Int J Fertil Steril
International Journal of Fertility & Sterility
Homepage: https://www.ijfs.ir/
Original Article
Vol 18, No 4, October-December 2024, Pages: 373-377

Altered Expression of Circulating *miR-223-3p* in Patients with Idiopathic Recurrent Pregnancy Loss: A Case and Control Study

Parisa Ostad Hasanzadeh Maleki, Ph.D.[1], Saeid Ghorbian, Ph.D.[1*], Jafar Mohseni, Ph.D.[2], Sedigheh Abdollahi Fard, Ph.D.[2], Changiz Ahmadizadeh, Ph.D.[3]

1. Department of Molecular Genetics, Ahar Branch, Islamic Azad University, Ahar, Iran
2. Genetics Errors of Fertility Research Center, ART Center of ACECR East Azerbaijan Branch, East Azerbaijan ACECR, Tabriz, Iran
3. Department of Microbiology, Ahar Branch, Islamic Azad University, Ahar, Iran

Abstract

Background: Circulating microRNAs (miRNAs or miRs) are key regulators of a wide array of biological processes that aberrantly express under physiological as well as pathological conditions. This study aims to determine whether altered expression of circulating *miR-223-3p* in plasma is associated with an increased risk of idiopathic recurrent pregnancy loss (iRPL), and if it could be a novel non-invasive biomarker for diagnosis of iRPL.

Materials and Methods: In this case and control study, Plasma samples were obtained from 60 women with a history of ≥ 2 consecutive miscarriages and 60 women with at least one full-term pregnancy and no history of miscarriage. The samples were used to assess the expression of circulating *miR-223-3p* by quantitative real-time polymerase chain reaction (qPCR) and determine the correlation between patient clinico-demographic parameters and *miR-223-3p* expression levels. We used receiver operating characteristic (ROC) curve analysis to evaluate the diagnostic accuracy of *miR-223-3p* for the diagnosis of iRPL.

Results: There was significant up-regulation in *miR-223-3p* expression in patients with iRPL compared with healthy controls (P=0.002). No significant correlation was found between the expression level of *miR-223-3p* and the number of miscarriages in iRPL patients (P=0.344). ROC curve analysis revealed that the area under the curve (AUC) value for *miR-223-3p* was 0.658 [95% confidence interval (95% CI): 0.5590.757, P=0.002).

Conclusion: These results suggest that a higher expression level of circulating *miR-223-3p* may be closely related with the increased risk of iRPL and possibly serve as a promising non-invasive diagnostic biomarker for iRPL.

Keywords: Biomarker, microRNAs, *miR-223-3p*, Recurrent Pregnancy Loss

Citation: Ostad Hasanzadeh Maleki P, Ghorbian S, Mohseni J, Abdollahi Fard S, Ahmadizadeh Ch. Altered expression of circulating miR-223-3p in patients with idiopathic recurrent pregnancy loss: a case and control study. Int J Fertil Steril. 2024; 18(4): 373-377. doi: 10.22074/ijfs.2023.2005351.1483

Introduction

The European Society of Human Reproduction and Embryology and the American Society of Reproductive Medicine guidelines define recurrent pregnancy loss (RPL) as two or more consecutive clinically recognised pregnancies that involuntarily end in the first trimester of gestation (1, 2). It is an emotionally challenging experience that affects approximately 2-5% of reproductive-aged couples who aim to conceive (3). There is a consensus that RPL is a multifactorial disorder influenced by changes in multiple genes in combination with lifestyle and environmental factors (4). Potential risk factors that have been identified are parental and embryonic chromosomal abnormalities, uterine anatomical defects, hormone imbalances, metabolic disorders, immune dysfunction, maternal inherited thrombophilias, and infections (5, 6). However, the underlying causes of RPL in the majority of cases are unexplained (3).

