IJMCM
Summer 2020, Vol 9, No 3
DOI: 10.22088/IJMCM.BUMS.9.3.180

Mini review

An Experimental Model for Peri-conceptual COVID-19 Pregnancy Loss and Proposed Interventions to Optimize Outcomes

Eric Scott Sills[1, 2*], Samuel Horace Wood[2, 3]

1. Reproductive Research Section, FertiGen/CAG, San Clemente, California, USA.
2. Department of Obstetrics and Gynecology, Palomar Medical Center; Escondido, California, USA.
3. Gen 5 Fertility Center, San Diego, California, USA.

Submmited 28 July 2020; Accepted 11 September 2020; Published 10 November 2020

Reports appear to give reassurance that vertical transmission near term is unlikely, but risks of incidental SARS-CoV-2 infection during fertility treatments, at embryo implantation, or in the first trimester remain unknown. If early pregnancy sequela in the current COVID-19 pandemic are modeled from the 2004 Coronavirus outbreak data, then SARS-CoV-2 infection proximate to blastocyst nidation is likely to cause implantation failure or spontaneous abortion. Our model explains why this outcome is less attributable to virus-associated maternal pulmonary distress and instead derives from systemic inflammation and interference with trophectoderm-endometrium molecular signaling required for implantation. COVID-19 is often accompanied by high levels of IL-6, IL-8, TNF-alpha and other cytokines, a process implicated in pulmonary collapse and systemic organ failure. Yet when regarded in an early reproductive context, this "cytokine storm" of COVID-19 triggers a pro-coagulative state hostile to normal *in utero* blastocyst/fetal development. Evidence from obstetrics is accumulating to show that mothers with SARS-CoV-2 deliver placentas with abnormal interstitial villi fibrin deposits, diffuse infarcts, and hemangiomatous changes. This model classifies such lesions as permissive at term but catastrophic near embryo implantation or early first trimester pregnancy. Clinical experience with recurrent pregnancy loss offers workable interventions to address this challenge, but success will depend on prompt and accurate SARS-CoV-2 diagnosis. Although no professional guidelines currently exist for SARS-CoV-2 in early pregnancy, this model would warrant a high-risk designation for such cases; these patients should receive priority access to screening and treatment resources.

Key words: SARS-CoV-2, hypercoagulation, inflammation, implantation

All coronaviruses are single-stranded, positive sense RNA viruses (Baltimore Class IV) within the family *Coronaviridae*, a viral type first described in the 1960's. Their ~30 Kb genome places coronaviruses among the largest known RNA viruses. The initial population of

*Corresponding author: Reproductive Research Section, FertiGen/ CAG, San Clemente, California, USA Email: drsills@CAGivf.com

[DOI: 10.22088/IJMCM.BUMS.9.3.180]

asymptomatic SARS-CoV-2 carriers permitted explosive contagion, leading to a crushing challenge for clinics and hospitals. While public awareness programs emphasizing hand hygiene, reduced travel, and social distancing can diminish the rate of viral spread, testing and treatment demands continue to outpace provision of basic services in many jurisdictions.

To develop a phylogenetic tree from multiple reference sources, Benvenuto et al. (1) used an unconstrained Bayesian analysis to find both nucleocapsid and S-protein under positive pressure, with 2019-nCoV having substantial homology with a previously isolated (2015) bat SARS-like coronavirus sequence. These data suggest the "novel Coronavirus" is distinct from the virus responsible for SARS and was likely acquired from bats after a mutation which conferred the ability to infect humans (1). Li et al. (2) identified three phylogenetic and transmission clusters via network analysis, with only one cluster identified from banked genome sequences of 2019-nCoV strains. An estimated mean evolutionary rate for 2019-nCoV has been calculated, providing useful information for disease mapping and public health guidance (2, 3). SARS-CoV-2 as a human pathogen is thought to be an independent emergence, distinct from the SARS-CoV infections of 2002-2003. Although SARS-CoV-2 is not a descendent of SARS-CoV, the two viruses are sufficiently similar that their shared evolutionary histories are informative (4). As efforts to develop an effective vaccine, treatment, and rapid screening for COVID-19 are redoubled, increased appreciation of underlying cytokine processes is gaining momentum. Perhaps the most intriguing of these involves a type of virus-induced hypofibrinolysis which brings the most relevance to the early reproductive sequence.

Early reproduction and coronavirus

While most COVID-19 research has focused on pulmonary angiotensin-converting enzyme 2 (ACE-2) receptor, *ACE-2* mRNA from endometrium localizes this receptor to the uterine interior as well (5). Our hypothesis incorporates data from endometrial samples assessed by immunoassay throughout the menstrual cycle (Figure 1). Such cells obtained by transcervical

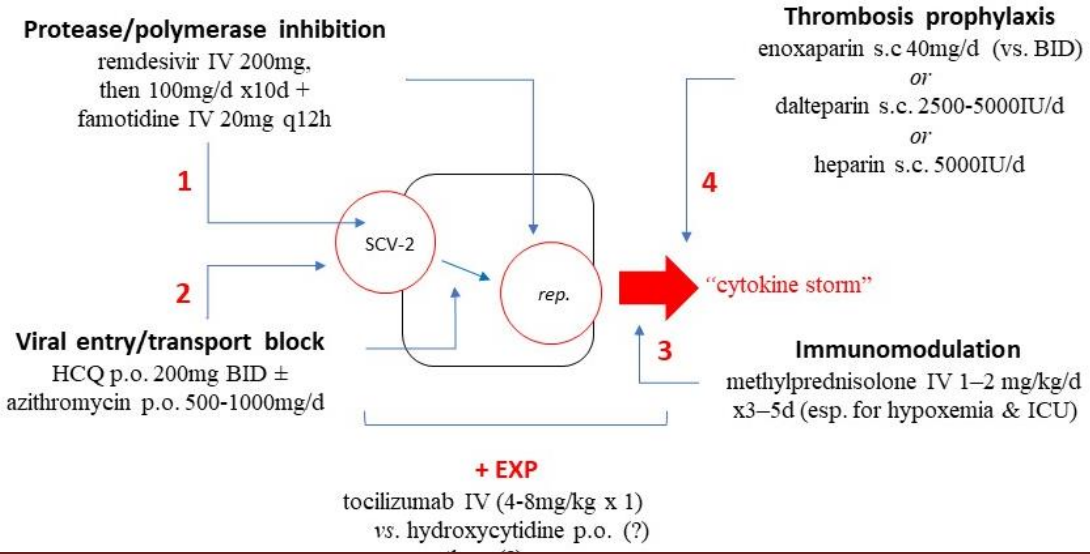

Fig. 1. Representative multifactorial treatment model for synergistic SARS CoV 2 (SCV 2) infection including antiviral, immunosuppressive, anticoagulant, and experimental (EXP) components. Some agents intervene by multiple mechanisms; exact dosing awaits multicenter clinical trial data. HCQ: hydroxychloroquine; rep: replication.

[DOI: 10.22088/IJMCM.BUMS.9.3.180]

lavage identified the angiotensin receptor (Ang1-7) in glandular epithelium of mid- to late secretory phase (5). This finding is relevant to the current SARS-CoV-2 pandemic because the virus uses the ACE-2 receptor for cell access, setting the stage for immune hyperactivity. Crucially, where embryo implantation defects occur in experimental knock-out animal models, pro-inflammatory Th-1 cytokines are usually involved (6). Threshold levels exist for IF- γ, IL-2, and TNF-α, designated as "abortifacients" by prior research (7, 8).

The effects of anti-inflammatory mediators such as IL-4, IL-10, IL-13, and TGF-β are physiologically offset by proinflammatory cytokines like IFN-γ, IL-1α, IL-1β, IL-6, and IL-12. This balance is recalibrated in pregnancy as a putative fetal protective effect, although such permissive immunotolerance comes with increased maternal susceptibility to pathogens (9-11). COVID-19 is characterized by augmented IFN-γ and IL-1β, IL-4, IL-10 and IFN-γ production (12). Preferential stimulation of Th1 immunity is often triggered in COVID-19 disease, yielding a sharp cytokine uptick for at least two weeks after initial infection (13). Interestingly, elevated IL-6 levels have been linked to increased mortality in COVID-19 (9, 14).

Xiong et al. (15) were among the first to report on a convalescing pregnant woman with COVID-19 in the third trimester. The uncomplicated vaginal delivery of a baby without SARS-CoV-2 infection suggested that intrauterine transmission of coronavirus is unlikely in late pregnancy. Italian experts (16) reviewed 13 studies, and found that vaginal delivery was performed in six cases and cesarean delivery (performed for worsening maternal status) in 31 cases (48.4%). Two newborns in this group were SARS-CoV-2 positive by RT-PCR assay, while IgG and IgM levels for SARS-CoV-2 were elevated with negative RT-PCR antigen for three offspring (16). Another summary identified 55 pregnant women infected with COVID-19 and their 46 neonates showing no vertical transmission (9). A more recent analysis of COVID-19 in pregnancy (17) found a vertical transmission rate of 11%. While limited, the available evidence tends to show that maternal-fetal SARS-CoV-2 passage is low (16-18). Placental histology reviewed at delivery from new mothers with confirmed SARS-CoV-2 infection found fibrin deposition near and within villi, with occasional increased local syncytial nodularity. Notably, chorionic hemangioma and massive placental infarction were also identified (19).

Available research on COVID-19 (published after peer-review or posted on early preprint sharing platforms) provides almost no data on SARS-CoV-2 infection impact on early pregnancy. One large meta-analysis of 266 COVID-19 pregnancies illustrates the problem (20) as time of when SARS-CoV-2 infection initially occurred in pregnancy is not reported. Curiously, two studies did include early pregnancy data (21, 22) and these were the only samples where any miscarriage or abortion occurred. These events align with our "catastrophic error" model of COVID-19, such that infection with this virus soon after implantation or in early gestation is antagonistic with pregnancy viability.

Since embryo orientation, apposition, docking and invasion are all under cytokine control, any excess of pro- or anti-inflammatory signaling is detrimental to pregnancy outcome (23). Moreover, the local chemokine milieu prevailing at implantation and shortly thereafter determines the maternal macrophage phenotype (24). Normally, first trimester decidual cells rely on IL-1β and TNF-α signaling via the NF-κB pathway for macrophage colony-stimulating factor-1 production. This results in increasing maternal macrophage CD163 expression and reducing signal-regulatory protein-α expression (24). While numerous other pathways must also remain operational to facilitate

[DOI: 10.22088/IJMCM.BUMS.9.3.180]

implantation, interference with any of these by cytokine excess can be sufficient to disrupt decidual receptivity or endometrial function.

The cytokine suite driving decidualization and implantation is impaired if supraphysiologic signaling arrives secondary to SARS-CoV-2 infection early in pregnancy. Measured trophoblast invasion into the decidua is contingent on pro- and anti-inflammatory inputs until the placenta is fully established (25), although this process is probably disrupted by the cytokine excess of COVID-19.

Risk reduction

Management of COVID-19 in early pregnancy must entail a multifront strategy including antiviral therapy, countering inflammation secondary to cytokine perturbations, and early antithrombosis prophylaxis (Figure 2). Term placentas are usually sufficiently developed to sustain local perfusion impairments without meaningful consequences, but the current model forecasts the hyperinflammatory state of COVID-19 as producing a toxic endometrial microenvironment counter to blastocyst implantation. Hypoperfusion secondary to microthrombus formation represents another major challenge to the first trimester pregnancy.

Antithrombin levels are greatly lowered among SARS-CoV-2 patients compared to healthy controls (26). Furthermore, those with the most severe infections had higher D-dimer and fibrinogen degradation products than patients with milder COVID-19 disease, suggesting that overall clinical severity of SARS-CoV-2 infection parallels the extent of coagulopathy (26). A recent multi-hospital study of Dutch ICU patients with COVID-19 pneumonia (n = 184) likewise reported thrombotic complications in > 30% of admissions. All these patients received thromboprophylaxis, yet 139 (76%) remained in intensive care by early April, 23 had died (13%), while 22 were discharged home (12%). Remarkably, venous or arterial thromboembolism was identified in 27% and 3.7% of cases, respectively, supporting aggressive thrombosis prophylaxis (27).

A separate study of 183 patients with confirmed SARS-CoV-2 infection found 71.4% of non-survivors (and only 0.6% of survivors) meeting criteria for disseminated intravascular coagulation (28). This underscores the priority of addressing the prothrombotic tendencies in SARS-CoV-2 infection. Experimental use of aerosolized plasminogen inhalation has shown promise in resolving lung lesions and improving oxygen saturation, suggesting a role for fibrinolytic strategies to treat pulmonary damage and associated hypoxemia in COVID-19 disease (29, 30).

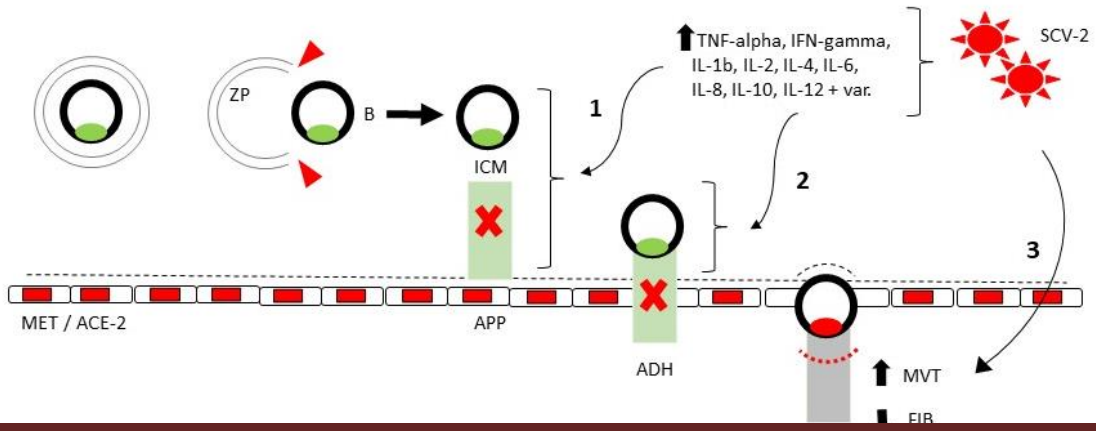

Fig. 2. Event outline for blastocyst (B) arrival and hatching (red arrows) from zona pellucida (ZP), near maternal endometrium (MET). SARS CoV 2 (SCV 2) gains cell access via ACE 2 receptor, present in MET. Next, cytokine excess interferes with (1) embryo apposition (APP) and (2) adhesion (ADH), while a COVID 19 associated procoagulant state (3) increases microvascular thrombus (MVT) formation and reduces fibrinolysis (FIB).

[DOI: 10.22088/IJMCM.BUMS.9.3.180]

Newer candidates? Mechanism and rationale

***Hydroxychloroquine (HCQ)* - FDA pregnancy category C**

Pathogen attachment to exposed respiratory cells requires action by a spike (S) protein, and marks the first step in viral replication. The viral S-protein facilitates entry through ACE-2 receptors as well as by exploiting specific ganglioside markers present on cell surfaces. It appears that HCQ metabolites attach avidly to a highly conserved viral ganglioside binding domain, causing a mismatch between sialic acids and/or viral S-protein and host/target cell (31, 32). HCQ also serves as a weak base to raise interior pH of normally acidic lysosomes and endosomes, structures needed by SARS-CoV-2 for membrane fusion and cellular access (33). Because low pH is crucial for proper endosomal action, it has been surmised (34) that endosome maturation might be blocked after endocytosis, resulting in failure of further transport of virions for subsequent exteriorization. Researchers recently confirmed that HCQ effectively blocks both the initial insertion step and post-entry stages of SARS-CoV-2 across certain organelles, thus severely limiting viral transport and release functions (35-37). HCQ may also restore defective trophoblast function impaired secondary to antiphospholipid antibodies, but clinical data remains inconclusive and awaits appraisal through clinical trials (38, 39). HCQ has been shown not to adversely affect cell turnover, nutrient transport, cytokine release or overall placental explant function *in vitro*, and thus HCQ may have a protective anti-inflammatory effect (40).

***Remdesivir* - FDA pregnancy category: not assigned**

An adenosine analog, remdesivir, causes early viral RNA truncation by interfering both with viral RNA polymerase and proofreading, resulting in dampened viral RNA production. Experience with remdesivir accumulated from its prior use in treating Ebola and Marburg virus infections, but it is also effective against other single stranded RNA viruses including Lassa fever virus, Hendra virus, and several coronaviruses. Almost no reproductive system safety data exist on remdesivir, although its use in a neonate who contracted Ebola virus by vertical transmission was successful (41).

***Tocilizumab* - FDA pregnancy category: not assigned**

Perhaps the most structurally complex of candidate drugs currently under study against COVID-19, tocilizumab is a humanized (genetically engineered) monoclonal antibody which targets the IL-6 receptor. IL-6 modulates immune response and is implicated in the pathogenesis of many diseases. Tocilizumab binds IL-6 receptors, blocking the pro-inflammatory action to attenuate the “cytokine storm” and associated pulmonary injury. Although reproductive health data are scant, preliminary reports agree on the safety of tocilizumab during pregnancy, and probably lactation. This medication likely has no negative effects on the embryo or fetus (42).

***Hydroxycytidine* - FDA pregnancy category: not assigned**

Sheahan et al. (43) recently reported on ribonucleoside analog β-D-N4-hydroxycytidine (NHC, EIDD-1931), having potent antiviral activity in a murine model against SARS-CoV-2, MERS-CoV, SARS-CoV, and related zoonotic group 2b or 2c Bat-CoVs. Because this investigational agent retained antiviral effectiveness across a wide range of therapeutic targets, and particularly in the setting of remdesivir resistance (43), hydroxycytidine could be useful in refractory or atypical COVID-19 cases.

Conclusion

Why have so few successful outcomes been reported when COVID-19 occurs early in pregnancy? The “cytokine storm” of COVID-19 complicates early blastocyst- endometrial signaling

[DOI: 10.22088/IJMCM.BUMS.9.3.180]

required for embryo docking and amplifies microvascular thrombus formation at the trophectoderm-endometrial interface, as discussed here. While both pathways are familiar in clinical reproductive medicine settings, there is no best standard to manage the problem. Our approach incorporates steroid immunomodulation and anticoagulation together with investigational antiviral therapy, and awaits findings from randomized controlled trial currently underway. The microvascular thrombosis in SARS-CoV-2 infection contrasts sharply with the consumptive coagulopathy encountered in Ebola and Lassa hemorrhagic fever (44, 45); available evidence supports our 'cytokine-mediated inflammation and disruption of maternal coagulation' model to explain the apparent adverse early reproductive outcomes with COVID-19.

Conflict of interest

The authors declare no conflict of interest.

References

1. Benvenuto D, Giovanetti M, Ciccozzi A, et al. The 2019-new coronavirus epidemic: Evidence for virus evolution. J Med Virol 2020;92:455-9.

2. Li X, Wang W, Zhao X, et al. Transmission dynamics and evolutionary history of 2019-nCoV. J Med Virol 2020;92: 501-11.

3. Giovanetti M, Benvenuto D, Angeletti S, et al. The first two cases of 2019-nCoV in Italy: Where they come from? J Med Virol 2020;92:518-21.

4. Coronaviridae Study Group of the International Committee on Taxonomy of V. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 2020;5:536-44.

5. Vaz-Silva J, Carneiro MM, Ferreira MC, et al. The vasoactive peptide angiotensin-(1-7), its receptor Mas and the angiotensin-converting enzyme type 2 are expressed in the human endometrium. Reprod Sci 2009;16:247-56.

6. Chaouat G, Dubanchet S, Ledee N. Cytokines: Important for implantation? J Assist Reprod Genet 2007;24:491-505.

7. Parant M, Chedid L. Protective Effect of Chlorpromazine against Endotoxin-Induced Abortion. Proc Soc Exp Biol Med 1964;116:906-9.

8. Tezabwala BU, Johnson PM, Rees RC. Inhibition of pregnancy viability in mice following IL-2 administration. Immunology 1989;67:115-9.

9. Dashraath P, Wong JLJ, Lim MXK, et al. Coronavirus disease 2019 (COVID-19) pandemic and pregnancy. Am J Obstet Gynecol 2020;222:521-31.

10. Kraus TA, Sperling RS, Engel SM, et al. Peripheral blood cytokine profiling during pregnancy and post-partum periods. Am J Reprod Immunol 2010;64:411-26.

11. Takeda S, Hisano M, Komano J, et al. Influenza vaccination during pregnancy and its usefulness to mothers and their young infants. J Infect Chemother 2015;21:238-46.

12. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506.

13. Wong SF, Chow KM, Leung TN, et al. Pregnancy and perinatal outcomes of women with severe acute respiratory syndrome. Am J Obstet Gynecol 2004;191:292-7.

14. Ruan Q, Yang K, Wang W, et al. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med 2020;46:846-8.

15. Xiong X, Wei H, Zhang Z, et al. Vaginal delivery report of a healthy neonate born to a convalescent mother with COVID--19. J Med Virol 2020.

16. Parazzini F, Bortolus R, Mauri PA, et al. Delivery in pregnant women infected with SARS-CoV-2: A fast review. Int J Gynaecol Obstet 2020;150:41-6.

17. Gajbhiye R, Modi D, Mahale S. Pregnancy outcomes, Newborn complications and Maternal-Fetal Transmission of SARS-CoV-2 in women with COVID-19: A systematic review of 441 cases (preprint). medRxiv 2020041120062356.

18. Li Y, Zhao R, Zheng S, et al. Lack of Vertical Transmission of Severe Acute Respiratory Syndrome Coronavirus 2, China. Emerg Infect Dis 2020;26:1335-6.

19. Chen S, Huang B, Luo DJ, et al. [Pregnancy with new coronavirus infection: clinical characteristics and placental pathological analysis of three cases]. Zhonghua Bing Li Xue Za Zhi 2020;49:418-23.

[DOI: 10.22088/IJMCM.BUMS.9.3.180]

20. Juan J, Gil MM, Rong Z, et al. Effects of Coronavirus Disease 2019 (COVID-19) on Maternal, Perinatal and Neonatal Outcomes: A Systematic Review of 266 Pregnancies. MedRxiv (preprint) May 6, 2020 doi: https://doiorg/ 101101/ 20200502-20088484.

21. Wu X, Sun R, Chen J, et al. Radiological findings and clinical characteristics of pregnant women with COVID-19 pneumonia. Int J Gynaecol Obstet 2020;150:58-63.

22. Yan J, Guo J, Fan C, et al. Coronavirus disease 2019 in pregnant women: a report based on 116 cases. Am J Obstet Gynecol 2020;223:111 e1- e14.

23. van Mourik MS, Macklon NS, Heijnen CJ. Embryonic implantation: cytokines, adhesion molecules, and immune cells in establishing an implantation environment. J Leukoc Biol 2009;85:4-19.

24. Li M, Piao L, Chen CP, et al. Modulation of Decidual Macrophage Polarization by Macrophage Colony-Stimulating Factor Derived from First-Trimester Decidual Cells: Implication in Preeclampsia. Am J Pathol 2016;186:1258-66.

25. Dimitriadis E, Nie G, Hannan NJ, et al. Local regulation of implantation at the human fetal-maternal interface. Int J Dev Biol 2010;54:313-22.

26. Han H, Yang L, Liu R, et al. Prominent changes in blood coagulation of patients with SARS-CoV-2 infection. Clin Chem Lab Med 2020;58:1116-20.

27. Klok FA, Kruip M, van der Meer NJM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res 2020;191:145-7.

28. Tang N, Li D, Wang X, et al. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost 2020;18: 844-7.

29. Wu Y, Wang T, Guo C, et al. Plasminogen improves lung lesions and hypoxemia in patients with COVID-19. QJM 2020;113:539-45.

30. Moore HB, Barrett CD, Moore EE, et al. Is there a role for tissue plasminogen activator as a novel treatment for refractory COVID-19 associated acute respiratory distress syndrome? J Trauma Acute Care Surg 2020;88:713-4.

31. Savarino A, Boelaert JR, Cassone A, et al. Effects of chloroquine on viral infections: an old drug against today's diseases. Lancet Infect Dis 2003;3:722-7.

32. Fantini J, Di Scala C, Chahinian H, et al. Structural and molecular modelling studies reveal a new mechanism of action of chloroquine and hydroxychloroquine against SARS-CoV-2 infection. Int J Antimicrob Agents 2020;55:105960.

33. Mauthe M, Orhon I, Rocchi C, et al. Chloroquine inhibits autophagic flux by decreasing autophagosome-lysosome fusion. Autophagy 2018;14:1435-55.

34. Ohkuma S, Poole B. Fluorescence probe measurement of the intralysosomal pH in living cells and the perturbation of pH by various agents. Proc Natl Acad Sci U S A 1978;75:3327-31.

35. Zheng N, Zhang X, Rosania GR. Effect of phospholipidosis on the cellular pharmacokinetics of chloroquine. J Pharmacol Exp Ther 2011;336:661-71.

36. Mingo RM, Simmons JA, Shoemaker CJ, et al. Ebola virus and severe acute respiratory syndrome coronavirus display late cell entry kinetics: evidence that transport to NPC1+ endolysosomes is a rate-defining step. J Virol 2015;89:2931-43.

37. Liu J, Cao R, Xu M, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discov 2020;6:16.

38. Marchetti T, Ruffatti A, Wuillemin C, et al. Hydroxychloroquine restores trophoblast fusion affected by antiphospholipid antibodies. J Thromb Haemost 2014;12:910-20.

39. Meroni PL. Prevention & treatment of obstetrical complications in APS: Is hydroxychloroquine the Holy Grail we are looking for? J Autoimmun 2016;75:1-5.

40. Scott RE, Greenwood SL, Hayes DJL, et al. Effects of hydroxychloroquine on the human placenta-Findings from in vitro experimental data and a systematic review. Reprod Toxicol 2019;87:50-9.

41. Dornemann J, Burzio C, Ronsse A, et al. First Newborn Baby to Receive Experimental Therapies Survives Ebola Virus Disease. J Infect Dis 2017;215:171-4.

42. Alijotas-Reig J, Esteve-Valverde E, Ferrer-Oliveras R. [Treatment with immunosuppressive and biologic drugs of pregnant women with systemic rheumatic or autoimmune disease]. Med Clin (Barc) 2016;147:352-60.

43. Sheahan TP, Sims AC, Zhou S, et al. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. Sci Transl Med 2020;12.

[DOI: 10.22088/IJMCM.BUMS.9.3.180]

44. Cha AE. A mysterious blood-clotting complication is killing coronavirus patients. Washington Post (newspaper)2020; Available from: https://www.msn.com/en-us/health/health-news/a-mysterious-blood-clotting-complication-is-killing-coronavirus-patients/ar-BB1336g0.

45. Spiezia L, Boscolo A, Poletto F, et al. COVID-19-Related Severe Hypercoagulability in Patients Admitted to Intensive Care Unit for Acute Respiratory Failure. Thromb Haemost 2020;120:998-1000.

[DOI: 10.22088/IJMCM.BUMS.9.3.180]