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Investigation of M3 muscarinic acetylcholine receptor expression in placentas of smoking women

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Abstract

This study examined M3 muscarinic acetylcholine receptor expression in placentas of women who smoked during pregnancy and explored the affected signaling pathways via network and functional annotation analyses. Placenta samples from 45 control subjects and 45 individuals who smoked during pregnancy were fixed in zinc-formalin and em- bedded in paraffin. Demographic data were recorded. Sections from the paraffin blocks were stained using Hematoxylin-Eosin and subjected to M3 muscarinic acetylcholine receptor immunostaining. Protein interaction networks were constructed with Cytoscape and analyzed with MCODE for module detection, while Enrichr was used for functional annotation. Histopathological analysis revealed significant degeneration in chorionic villi, increased fibrin deposition, a rise in syncytial knots, and vascular alterations, indicating that smoking adversely affects placental structure. In placental components, M3 muscarinic acetylcholine receptor immunoreactivity was mostly absent in the trophoblastic layer, syncytial knots, villous stroma, chorionic capillaries, and fibrin-rich areas. In the control group, moderate M3 muscarinic acetylcholine receptor expression was noted in connective tissue cells, whereas the trop- hoblastic layer and vascular structures displayed little reactivity. Conversely, placental sections from the smoking group showed a pronounced reduction in M3 muscarinic acetylcholine receptor expression, with a negative immunoreaction in key areas. Module and reactome pathway analysis indicated that Module 2, enriched in nicotinic acetylcholine receptor signaling, may mediate smoking-induced placental dysfunction. Additionally, Modules 1 and 3 were linked to GPCR and neurotransmitter pathways, respectively ($p<0.05$). The diminished M3 muscarinic acetylcholine receptor expression appears to disrupt placental function via altered nicotinic receptor signaling, potentially affecting vascular tone and nutrient exchange.

Keywords: Pregnancy, Smoking, Muscarinic, Placenta, Nicotine

Introduction

Smoking during pregnancy is a major public health con- cern, negatively impacting maternal and fetal health. Cigarette smoke toxins, including nicotine and carbon monoxide, impair fetal development, leading to comp- lications such as low birth weight, intrauterine growth restriction (IUGR), preterm birth, and placental abnor- malities .[1] A key mechanism underlying these effects is smoking's direct impact on the placenta, which regulates oxygen and nutrient supply to the fetus. Cigarette smoke toxins disrupt placental blood flow, restricting fetal oxy- gen and nutrient availability, leading to growth restricti- on and health complications [2, 3].

Muscarinic acetylcholine receptors (mAChRs), G- prote- in-coupled receptors activated by acetylcholine, regulate autonomic functions such as heart rate, smooth muscle contraction, and glandular secretion. These receptors, classified into five subtypes (M1–M5), are expressed in various tissues, including the placenta, where they may regulate vascular tone and nutrient exchange.[4, 5].. Acetylcholine in the placenta modulates uterine functi- on, prostaglandin production, and amino acid transport between mother and fetus.[6]. . Additionally, muscarinic receptors in ovarian tissue influence follicular develop- ment and ovulation, playing a vital role in fertility.[7].

Studies suggest muscarinic receptors are essential for placental function. Pavia et al. (1997) identified mAChR subtypes in syncytiotrophoblast membranes, with M1 re- ceptors in brush-border membranes and M2 receptors in basal membranes.[8].. Bhuiyan et al.

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(2006) reported that placental acetylcholine modulates nitric oxide (NO) pro- duction via muscarinic receptors, where carbachol (CCh) increases intracellular calcium levels, activating endothe- lial nitric oxide synthase (eNOS) and NO release.[5].. As NO regulates placental blood flow, muscarinic receptors likely contribute to vascular tone modulation.

Nicotine affects acetylcholine receptors, potentially dis- rupting muscarinic receptor function, leading to vascular dysregulation and impaired placental nutrient exchange. [9].. Altered M3 mAChR expression in smoking-exposed placentas may impair placental function.[10].. Additional- ly, muscarinic receptors influence hematopoietic system development, highlighting their broader impact on pla- cental function .[11].

This study investigates M3 mAChR expression in smo- king-exposed placentas and assesses smoking's effects on placental histology and in silico pathways.

Methods

Study design

Ethical approval was granted by Dicle University Faculty of Medicine Non-Interventional Studies Ethics Commit- tee (Date: 20/11/2024, Issue: 21). Placental tissues were collected from 45 healthy non-smoking pregnant women and 45 women who smoked at least five cigarettes per day. Participants were recruited from the Gynecology and Obstetrics Clinic at Dicle University. Demographic data, ultrasonographic findings, pregnancy outcomes, and blo- od samples collected before delivery were documented. Inclusion criteria were maternal age between 18–40 years, absence of pregnancy complications, systemic conditions, or chronic diseases. Exclusion criteria included preeclam- psia, severe anemia, diabetes, systemic lupus erythemato-sus (SLE), heart disease, and fetal anomalies. The control group consisted of healthy non-smokers with no pregnan- cy complications and fetal weight within the 10th to 90th percentile .[12].

Histological tissue protocol

Placentas were obtained from Dicle University Faculty of Medicine, Department of Gynecology and Obstetrics. Tissue samples were excised, fixed in zinc-formalin, and processed through tap water, alcohol series, and xylene stages before being embedded in paraffin. 5 µm sections were cut, stained with Hematoxylin-Eosin, and immunostained for M3 mAChR. [13, 14].

Immuno histochemical Staining

Placental sections from paraffin blocks were placed onto polylysine-coated slides at 37°C using a double boiler. Excess paraffin was removed by incubating slides at 58- 62°C for 6 hours. Sections were deparaffinized in xylene, rehydrated through decreasing alcohol concentrations, and rinsed in distilled water. Hydrogen peroxide was app- lied for 20 minutes, followed by PBS wash and treatment with Ultra V Block solution for 7 minutes. Sections were incubated overnight at +4°C with the primary antibody against M3 mAChR (Catalog no: ab-87199, Abcam, US). After washing with PBS, sections were exposed to a bio- tin-conjugated secondary antibody for 14 minutes, then incubated with streptavidin-peroxidase for 15 minutes. Diaminobenzidine (DAB) was used to visualize the anti- body-antigen reaction, and sections were observed under a microscope. After three PBS rinses (15 minutes each), sections were counterstained with Harris hematoxylin. Coverslips were mounted, and slides were examined with a Zeiss Imager A2 photomicroscope.[15].

Module and Pathway Analysis of M3 mAChR

To investigate the signaling pathways potentially affec- ted by the significant reduction in M3 mAChR expression observed in placenta samples of smoking women and to identify the proteins involved in these pathways, modu- le and functional annotation analyses were performed. Initially, the protein interaction network of M3 mAChR was constructed using the Cytoscape software (v.3.10.3), including up to 100 additional interactors at a confidence level of 0.400. To identify clusters within the network, the MCODE plugin was utilized, which enabled the detecti- on of densely interconnected regions indicative of poten- tial functional modules. Subsequently, proteins identified within different modules were subjected to Reactome pathway analysis using the Enrichr platform [a]. The ob- tained pathways were ranked in ascending order based on their p-values, and the top five statistically significant

pat- hways (p<0.05) were selected for further interpretation.
[16, 17].

Statistical analysis

Statistical analysis was performed using IBM SPSS 25.0 software (IBM, Armonk, New York, USA). Data were recorded as mean±standard deviation. Statistical distri- bution was evaluated with the Shapiro-Wilk test. Paired group comparisons were made with the independent t test. Significance was considered for p values <0.05. The number of patients for each group was calculated by G Power analysis (version 3.1). Cohen criteria were defined according to the study of Alviggi et al.[18].

Results

Demographic findings

Table 1 presents data on characteristics of pregnant smo- kers and non-smokers and their newborns. Measurements like weight, length, and head circumference highlight the effects of smoking. Newborns of smokers have signifi- cantly lower birth weight, length, and head circumferen- ce.

Table 1. Demographic parameters, measurements and pregnancy outcomes of the patients

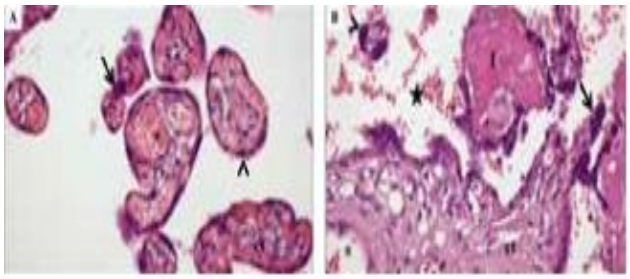
Parameters	Control Group (n=45)	Smoking grou p (n=45)	Significance (p-value)
Maternal age, years	25.15±4.62	24.89±5.6	>0.05
GW [1]	37.50±1.39	37.38±2.09	>0.05
Birth weight, g	3340.20±511.37	2685.42±622.83	<0.0001
Birth length, cm	49.90±2.44	46.69±2.31	<0.0001

GW: Gestational Week

2 APGAR: Appearance (skin color), Pulse (heart rate), Grimace (reflex irritability), Activity (muscle tone), and Respira- tion (breathing effort). Each criterion is scored from 0 to 2, with a maximum total score of 10.

Histopathological findings

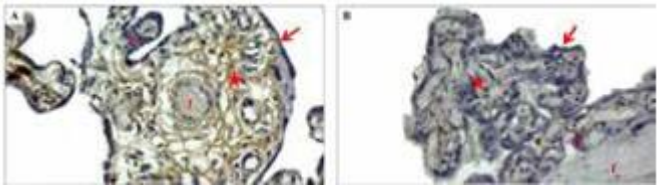
The histological analysis of placental sections revealed distinct structural differences between the control group and smokers, highlighting the impact of smoking on pla- cental morphology. Figure 1 presents the stained placental sections used for histological evaluation. In the placental sections of the control group consisting of healthy indivi- duals, the chorionic villi were observed to have a regular, oval structure with well-defined borders. No signs of dege- neration were observed in the structures of the chorionic villi. The syncytiotrophoblast cells were regularly arranged at the periphery of the villi, and the structural integrity of the chorionic vessels and villous connective tissue was ma- intained. Fibrin accumulation and the number of syncytial knots were minimal. The trophoblastic cells exhibited a ho- mogeneous distribution within the placental section. No congestion, dilation, or any pathological changes were de- tected in the vascular structures (Figure 1A). The placental sections of the smokers are shown in Figure 1B. Histopat- hological findings in this group revealed degeneration and morphological alterations in the chorionic villi structures.



A significant increase in fibrin accumulation and syncytial knot count was observed. Dilation of the vascular structu- res was prominent. Intense hemorrhage and the presence of inflammatory cells were noted in the intervillous space. Pyknotic nuclei were observed in the connective tissue cel- ls.

The immunostaining analysis of M3 mAChR in placental sections revealed distinct differences between the control and smoker groups, highlighting a reduction in M3 mAChR expression in the placentas of smokers. Figure 2 illustrates the M3 mAChR immunostaining pattern observed in the placental sections of the control group. The M3 mAChR immunoreactivity was primarily observed at a moderate level in the cytoplasmic regions of the connective tissue cells of the villi. M3 mAChR expression was negative in the trophoblastic layer cells and the chorionic capillary endo- thelium. Negative M3 mAChR immunoreactivity was also

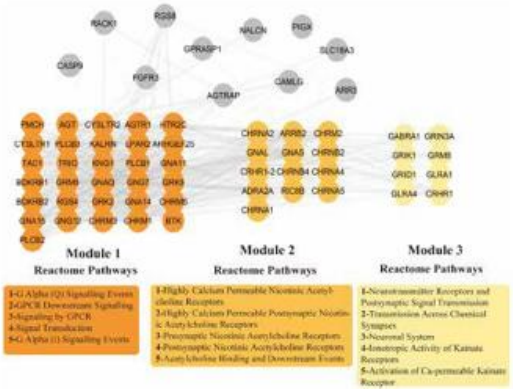
observed in fibrin deposit areas and syncytial knots (Figure 2A). Placental sections of the smokers were stained using the M3 mAChR immunostaining method (Figure 4).



A decrease in M3 mAChR expression was observed compared to the control group. In the components of the placenta, M3 mAChR immunoreactivity was generally negative in the trophoblastic layer, syncytial knots, villous stroma, cho- rionic capillaries, and fibrin deposit areas. Very slight M3 mAChR expression was observed in the connective tissue stromal areas (Figure 2B).

Identification of Functional Modules and Pathway Enrichment Analysis

The M3 mAChR protein interaction network analysis iden- tified three distinct modules using the MCODE plugin. Module 1, comprising 31 nodes and 379 edges, was the largest and most densely connected cluster and was sig- nificantly enriched in pathways related to G-protein coup- led receptor (GPCR) signaling, including G Alpha (Q) sig- naling events, GPCR downstream signaling, and signaling by GPCR ($p<0.05$). Module 2, containing 13 nodes and 30 edges, was predominantly associated with nicotinic acety- lcholine receptor signaling, highlighting pathways such as highly calcium permeable nicotinic acetylcholine recep- tors, presynaptic nicotinic acetylcholine receptors, and postsynaptic nicotinic acetylcholine receptors ($p<0.05$).



Module 3, the smallest cluster with 8 nodes and 19 edges, was enriched in neurotransmitter receptor pathways, inc- luding neurotransmitter receptors and postsynaptic signal transmission, transmission across chemical synapses, and neuronal system ($p<0.05$) (Figure 3).

Discussion

Smoking during pregnancy leads to numerous histologi- cal changes in the placenta (19). Toxic substances in ciga- rettes, such as nicotine, carbon monoxide, polycyclic aro- matic hydrocarbons (PAHs), heavy metals (e.g., cadmium, lead), and reactive oxygen species (ROS), negatively affect placental structure and function, thereby threatening fetal development. Studies have demonstrated that nicotine in cigarettes induces morphological and functional alterati- ons in the placenta. Due to its small molecular size and lipophilic nature, nicotine is rapidly absorbed (20), qui- ckly reaching the brain and crossing the placenta, thereby exerting effects on the fetus as well. Nicotine impairs the differentiation and migration of trophoblast cells, adver- sely affecting their functions(3, 21). Additionally, nicotine may increase endoplasmic reticulum (ER) stress by affec- ting pathways such as PERK and JNK (22).

Smoking can trigger various mechanisms that impair pla- cental angiogenesis and vascular integrity, leading to ad- verse effects on fetal development. Maternal smoking inc- reases the risk of placental abruption and placenta previa, jeopardizing oxygen and nutrient supply to the fetus and potentially causing severe health complications (23). Se- veral studies have reported reduced placental blood flow,

edematous plasma, and increased trophoblast cell dama- ge in pregnant smokers (24). Furthermore, smoking has been shown to cause abnormal placental vascular develop- ment and an increased tendency for hemorrhage, which contributes to reduced fetal oxygen and nutrient supply (25). Additionally, placental tissue from smoking mo- thers frequently exhibits signs of inflammation and cel- lular necrosis (19). Moreover, maternal smoking during pregnancy has been associated with intrauterine growth restriction and an increased risk of low birth weight (26). Our findings demonstrated that maternal smoking during pregnancy was associated with significantly lower birth weight, length, and head circumference in newborns compared to the control

group. These results align with previous studies reporting that smoking impairs placen- tal angiogenesis, disrupts vascular integrity, and reduces oxygen and nutrient supply to the fetus, contributing to intrauterine growth restriction and low birth weight. Histopathological alterations observed in the placentas of smoking mothers, such as increased fibrin deposition and inflammatory infiltration, likely underlie these adverse perinatal outcomes (2, 3, 27).

In this study, significant differences were observed in the histopathology and muscarinic acetylcholine recep- tor (M3 mAChR) immunoexpression of placental tissu- es from smoking mothers compared to healthy controls. Histopathological examination revealed that placental se- ctions from the control group maintained the structural integrity of chorionic villi, exhibited minimal syncytial knot formation, and preserved vascular integrity. In cont- rast, placental sections from smoking mothers displayed pronounced degenerative changes, including structural abnormalities in chorionic villi, increased fibrin depositi- on, a significant rise in syncytial knot formation, and ex- tensive hemorrhage and inflammatory cell infiltration in the intervillous space. Additionally, a notable dilation and congestion of vascular structures were observed. These findings support the detrimental effects of smoking on placental morphology and are consistent with previous studies.

Nicotine may disrupt the placental cholinergic system by increasing acetylcholine release and causing excessive sti- mulation of muscarinic receptors (M3 mAChRs) (28). The cholinergic system in the human placenta is characterized by the expression of acetylcholine and choline acetyltrans- ferase (ChAT), the enzyme responsible for acetylcholine synthesis. This system regulates various cellular processes, including proliferation, differentiation, cytoskeletal orga- nization, cell-cell interactions, motility, migration, cilia ac- tivity, and immune functions (29). Muscarinic receptors, which mediate the effects of acetylcholine through intra- cellular signaling, play a crucial role in modulating cellular responses. In the placenta, the activation of muscarinic receptors contributes to the regulation of cell growth and differentiation, thereby influencing placental functions. However, exposure to cigarette smoke has been shown to significantly disrupt these mechanisms.

Our study's M3 mAChR immunostaining results further emphasize the impact of smoking on placental neurot- ransmitter regulatory mechanisms. In the control group, moderate M3 mAChR expression was observed in conne- ctive tissue cells, with minimal reactivity in the trophob- lastic layer or vascular structures. In contrast, placental sections from smokers exhibited a marked reduction in M3 mAChR expression, with negative immunoreactivity observed in the trophoblastic layer, syncytial knots, cho- rionic capillaries, and fibrin accumulation sites. Literature suggests that alterations in muscarinic receptor expressi- on within the placenta may lead to disruptions in acetyl- choline-mediated signaling, which plays a critical role in fetal development (30). This decrease in expression may reflect the impact of smoking on acetylcholine-mediated signal transmission in placental cells and may also indica- te alterations in placental stress responses.

To elucidate the molecular mechanisms underlying the reduced M3 mAChR expression and associated histo- pathological changes observed in the placental tissues of smoking women, pathway and protein interaction analyses were conducted, identifying three distinct mo- dules(16). Module 1 included pathways related to GPCR signaling and G-protein-mediated signal transduction, which may be linked to smoking-induced vascular dysfun- ction through altered receptor modulation(17) . Module 3 encompassed neurotransmitter receptor and synaptic transmission pathways, suggesting potential disruptions in neurotransmission dynamics due to nicotine exposu- re(31). Notably, Module 2 was significantly enriched in ni- cotinic acetylcholine receptor (nAChR)-related pathways, including highly calcium-permeable nAChRs, presynaptic and postsynaptic nAChRs, and downstream acetylcholine binding events. Given that nicotine from cigarette smoke directly activates nAChRs, this finding suggests that redu- ced M3 mAChR expression may lead to a compensatory upregulation or increased activation of nAChR-media- ted signaling (6). Such alterations could disrupt calcium homeostasis and neurotransmitter transmission, crucial for maintaining placental function and vascular integrity Studies have shown that calcium homeostasis is vital for fetal skeletal mineralization and neurotransmitter release, and its disruption is associated with placental dysfuncti- on and pregnancy complications, including preeclampsia, where calcium transport is

compromised by oxidati- ve stress and ATP deficiency (32, 33). Additionally, it has been demonstrated that the placenta actively metaboli- zes and transports neurotransmitters like dopamine, no- repinephrine, and serotonin, which are essential for fetal development and programming, further highlighting the potential impact of disrupted signaling pathways in smoking-related placental dysfunction (34). These results underscore the pivotal role of nAChRs in mediating the detrimental effects of smoking on placental function and fetal development. Thus, identifying the protein targets within Module 2 provides valuable insights into the spe- cific signaling networks affected by reduced M3 mAChR expression, offering potential biomarkers and therapeutic targets to mitigate the adverse effects of smoking on pla- cental health.

A reduction in muscarinic receptor expression may dis- rupt physiological processes such as placental vascular tone and blood flow, adversely affecting oxygen and nut- rient transport to the fetus. Moreover, oxidative stress and inflammatory responses induced by nicotine and other ci- garette components are proposed to exert a suppressive effect on the placental cholinergic system, contributing to these alterations (35). Particularly, the loss of immuno- reactivity observed in the syncytiotrophoblast layer may weaken the placental barrier function, thereby compromi- sing fetal circulatory homeostasis.

Conclusion

This study demonstrates that histological and immuno- histochemical changes in the placental tissues of smoking mothers may impair placental function. The observed re- duction in M3 mAChR expression suggests a disruption in placental acetylcholine signaling, potentially contributing to vascular abnormalities. In silico analyses highlight the crucial impact of reduced M3 mAChR expression on pla- cental function through disrupted signaling networks, particularly those mediated by nicotinic acetylcholine receptors. Further experimental validation of these pat- hways could pave the way for novel therapeutic strategies to improve pregnancy outcomes in smoking women.

References

1. Delcroix-Gomez C, Delcroix MH, Jamee A, Gauthier T, Marquet P, Aubard Y. Fetal growth restriction, low birth weight, and preterm birth: Effects of active or passive smoking evaluated by maternal expired CO at delivery, impacts of cessation at different trimesters. Tob Induc Dis 2022;20:70.
2. Wickström R. Effects of nicotine during pregnancy: human and experimental evidence. Curr Neuropharmacol 2007;5(3):213-22.
3. Suter MA, Aagaard KM. The impact of tobacco chemicals and nicotine on placental development. Prenat Diagn 2020;40(9):1193-200.
4. Tizabi Y, Getachew B, Tsytsarev V, Csoka A, Copeland R, Heinbockel T. Central Nicotinic and Muscarinic Receptors in Health and Disease. 2023.
5. Bhuiyan MB, Murad F, Fant ME. The placental cholinergic system: localization to the cytotrophoblast and modulation of nitric oxide. Cell Commun Signal 2006;4:4.
6. Lips KS, Brüggmann D, Pfeil U, Vollerthun R, Grando SA, Kummer W. Nicotinic acetylcholine receptors in rat and human placenta. Placenta 2005;26(10):735-46.
7. Cuevas FC, Bastias D, Alanis C, Benitez A, Squicciarini V, Riquelme R, et al. Muscarinic receptors in the rat ovary are involved in follicular development but not in steroid secretion. Physiological Reports 2022;10(21):e15474.
8. Pavía J, Muñoz M, Jiménez E, Martos F, Gonzalez-Correa JA, De la Cruz JP, et al. Pharmacological characterization and distribution of muscarinic receptors in human placental syncytiotrophoblast brush-border and basal plasma membranes. Eur J Pharmacol 1997;320(2-3):209-14.
9. Machaalani R, Ghazavi E, Hinton T, Waters KA, Hennessy A. Cigarette smoking during pregnancy regulates the expression of specific nicotinic acetylcholine receptor (nAChR) subunits in the human placenta. Toxicol Appl Pharmacol 2014;276(3):204-12.
10. Beltrán-Castillo S, Bravo K, Eugenín J. Impact of Prenatal Nicotine Exposure on Placental Function and Respiratory Neural Network Development. Adv Exp Med Biol 2023;1428:233-44.

11. Trivedi G, Inoue D, Chen C, Bitner L, Chung YR, Taylor J, et al. Muscarinic acetylcholine receptor regulates self- renewal of early erythroid progenitors. Sci Transl Med 2019;11(511).
12. Asir F, Oglak SC, Korak T, Tas F, Yilmaz M, Erdemci F, et al. Placental vimentin expression in preeclampsia and gestational diabetes mellitus. Gynecology Obstetrics & Reproductive Medicine 2024;30(1):10-8.
13. Aşır F, Oğlak SC, Ağaçayak E, Alabalık U. Homeobox A Cluster 7 (HOXA7) protein expression increased in the placentas of patients with preterm delivery. Perinatal Journal 2023;31(3):213-8.
14. Aşır F, Ağaçayak E, Oğlak S, Deveci E, Akkuş M. WIPI- 2 protein expression increases in the placentas of patients with preeclampsia. Perinatal Journal 2023;31(3):219-23.
15. Aydeniz Acar GE, Akdeniz AS, Türe Z, Aşır A, Acar M, Aşır F, et al. HOXA1 expression in placentas of woman with fetal growth restriction. Perinatal Journal 2024;32(2).
16. Xie Z, Bailey A, Kuleshov MV, Clarke DJB, Evangelista JE, Jenkins SL, et al. Gene Set Knowledge Discovery with Enrichr. Curr Protoc 2021;1(3):e90.
17. Wong TS, Li G, Li S, Gao W, Chen G, Gan S, et al. protein-coupled receptors in neurodegenerative diseases and psychiatric disorders. Signal Transduct Target Ther 2023;8(1):177.
18. Alviggi C, Conforti A, De Rosa P, Strina I, Palomba S, Vallone R, et al. The Distribution of Stroma and Antral Follicles Differs between Insulin-Resistance and Hyperandrogenism-Related Polycystic Ovarian Syndrome. Front Endocrinol (Lausanne) 2017;8:117.
19. Ganer Herman H, Miremberg H, Nini N, Feit H, Schreiber L, Bar J, Kovo M. The effects of maternal smoking on pregnancy outcome and placental histopathology lesions. Reprod Toxicol 2016;65:24-8.
20. McKinney DL, Vansickel AR. Chapter 9 - Nicotine Chemistry, Pharmacology, and Pharmacokinetics. In: Preedy VR, editor. Neuropathology of Drug Addictions and Substance Misuse. San Diego: Academic Press; 2016. p. 93-103.
21. Kim CW, Lee HM, Lee K, Kim B, Lee MY, Choi KC. Effects of cigarette smoke extracts on cell cycle, cell migration and endocrine activity in human placental cells. Reprod Toxicol 2017;73:8-19.
22. He B, Chen Q, Zhou D, Wang L, Liu Z. Role of reciprocal interaction between autophagy and endoplasmic reticulum stress in apoptosis of human bronchial epithelial cells induced by cigarette smoke extract. IUBMB Life 2019;71(1):66-80.
23. Lal A, Watson W. Placenta previa and placental abruption. 2021. p. 9.1-9.8.
24. Kumar SN, Bastia B, Borgohain D, Agrawal U, Raisuddin S, Jain AK. Structural changes, increased hypoxia, and oxidative DNA damage in placenta due to maternal smokeless tobacco use. Birth Defects Res 2021;113(16):1198-214.
25. Pintican D, Poienar AA, Strilciuc S, Mihu D. Effects of maternal smoking on human placental vascularization: A systematic review. Taiwan J Obstet Gynecol 2019;58(4):454-9.
26. Ko TJ, Tsai LY, Chu LC, Yeh SJ, Leung C, Chen CY, et al. Parental smoking during pregnancy and its association with low birth weight, small for gestational age, and preterm birth offspring: a birth cohort study. Pediatr Neonatol 2014;55(1):20-7.
27. Tsai TH, Huang WC, Ying HT, Kuo YH, Shen CC, Lin YK, Tsai PJ. Wild Bitter Melon Leaf Extract Inhibits Porphyromonas gingivalis-Induced Inflammation: Identification of Active Compounds through Bioassay- Guided Isolation. Molecules 2016;21(4):454.
28. Machaalani R, Ghazavi E, Hinton T, Makris A, Hennessy A. Immunohistochemical expression of the nicotinic acetylcholine receptor (nAChR) subunits in the human placenta, and effects of cigarette smoking and preeclampsia. Placenta 2018;71:16-23.
29. Wessler I, Kilbinger H, Bittinger F, Unger R, Kirkpatrick CJ. The non-neuronal cholinergic system in humans: expression, function and pathophysiology. Life Sci 2003;72(18-19):2055- 61.
30. Wang Z, Zibrila AI, Liu S, Zhao G, Li Y, Xu J, et al. Acetylcholine ameliorated TNF-α-induced primary trophoblast malfunction via muscarinic receptors†. Biol Reprod 2020;103(6):1238-48.
31. Lucente E, Söderpalm B, Ericson M, Adermark

L. Acute and chronic effects by nicotine on striatal neurotransmission and synaptic plasticity in the female rat brain. Front Mol Neurosci 2022;15:1104648.
32. Haché S, Takser L, LeBellego F, Weiler H, Leduc L, Forest JC, et al. Alteration of calcium homeostasis in primary preeclamptic syncytiotrophoblasts: effect on calcium exchange in placenta. J Cell Mol Med 2011;15(3):654-67.
33. Lafond J, Simoneau L. Calcium homeostasis in human placenta: role of calcium-handling proteins. Int Rev Cytol 2006;250:109-74.
34. Horackova H, Karahoda R, Vachalova V, Turkova H, Abad C, Staud F. Functional characterization of dopamine and norepinephrine transport across the apical and basal plasma membranes of the human placental syncytiotrophoblast. Sci Rep 2022;12(1):11603.
35. Resende RR, Adhikari A. Cholinergic receptor pathways involved in apoptosis, cell proliferation and neuronal differentiation. Cell Commun Signal 2009;7:20. [PubMed] [CrossRef].