Our understanding of the pathogenesis of human diseases and underlying cellular and molecular pathways and processes has grown exponentially since the discovery of the first small non-coding RNA in the nematode Caenorhabditis elegans (7). MicroRNAs (miRNAs or miRs) are a class of highly evolutionarily conserved small (~2025 nucleotides) single-stranded non-coding RNA molecules that strictly regulate the expression of protein-coding genes at the post-transcriptional level (8, 9). miRNAs exert their regulatory effects by repressing translation and/or inducing mRNA degradation through interacting in a sequence-specific manner with comple-

Received: 22/June/2023, Revised: 13/August/2023, Accepted: 15/October/2023
*Corresponding Address: P.O.Box: 5451116714, Department of Molecular Genetics, Ahar Branch, Islamic Azad University, Ahar, Iran
Emails: s_ghorbian@iau-aharac.ir, ghorbian20@yahoo.com

Royan Institute
International Journal of Fertility & Sterility

mentary sites located predominantly in the 3′-untranslated regions of their target mRNAs (10). Both experimental and computational studies show that a single mRNA can be targeted by multiple miRNAs and each miRNA has the potential to regulate up to hundreds of different mRNA molecules (11). At least 60% of the protein-coding genes in the human genome are predicted to be under direct control of miRNAs (12). Therefore, it is conceivable that miRNAs may play crucial roles in coordinating the fundamental biological processes of cell growth, proliferation, differentiation, survival, and apoptosis (13-16). miRNAs are crucial mediators of maternal communication during pregnancy, and they play a role in the establishment and maintenance of pregnancy (17, 18). It is not surprising that altered miRNAs expression patterns can contribute to various pathophysiological conditions, especially RPL (19, 20). Although miRNAs are abundant intracellularly, they are also present in extracellular body fluids such as plasma, serum, urine, saliva, amniotic fluid and seminal fluid in remarkably stable forms (21). Intriguingly, extracellular miRNAs have been shown to influence expression levels of genes related to physiological and pathological events (22). Recent studies show the significance of extracellular miRNAs as reliable non-invasive biomarkers for the diagnosis of human diseases (22, 23).

A series of circulating miRNAs with aberrant expression levels in the plasma of patients who suffer from RPL has been reported (24-26). In humans, the gene encoding *miR-223-3p* is located within the q12 locus of the X chromosome (27). Recent studies have shown that over-expression of *miR-223-3p* during implantation may contribute to the pathogenesis of idiopathic RPL (iRPL) (28-32). To the best of the author's knowledge, no study to date has investigated the relation between the altered expression of circulating *miR-223-3p* and RPL in Iranian women. So, the present study intends to quantify the expression levels of circulating *miR-223-3p* in plasma samples of Iranian women with iRPL compared to healthy subjects and determine its correlation with clinico-demographic features.

Materials and Methods

Study subjects

This was a case-control study. We recruited 60 women, age 34.60 ± 3.96 years (range: 20-42) who had a history of at least two consecutive failed clinically recognised pregnancies and live births miscarriages: 3.27 ± 0.86, range 25) for the experimental group. The control group consisted of 60race/ethnicity-matched women, age 29.97 ± 5.54 years (range: 18-41), who had at least one previous uncomplicated term pregnancy and no history of miscarriage successful pregnancies: 1.91 ± 0.67, range 13). The women were evaluated at the assisted reproductive technology (ART) and Stem Cell Research Centre, Tabriz, Iran, between April 2018 and March 2020. Patients who had any of the following criteria were excluded from the study: chromosomal abnormalities, uterine anatomical anomalies, autoimmune diseases, endocrine and metabolic disorders, hereditary thrombophilia or genital infections (TORCH syndrome). A structured questionnaire form was designed to collect demographic and medical data such as age, number of live births, stillbirths, miscarriages, ectopic pregnancies and history of infertility treatment from all participants. The Institutional Review Board/Ethics Committee of the Tabriz Islamic Azad University of Medical Science approved the study protocol (IR.IAU.TABRIZ.REC.1398.084). Written informed consent was obtained from all subjects prior to the interview and blood sample collection in accordance with the provisions of the Declaration of Helsinki.

