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An update on the place of ART with SARS CoV2-around and influence on Both Reproduction, transmission possibilities to, Progeny, Partners and Staff - What is the Answer

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Abstract

Since the initiation of COVID 19 infection in Wuhan China, in November 2019 the whole world has been battling with the COVID 19 Disease. We have earlier reviewed COVID 19 with respect to viral structure similarities with other Corona viruses, main clinical features and kept on updating management as and when some insight is gained. We briefly covered the aspect of influence on pregnancy, vertical transmission, lactation safety, along with presence of controversial findings regarding presence of the virus in semen or absence and safety of intercourse with regards to transmission. It had been observed males around 50+ are more affected by the disease with mortality greater in them both in Chinese studies along with New York statistics probably due to lack of exposure of male antigens getting recognized as self by the immune system. With the spread of COVID 19 to European Countries with so many ICU admissions they faced shortage of OT's, ICU'S and hence all routine procedures were banned in Italy, Spain and then whole world European Society of Human Reproduction and Embryology (ESHRE), American Society of Reproductive Medicine (ASRM) recommendations banned ART worldwide but gradually reintroduction is being considered. Hence it became vital to get an insight into how the virus affects both the male and female reproduction, probability of involving the next generation through embryos and transmission to staff with pandemic not subsiding. Here we tried to update the present day knowledge on the same utilizing a PubMed search engine with the MeSH terminology; SARS-CoV2; male reproduction; female reproduction; gametes; embryos; transmission through placenta till date along with utilizing the WHO worldometer site to update the current disease status in world and the current recommendations for prospective ART introduction back gradually and how to ensure safety of patients, health care personnel dealing with secretions like semen follicular fluid along with safety of embryos and precautions needed in embryology laboratory to be back on the right path as earlier be it with regards to vitrification, medication cycles along with IVF/ICSI Procedures.

Keywords: COVID-19; Male gametes; Female gametes; Endometrium; Vitrification; ART

Introduction

Earlier we have reviewed the pathophysiology of COVID-19 along with possible therapies as and when we are getting updated on this pandemic and have covered step by step presentation, prophylaxis as well as treatment [1-4]. As of 21tst June the global incidence was 8,992,0723 cases with United States of America (USA) accounting for 2.33 million, and then with 1,070,139 cases in Brazil to be followed by Russia with 5,76,952 cases and India was 4th with 411,773 cases (Table 1). However Today on 7-7-2020 in USA it has escalated to 3,041,950, as well as 1,628,283 in Brazil. To start with India started with social distancing and had slow rise but after the lockdown over rate has been escalating in India as well reaching from 3.44 lakhs on 21st June to 7.23 lakhs on 7-7-2020, 694,230 in Russia where US population is 331,034037 and Indian is 1380196,747, although worldwide escalation keeps continuing with this virus defying all norms, be it temperature, in coolest to hottest countries displaying temperatures of up to 50 degree Celsius in India to cooler USA. On 4th July there was a 50,000 increase in USA in 24 hrs alone. Although there was massive rise in some European countries, initially with lot of mortality like Italy, Spain and UK the process seems to have halted there now with better awareness. Percentage deaths continue to be low in India as compared to America and European countries. Earlier we have tried to update with the controversial reports on male with some studies showing the virus in sperms others contradicting it here we further update on latest work done with reproduction not being given a priority in view of ICU settings of the severe cases and daily new kinds of presentation being unfolded. On 8/4-USA-418,803 whereas India had 5745 cases [see Table 1 published in references [1].

Table 1: Worldwide Epidemiology as on 21/6/2020 at 11.30 pm.

Country	Total Cases	New Cases	New Deaths	Total Recovered	Active Cases
World					
USA	89220723	13,518	5258	47,43,658	37,11,544
	2330578		1373	9,72,941	12,35,657
Brazil	1070139		621	5,43,186	4,76,895
Russia	5,76,952		542	3,34,592	2,34,358
India	4,11,773	46	4	2,28,307	2,34,358
UK	3,03,110			N/A	N/A
Spain	2,93,018			N/A	N/A
Peru	2,51,338			7,861	1,38,763
Italy	2,38,275			1,82,453	21,212
Chile	2,36,748			1,96,609	35,844
Iran	2,02,584		9507	1,61,384	31,693
Germany	1,91,216		147	1,74,700	7555
Turkey	1,86,493			1,58,828	22,738
Pakistan	1,76,617	4951	119	67,892	1,05,224
Mexico	1,75,202	20,781	387	1,31,686	22,735
Russia	8672	1175	5	580	8029
Sweden	8419	726	96	205	7527
Norway	6086		12	32	5953
India	5745	298	18	506	5065

Structure of SARS-CoV along with SARS-CoV-2

Corona viruses represent a family of large enveloped, positive stranded RNA viruses which cause upper respiratory, Gastrointestinal Tract (GIT), as well as Central Nervous System (CNS) diseases in humans as well as other animals [2-5]. Human Corona viruses H CoV-OC43, H CoV-229E, H CoV-NL63 as well as H CoV-HKU1 circulate in humans and cause mild respiratory diseases [6]. But the

outbreak of SARS-CoV2 in 2002 as well as MERS-CoV in 2012 demonstrated that Corona viruses can cross the species barrier and come out as highly pathogenic viruses [7]. The high mortality rate along with wide spread nature of these new emerging Corona viruses point that they are a marked threat to global health.

The Spike (S) glyco protein of the Corona viruses is a class I viral fusion protein present on the outer envelope of the virion having a key role in viral infection by recognizing host cell receptors and modulating fusion of the viral as well as cellular membranes [8]. The Coronavirus S glyco protein gets synthesized as a precursor protein made up of ~1300 amino acids which then get cleaved into an amino (N) terminal S1 subunit (~700 amino acids) as well as carboxy (C) terminal S2 subunit (600 amino acids). 3 S1/S2 heterodimers assemble to develop a trimer spike that protrudes *via* the envelope. The S1 subunit contains a Receptor Binding Domain (RBD), while the S2 sub-unit possesses a hydrophobic fusion peptide as well as 2 heptad repeat regions. Triggered by receptor binding, proteolytic processing as well as acidic p^H in the cellular compartments, the class I viral fusion proteins undergo a transition through a metastable prefusion state to a stable prefusion state at the time of infection. Receptor binding subunit gets cleaved as well as the fusion subunits undergoes large conformational rearrangements for exposure of the hydrophobic fusion peptide, stimulating the development of a 6-helix bundle, and bring the viral along with cellular membranes nearer for fusion [9]. Song et al. [10] reported the structure of the SARS-CoV S glyco protein in complex with the host cell receptor ACE2 as shown by cryo-EM [10]. The complicated structure revealed that only one receptor binding domain of the trimeric S glyco protein binds CE2 and takes a protruding up'' conformation. Additionally, they evaluated the structure of the SARS-CoV S glyco protein and its complexes with ACE2 in separate *in vitro* conditions, that might simulate various conformational states of the S glyco protein at the time of viral entry. Dissociation of the S1-ACE2 complex from certain prefusion spikes was seen and characterized. Further, they characterized the rosette-like structures of the clustered SARS-CoV S2 trimers in the post fusion state seen on electron micrographs. Structural comparisons pointed that the SARS-CoV S glyco protein retains a prefusion architecture following trypsin cleavage into the S1 and S2 subunits along with acidic p^H treatment. But binding to the receptor opens up the receptor binding domain of S1, that might aid in the liberation of the S1-ACE2 complex as well as S1 monomers from the prefusion spike and trigger the pre to post fusion conformation transition [10] (Figure1). The Cryo-EM structure of the 2019-nCoV spike in the pre-fusion conformation is demonstrated in Figure 2 [11].

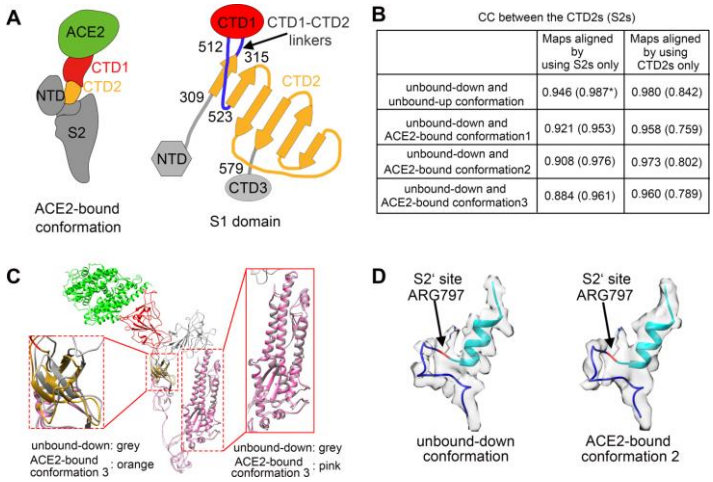


Figure 1: Courtesy ref no: 10- Structural comparisons of the ACE2-bound and ACE2-free SARS-CoV spikes.

(A) Schematic and topology diagrams showing the domain organization. NTD, CTD3 and S2 are colored grey, CTD1 is colored red, CTD2 is colored orange, and CTD1-CTD2 linkers are colored blue.

(B) (B) Cross-Correlation coefficients (CCs) between the CTD2s or the S2s of different conformations. *Values in the parentheses are the CCs between the S2s of different conformations. Density maps were low-pass filtered to 5.5 Å and was compared at a contouring level of 4.0 σ.

(C) (C) Ribbon-diagram structural comparisons of the ACE2-bound conformation 3 and the unbound-down conformation. ACE2, CTD1, CTD2, CTD3 and S2 of the ACE2-bound conformation 3 are colored green, red, yellow, pink and pink respectively. The unbound-down conformation is colored grey. CTD2 and S2 domain are zoomed in to show the receptor-binding induced hinge motion of CTD2.

(D) (D) EM densities and corresponding atomic models represented in ribbon diagrams around the S2' protease cleavage site: unbound-down conformation (left) and ACE2-bound conformation (right). The S2' site is colored red and position of the S2' site is indicated with black arrows. The fusion peptide is colored cyan. The "C" shape loop covering the S2' site is colored blue.

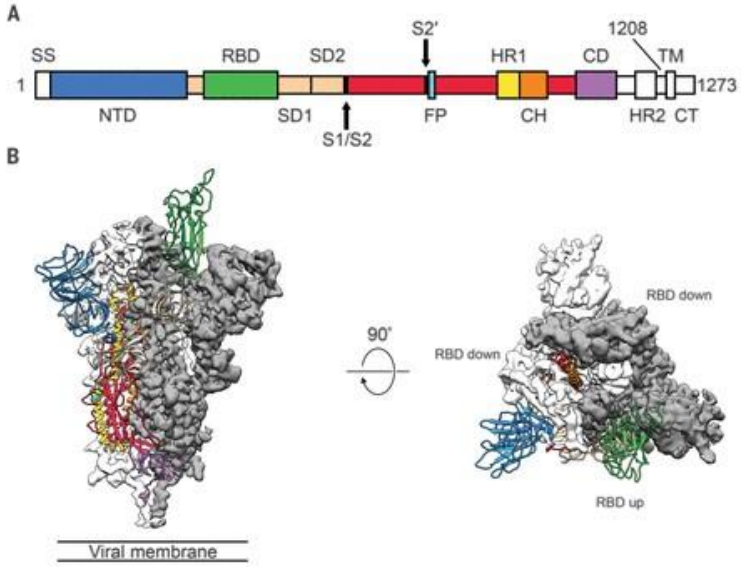


Figure 2: Courtesy ref no: 11-Structure of SARS-COV2.

Role in Human Reproduction

Further we had evaluated the contradictory findings of presence or absence of SARS-CoV2 infection in semen earlier and hence the fear of transmission of the virus by coital transmission, besides pregnancy outcomes and influence of lactation in SARS-CoV2 positive women [4].

Role in Male and Female Gametes

Evaluation into the molecular details of SARS-CoV2 infection have been started at a fast pace, with various crucial truths already found out. The ones we have already highlighted earlier, briefly the viral entry needs the binding of SARS-CoV2 (S) glycoprotein to the host receptor ACE2 [12-15]. Host proteases like Transmembrane Serine Protease2 (TMPRSS2) are then required to cleave the viral S protein for stimulating conformational alteration to S that lets the permanent fusing of the viral as well as host cell membranes [13,15]. The significance of TMPRSS2 has got corroborated as well as is clear in studies projecting that its inhibition blocks the entry of SARS-CoV2 as well as transmission in targeted lung cells [15].

TMPRSS2 gets more widely expressed within human tissue as compared to ACE2, pointing that ACE2 might be one of the major determinants, regarding a particular cell type can get infected *via* the virus [5]. Single-cell RNA sequencing (scRNAseq) in both human as well as nonhuman primates' respiratory tissues correlated with COVID-19. This co-expression has further been demonstrated in

different tissue kinds like the ileum, heart as well as kidney for which there are proven COVID-19 symptoms [16,17-22]. Co-expression of ACE2 as well as TMPRSS2 in pneumocytes within the lungs as well as goblet liberating cells in the nose [18], pointing that these cell kinds might act as foci for infection as well as potentially reasoning out the range of respiratory symptoms correlated with COVID-19 symptoms [16,18-22]. Finding the virus in blood, faeces as well as probably urine points that the influence of SARS-CoV2 on cardiac, enteric as well as renal function might be secondary to the direct infection of cells in these tissues instead of being following Acute Respiratory Distress Syndrome (ARDS) [23-25].

With the knowledge that the clinical features of COVID-19 seem to be mainly influenced by the tissue that display co-expression of ACE2 as well as TMPRSS2 in the cells that make them, it can be posited that viral infection might influence the reproductive function. If cells of the male as well as/or female reproductive systems express these genes too. ACE2 as well as TMPRSS2 expression has been demonstrated in testicular, endometrial, as well as placental cells with different interpretations [18,22]. Till date the expression of SARS-CoV2 host entry protein have not been evaluated in human or nonhuman primate ovaries, particularly in the outer ovarian cortex, that are the location sites of germ cells. These reproductive indices are not classically evaluated in Intensive Care Unit (ICU) setting as well as any actions of COVID-19 on fertility might not be easily appreciated till epidemiological results are there. Hence use of publicly data easily accessible (scRNAseq data), the unpublished transcriptomic data, as well publicly available bulk RNA as well as proteomics data, for evaluation of the expression patterns of viral host entry protein in reproductive cells population can be done. Further consideration was given to the expression of the receptor basigin (BSG/CD147), with the minimal data point that it might have the ability to modulate viral host entry [26], as well as cysteine protein cathepsinL (Ca L; gene symbol, CTSL) that potentially cleaves viral S protein [13,15]. This Ca L is not necessary for viral infection, but it is probable that residual infection in cells that get therapy with camosal mesylate, a TMPRSS2 inhibitor, might show that S protein priming *via* Ca L [15,27].

With the expression patterns of ACE2 as well as TMPRSS2 in tissue examined up to date are cell type specific [16], scRNA seq evaluation that shows the co-expression of these genes within individual cells, are anticipated to be especially significant in getting insight in the etiology of the disease. Cell kinds in the reproductive system have different lifespans as well as cells which live for smaller time might not be that harmful for a person's lifetime reproductive potential on infection. Like, somatic spermatogonial cells in the testis are constantly self-renewing cells which remain throughout male reproductive life, in contrast to differentiated spermatocytes which are removed from the reproductive system in about 60 days [28]. In the ovary a cohort of approximately ~2000,000 oocytes are present at birth, a declining subset of whom will stay throughout female reproductive life that is a cellular span that is measurable in decades. No new oocytes get generated. In comparison, the cells of the deciduas (or outermost lining of the endometrium) differentiating as well as shed in successive menses (usually 21 days to 40 days in case of regular cycles). Cell-kind particular expression patterns of genes which generate viral entry host entry protein, as well as isolation of potential infection loci within the reproductive system, are hence essential to anticipate if SARS-CoV2 will have any influence over fertility. Hence studies carried out by Stanley et al. [29], might anticipate the chances of human embryos getting infected with SARS-CoV2. During the pandemic, fertility therapies in a lot of countries have been slowed down, cancelled or even banned straight. Knowing the decreased percentage of success with escalating female age, an urgent requirement is to restart these therapies as early as possible once safety is proven. Having insight if SARS-CoV2 has the ability to infect gametes as well as embryos generated is of vital significance when thinking of risks on naturally as well as Assisted Reproductive Technology (ART) during this COVID-19 pandemic.

Thus Stanley et al. [27] tried to isolate cell types in both male as well as female reproductive systems at risk for SARS-CoV2 infection in view of the expression of host genes as well as proteins utilized by the virus for cell entry. For this they utilized a descriptive evaluation

of both transcriptomic as well as proteomic data in an academic research department as well as clinical diagnostic laboratory. They utilized the isolation of cell types that co-express the crucial ACE2 as well as TMPRSS2 genes along with proteins in addition to other candidates that are probably responsible for SARS-CoV2 cell entry. Depending on the single cell RNA-sequencing data this co-expression of ACE2 as well as TMPRSS2 was not found in testicular cells that included sperms. A sub-population of oocytes in nonhuman primate ovarian tissue was detected to express ACE2 as well as TMPRSS2, but no co-expression was seen in somatic cells. RNA expression of TMPRSS2 in 18 samples of human cumulus cells was demonstrated to be low or absent. A general consensus was found among publicly present bulk RNA as well as protein datasets regarding ACE2 as well as TMPRSS2 expression patterns in testis, ovary, endometrial along with placental cells. Hence concluding that this examination points that SARS-CoV2 infection is not likely to exert longtime actions on male as well as female reproductive function. Though one can't assume that these outcomes are definitive, they indicate that certain techniques where oocytes get retrieved as well as fertilized *in vitro* are correlated with minimal risk of viral transmission from gametes to embryos and might actually have the potential of reducing the exposure of reproductive cell types that are vulnerable to infection in contrast to natural conception (Figure 3-5) [29].

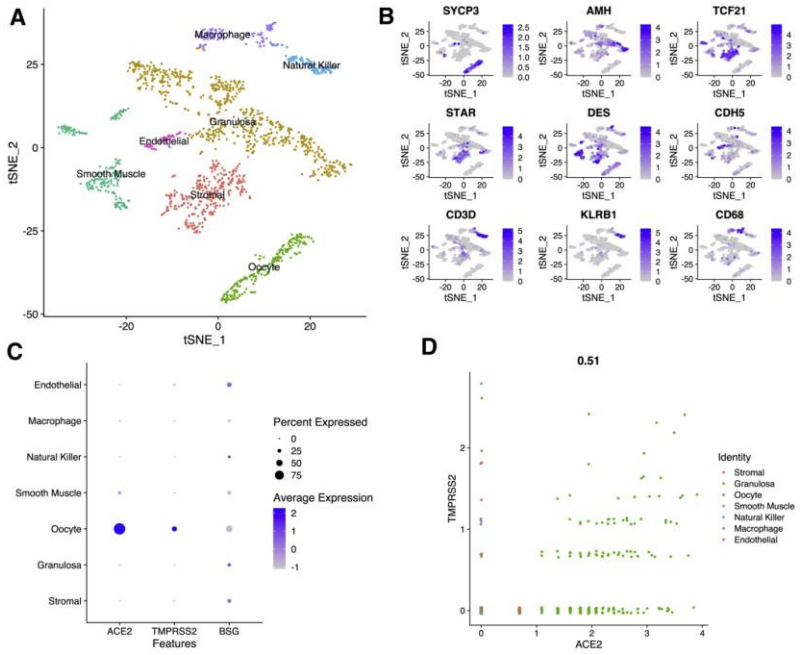


Figure 3: Courtesy ref no: 29-Clustering of distinct ovarian cell populations and transcriptional signatures. (A) Clustering was performed on the normalized and scaled data, and (B) Clusters were manually assigned cell types based on well-known markers. Legend bars reflect the normalized and log-transformed gene expression values for each cell based on the raw expression matrix (mRNA transcript counts) and therefore contain zero or positive values. (C) The dot plot depicts the scaled (Pearson residuals) and centered (mean zero) expression of an average cell in each cluster and therefore contains negative and positive values. The average expression reflects the mean expression in each cluster compared with all other cells. The size of the dot reflects the percentage of cells with mRNA transcripts detected. (D) The feature scatterplot shows the Pearson correlation value for expression of Angiotensin-Converting Enzyme 2 (ACE2) and Transmembrane Serine Protease 2 (TMPRSS2) in all ovarian cells with color-coded cell types.

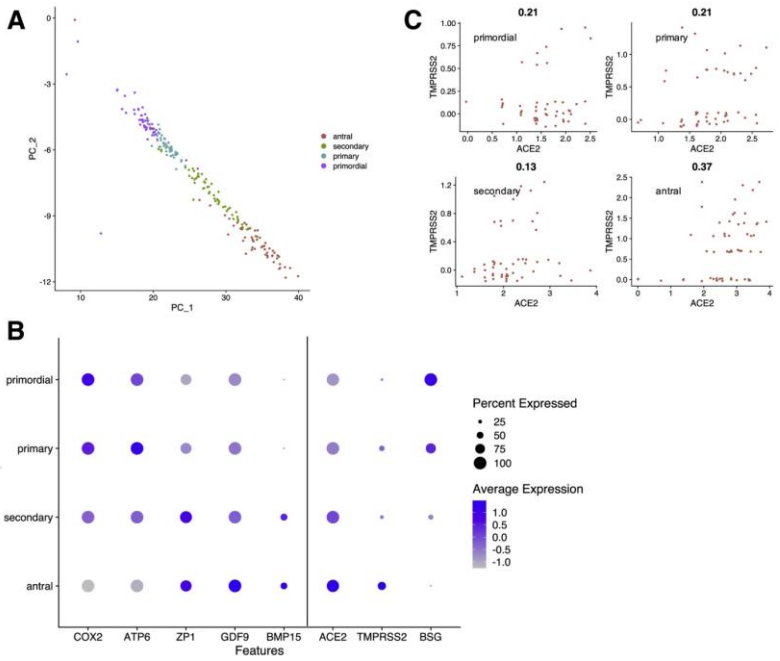


Figure 4: Courtesy ref no: 29-Clustering of distinct stages of folliculogenesis. (A) Principal Components Analysis (PCA) revealed four stages of folliculogenesis within the oocyte cluster. PC1 is plotted against PC2, and clusters were manually assigned cell types using known markers of follicular development. (B) The dot plot depicts the expression distribution of follicular development markers and expression of Transmembrane Serine Protease 2 (TMPRSS2) and Angiotensin-Converting Enzyme 2 (ACE2) across oocyte subclusters. Mitochondrially encoded ATP synthase membrane subunit 6 (ATP6) and Cyclooxygenase 2 (COX2) are markers of primordial follicles and are progressively downregulated during folliculogenesis. Zona Pellucida glycoprotein 1 (ZP1), Growth Differentiation Factor 9 (GDF9), and Bone Morphogenetic Protein 15 (BMP15) are promoters of follicular development and are progressively upregulated during folliculogenesis. Average expression represents the scaled (Pearson residuals) and centered (mean zero) expression of an average cell in each cluster and therefore contains negative and positive values. The size of the dot reflects the percentage of cells with detected mRNA transcripts. Primordial = oocytes within primordial follicles (also known as the ovarian reserve). Primary = oocytes within primary follicles. Secondary = oocytes within secondary follicles. Antral = oocytes within antral follicles.

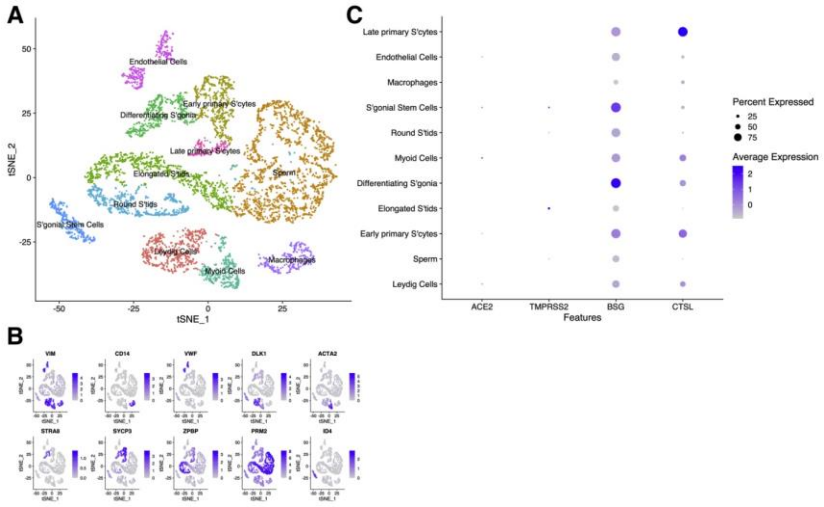


Figure 5: Courtesy ref no: 29-Clustering of distinct testicular cell populations and transcriptional signatures. (A) Clustering was performed on the normalized and scaled data, and (B) clusters were manually assigned cell types based on well-known markers. Legend bars reflect the normalized and log-transformed gene expression values for each cell based on the raw expression matrix (mRNA transcript counts) and therefore contain zero or positive values. (C) The dot plot depicts the scaled (Pearson residuals) and centered (mean zero) expression of an average cell in each cluster and therefore contains negative and positive values. The average expression reflects the mean expression in each cluster compared with all other cells. The size of the dot reflects the percentage of cells with mRNA transcripts detected. VIM = Somatic Cell Marker, CD14 = Macrophage Marker, VWF = Endothelial Cell Marker, DLK1 = Leydig Cell Marker, ACTA2 = Myoid Cell Marker, STRA8 = Retinoic Acid target gene that marks the transition between differentiating spermatogonia and spermatocytes, SYCP3 = Meiosis Marker, ZPBP = Spermatid Structure Protein, PRM2 = Nuclear Condensation/Protamine Repackaging Factor, ID4 = Spermatogonial Stem Cell Marker, S'cytes = Spermatocytes, S'gonial = Spermatogonial, ACE2 = Angiotensin-Converting Enzyme 2, TMPRSS2 = Transmembrane Serine Protease 2, BSG = Receptor Basigin, CTSL = Cysteine Protease Cathepsin L.

Role in Human Endometrium

TMPRSS2 is essential for the virus to bind ACE2 as well as spread right through the infected host [15], *via* cleavage that is essential for the virus to bind ACE2 as well as spread [15]. Other proteases being evaluated having association with SARS-CoV-2 infectivity, in relation to the S protein getting cleaved. TMPRSS2 escalates the virus infectivity by itself, in gut epithelial cells [30], whereas cathepsin B as well as L (CTSB as well as CTSL, respectively possessed residual cleavage action of viral S protein in TMPRSS2 cells [15].

FURIN, is another protease that is anticipated to cleave S protein, that is located besides ACE2 in the epithelial layers of numerous oral mucosal tissues [31,32]. MX dynamain like-GTPase control neutrophil invading that promotes infection *via* protein S modulation through neutrophil elastase [33].

Minimal studies have concentrated on the virus's action on fertility as well as how it influences fertility along with causing injury to reproductive therapies. Leydig as well as sertoli cells [34-37], oocytes [29], as well as ovarian tissue [37], have the probability of getting damaged in view of medium-high expression of the ACE2 receptor. The endometrium is key for human reproduction as well as embryo getting implanted, although studies had not been done on SARS-CoV-2 infection on menstrual cycle propagation. Finding this has significance for evaluation of the risk of Assisted Reproductive Technology (ART), knowing that a healthy endometrium is required for embryo implantation as well as growth.

This endometrium is a complicated tissue, undergoing a cycle of cell death as well as renewal about each 28 days [38]. Various transcriptomic studies have tried to get insight in gene expression alterations right through menstrual cycle [39], with maximum data being present in public places like Gene Expression Omnibus (GEO) database [40]. As per the Human Protein Atlas [41], ACE transcript is low in endometrium as well as not located as a protein. As per the HPA expression amounts, TMPRSS4 as well as FURIN RNA expression is small as well as protein amounts are medium while CTSB, MX1, as well as BSG have medium RNA expression as well as larger amounts of protein expression [42]. Nevertheless, little knowledge regarding how the virus could influence endometrial receptivity as well as embryo implantation has been available. Thus Henarejes-Castello et al. [43], evaluated the influence of SARS-CoV-2 infection on the endometrium by measurement of endometrial ACE2, TMPRSS2, TMPRSS4, CTSB, CTSL, FURIN, MX1 as well as BSG gene expression. Gene expression data from 5 studies that included 112 women having normal endometrial pathology was utilized to characterize receptor expression throughout the menstrual cycle. Their study population comprised of 29 samples in the proliferative phase, 29 samples in early secretory phase, 43 in the medium secretory phase, and 8 in the late secretory

phase. A relative expression value of low, medium, and high expression was established. The thresholds were equivalent to 1% - 10%, 11% - 50% and 51% - 100% categories of gene expression values of the total integrated data set. