Journal of Pediatric Genetics

Manuscript 1021

Short-Term Effects of Extremely Low-Frequency Electromagnetic Fields on Neurogenesis and Wnt Signaling in Amniotic Fluid Cells

Coşar Uzun

Ümit Karakaş

Mustafa Ertan Ay

Özlem İzci Ay

Didem Derici Yıldırım

See next page for additional authors

Short-Term Effects of Extremely Low-Frequency Electromagnetic Fields on Neurogenesis and Wnt Signaling in Amniotic Fluid Cells

Authors

Coşar Uzun, Ümit Karakaş, Mustafa Ertan Ay, Özlem İzci Ay, Didem Derici Yıldırım, Nurten Erdal, and Mehmet Emin Erdal

Volume: 14 Issue: 3 Year: 2025 Original Study e-ISSN: 2146-4596

Short-Term Effects of Extremely Low-Frequency Electromagnetic Fields on Neurogenesis and Wnt Signaling in Amniotic Fluid Cells

Coşar Uzun[1], Ümit Karakaş[2]*, Mustafa Ertan Ay[3], Özlem İzci Ay[3], Didem Derici Yıldırım[4], Nurten Erdal[2], Mehmet Emin Erdal[3]

[1] Bayburt University Pharmacy Service Department, Vocational School of Health Services, Bayburt, Türkiye

[2] Mersin University, Department of Biophysics, Faculty of Medicine, Mersin, Türkiye

[3] Mersin University, Faculty of Medicine, Department of Medical Biology and Genetics, Mersin, Türkiye

[4] Mersin University, Faculty of Medicine, Department of Biostatistics and Medical Informatics, Mersin, Türkiye

Article Info

Received: October 23, 2024
Accepted: October 11, 2025
Published: November 15, 2025

Keywords:
Amniotic fluid cells,
Extremely low-frequency electromagnetic fields,
Neurogenesis,
Wnt signaling,
50 Hz.

ABSTRACT

Background: Extremely low-frequency electromagnetic fields (ELF-EMF) at 50 Hz are prevalent in household electrical systems. Although various studies have examined the effects of ELF-EMF on cell proliferation and gene expression, its impact on amniotic fluid cells (AFCs) remains unclear.

Objectives: This study aimed to assess the potential effects of ELF-EMFs on gene expression related to neurogenesis and the Wnt signaling pathway in AFCs.

Methods: AFCs were isolated from amniotic fluid obtained via amniocentesis and divided into five groups: control, sham, and three groups exposed to different ELF-EMF intensities (1 mT, 2 mT, 3 mT for 30 minutes/day for 7 days). Expression levels of genes involved in neurogenesis (HES1, Neurog1, Neurog2, Neurod1) and Wnt signaling (SFRP2, SFRP4, SFRP5, APC1) were analyzed using real-time PCR.

Results: ELF-EMF exposure did not result in significant changes in gene expression among the experimental groups compared to controls.

Conclusion: Short-term exposure (Acute exposure) to ELF-EMF at moderate intensities does not significantly impact gene expression related to neurogenesis or Wnt signaling in AFCs. Future studies should explore prolonged exposure (chronic exposure) and a broader range of intensities to evaluate developmental impacts more comprehensively.

To cite this article:

Coşar Uzun, C., Karakaş, Ü., Ay, M. E., İzci Ay, Ö., Derici Yıldırım, D., Erdal, N., & Erdal, M. E. (2025). Short-term effects of extremely low-frequency electromagnetic fields on neurogenesis and Wnt signaling in amniotic fluid cells. *Journal of Pediatric Genetics, 14*(3), 172-180.

*** Corresponding Author:** Ümit Karakaş, *umitkarakas@bayburt.edu.tr*

Introduction

Extremely low-frequency electromagnetic fields (ELF-EMF) range between 3 and 300 Hz and are predominantly encountered through household and workplace electrical systems, operating at 60 Hz in North America and 50 Hz elsewhere. While higher frequency EMFs are well studied due to their use in telecommunications and medical devices, the biological impact of ELF-EMF, particularly in relation to human health, remains a subject of ongoing research. The International Agency for Research on Cancer (IARC) and other regulatory bodies have recognized ELF-EMFs as potentially carcinogenic, though evidence linking ELF-EMF exposure to diseases like leukemia remains inconclusive.[1,2] Beyond carcinogenesis, there is growing interest in understanding how ELF-EMFs affect gene expression, particularly genes involved in neurogenesis and developmental pathways like Wnt signaling.[3,4] *APC1, SFRP2, SFRP4*, and *SFRP5* stand out among the methylation-associated genes. These genes are associated with WNT signaling pathways, critical in cell proliferation, differentiation, and tumorigenesis. *APC1* plays an essential role in the control of WNT signaling through β-catenin regulation, while *SFRP2, SFRP4*, and *SFRP5* are involved in suppressing the signaling pathway by inhibiting the binding of WNT ligands. In addition, the methylation status of these genes may affect the activation of the WNT signaling pathway, especially in colorectal and other types of cancer. These genes, silenced through methylation, interact with AXIN and CTNNB1, which are important components of the WNT pathway, thereby directing cell cycle regulation and cancer biology.[5,6]

Previous research has shown that ELF-EMFs can influence neural stem cell (NSC) proliferation, differentiation, and neurogenesis in both in vitro and in vivo models.[7,8] Studies using human neuroblastoma cells (SHSY5Y) and embryonic neural stem cells (eNSCs) have demonstrated altered protein expression and enhanced neuronal differentiation following ELF-EMF exposure[9,10]. However, the effects of ELF-EMF on human amniotic fluid cells (AFCs) remain largely unexplored. Given that AFCs play a critical role in fetal development and serve as a source for diagnostic and therapeutic applications, it is essential to investigate how ELF-EMF exposure might influence gene expression in these cells.

In this study, we aimed to assess the effects of 50 Hz ELF-EMF at different intensities (1 mT, 2 mT, 3 mT) on gene expression related to neurogenesis and the Wnt signaling pathway in AFCs. By focusing on short-term exposure, we sought to elucidate the potential impact of ELF-EMF on embryonic development. Future studies will be necessary to determine the long-term effects of ELF-EMF on AFCs and their potential implications for fetal health.

Materials and Methods

Extremely Low-Frequency Electromagnetic Field Exposure System

A homogeneous sinusoidal ELF-EMF was generated using a custom-built exposure system consisting of a pair of Helmholtz coils (25 cm inner diameter) positioned to create a vertical magnetic field within a Faraday cage (130x65x80 cm) to shield against electrical noise and vibrations. The system was calibrated based on previous studies[11–13]. The magnetic field intensity (1, 2, and 3 mT) was measured using an axial Hall-effect Gaussmeter (F.W. Bell Model 6010 & Sypris Test).

Amniotic fluid cells (AFCs) were exposed to ELF-EMF outside the incubator under standard culture conditions at room temperature. Sham and exposed AFCs were positioned outside and inside the Helmholtz coils, respectively. The cells were exposed to 50 Hz ELF-EMF for 30 minutes daily over 7 days. No temperature differences were observed between the groups. AFC cultures were divided into five groups: (1) Control (kept in the incubator), (2) Sham (placed outside the incubator for 30 min/day without ELF-EMF). In this way, the possible stress factor resulting from the change in environment was ensured to be similar to the experimental groups and three experimental groups exposed to ELF-EMF: (3) G1 (1 mT), (4) G2 (2 mT), (5) G3 (3 mT).

Cell Culture

Ethical approval was obtained from Mersin University Clinical Research Ethics Committee (dated 05.10.2017, no: 2017/291). AFCs were collected from patients undergoing amniocentesis for cytogenetic analysis and cultured in the Medical Biology Department. Population groupings of undifferentiated cells connected to the amniotic membrane (AM) are found in amniotic tissues. Two distinct groups, AM epithelial cells (AECs) and AM stromal cells, derived from the embryonic

mesoderm, are found within the membrane. The first layer of the AM comprises amniotic epithelial cells, which exhibit numerous cytokeratins and are situated on top of the basement membrane, the thickest membrane seen in human tissues. AECs can differentiate into each of the three germ layers. Three primary cell types prevail in the diverse combination of amniotic fluid. While E-type (epithelioid type) cells have a rounded morphology, two cell types—known as AF-type (amniotic fluid specific) cells and F-type (fibroblast type) cells—have spindle-shaped morphologies[14],[15]. In this study, mesenchymal cells were analyzed using an inverted microscope and used for experiments. Cells were grown in BioAmf-1 medium (Biological Industries, Israel) and expanded using standard techniques. Upon reaching confluency, cells were harvested using Hank's solution and 0.05% Trypsin-EDTA (Gibco). Cultures were monitored with an inverted phase-contrast microscope (Olympus CK40, Germany) and maintained at 37°C in 5% CO2.

Table 1. Primer-probe sequences for Real-Time PCR expression analysis. These primer-probe arrays were designed by Prof. Dr. Mehmet Emin ERDAL

Gene	Primer/Prob genre	Primer/Prob arrays*
SFRP2	F Primer	5'-TGCTTGAGTGCGACCGTTT-3'
	R Primer	5'-CAGGAGGTGGTCGCTGCTA-3'
	Prob	5'-FAM-ACAACGACCTTTGCATCCCCCTCG-BHQ-1-3'
SFRP4	F Primer	5'-GCCGTGCTGCGCTTCTT-3'
	R Primer	5'-TGATAGGGTCGTGCAGGAACT-3'
	Prob	5'-FAM-CCATGTACGCGCCCATTTGCAC-BHQ-1-3'
SFRP5	F Primer	5'-TGATTGGAGCCCAGAAAAAGA-3'
	R Primer	5'-TGGTGTCCTTGCGCTTCAG-3'
	Prob	5'-FAM-AAGCTGCTCAAGCCGGGCCC-BHQ-1-3'
APC1	F Primer	5'-GAACCTGGAGGTACTTCATGTGAA-3'
	R Primer	5'-GCAAATGTTTGGCCCACTAAA-3'
	Prob	5'-FAM-AGCGCTTCGTTCAAACAGCAATCCA-BHQ-1-3'
HES1	F Primer	5'-TTGGAGGCTTCCAGGTGGTA-3'
	R Primer	5'-GCCCCGTTGGGAATGAG-3'
	Prob	5'-FAM-CT(pdC)CCGATGG(pdC)CAGTT-ZNA4-BHQ-1-3'
Neurog1	F Primer	5'-CACCAAGCTCACCAAAATCGA-3'
	R Primer	5'-TCGGCCAGAGCCCAGAT-3'
	Prob	5'-FAM-TGCG(pdC)TTCGC(pdC)TACA-ZNA4-BHQ-1-3'
Neurog2	F Primer	5'-CAGGGCAGGTGTAGCCTTTC-3'
	R Primer	5'-GAAGGATACCCAAAGCCAAGAA-3'
	Prob	5'-FAM-ATT(pdC)(pdC)T(pdC)GGTTGTTT(pdC)T-ZNA4-BHQ-1-3'
Neurod1	F Primer	5'-AAGGTGGTGCCTTGCTATTCTAA-3'
	R Primer	5'-CCAAGCGCAGAGTCTCGATT-3'
	Prob	5'-FAM-ACG(pdC)AGAAG(pdC)TGT(pdC)CA-ZNA4-BHQ-1-3'
ACTB (β-Actin)	F Primer	F 5'-GGCACCCAGCACAATGAAG-3'
	R Primer	R 5'-GCCGATCCACACGGAGTACT-3'
	Prob	Pr 5'-Yakima Yellow-TCAAGATCATTGCTCCTCCTGAGCGC-BHQ-1-3'

*pdC: Substitution of C-5 propynyl-dC (pdC) for dC is an effective strategy to enhance base pairing. These base substitutions raise duplex stability and

RNA Extraction and RT-PCR

Total RNA was extracted using Tri-Reagent (Sigma Aldrich, Turkey) following the acid guanidinium thiocyanate-phenol-chloroform method[16]. RNA purity and concentration were measured using a micro-drop spectrophotometer (CapitalBio NanoQ) at Mersin University Advanced Technology Education, Research, and Application Center (MEITAM). Complementary DNA (cDNA) was synthesized using the isolated RNA, and gene expression was assessed via Real-Time PCR (ABI 7500, Applied Biosystems, USA). Specific gene

Table 2. Genes for analyzing gene expression

	Gene	Explicit/Official name	Reference No **	Gene ID*
Wnt Signaling Pathway Associated Genes	*SFRP2*	Secreted Frizzled Related Protein 2	NM_003013.2	6423
	SFRP4	Secreted Frizzled Related Protein 4	NM_003014.3	6424
	SFRP5	Secreted Frizzled Related Protein 5	NM_003015.3	6425
	APC1	Adenomatous polyposis coli 1	NM_022662.2	324
Neurogenesis Related Genes	*HES1*	hes family bHLH transcription factor 1	NM_005524.3	3280
	Neurog1	Neurogenin-1	NM_006161.2	4762
	Neurog2	Neurogenin-2	NM_024019.3	63973
	Neurod1	Neuronal Differentiation-1	NM_002500.4	4760

*http://www.ncbi.nlm.nih.gov/gene ** http://www.ncbi.nlm.nih.gov/RefSeq/

primers for neurogenesis and Wnt signaling pathway components are summarized in **Table.1 and 2**. Reactions were run with the following conditions: initial denaturation at 50°C for 2 min, followed by amplification at 95°C for 10 min, and cycling at 95°C for 15 s, 60°C for 1 min (50 cycles). Relative gene expression levels were calculated using the 2-ΔΔCt method, normalized to β-actin as the internal control.[17]

Statistical Analysis

Data were analyzed using STATISTICA version 13.3 (TIBCO Software Inc., USA). Normality was assessed using the Shapiro-Wilk test. Due to non-normal distributions, $2^{-\Delta\Delta Ct}$ values were presented as medians and percentiles (25th–75th). Group comparisons were performed using the Kruskal-Wallis test. Box-and-whisker plots were generated to visualize group differences. A two-tailed p-value < 0.05 was considered statistically significant.

Results

To learn more about the molecular processes underlying how ELF-EMFs affect cells in amniotic fluid, we studied the gene expression patterns of bHLH transcription factors including, *Hes1* (a component of the neurogenesis that regulates cell proliferation and fate) and *Neurog1, Neurog2* and *Neurod1* (which play critical roles in fate determination and neuronal differentiation)[18,19]. In addition, we looked into the gene expression patterns of *APC1, SFRP2, SFRP4*, and *SFRP5*, which are the

Table 3. Expression changes of Wnt signaling pathway and neurogenesis related genes. Data are given as median and Interquartile range (IQR) [25%-%75].

	Groups					
	C (n=6)	SH (n=6)	G1 (n=6)	G2 (n=6)	G3 (n=6)	
Neurogenesis Related Genes						**p**
HES1	1.136 [0.645-1.837]	1.283 [0.886-1.961]	1.514 [0.840-2.606]	1.316 [0.832-1.937]	1.481 [0.538-2.904]	0.959
Neurog1	0.105 [0.003-11.490]	0.597 [0.019-8.725]	0.152 [0.011-0.755]	0.228 [0.044-0.710]	0.421 [0.003-2.208]	0.978
Neurog2	0.050 [0.027-0.184]	0.086 [0.061-0.111]	0.092 [0.036-0.339]	0.075 [0.032-0.185]	0.036 [0.008-0.544]	0.746
Neurod1	0.168 [0.110-0.593]	0.335 [0.248-0.429]	0.290 [0.206-0.971]	0.389 [0.193-0.880]	0.115 [0.045-1.262]	0.408
Wnt Signaling Pathway Associated Genes						**p**
SFRP2	0.069 [0.055-0.215]	0.144 [0.127-0.202]	0.112 [0.077-0.559]	0.210 [0.114-0.435]	0.037 [0.015-0.832]	0.326
SFRP4	0.113 [0.048-0.392]	0.194 [0.111-0.548]	0.143 [0.091-0.375]	0.206 [0.108-0.404]	0.126 [0.019-0.752]	0.820
SFRP5	0.077 [0.033-0.471]	0.128 [0.069-0.205]	0.074 [0.063-0.769]	0.155 [0.090-0.688]	0.018 [0.008-1.016]	0.592
APC1	0.030 [0.023-0.126]	0.067 [0.060-0.086]	0.041 [0.031-0.198]	0.066 [0.024-0.293]	0.034 [0.012-0.427]	0.557

C (Control): Incubated cells were allowed to go through their usual life cycle. SH (Sham): Cells were kept in the device for 30 min. with the ELF-EMF device off. Group 1 (G1): the group exposed to an electromagnetic field at a frequency of 50 Hz and intensity value of 1 mT. Group 2 (G2): the group exposed to an electromagnetic field at a frequency of 50 Hz and intensity value of 2 mT. Group 3 (G3): the group exposed to an electromagnetic field at a frequency of 50 Hz and intensity value of 3 mT. *APC1; Adenomatous Polyposis Coli 1, sFRP1; Secreted Frizzled Related Protein 1, SFRP2; Secreted Frizzled Related Protein 2, SFRP4; Secreted Frizzled Related Protein 4, SFRP5; Secreted Frizzled Related Protein 5, HES1; Hes Family BHLH Transcription Factor 1, Neurod1; Neuronal Differentiation 1, Neurog1; Neurogenin 1, Neurog2; Neurogenin 2*. C and SH groups compared to G1, G2 and G3 experimental groups.

components of the Wnt signal pathway. The amniocyte cells were exposed to 50 Hz ELF-EMF (1, 2, 3 mT) for 30 minutes each day for 7 days to observe whether exposure to ELF-EMF can influence gene expression. Gene expression levels of 4 Wnt signaling pathway-associated genes (*APC1, SFRP2, SFRP4, SFRP5*) and 4 neurogenesis-related genes (*HES1, Neurog1, Neurod1, Neurod2*) of the control, sham and 3 ELF-EMF application groups are shown in **Figure 1**, and the quantity of change in gene expression levels is depicted in **Table 3**. Neurogenesis and wnt signal pathways-related genes were examined to investigate the alterations in AFCs induced by ELF-EMF exposure. The main findings of the current study were that there were no significant differences in the amniocyte cells' gene expression levels for any of the genes associated with neurogenesis and wnt signaling pathways. As shown in **Figure 1**, gene expressions in ELF-EMF exposed cells did not show significant differences.

Discussion

In this study, we evaluated the effects of 50 Hz ELF-EMF exposure at varying intensities (1, 2, 3 mT) on the gene expression of amniotic fluid cells (AFCs). Our results showed that short-term ELF-EMF exposure did not significantly alter the expression of genes associated with neurogenesis or the Wnt/β-catenin signaling pathway. These findings are consistent with several studies in the literature that have reported minimal or no effects of ELF-EMF on certain cell lines *in vitro*.[20,21] The variability in responses to ELF-EMF exposure seen in different studies is likely due to differences in experimental conditions, such as exposure duration, field intensity, and cell type.[8,22]

Hes1, an important regulator of neurogenesis, acts as a transcriptional repressor that maintains neural stem cells (NSCs) by inhibiting pro-neural genes such as *Neurog1* and *NeuroD1*[23]. In our study, no significant change was observed in the expression of these genes, suggesting that short-term exposure

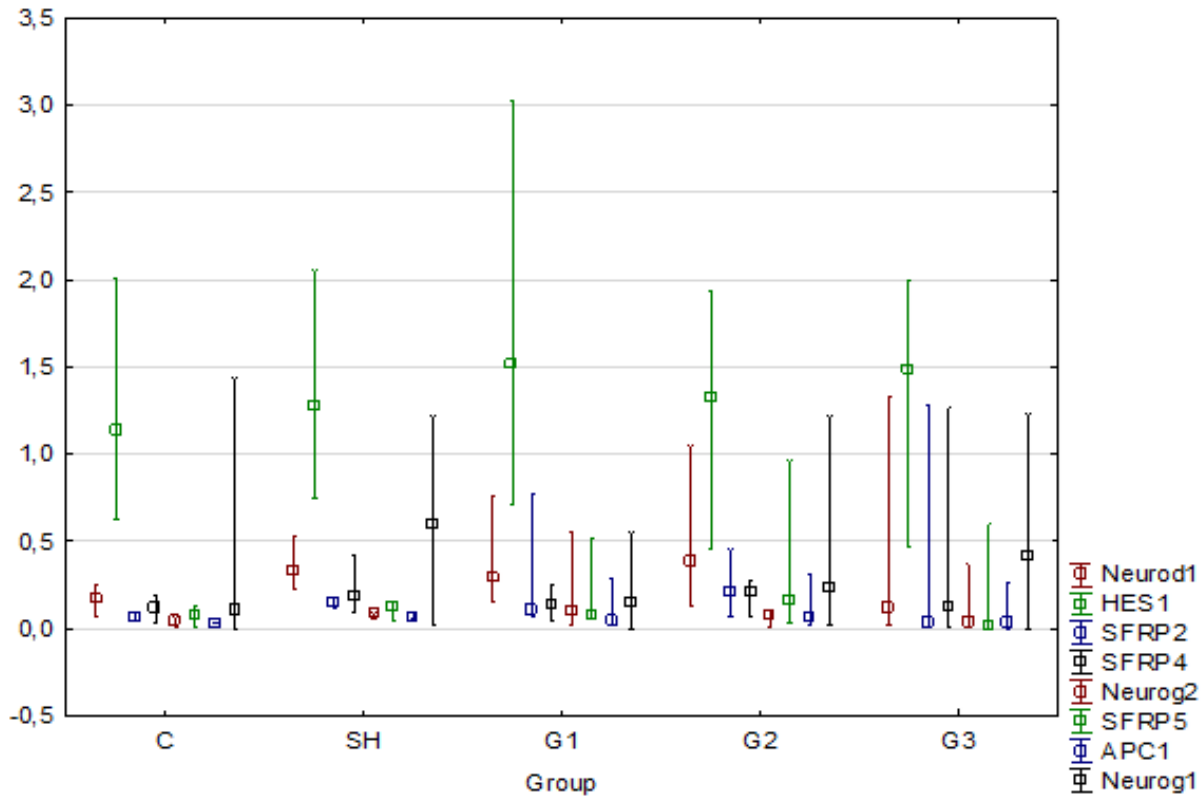

Figure 1. Expression changes of Wnt signaling pathway and neurogenesis related genes. (C: Control, SH: Sham, Group 1 (G1; 50 Hz, 1 mT), Group 2 (G2; 50 Hz, 2 mT), Group 3 (G3; 50 Hz, 3 mT). APC1: Adenomatous Polyposis Coli 1, sFRP1: Secreted Frizzled Related Protein 1, sFRP2: Secreted Frizzled Related Protein 2, sFRP4: Secreted Frizzled Related Protein 4, sFRP5: Secreted Frizzled Related Protein 5, HES1: Hes Family BHLH Transcription Factor 1, Neurod1: Neuronal Differentiation 1, Neurog1: Neurogenin 1, Neurog2: Neurogenin 2. No significant difference. Data are given as median and Interquartile range (IQR) (25%-%75).

to 50 Hz ELF-EMF at the tested intensities does not induce notable alterations in NSC-related gene expression. Previous studies have shown that ELF-EMF exposure can enhance NSC proliferation and differentiation, but these effects were typically observed after prolonged exposure or at higher intensities.[24,25] For example, Oda and Koike (2004) demonstrated that exposure to 50 Hz ELF-EMF at 300 mT significantly reduced apoptosis and increased neuronal survival in rat cerebellar neurons.[26] These differences highlight the importance of considering both exposure intensity and duration in future studies.The Wnt/β-catenin pathway is critical for embryonic development and tissue regeneration, and several studies have suggested that ELF-EMF can modulate this pathway.[27] *APC1, SFRP2, SFRP4*, and *SFRP5* are methylation-related genes that regulate the Wnt signaling pathway. Aberrant methylation of these genes could influence cellular differentiation and neurogenesis.[28] However, our results did not show significant changes in the expression of Wnt-related genes (*APC1, SFRP2, SFRP4, SFRP5*) following short-term exposure, consistent with other studies that found no immediate effects of ELF-EMF on gene expression.[29] The sFRP proteins, which inhibit Wnt signaling by preventing Wnt ligands from binding to their receptors, were unaffected in our experimental conditions. This suggests that more prolonged exposure or higher intensities may be required to observe modulation of this pathway, as seen in studies with longer-term ELF-EMF exposure.[10,30]

The absence of significant changes in gene expression in our study may be due to the relatively short duration (7 days) and moderate intensity (1-3 mT) of the exposure. Studies using higher intensities or longer exposure times have reported more pronounced effects on gene expression and cellular processes.[31] This suggests that while short-term ELF-EMF exposure may not have immediate biological effects on AFCs, long-term exposure may elicit more significant changes. Further studies are needed to explore the long-term effects of ELF-EMF on neurogenesis and Wnt signaling pathways, particularly during critical stages of embryonic development.

In conclusion, our findings suggest that short-term exposure (Acute exposure) to 50 Hz ELF-EMF does not significantly impact gene expression related to neurogenesis or Wnt signaling in amniotic fluid cells. Inconsistencies in the literature regarding the effects of ELF-EMF on cellular functions can be attributed to cell-type specific responses, variations in exposure parameters (intensity, frequency, duration), and differences in experimental conditions. These factors emphasize the need for standardized protocols in future studies[32]. Future research should focus on longer exposure periods and higher field intensities to fully understand the potential biological effects of ELF-EMF on these pathways, particularly in the context of fetal development. This study is limited because it was planned in vitro, which can only partially explain the complexity of in vivo systems. Therefore, demonstrating the study using in-vivo models may provide a more meaningful explanation. In addition, the long-term effects of ELF-EMF exposure on neurogenesis have not been evaluated and warrant further investigation.

Conclusion

In this study, we investigated the effects of short-term exposure (Acute exposure) to 50 Hz ELF-EMF on the gene expression of neurogenesis and Wnt signaling pathways in amniotic fluid cells. Our results demonstrated no significant changes in the expression of key genes related to these pathways, suggesting that brief exposure at moderate intensities does not induce notable biological effects in AFCs. These findings align with previous studies, highlighting the variability of ELF-EMF effects depending on exposure duration and intensity. Given the importance of the Wnt pathway and neurogenesis in fetal development, future research should focus on longer-term exposures and higher field intensities to fully elucidate the potential impact of ELF-EMF on embryonic development. Short-term exposure to ELF-EMF does not induce significant changes in AFCs. Long-term studies are needed to assess developmental impacts. Further exploration is necessary to determine whether sustained ELF-EMF exposure could influence these critical processes, with implications for both fetal health and therapeutic applications.

Declarations

Conflict of interest

There is no conflict of interest for any of the authors.

Acknowledgements

This work was supported by the Researchers

themselves.

References

1 Ahlbom A, Day N, Feychting M, et al. A pooled analysis of magnetic fields and childhood leukaemia. *British journal of cancer*. 2000;83(5):692-698. doi:https://doi.org/10.1054/bjoc.2000.1376

2 Savitz DA, Loomis DP. Magnetic field exposure in relation to leukemia and brain cancer mortality among electric utility workers. *American Journal of Epidemiology*. 1995;141(2):123-134. doi:10.1093/oxfordjournals.aje.a117400

3 Eichholz GG. *Non-Ionizing Radiation, Part 1: Static and Extremely Low-Frequency (ELF) Electric and Magnetic Fields*. IARC Press, Lyon France; 2002.

4 International Agency for Research on Cancer. IARC monographs on the identification of carcinogenic hazards to humans. Agents classified by the IARC Monographs, Volumes 1–136. World Health Organization,. September 22, 2023. Accessed November 27, 2024. https://monographs.iarc.who.int/list-of-classifications. Accessed November 27, 2024

5 Yu M, Qin K, Fan J, et al. The evolving roles of Wnt signaling in stem cell proliferation and differentiation, the development of human diseases, and therapeutic opportunities. *Genes & Diseases*. 2024;11(3):101026. https://doi.org/10.1016/j.gendis.2023.04.042

6 Yang K, Wang X, Zhang H, et al. The evolving roles of canonical WNT signaling in stem cells and tumorigenesis: implications in targeted cancer therapies. *Laboratory investigation*. 2016;96(2):116-136. https://doi.org/10.1038/labinvest.2015.144

7 Czéh B, Welt T, Fischer AK, et al. Chronic psychosocial stress and concomitant repetitive transcranial magnetic stimulation: effects on stress hormone levels and adult hippocampal neurogenesis. *Biological psychiatry*. 2002;52(11):1057-1065.

8 Piacentini R, Ripoli C, Mezzogori D, Azzena GB, Grassi C. Extremely low-frequency electromagnetic fields promote in vitro neurogenesis via upregulation of Cav1-channel activity. *Journal of cellular physiology*. 2008;215(1):129-139. doi:https://doi.org/10.1002/jcp.21293

9 Ma Q, Chen C, Deng P, et al. Extremely low-frequency electromagnetic fields promote in vitro neuronal differentiation and neurite outgrowth of embryonic neural stem cells via up-regulating TRPC1. *PloS one*. 2016;11(3):e0150923. doi:https://doi.org/10.1371/journal.pone.0150923

10 Rezaie-Tavirani M, Hasanzadeh H, Seyyedi S, Zali H. Proteomic analysis of the effect of extremely low-frequency electromagnetic fields (ELF-EMF) with different intensities in SH-SY5Y neuroblastoma cell line. *Journal of Lasers in Medical Sciences*. 2017;8(2):79. doi:10.15171/jlms.2017.14

11 Akdag MZ, Dasdag S, Erdal N, Buyukbayram H, Gurgul S. The effect of long-term extremely low-frequency magnetic field on geometric and biomechanical properties of rats' bone. *Electromagnetic Biology and Medicine*. 2010;29(1-12):9-18. doi:https://doi.org/10.3109/15368371003635343

12 Erdal ME, Yılmaz SG, Gürgül S, Uzun C, Derici D, Erdal N. miRNA expression profile is altered differentially in the rat brain compared to blood after experimental exposure to 50 Hz and 1 mT electromagnetic field. *Progress in biophysics and molecular biology*. 2018;132:35-42. doi:https://doi.org/10.1016/j.pbiomolbio.2017.08.001

13 Gürgül S, Erdal N, Yılmaz ŞN, Yıldız A, Ankaralı H. Deterioration of bone quality by long-term magnetic field with extremely low frequency in rats. *Bone*. 2008;42(1):74-80. doi:https://doi.org/10.1016/j.bone.2007.08.040

14 Friel NA, de Girolamo L, Gomoll AH, Mowry KC, Vines JB, Farr J. Amniotic fluid, cells, and membrane application. *Operative Techniques in Sports Medicine*.

2017;25(1):20-24. doi:https://doi.org/10.1053/j.otsm.2016.12.004

15 Rooney DE. *Human Cytogenetics: Constitutional Analysis: A Practical Approach*. Vol 1. Practical Approach; 2001.

16 Chomczynski P, Sacchi N. The single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction: twenty-something years on. *Nature protocols*. 2006;1(2):581-585. doi:https://doi.org/10.1038/nprot.2006.83

17 Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *methods*. 2001;25(4):402-408. doi:https://doi.org/10.1006/meth.2001.1262

18 Boutin C, Hardt O, De Chevigny A, et al. NeuroD1 induces terminal neuronal differentiation in olfactory neurogenesis. *Proceedings of the National Academy of Sciences*. 2010;107(3):1201-1206. doi:https://doi.org/10.1073/pnas.0909015107

19 Sun Y, Nadal-Vicens M, Misono S, et al. Neurogenin promotes neurogenesis and inhibits glial differentiation by independent mechanisms. *Cell*. 2001;104(3):365-376. doi:https://doi.org/10.1016/S0092-8674(01)00224-0

20 Falone S, Grossi MR, Cinque B, et al. Fifty hertz extremely low-frequency electromagnetic field causes changes in redox and differentiative status in neuroblastoma cells. *The international journal of biochemistry & cell biology*. 2007;39(11):2093-2106. doi:https://doi.org/10.1016/j.biocel.2007.06.001

21 Reale M, Kamal MA, Patruno A, et al. Neuronal cellular responses to extremely low frequency electromagnetic field exposure: implications regarding oxidative stress and neurodegeneration. *PloS one*. 2014;9(8):e104973. doi:https://doi.org/10.1371/journal.pone.0104973

22 Winker R, Ivancsits S, Pilger A, Adlkofer F, Rüdiger HW. Chromosomal damage in human diploid fibroblasts by intermittent exposure to extremely low-frequency electromagnetic fields. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 2005;585(1-2):43-49. doi:https://doi.org/10.1016/j.mrgentox.2005.04.013

23 Hatakeyama J, Kageyama R. Retinal cell fate determination and bHLH factors. In: *Seminars in Cell & Developmental Biology*. Vol 15. Elsevier; 2004:83-89. doi:https://doi.org/10.1016/j.semcdb.2003.09.005

24 Cuccurazzu B, Leone L, Podda MV, et al. Exposure to extremely low-frequency (50 Hz) electromagnetic fields enhances adult hippocampal neurogenesis in C57BL/6 mice. *Experimental neurology*. 2010;226(1):173-182. doi:https://doi.org/10.1016/j.expneurol.2010.08.022

25 Leone L, Fusco S, Mastrodonato A, et al. Epigenetic modulation of adult hippocampal neurogenesis by extremely low-frequency electromagnetic fields. *Molecular neurobiology*. 2014;49:1472-1486. doi:https://doi.org/10.1007/s12035-014-8650-8

26 Oda T, Koike T. Magnetic field exposure saves rat cerebellar granule neurons from apoptosis in vitro. *Neuroscience letters*. 2004;365(2):83-86. doi:https://doi.org/10.1016/j.neulet.2004.04.068

27 Du L, Fan H, Miao H, Zhao G, Hou Y. Extremely low frequency magnetic fields inhibit adipogenesis of human mesenchymal stem cells. *Bioelectromagnetics*. 2014;35(7):519-530. doi:https://doi.org/10.1002/bem.21873

28 Guan H, Zhang J, Luan J, et al. Secreted frizzled related proteins in cardiovascular and metabolic diseases. *Frontiers in Endocrinology*. 2021;12:712217. https://doi.org/10.3389/fendo.2021.712217

29 Zhou J, Gao YH, Zhu BY, et al. Sinusoidal electromagnetic fields increase peak bone mass in rats by activating Wnt10b/β-catenin in primary cilia of osteoblasts. *Journal of bone and mineral research*. 2019;34(7):1336-1351. doi:https://doi.org/10.1002/jbmr.3704

30 Azim K, Fischer B, Hurtado-Chong A, et al. Persistent Wnt/β-catenin signaling determines dorsalization of the postnatal subventricular zone and neural stem cell specification into oligodendrocytes and glutamatergic neurons. *Stem cells*. 2014;32(5):1301-1312. doi:https://doi.org/10.1002/stem.1639

31 Martínez M, Úbeda A, Martínez-Botas J, Trillo M. Field exposure to 50 Hz significantly affects wild-type and unfolded p53 expression in NB69 neuroblastoma cells. *Oncol Lett*. 2022;24(3):295-304. doi:10.3892/ol.2022.13415

32 Tian H, Zhu H, Gao C, et al. System-level biological effects of extremely low-frequency electromagnetic fields: An in vivo experimental review. *Frontiers in Neuroscience*. 2023;17:1247021. doi:https://doi.org/10.3389/fnins.2023.1247021