RNA extraction and microRNA reverse transcription

Peripheral blood samples (~5 mL) were obtained from all subjects by venipuncture and placed in EDTA-containing vacutainers. Blood samples were centrifuged at 12000 xg for 10 minutes at 4°C to separate the plasma and the resultant supernatant was transferred to another microtube. All cellular debris and/or residual intact cells were removed by centrifuging the harvested plasma samples at 3000 xg for 5 minutes at 4°C. The pure plasma samples were transferred to new 1.5 ml DNase/RNase-free microtubes and stored at -80°C until further use. In order to extract the circulating RNAs, plasma samples were incubated on ice until they completely thawed. The circulating RNAs were extracted using the miRCURYTM RNA Isolation Kit-Biofluids (Exiqon Vedbaek, Denmark) according to the manufacturer's instructions and then treated with RNase-free DNase I (Thermo Fisher Scientific, USA). The quantity, quality, and integrity of the extracted RNA were determined using a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, LLC, Wilmington, DE, USA) and agarose gel electrophoresis. Finally, 1 µg of the extracted RNAs were reverse-transcribed into cDNA using a Reverse Transcription System Kit (ZistRoyesh, Iran) with miRNA specific stem-loop primers according to the manufacturer's recommendations.

Quantitative real-time polymerase chain reaction

The expression level of circulating *miR-223-3p* in all of the plasma samples was examined by SYBR® Green I-based qPCR in a StepOnePlusTM Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). All quantitative real-time polymerase chain reaction (qPCR) assays were performed independently, in triplicate using an AccuPower® 2X GreenStar™ qPCR Master Mix (Bioneer, Inc., Seoul, South Korea) and miRNA-specific primers, according to the manufacturer's protocol. The PCR reaction mixture final volume: 25 µl consisted of 100 ng cDNA, 12.5 µL of 2X SYBR Green PCR Master Mix (Bioneer Inc., South Korea), and 10 pmol of specific primer pairs for *miR-*

223-3p and the housekeeping gene *U6* small nuclear RNA (snRNA). The desired fragments were amplified using the following thermal cycling program: an initial denaturation step at 95°C for 30 seconds, 40 cycles at 94°C for 5 seconds, and 60°C for one minute. The following primer sequences were used:

miR-223-3p-

F: 5′-TAAAGCAACCGAGCACTGAGA-3′
R: 5′-ACGGTAGAGGTCCTTTCCTTTG-3′

U6 snRNA-
F: 5'-CTCGCTTCGGCAGCACA-3'
R: 5'-AACGCTTCACGAATTTGCGT-3'

The housekeeping gene *U6 snRNA*, as an internal reaction control, was used to normalise the variability of *miR-223-3p* expression. In order to avoid possible contamination, a no template control was used as a negative control. Relative changes in expression levels of *miR-223-3p* were determined by the comparative $2^{-\Delta\Delta CT}$ method.

Statistical analysis

Statistical analysis was performed by IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA) and all continuous variables were expressed as mean ± standard deviation (SD). The distribution of the data was checked for normality using the Kolmogorov-Smirnov test. The student's t test was used to compare mean expression levels of circulating *miR-223-3p* between the iRPL patients and healthy controls. The correlations between the expression levels of circulating *miR-223-3p* and clinico-demographic parameters were determined by Pearson's correlation coefficient. P<0.05 were considered to be statistically significant. Finally, the diagnostic accuracy of circulating *miR-223-3p* was assessed by GraphPad Prism version 9.1.1 (GraphPad Software Inc., San Diego, CA, USA) to discriminate between patients with iRPL and healthy individuals.

Results

Expression levels of circulating *miR-223-3p* in idiopathic recurrent pregnancy loss patients and healthy subjects

We quantified the expression levels of circulating *miR-223-3p* in plasma samples of women with iRPL and healthy controls by qPCR. The Kolmogorov-Smirnov test results showed that the expression levels of circulating *miR-223-3p* had a normal distribution (P>0.05). Figure 1 shows a significant upregulation inexpression levels of circulating *miR-223-3p* in the plasma samples from the iRPL patients (18.32 ± 2.84) compared with the healthy controls (8.34 ± 1.24) with a 95% confidence interval (95% CI) of 3.847-16.195 (P=0.002. The fold change in circulating *miR-223-3p* expression was assessed by the $2^{-\Delta\Delta CT}$ method. As shown in Figure 2, the relative expression levels of circulating *miR-223-3p* were significantly higher in the iRPL group compared with healthy subjects (fold change: 7.068, P=0.013).

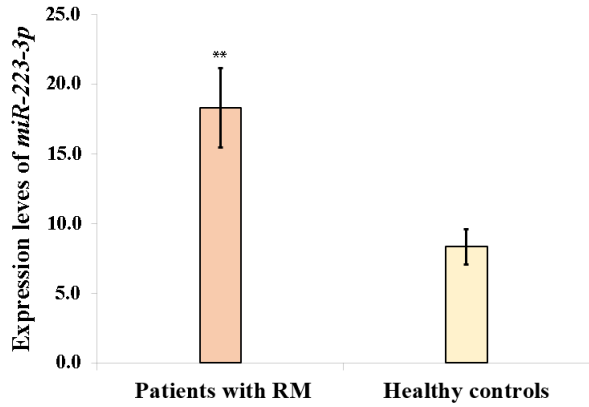

Fig.1: Expression of circulating *miR-223-3p* in iRPL patients and healthy controls. The plasma levels of *miR-223-3p* in patients with iRPL are significantly higher than those in healthy controls (CI= 0.559-0.757, P=0.002). iRPL; Idiopathic recurrent pregnancy loss and RM; Recurrent miscarriage (**; P<0.01).

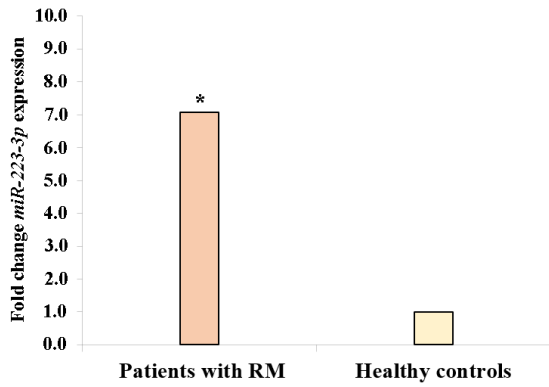

Fig.2: The fold change in expression of circulating *miR-223-3p* in iRPL patients and healthy controls. There is a higher relative expression of circulating *miR-223-3p* in the iRPL patients compared with healthy controls (fold change=7.068, P=0.013). iRPL: Idiopathic recurrent pregnancy loss and RM; Recurrent miscarriage (*; P<0.05).

Correlation between plasma levels of *miR-223-3p* and clinico-demographic parameters

We analysed the correlations of circulating *miR-223-3p* expression in iRPL patients with age and the number of miscarriages. Our data demonstrated that there was no significant correlation between the expression levels of circulating *miR-223-3p* in iRPL patients in terms of age (r=0.165, P=0.208). No correlation was found between the number of abortions and expression levels of circulating *miR-223-3p* in iRPL patients (r=0.124, P=0.344).

Diagnostic accuracy of circulating *miR-223-3p*

The diagnostic value of circulating *miR-223-3p*, as a potential non-invasive biomarker for diagnosis of iRPL, was assessed by receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) value of circulating *miR-223-3p* was 0.658 (95% CI: 0.5596-0.7579, P=0.002), with a sensitivity of 60% and specificity of 70% at a cut-off of 8.545 (Fig.3).

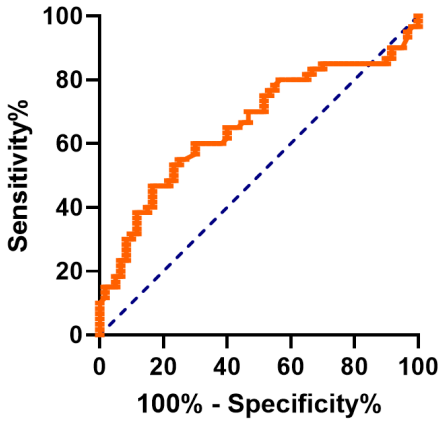

Fig.3: Diagnostic value of circulating *miR-223-3p* according to ROC curve analysis. The AUC value of circulating *miR-223-3p* is 0.658 (95% CI: 0.55960.7579, P=0.002), with a sensitivity of 60% and specificity of 70% at a cut-off of 8.545. ROC; Receiver operating characteristic, AUC; Area under the curve, and CI; Confidence interval.

Discussion

Extensive efforts have been made to better understand the molecular mechanisms involved in the pathogenesis of RPL with a special focus on polymorphisms and expression levels of certain genes. Among them, miRNAs have attracted substantial attention in the field of reproductive medicine because of their fine-tuning properties (33, 34). A set of circulating miRNAs have been recently identified as potential non-invasive biomarkers for more effective clinical diagnosis and management of RPL (24-26). *miR-223-3p* is initially thought to be a hematopoietic-specific miRNA that regulates myeloid lineage development, but it has been suggested that *miR-223-3p* expresses in many different cell types and is closely related to a broad range of pathological processes (35-37). To date, there is limited information regarding the role of *miR-223-3p* in reproductive functions. Chen et al. (38) reported that *miR-223-3p* alleviates TGF-β-induced cell migration, invasion, the epithelial-mesenchymal transition process, and extracellular matrix deposition in endometrial epithelial cells by targeting SP3. Dong et al. (28) reported that *miR-223-3p* down-regulated the expression of leukaemia inhibitory factor (LIF) during embryo implantation in mice. LIF is a multi-functional cytokine that belongs to the interleukin-6 superfamily that is expressed in the endometrial glandular epithelium. LIF acts on the endometrial epithelium to increase epithelial adhesiveness and receptivity to blastocyst attachment, and on the endometrial stroma to decidualise it in preparation for implantation and subsequent placenta development (20). Dong et al. (28) reported overexpression of LIF in mice endometrium on day 4 of pregnancy along with reduced expression of *miR-223-3p*. They concluded that *miR-223-3p* directly targeted the LIF gene, and this led to a decrease in its expression and a reduction in the number of implanted embryos. Low expression levels of *miR-223-3p* during the implantation window might offset its inhibitory effect on *LIF* gene expression, which would cause an increase in *LIF* expression and facilitate embryo implantation during early pregnancy. In addition, these researchers studied the role of *miR-223-3p* in pinopode formation during embryo implantation in mice. Pinopodes, also called uterodomes, are smooth mushroom or balloon-like projections that arise from the apical surface of the endometrial luminal epithelium in mice and humans (29). These microscopic structures are formed at the beginning of the implantation window and most likely have a strong relationship to different endometrial receptivity-associated factors (30, 31). Dong et al. (28) reported that *miR-223-3p* might adversely affect embryo implantation in the endometrium by preventing pinopode formation. A study conducted by Niknafs et al. (32) on mice suggested that up-regulation of *miR-223-3p* inhibit the mTOR signalling pathway, and thereby halt expression of the endometrial receptivity-associated genes. Taken together, these data strongly suggest that over-expression of *miR-223-3p* during the implantation window may contribute to the pathogenesis of iRPL. Thus, it is necessary to further research the effects of *miR-223-3p* in female reproduction. In the current study, we evaluated the expression levels of *miR-223-3p* in plasma samples of iRPL patients and healthy individuals in order to determine if circulating *miR-223-3p* is dysregulated in women with iRPL. We also explored the diagnostic performance of circulating *miR-223-3p*, as a novel non-invasive biomarker, for diagnosis and clinical management of iRPL. Our results clearly show up-regulated plasma levels of *miR-223-3p* in patients with iRPL compared with healthy subjects. ROC curve analysis indicated that circulating *miR-223-3p* may be a valuable biomarker for distinguishing iRPL women from healthy women. To the best of our knowledge, this is the first study to investigate the expression levels of *miR-223-3p* in plasma samples of Iranian patients with iRPL and healthy subjects. In agreement with our findings, a previous study reported over-expression of *miR-223-3p* in iRPL patients (20). Given the important biological function of *miR-223-3p* in embryo implantation, it is conceivable that its up-regulation has a profound effect on the success of a pregnancy. Further studies should be designed to validate these findings.

