagulant relationship of APC is known (griffin 2012), but more extensive research is required for a more definitive conclusion. In addition, APC, which acts as a negative regulator in the Wnt/ β -catenin signalling pathway, plays an important role in many events such as cell migration. In this study, we observed that APC expression level was higher in low samples with normal karyotype than in low samples with abnormal karyotype.

This indicates that this signalling pathway may be down-regulated, and downregulation of this signalling pathway may be associated with miscarriage.

Furthermore, the gene expression analysis of WNT signal genes (WNT3A, WNT4, WNT5A, APC, AXIN2, DVL1, β -catenin, and GSK3 β) demonstrated to be associated with spontaneous abortion in this study. However, the changes in RNA level may not be reflected in the protein level, as this should be further confirmed with western blotting in the same samples and preferentially with a larger cohort of samples.

The limitations of this study were a small sample sizes in both groups and the RNA concentration measurements, as the samples were not freshly collected. Further research must be carried out to investigate factors related to spontaneous abortion associated with Wnt signal genes in terms of implantation. Furthermore, a better understanding of the role of Wnt genes during implantation can be implored and rectifying errors using IVF techniques.

CONCLUSION

This study highlights the potential role of Wnt/ β -catenin signalling dysregulation in the pathogenesis of miscarriage, particularly in cases with normal fetal karyotypes. The observed alterations in gene expression suggest disruption in key implantation and placental development pathways, offering a possible molecular explanation for pregnancy loss in genetically normal cases. These findings may inspire future research and potentially inform diagnostic and preventive strategies for affected couples.

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CONFLICT OF INTEREST

The authors do not have any conflict of interest to declare.

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DATA AVAILABILITY STATEMENT

The data is available upon request.

AUTHOR CONTRIBUTION

Conceived and designed the analysis: E.G., A.B.C.G., T.A.H., G.M., S.G.T., M.C.E. ; Collected the data: E.G., A.B.C.G., T.A.H., G.M., S.G.T., M.C.E.; Performed the analysis: E.G., A.B.C.G., T.A.H., G.M., S.G.T., M.C.E.; Wrote the paper: E.G., A.B.C.G., T.A.H., G.M., S.G.T., M.C.E.; revised the paper: E.G., A.B.C.G., T.A.H., G.M., S.G.T., M.C.E.; supervised the project: M.C.E.

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