Sys Rev Pharm 2022; 13(3): 135-140
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453 / DOI: 10.31858/0975-8453.13.3.135-140

Research Article

The Evaluation of COVID-19 Effect on Pregnancy Loss-Molecular and Diagnostic Approach: A Narrative Review

Seyedeh Kosar Mahmoudi[1], Mina yazdizadeh[2], Zahra Jahanshahiafshar[3], Marzieh Rohaninasab[4*]

[1]Department of Cellular and Molecular Biology, Shahid Beheshti University, Tehran, Iran
[2]Department of Pediatric Dentistry, Babol University of Medical Sciences, Babol, Iran
[3]Department of Oral and Maxillofacial Radiology, Babol University of Medical Sciences, Babol, Iran
[4]Department of Aestetic and Restorative Dentistry, Tehran University of Medical Sciences, Tehran, Iran

Article History: *Submitted: 07.02.2022* *Accepted: 22.02.2022* *Published: 01.03.2022*

ABSTRACT

The new severe respiratory disease causing virus (SARS-CoV-2) originates from Wuhan, China and has spread around the world. According to studies, the results of the SARS-CoV-2 test have been reported to be positive for a number of pregnant women. However, not much is known about the effect of this virus on pregnancy and the outcome of the baby. The aim of this study was to evaluate the molecular and diagnostic approach in evaluating the effect of Coronavirus disease 2019 (COVID-19) on pregnancy loss.

The entry of COVID-19 virus into the pregnant mother's body through various channels, including the Angiotensin-Converting Enzyme receptor (ACE2), affects the immune and coagulation systems and hormone levels. These changes include increased D-Dimer, platelet and decreased Protein C (PC) levels, increased antithrombin III (AT-III) and elevated levels of proinflammatory cytokines, including IL-6, followed by disruption of various signaling pathways such as JAK/STAT and PI3K Is. And SMAD factors 1/3; Decreased regulation of the expression of cyclooxygenase 1 (COX1) and prostaglandin E2 (PGE2) and hormones such as progesterone was observed. These changes disrupt decision-making in the endometrium and ultimately lead to serious pregnancy risks, including miscarriage. Due to the important role of signaling pathways in maintaining the body's homeostasis and regulating the body's immune functions, disrupting the pathways through the entry of the virus into the body disrupts the body's homeostasis increases the risk of miscarriage in pregnant women.

Keywords: COVID-19, Pregnancy loss, Pathogenesis, Diagnosis

***Correspondence:** Marzieh Rohaninasab, Department of Aestetic and Restorative Dentistry, Tehran University of Medical Sciences, Tehran, Iran, E-mail: hadi.rezaeeyan@yahoo.com

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a virus from the coronavirus family, whose study of clinical effects has become one of the main topics of research in the world scientific community. The pandemic of the virus began in late 2019 in Wuhan, China (Najafi S, *et al.*, 2020). Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2) is the name now given to this new virus (Ahn DG, *et al.*, 2020). It is named by the International Committee on Taxonomy of Viruses, as well as other virologists based on the similarity of this new virus to SARS-COV, which broke out in 2002 (Li H, *et al.*, 2020).

LITERATURE REVIEW

COVID-19 can infect all age groups; it is easily transmitted through the respiratory tract (Ahn DG, *et al.*, 2020). Clinical signs of the virus include shortness of breath, fever, cough, headache, gastrointestinal and heart problems, blood clotting disorders and other complications (Guo YR, *et al.*, 2020).

Many studies have focused on the effect of this disease on the general population, but not enough research has been done to investigate the effect of this disease on people with certain conditions, including pregnancy conditions, while pregnant women due to physiological changes is considered as in danger group (Juan J, *et al.*, 2020). Including these physiological changes hematological, cardiac, renal, respiratory, metabolic, gastrointestinal and hormonal changes (Pillay SP, *et al.*, 2016). In one case, placental infection was observed with COVID-19, which may cause preeclampsia and worsening the mother's physiological condition, and premature termination of pregnancy or abortion (Lu-Culligan A, *et al.*, 2021). Another study of 116 pregnant women with COVID-19 found clinical findings of preterm delivery and miscarriage, but found no significant association between the Infection with the virus and those reported (Yan J, *et al.*, 2020).

In another case, the traces of COVID-19 left in placenta and miscarriage for no reason in the second trimester in a 28-year-old mother can bring us closer to understand the relationship between viral placental infection and miscarriage (Baud D, *et al.*, 2020). Due to the clinical effects of this virus on various organs of the body and to improve the control and management of this pandemic, and increase the knowledge about the pathogenesis of this disease, it is necessary to conduct researches about the effect of virus on different populations in different conditions such as pregnancy (Afshar Y, *et al.*, 2020).Therefore, in the preset study we evaluated the clinical effects of this virus on pregnant women and their specific physiological conditions, as well as the relationship between maternal infection with this virus and abortion.

COVID-19 and coagulation disorder

Coagulation disorders are one of the clinical symptoms in COVID-19 infected individuals, which cause an imbalance in homeostasis. Among the related factors to the coagulation system, D-Dimer has been frequently observed in pregnant women with COVID (Zou Y, *et al.*, 2020). D-Dimer is a protein that has been shown to increase plasma levels as a result of blood clotting, and high levels of D-Dimer can be explained by a local increase in fibrin formation (Wells PS, *et al.*, 2003). Coagulation disorders and increased risk of Disseminated Intravascular Coagulation (DIC) are the results of D-dimer and inflammatory cytokines increment; they can lead to clot formation and deposition of Fibrin in the bloodstream of the placenta, and prevent proper blood supply to placenta and abortion (Kumano O, *et al.*, 2020).

Also, according to studies, antithrombin III (AT-III), which

has a binding site for Thrombin and Heparin, is an important inhibitor of coagulation cascade. AT-III can inhibit serine-activated proteases such as FXa, FIXa, FXIa, and FXIIa (Yang S, *et al*., 2021). It also potentially prevents the activation of Nuclear Factor-κB (NF-KB), which is involved in inflammation (Rezaie AR and Giri H, 2020). When the Corona virus enters the body, ACE-2 glycosylation facilitates the entry of the virus into the body. On the other hand, glycosylation of AT III causes thrombosis. In the virus infected pregnant women, who have recurrent miscarriages, we see an increase in AT-III levels (Ceriello A, *et al*., 2020).

On the other hand, protein S (PS) and protein C (PC) also have a vitamin K-dependent anticoagulant role and can bind to Activated Protein C (APC) attached to the phospholipid platelet surface and reduce the effect of FXa on FVa in the coagulation cascade. As a result, PS and PC significantly prevent Fibrin from clotting and forming (Medfisch SM, *et al*., 2020; Amiral J and Seghatchian J, 2019). PS and PC also impair the expression of thrombomodulin, and the endothelial PC receptor on the endothelium. Studies have shown that thrombomodulin has anticoagulant function by binding to Thrombin and activating the PC. Decreased PC levels in people with COVID are associated with an imbalance in thrombin regulation (Okada M, *et al*., 2020; Tiscia GL, *et al*., 2020).

COVID-19 entry the body is associated with an increase in interleukin-6 (IL-6) and Tumor necrosis factor α (TNF α), which are linked to insulin resistance, endothelial dysfunction and blood clotting. Insulin resistance is associated with increased plasminogen activator inhibitor-1 (PAI-1) (Yanai H, 2020). Increasing PAI-1 has a significant effect on the fibrinolytic process by increasing platelet count and decreasing Prothrombin activity time and Thromboplastin activation (Bigdeli R, *et al*., 2018). Therefore, maintaining balance in PAI-1, especially in pregnant women with COVID, is essential for a successful pregnancy. Platelet Lymphocyte Ratio (PLL) and Neutrophil Lymphocyte Ratio (NLR) levels also change in people with COVID And the increase in PLR and NLR levels with the effect on inflammatory factors and ROS, causes a lack of coagulation factors, followed by bleeding and miscarriage (Biyik I, *et al*., 2020).

It can now be concluded that the COVID-19 can affects coagulation factors; it increases D-Dimer, platelets and prothrombin time and also decreases PC and vitamin K levels, especially in pregnant women. It would be a risk for mother and fetus, and finally it may lead to abortion.

COVID-19 and immune system disorder

The immune system plays an important role in the body homeostasis; its irregularities can trigger systemic immune responses, following the rapid spread of the virus, which in turn can lead to COVID-19. The spread of viral agents and inflammation can cause tissue damage that result in damaged cells releasing cytokines. Due to the activated signaling cascade, these cytokines affect gene expression. High levels of cytokine in association with variety of infectious conditions are often called cytokine storms, indicating an immune response characterized by the release of interferons, interleukins, tumor necrosis factors, chemokines, and several other mediators (Wong YP, *et al*., 2021).

In COVID-19 infected pregnant women, pro-inflammatory cytokines including IL-6 increase. This factor causes the adverse consequences of abortion or abnormal fetal development. Modulation of the mother's immune system during pregnancy may affect the response to infections, particularly viruses. In general, the immune system during pregnancy adapts to the growth of semi-allogeneic fetus, resulting in an altered immune response to infection during pregnancy (Giri TK, 2021). In the placental trophoblast cells of women with the abortion history, this response includes increased production of pro-inflammatory cytokines including Interferon gamma (*IFN-γ*)/IL-2/IL-6/IL-7/TNF-α/IL-1, and decreased concentrations of anti-inflammatory cytokines (Han H, *et al*., 2020).

In other words, in the inflammatory and stimulated conditions, IL-1, TNF-a and IL-6 also increase, followed by an increase in IL-17; and this increase in cytokines activates the NFKB, MAPK, PI3K and JAK/STAT pathways (Noack M and Miossec P, 2017). By activating these signaling pathways, serum and urinary concentrations of Progesterone-Induced Blocking Factor (PIBF) expressed by lymphocytes during pregnancy are affected and decreased; As a result, by increasing NK cells and producing TNF, NK cells target trophoblast cells and cause spontaneous abortion in pregnancy (Bartho SJ, *et al*., 2018).

The proliferation, differentiation, and function of NK cells are regulated by the direct action of progesterone and estrogen on intracellular nuclear receptors, or by intermediate cells in the uterus in early pregnancy. In normal pregnancy, lymphocytes are able to produce progesterone and PIBF during pregnancy; they increase fetal immunity, inhibit NK cell activity and prevent the risk of miscarriage by increasing the production of anti-inflammatory cytokines. But when the immune system becomes abnormal due to the virus spread, this ability is lost; NK cells spread is associated with an increase in spontaneous abortions (Widya AC, *et al*., 2017; Bogdan A, *et al*., 2017). Meanwhile, IL-10 modulates resistance to inflammatory stimuli by reducing the regulation of pro-inflammatory cytokines expression, such as IL-1A, IL-12, IL-6 and TNF α (Darif D, *et al*., 2021). In general, with the increase of these inflammatory cytokines, especially IL-6, we see the activation of *STAT3* and *STAT1* in the JAK/STAT pathway and the imbalance created between Treg/Th17, and cellular immunodeficiency; On the other hand, increasing IL-6 disrupts the expression of genes in this pathway (Venkatesan T, *et al*., 2017).

For example, the Sprouty 4 *(SPRY4)* gene is called *IFN-γ*, which causes the expression of this gene and *STAT1* phosphorylation *via* the PI3K/AKT pathway. Increased expression of *SPRY4* gene inhibits trophoblast proliferation and increases the apoptosis (Qin S, *et al*., 2020). Also, the *IRF1* gene, which in collaboration with *STAT1* reduces *miR-103*, leads to repeated spontaneous abortions by increasing the M1 macrophage activity (Zhu X, *et al*., 2020). On the other hand, *STAT3* factor increment in large quantities, can suppress GP130 through the suppressor of cytokine signaling 3 *(SOCS3)*, which activates the MAP kinase pathways, PI3 kinase (Liu X, *et al*., 2019). In these pathways, overexpression of TNF-a and matrix metalloproteinase (MMP)-3 and MMP-9 factors by altering B-catenin/wnt pathway activity, inhibiting the Integrin-Linked Kinase (ILK) and also by acting on caspases and activating caspase3 causes apoptosis of trophoblast cells (Chen Q, *et al*., 2020).

On the other hand, activation and increase of NFKB pathway by affecting on hypertension, causes a significant decrease in uterine endometrial immunity and the immunological mechanism of gestational hypertension syndrome. Eventually, these pathways affect estradiol and intracellular hormones and PIBF concentrations, increasing the risk of miscarriage (Arck PC, 2021). On the other hand, it has been shown that inflammation in the dental pulp can also affect the incidence of abortion in patients. It has been shown that inflammation in the dental pulp can stimulate the immune system and produce inflammatory mediators through dysregulation of some miRs (*Table 1*). Considering that one of the complications of COVID-19 infection is the occurrence of cytokine storm and also since cytokine storm can play a role in the occurrence of abortion due to dental pulp, possibly COVID-19 infection can be caused by dental pulp Abortion in patients (Shen Z, *et al*., 2020).

On the other hand, this stimulatory effect of IL-6 increases MicroRNA-223-3p (*miR-223-3p*) and *mir-184*. Elevation of *mir-184* by targeting WIG1 increases apoptosis of trophoblast cells by regulating FAS expression. *mir-223-3p* increment also has a regulatory effect on *FOX1* and *APOL1* genes, which are involved in immune cell homeostasis and regulation of inflammation and apoptosis. Finally, they form a complex monitoring network with pro-inflammatory agents and signaling pathways (Zhang Y, *et al*., 2019; Wu J, *et al*., 2019).

Table 1: Summary of miR's involved in inflammation

miR	Target	Mechanism	Reference
miR-21	KBTBD7	Cause activation NF-kB and lead to inflammation	(Song J, *et al.*, 2020)
miR-410	MMP-14	Decreased expression *miR-410* cause increased MMP-14 production and inflammation	(Brodzikowska A, *et al.*, 2019)
miR-146a	bFGF	Cause production inflammation mediators	(Liu L, *et al.*, 2016)
miR-Let-7c-5p	DMP1	Down regulation of Let-7c-5p lead to active NF-kB pathway and inflammation	(Yuan H, *et al.*, 2018)
miR-223-3p	DSPP DMP1	Cause stimulate immune response and inflammation	(Huang X, *et al.*, 2019)
Note: Where bFGF: Basic Fibroblast Growth Factor; DMP1: Dentin Matrix Protein-1; MMP-14: Matrix Metalloproteinase; DSPP: Dentine sialophosphoprotein			

It can now be concluded that due to the increase in these inflammatory cytokines in pregnant women undergoing abortion, and given that these inflammatory cytokines including IL-6 also increase in pregnant women with COVID. It can be concluded that these cytokines, by activating the signaling pathways and the changes mentioned, cause adverse consequences in pregnancy, such as abortion for the mother or abnormal growth and development for the fetus (*Table 2*).

Table 2: Evaluation of function of some cytokines in pathogenesis of COVID-19

Cytokine	Increase/decrease	Mechanism	Potential therapeutic	Reference
IL-1	Increase	Activation the MAPK and PI3K pathways and proliferative functions, inflammation	Interferon-I- • Inhibition of viral replication, mitigation of host inflammatory response • Slowdown the cell metabolism • Inhibition the production of IL-1b and TNF-α in host	(Ramaiah MJ, 2020; Hiratsuka M, *et al.*, 2000)
TNF-a	Increase	Activation of the MAPK pathway. inflammation and proliferation of acute phase proteins	Azithromycin- • Inhibition of viral cell penetration • Inhibition of terminal ACE-2 glicosilation • Endosomal pH increase	(Hariharan A, *et al.*, 2021; Favilli A, *et al.*, 2020)
IL-5	Decrease	Decreased production of B cell antibodies, activation of macrophages and neutrophils	-	(Bonaventura A, *et al.*, 2020)
IL-4	Decrease	-	-	(Renu K, *et al.*, 2020)
TGFB	Decrease	Increased expression of MHC II at the level of monocytes, macrophages and proinflammatory cytokine production	-	(Liu J, *et al.*, 2020)
IL-10	Decrease	Increased expression of pro-inflammatory macrophage marker/effect on TH1 and increases the production of pro-inflammatory cytokines IL-2 and IFNY and increases inflammation.	-	(Abers MS, *et al.*, 2021)
IL-8	Increase	With its chemokine activity, it causes the lymphocytes and neutrophils release into the area of inflammation.	Pioglitazon- • Mitigation of host inflammatory response • Inhibition of host IL-8 production	(Tatum D, *et al.*, 2020; Ota H, *et al.*, 2008)
Il-6	Increase	Activation of the JAK/STAT pathway and *STAT 3* and *STAT 1*. Increase the trophoblast apoptosis	Tocilizumab- • Mitigation of host inflammatory response • Soluble and membrane-bound IL-6 receptor blockage	(Magro G, 2020; Nakajima K, *et al.*, 2016)
IL-12	Increase	Activate the JAK/STAT pathway and *Stat4* and *Stat1*, and cause inflammation	Betamethasone- • Mitigation of host inflammatory response • Inhibition of host IL-12 production	(Lusty E, *et al.*, 2017; Kakoulidis I, *et al.*, 2020)
Note: MAPK: A Mitogen-Activated Protein Kinase; PI3K: Phosphoinositide 3-Kinase; TNF-a: Tumor Necrosis Factor Alpha, ACE-2: Angiotensin-Converting Enzyme 2; LPS: Lipopolysaccharides; NK cell: Natural Killer cell, TGFB: Transforming Growth Factor Beta; MHC II: Major Histocompatibility Complex Class II; IFNY: Interferon Gamma, jak/stat: The Janus Kinase/Signal Transducer and Activator of Transcription; IL: Interleukin; TH1: T Helper1				

COVID-19 and endometrial decidualization

Endometrial decidualization, a vital multicellular process for pregnancy progression, is one of the first changes that the uterus adapts to. The interaction between pregnancy-related hormones and cytokines produced by embryonic and uterine cells has been identified as an essential event for decidualization (Oestreich AK, *et al*., 2020). In COVID-19 infected pregnant women, due to the lack of coordination and balance between cytokines and hormones, a disorder is observed in the decidualization process (Sills ES and Wood SH, 2020). Endometriosis, one of the most common diseases in pregnant women, has been partly explained by resistance to progesterone and decreased intracellular progesterone receptor expression in the extra uterine endometrium (Naidoo N, *et al*., 2021). However, overproduction of progesterone can also impair decidualization by inhibiting Leukemia Inhibitory Factor (LIF)/Signal transducer and transcription activator 3 *(STAT3)* (Liang YX, *et al*., 2018).

Heart- and neural crest derivatives-expressed transcript 2 (*HAND2*), which is a major factor in the transcription of progestin-induced human endometrial stromal cells, also play a key role in activation and surviving the Uterine Natural Killer (UNK) by regulating IL-15. *HAND2* balances the production of Vascular Endothelial Growth Factor (VEGF) and Placental Growth Factor (PlGF), resulting in a balance in Endometrial Stromal Cells (ESCs) (Murata H, *et al*., 2020). COVID-19 infected pregnant women are at the risk of miscarriage, due to decreased level of progestin production (Cui L, *et al*., 2021).

RESULTS AND DISCUSSION

Studies have shown that ACE2, known as a receptor for coronavirus entry, is essential for decidualization of endometrial stromal cells (Albini A, *et al*., 2020). ACE2 is increased during primary Human Endometrial Stromal Cell (HESC). Due to the increase in ACE2 in various tissues, such as ovaries and uterus, there is a potential risk to the reproductive system and pregnancy (Wang N, *et al*., 2021). In addition to ACE2, other factors affecting human endometrial stromal cells include prolactin (*PRL*) and insulin-like growth factor binding protein-1 (*IGFBP1*), which is produced by decidual cells. *PRL* and *IGFBP1* are two known factors for decidual cell maturation and proteoglycan decorin (DCN) (Buzzio OL, *et al*., 2006). DCN inhibits human trophoblast regeneration, migration, invasion, and differentiation needed to regenerate uterine arteries during normal pregnancy. According to studies, some cytokines increment such as IL-11, is associated with change in the regulation of expression and an increase in *IGFBP1* and *PRL*. This increase is also achieved through the entry of virus to the pregnant women, followed by the activation of other cytokines and changes in the regulation of their expression. As a result, there would be the miscarriage risk during pregnancy (Eyal O, *et al*., 2007).

Morphogenetic protein-2 (*bmp2*) has also been shown to increase the production of pro-inflammatory cytokines. It also reduces the regulation of cyclooxygenase 1 (cox1) expression, followed by a decrease in prostaglandin E2 (PGE2), which is disrupted by the signaling pathway smad1/smad5/(Alk3) activin receptor-like kinase 3 in ESCs. An increase in the amount of pro-inflammatory cytokines without any controlling effect increases the risk of miscarriage for COVID-19 infected pregnant women (Zhang Y, *et al*., 2020; Arikawa T, *et al*., 2004).

According to studies, Sirtuin 1 (*SIRT1*) can also be introduced as an effective factor in regulating homeostasis and ESC decidualization (Li J, *et al*., 2021). *SIRT1* through regulating the expression of superoxide dismutase2 *SOD2*) and The nuclear factor erythroid 2-related factor 2 NRF2) as well as by deacetylation Forkhead Box O1 (*FOXO1*), makes adjustments homeostasis Reactive Oxygen Species (ROS) and NAD^+ and enhanced protection Equals oxidative stress. Decreased *SIRT1* followed by decreased *FOXO1* deacetylation and imbalances in ROS and NAD^+ observed in patients with COVID cause Recurrent Implantation Failure (RIF) (Madkour MI, *et al*., 2019; Yang Y, *et al*., 2014). *FOXO1* is also considered as an ESC decidualization marker, which acts as a transcriptional regulator of *PRL* and Insulin (Yoshino O, *et al*., 2003).

Studies have also shown that the effect of Norepinephrine (NEP) on decidualization can be investigated. NEP prevents endometrial decidualization by activating the PKC signaling pathway *via* a1B-adrenergic receptor regulation (Wang J, *et al*., 2021).

Given the changes mentioned, and the imbalance between hormones, cytokines and factors involved in pregnancy in COVID-19 infected pregnant women, we can conclude that the process of endometrial decidualization is disrupted and subsequently abortion may happen.

CONCLUSION

Pregnant women are in a state of suppressed immune system due to physiological changes; they are considered as COVID high-risk group, due to susceptibility to infections and mechanical functions. Immune system suppression disrupts the pregnancy process by affecting the profiles of cytokines and various coagulation systems and hormones; these disorders are associated with the risk of miscarriage. We hope this review be useful for pregnancy and neonatal services, seeking to respond to COVID-19.

ACKNOWLEDGEMENT

We wish to thank all our colleagues in Tehran University of Medical Sciences.

REFERENCES

1. Najafi S, Rajaei E, Moallemian R, Nokhostin F. The potential similarities of COVID-19 and autoimmune disease pathogenesis and therapeutic options: New insights approach. Clin Rheumatol. 2020: 1-13.
2. Ahn DG, Shin HJ, Kim MH, Lee S, Kim HS, Myoung J, *et al*. Current status of epidemiology, diagnosis, therapeutics, and vaccines for novel coronavirus disease 2019 (COVID-19). J Microbiol Biotechnol. 2020; 30(3): 313-324.
3. Li H, Liu SM, Yu XH, Tang SL, Tang CK. Coronavirus disease 2019 (COVID-19): Current status and future perspectives. Int J Antimicrob Agents. 2020; 55(5): 105951.
4. Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, *et al*. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak-an update on the status. Mil Med Res. 2020; 7(1): 1-10.
5. Juan J, Gil MM, Rong Z, Zhang Y, Yang H, Poon LC. Effect of coronavirus disease 2019 (COVID-19) on maternal, perinatal and neonatal outcome: Systematic review. Ultrasound Obstet Gynecol. 2020; 56(1): 15-27.
6. Pillay SP, Piercy NC, Tolppanen H, Mebazaa A. Physiological changes in pregnancy: Review articles. Cardiovasc J Afr. 2016; 27(2): 89-94.
7. Lu-Culligan A, Chavan AR, Vijayakumar P, Irshaid L, Courchaine EM, Milano KM, *et al*. SARS-CoV-2 infection in pregnancy is associated with robust inflammatory response at the maternal-fetal interface. medRxiv. 2021.
8. Yan J, Guo J, Fan C, Juan J, Yu X, Li J, *et al*. Coronavirus disease 2019 in pregnant women: A report based on 116 cases. Am J Obstet Gynecol. 2020; 223(1): e1-e14.
9. Baud D, Greub G, Favre G, Gengler C, Jaton K, Dubruc E, *et al*. Second-trimester miscarriage in a pregnant woman with SARS-CoV-2 infection. Jama. 2020; 323(21): 2198-2200.
10. Afshar Y, Gaw SL, Flaherman VJ, Chambers BD, Krakow D, Berghella V, *et al*. Clinical presentation of coronavirus disease 2019 (COVID-19) in pregnant and recently pregnant people. Obstet Gynecol. 2020; 136(6): 1117.

11. Zou Y, Guo H, Zhang Y, Zhang Z, Liu Y, Wang J, *et al*. Analysis of coagulation parameters in patients with COVID-19 in Shanghai, China. Biosci trends. 2020; 14(4).
12. Wells PS, Anderson DR, Rodger M, Forgie M, Kearon C, Dreyer J, *et al*. Evaluation of D-dimer in the diagnosis of suspected deep-vein thrombosis. N Engl J Med. 2003; 349(13): 1227-1235.
13. Kumano O, Ieko M, Komiyama Y, Naito S, Yoshida M, Takahashi N, *et al*. Characterization of fibrin/fibrinogen degradation products reagents and their utility in critical care patients with enhanced fibrinolysis. Int J Lab Hematol. 2020; 43(4): 813-820.
14. Yang S, Chen D, Wang J, Qiao R, Cui L. Coagulation status in women with missed abortion. 2021.
15. Rezaie AR, Giri H. Antithrombin: An anticoagulant, anti-inflammatory and anti-bacterial serpin. J Thromb Haemost. 2020; 18(3): 528.
16. Ceriello A, de Nigris V, Prattichizzo F. Why is hyperglycaemia worsening COVID-19 and its prognosis? Diabetes Obes Metab. 2020; 22(10): 1951-1952.
17. Medfisch SM, Muehl EM, Morrissey JH, Bailey RC. Phosphatidylethanolamine-phosphatidylserine binding synergy of seven coagulation factors revealed using nanodisc arrays on silicon photonic sensors. Sci rep. 2020; 10(1): 1-7.
18. Amiral J, Seghatchian J. Revisiting the activated protein C-protein S-thrombomodulin ternary pathway: Impact of new understanding on its laboratory investigation. Transfus Apher Sci. 2019; 58(4): 538-544.
19. Okada M, Tominaga N, Honda G, Nishioka J, Akita N, Hayashi T, *et al*. A case of thrombomodulin mutation causing defective thrombin binding with absence of protein C and TAFI activation. Blood adv. 2020; 4(12): 2631-2639.
20. Tiscia GL, Favuzzi G, de Laurenzo A, Cappucci F, Fischetti L, di Mauro L, *et al*. Reduction of ADAMTS13 levels predicts mortality in SARS-CoV-2 patients. TH Open. 2020; 4(03): e203-e206.
21. Yanai H. Adiposity is the crucial enhancer of COVID-19. Cardiol Res. 2020; 11(5): 353.
22. Bigdeli R, Younesi MR, Panahnejad E, Asgary V, Heidarzadeh S, Mazaheri H, *et al*. Association between thrombophilia gene polymorphisms and recurrent pregnancy loss risk in the Iranian population. Syst Biol Reprod Med. 2018; 64(4): 274-282.
23. Biyik I, Albayrak M, Keskin F. Platelet to lymphocyte ratio and neutrophil to lymphocyte ratio in missed abortion. Rev Bras Ginecol Obstet. 2020; 42: 235-239.
24. Wong YP, Khong TY, Tan GC. The effects of COVID-19 on placenta and pregnancy: What do we know so far? Diagnostics. 2021; 11(1): 94.
25. Giri TK. Severity of maternal illness in pregnancy during COVID-19 pandemic. J Adv Res Med Sci Technol. 2021; 8(1): 3-6.
26. Han H, Ma Q, Li C, Liu R, Zhao L, Wang W, *et al*. Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. Emerg microbes infect. 2020; 9(1): 1123-1130.
27. Noack M, Miossec P. Selected cytokine pathways in rheumatoid arthritis. Semin Immunopathol. 2017; 39(4): 365-383.
28. Bartho SJ, Šućurović S, Jeričević MB. The role of extracellular vesicles and PIBF in embryo-maternal immune-interactions. Front Immunol. 2018; 9: 2890.
29. Widya AC, Loho MF, Wantania JJ. The role of progesterone induced blocking factor in threatened abortion. Indones J Soc Obstet Gynecol. 2017; 5(4): 193-198.
30. Bogdan A, Berta G, Bartho SJ. PIBF positive uterine NK cells in the mouse decidua. J Reprod Immunol. 2017; 119: 38-43.
31. Darif D, Hammi I, Kihel A, Saik IEI, Guessous F, Akarid K. The pro-inflammatory cytokines in COVID-19 pathogenesis: What goes wrong? Microb Pathog. 2021:104799.
32. Venkatesan T, Choi YW, Lee J, Kim YK. *Pinus densiflora* needle supercritical fluid extract suppresses the expression of pro-inflammatory mediators iNOS, IL-6 and IL-1β, and activation of inflammatory *STAT1* and *STAT3* signaling proteins in bacterial lipopolysaccharide-challenged murine macrophages. DARU. 2017; 25(1): 1-10.
33. Qin S, Zhang Y, Zhang J, Tian F, Sun L, He X, *et al*. *SPRY4* regulates trophoblast proliferation and apoptosis *via* regulating *IFN*-γ-induced *STAT1* expression and activation in recurrent miscarriage. Am J Reprod Immunol. 2020; 83(6): e13234.
34. Zhu X, Liu H, Zhang Z, Wei R, Zhou X, Wang Z, *et al*. *MiR-103* protects from recurrent spontaneous abortion *via* inhibiting *STAT1* mediated M1 macrophage polarization. Int J Biol Sci. 2020; 16(12): 2248.
35. Liu X, D'Cruz A, Hansen J, Croker B, Lawlor K, Sims N, *et al*. Deleting suppressor of cytokine signaling-3 in chondrocytes reduces bone growth by disrupting mitogen-activated protein kinase signaling. Osteoarthritis Cartilage. 2019; 27(10): 1557-1563.
36. Chen Q, Ni Y, Han M, Zhou WJ, Zhu XB, Zhang AJ. Integrin-linked kinase improves uterine receptivity formation by activating Wnt/β-catenin signaling and up-regulating MMP-3/9 expression. Am J Transl Res. 2020; 12(6): 3011.
37. Arck PC. Stress and pregnancy loss: Role of immune mediators, hormones and neurotransmitters. Am J Reprod Immunol. 2001; 46(2): 117-23.
38. Shen Z, Kuang S, Zhang Y, Yang M, Qin W, Shi X, *et al*. Chitosan hydrogel incorporated with dental pulp stem cell-derived exosomes alleviates periodontitis in mice *via* a macrophage-dependent mechanism. Bioact mater. 2020; 5(4): 1113-1126.
39. Song J, Wu Q, Jiang J, Sun D, Wang F, Xin B, *et al*. Berberine reduces inflammation of human dental pulp fibroblast *via* miR-21/KBTBD7 axis. Arch oral biol. 2020; 110: 104630.
40. Brodzikowska A, Gondek A, Rak B, Paskal W, Pełka K, Cudnoch-Jędrzejewska A, *et al*. Metalloproteinase 14 (MMP-14) and *hsa-miR-410-3p* expression in human inflamed dental pulp and odontoblasts. Histochem cell biol. 2019; 152(5): 345-353.
41. Liu L, Shu S, Cheung GS, Wei X. Effect of *miR-146a*/bFGF/PEG-PEI nanoparticles on inflammation response and tissue regeneration of human dental pulp cells. Biomed Res Int. 2016; 2016: 3892685.
42. Yuan H, Zhang H, Hong L, Zhao H, Wang J, Li H, *et al*. *MicroRNA let-7c-5p* suppressed lipopolysaccharide-induced dental pulp inflammation by inhibiting dentin matrix protein-1-mediated nuclear factor kappa b (nf-κb) pathway *in vitro* and *in vivo*. Med sci moni. 2018; 24: 6656-6665.
43. Huang X, Liu F, Hou J, Chen K. Inflammation-induced overexpression of microRNA-223-3p regulates odontoblastic differentiation of human dental pulp stem cells by targeting *SMAD3*. Int Endod J. 2019; 52(4): 491-503.
44. Zhang Y, Zhou J, Li Mq, Xu J, Zhang Jp, Jin Lp. *MicroRNA-184* promotes apoptosis of trophoblast cells *via* targeting WIG1 and induces early spontaneous abortion. Cell death dis. 2019; 10(3): 1-9.
45. Wu J, Niu P, Zhao Y, Cheng Y, Chen W, Lin L, *et al*. Impact of *miR-223-3p* and *miR-2909* on inflammatory factors IL-6, IL-1ß, and TNF-α, and the TLR4/TLR2/NF-κB/*STAT3* signaling pathway induced by lipopolysaccharide in human adipose stem cells. PLoS One. 2019; 14(2): e0212063.

46. Ramaiah MJ. mTOR inhibition and p53 activation, microRNAs: The possible therapy against pandemic COVID-19. Gene rep. 2020: 100765.
47. Hiratsuka M, Minakami H, Koshizuka S, Sato I. Administration of interferon-α during pregnancy: Effects on fetus. J Perinat Med. 2000; 28(5): 372-376.
48. Hariharan A, Hakeem AR, Radhakrishnan S, Reddy MS, Rela M. The role and therapeutic potential of NF-kappa-B pathway in severe COVID-19 patients. Inflammopharmacology. 2021; 29(1): 91-100.
49. Favilli A, Mattei Gentili M, Raspa F, Giardina I, Parazzini F, Vitagliano A, *et al*. Effectiveness and safety of available treatments for COVID-19 during pregnancy: A critical review. J Matern Fetal Neonatal Med. 2020: 1-14.
50. Bonaventura A, Vecchié A, Wang TS, Lee E, Cremer PC, Carey B, *et al*. Targeting GM-CSF in COVID-19 pneumonia: Rationale and strategies. Front immunol. 2020; 11: 1625.
51. Renu K, Subramaniam MD, Chakraborty R, Haritha M, Iyer M, Bharathi G, *et al*. The role of Interleukin-4 in COVID-19 associated male infertility-a hypothesis. J reprod immunol. 2020; 142: 103213.
52. Liu J, Wan M, Lyon CJ, Hu TY. Nanomedicine therapies modulating macrophage dysfunction: A potential strategy to attenuate cytokine storms in severe infections. Theranostics. 2020; 10(21): 9591.
53. Abers MS, Delmonte OM, Ricotta EE, Fintzi J, Fink DL, de Jesus AAA, *et al*. An immune-based biomarker signature is associated with mortality in COVID-19 patients. JCI insight. 2021; 6(1): e144455.
54. Tatum D, Taghavi S, Houghton A, Stover J, Toraih E, Duchesne J. Neutrophil-to-lymphocyte ratio and outcomes in Louisiana COVID-19 patients. Shock. 2020; 54(5): 652-658.
55. Ota H, Goto T, Yoshioka T, Ohyama N. Successful pregnancies treated with pioglitazone in infertile patients with polycystic ovary syndrome. Fertil steril. 2008; 90(3): 709-713.
56. Magro G. SARS-CoV-2 and COVID-19: Is interleukin-6 (IL-6) the 'culprit lesion'of ARDS onset? What is there besides tocilizumab? SGP130Fc. Cytokine X. 2020; 2(2): 100029.
57. Nakajima K, Watanabe O, Mochizuki M, Nakasone A, Ishizuka N, Murashima A. Pregnancy outcomes after exposure to tocilizumab: A retrospective analysis of 61 patients in Japan. Mod rheumatol. 2016; 26(5): 667-671.
58. Lusty E, Poznanski SM, Kwofie K, Mandur TS, Lee DA, Richards CD, *et al*. IL-18/IL-15/IL-12 synergy induces elevated and prolonged *IFN-γ* production by *ex vivo* expanded NK cells which is not due to enhanced *STAT4* activation. Mol immunol. 2017; 88: 138-147.
59. Kakoulidis I, Ilias I, Koukkou E. SARS-CoV-2 infection and glucose homeostasis in pregnancy. What about antenatal corticosteroids? Diabetes Metab Syndr. 2020; 14(4): 519-520.
60. Oestreich AK, Chadchan SB, Popli P, Medvedeva A, Rowen MN, Stephens CS, *et al*. The autophagy gene *Atg16L1* is necessary for endometrial decidualization. Endocrinology. 2020; 161(1): bqz039.
61. Sills ES, Wood SH. An experimental model for peri-conceptual COVID-19 pregnancy loss and proposed interventions to optimize outcomes. Int J Mol Cell Med. 2020; 9(3): 180.
62. Naidoo N, Moodley J, Naicker T. Maternal endothelial dysfunction in HIV-associated preeclampsia comorbid with COVID-19: A review. Hypertens Res. 2021; 44(4): 386-398.
63. Liang YX, Liu L, Jin ZY, Liang XH, Fu YS, Gu XW, *et al*. The high concentration of progesterone is harmful for endometrial receptivity and decidualization. Sci rep. 2018; 8(1): 1-12.
64. Murata H, Tanaka S, Tsuzuki-Nakao T, Kido T, Kakita-Kobayashi M, Kida N, *et al*. The transcription factor *HAND2* up-regulates transcription of the *IL15* gene in human endometrial stromal cells. J Biol Chem. 2020; 295(28): 9596-9605.
65. Cui L, Xu F, Wang S, Li X, Lin H, Ding Y, *et al*. Pharmacological activation of rev-erb α suppresses LPS-induced macrophage M1 polarization and prevents pregnancy loss. BMC immunol. 2021; 22(1): 1-11.
66. Albini A, di Guardo G, Noonan DM, Lombardo M. The SARS-CoV-2 receptor, ACE-2, is expressed on many different cell types: Implications for ACE-inhibitor-and angiotensin II receptor blocker-based cardiovascular therapies. Intern Emerg Med. 2020; 15: 759-766.
67. Wang N, Qin L, Ma L, Yan H. Effect of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) on reproductive system. Stem Cell Res. 2021:102189.
68. Buzzio OL, Lu Z, Miller CD, Unterman TG, Kim JJ. *FOXO1*A differentially regulates genes of decidualization. Endocrinology. 2006; 147(8): 3870-3876.
69. Eyal O, Jomain JB, Kessler C, Goffin V, Handwerger S. Autocrine prolactin inhibits human uterine decidualization: A novel role for prolactin. Biol Reprod. 2007; 76(5): 777-783.
70. Zhang Y, Zhu H, Chang H-M, Leung PC. ALK3-SMAD1/5 signaling mediates the *BMP2*-induced decrease in PGE2 production in human endometrial stromal cells and decidual stromal cells. Front Cell Dev Biol. 2020; 8.
71. Arikawa T, Omura K, Morita I. Regulation of bone morphogenetic protein-2 expression by endogenous prostaglandin E_2 in human mesenchymal stem cells. J Cell Physiol. 2004; 200(3): 400-406.
72. Li J, Qi J, Yao G, Zhu Q, Li X, Xu R, *et al*. Deficiency of sirtuin 1 impedes endometrial decidualization in recurrent implantation failure patients. Front Cell Dev Biol. 2021; 9: 98.
73. Madkour MI, El-Serafi AT, Jahrami HA, Sherif NM, Hassan RE, Awadallah S. Ramadan diurnal intermittent fasting modulates *SOD2, TFAM*, Nrf2, and sirtuins (*SIRT1*, *SIRT3*) gene expressions in subjects with overweight and obesity. Diabetes Res Clin Pract. 2019; 155: 107801.
74. Yang Y, Li W, Liu Y, Sun Y, Li Y, Yao Q, *et al*. Alpha-lipoic acid improves high-fat diet-induced hepatic steatosis by modulating the transcription factors SREBP-1, *FoxO1* and Nrf2 *via* the *SIRT1*/LKB1/AMPK pathway. The J Nutr Biochem. 2014; 25(11): 1207-1217.
75. Yoshino O, Osuga Y, Hirota Y, Koga K, Yano T, Tsutsumi O, *et al*. Akt as a possible intracellular mediator for decidualization in human endometrial stromal cells. Mol Hum Reprod. 2003; 9(5): 265-269.
76. Wang J, Tang Y, Wang S, Cui L, Li D, Du M. Norepinephrine exposure restrains endometrial decidualization during early pregnancy. J Endocrinol. 2021; 248(3): 277-288.