Conclusion

The existence of quantitative differences in the levels of circulating miRNAs between patients and healthy individuals suggest that changes in circulating miRNAs may be used as a promising non-invasive biomarker for diagnosis, prognosis, screening, and monitoring of various diseases. Despite extensive research, the clinical applicability of circulating miRNAs as biomarkers is limited. Circulating miRNAs could possibly be used to complement other types of markers. Our data indicate that up-regulation of circulating *miR-223-3p* may be associated with an increased risk of iRPL, and may serve as a promising non-invasive diagnostic biomarker for iRPL. However, it is necessary to conduct additional research in order to determine if a causal association exists between the expression level of circulating *miR-223-3p* and increased risk of iRPL.

Acknowledgements

The authors would like to express their sincerest appreciation to all individuals who participated in this study. There is no finantial support and conflict of interest in this study.

Authors' Contributions

P.O.H.M., S.Gh., J.M., S.A.F., Ch.A.; Designed this study and Analysed the data. P.O.H.M.; Carried out the experiments and Wrote the draft of the manuscript. S.Gh., J.M.; Supervised the project. All authors critically reviewed and approved the final version of this manuscript.

References

1. ESHRE Guideline Group on RPL; Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, et al. ESHRE guideline: recurrent pregnancy loss. Hum Reprod Open. 2018; 2018(2): hoy004.
2. Practice Committee of the American Society for Reproductive Medicine. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. Fertil Steril. 2012; 98(5): 1103-1111.
3. El Hachem H, Crepaux V, May-Panloup P, Descamps P, Legendre G, Bouet PE. Recurrent pregnancy loss: current perspectives. Int J Womens Health. 2017; 9: 331-345.
4. Christiansen OB, Steffensen R, Nielsen HS, Varming K. Multifactorial etiology of recurrent miscarriage and its scientific and clinical implications. Gynecol Obstet Invest. 2008; 66(4): 257-267.
5. Page JM, Silver RM. Genetic causes of recurrent pregnancy loss. Clin Obstet Gynecol. 2016; 59(3): 498-508.
6. Blue NR, Page JM, Silver RM. Genetic abnormalities and pregnancy loss. Semin Perinatol. 2019 Mar;43(2):66-73.
7. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993; 75(5): 843-854.
8. Mohr AM, Mott JL. Overview of microRNA biology. Semin Liver Dis. 2015; 35(1): 3-11.
9. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet. 2010; 11(9): 597-610.
10. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front Endocrinol (Lausanne). 2018; 9: 402.
11. Li Y, Kowdley KV. MicroRNAs in common human diseases. Genomics Proteomics Bioinformatics. 2012; 10(5): 246-253.
12. Saito T, Saetrom P. MicroRNAs--targeting and target prediction. N Biotechnol. 2010; 27(3): 243-249.
13. Bueno MJ, Pérez de Castro I, Malumbres M. Control of cell proliferation pathways by microRNAs. Cell Cycle. 2008; 7(20): 3143-3148.
14. Bueno MJ, Malumbres M. MicroRNAs and the cell cycle. Biochim Biophys Acta. 2011; 1812(5): 592-601.
15. Song L, Tuan RS. MicroRNAs and cell differentiation in mammalian development. Birth Defects Res C Embryo Today. 2006; 78(2): 140-149.
16. Subramanian S, Steer CJ. MicroRNAs as gatekeepers of apoptosis. J Cell Physiol. 2010; 223(2): 289-298.
17. Liu W, Niu Z, Li Q, Pang RT, Chiu PC, Yeung WS. MicroRNA and embryo implantation. Am J Reprod Immunol. 2016; 75(3): 263-271.
18. Ivey KN, Srivastava D. microRNAs as developmental regulators. Cold Spring Harb Perspect Biol. 2015; 7(7): a008144.
19. Karami N, Mirabutalebi SH, Montazeri F, Kalantar SM, Sheikhha MH, Eftekhar M. Aberrant expression of microRNAs 16 and 21 and gene targets in women with unexplained recurrent miscarriage: a case-control study. Int J Reprod Biomed. 2018; 16(10): 617-622.
20. Bahia W, Soltani I, Abidi A, Haddad A, Ferchichi S, Menif S, et al. Identification of genes and miRNA associated with idiopathic recurrent pregnancy loss: an exploratory data mining study. BMC Med Genomics. 2020; 13(1): 75.
21. Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, et al. The microRNA spectrum in 12 body fluids. Clin Chem. 2010; 56(11): 1733-1741.
22. Liang H, Gong F, Zhang S, Zhang CY, Zen K, Chen X. The origin, function, and diagnostic potential of extracellular microRNAs in human body fluids. Wiley Interdiscip Rev RNA. 2014; 5(2): 285-300.
23. Kim YK. Extracellular microRNAs as biomarkers in human disease. Chonnam Med J. 2015; 51(2): 51-57.
24. Jairajpuri DS, Malalla ZH, Mahmood N, Khan F, Almawi WY. Differentially expressed circulating microRNAs associated with idiopathic recurrent pregnancy loss. Gene. 2021; 768: 145334.
25. Qin W, Tang Y, Yang N, Wei X, Wu J. Potential role of circulating microRNAs as a biomarker for unexplained recurrent spontaneous abortion. Fertil Steril. 2016; 105(5): 1247-1254. e3.
26. Yang Q, Gu WW, Gu Y, Yan NN, Mao YY, Zhen XX, et al. Association of the peripheral blood levels of circulating microRNAs with both recurrent miscarriage and the outcomes of embryo transfer in an in vitro fertilization process. J Transl Med. 2018; 16(1): 186.
27. Du T, Wang D, Wan X, Xu J, Xiao Q, Liu B. Regulatory effect of microRNA-223-3p on breast cancer cell processes via the Hippo/Yap signaling pathway. Oncol Lett. 2021; 22(1): 516.
28. Dong X, Sui C, Huang K, Wang L, Hu D, Xiong T, et al. MicroRNA-223-3p suppresses leukemia inhibitory factor expression and pinopodes formation during embryo implantation in mice. Am J Transl Res. 2016; 8(2): 1155-1163.
29. Quinn CE, Casper RF. Pinopodes: a questionable role in endometrial receptivity. Hum Reprod Update. 2009; 15(2): 229-236.
30. Nardo LG, Nikas G, Makrigiannakis A, Sinatra F, Nardo F. Synchronous expression of pinopodes and alpha v beta 3 and alpha 4 beta 1 integrins in the endometrial surface epithelium of normally menstruating women during the implantation window. J Reprod Med. 2003; 48(5): 355-361.
31. Xu B, Sun X, Li L, Wu L, Zhang A, Feng Y. Pinopodes, leukemia inhibitory factor, integrin-β3, and mucin-1 expression in the periimplantation endometrium of women with unexplained recurrent pregnancy loss. Fertil Steril. 2012; 98(2): 389-395.
32. Niknafs B, Hesam Shariati MB, Shokrzadeh N. miR223-3p, HAND2, and LIF expression regulated by calcitonin in the ERK1/2-mTOR pathway during the implantation window in the endometrium of mice. Am J Reprod Immunol. 2021; 85(1): e13333.
33. Eisenberg I, Kotaja N, Goldman-Wohl D, Imbar T. microRNA in human reproduction. Adv Exp Med Biol. 2015; 888: 353-387.
34. Imbar T, Galliano D, Pellicer A, Laufer N. Introduction: MicroRNAs in human reproduction: small molecules with crucial regulatory roles. Fertil Steril. 2014; 101(6): 1514-1515.
35. Ye D, Zhang T, Lou G, Liu Y. Role of miR-223 in the pathophysiology of liver diseases. Exp Mol Med. 2018; 50(9): 1-12.
36. Zhang MW, Shen YJ, Shi J, Yu JG. MiR-223-3p in cardiovascular diseases: a biomarker and potential therapeutic target. Front Cardiovasc Med. 2021; 7: 610561.
37. Mancuso R, Agostini S, Hernis A, Zanzottera M, Bianchi A, Clerici M. Circulatory miR-223-3p discriminates between parkinson's and alzheimer's patients. Sci Rep. 2019; 9(1): 9393.
38. Chen Y, Sun D, Shang D, Jiang Z, Miao P, Gao J. miR-223-3p alleviates TGF-β-induced epithelial-mesenchymal transition and extracellular matrix deposition by targeting SP3 in endometrial epithelial cells. Open Med (Wars). 2022;17(1): 518-526.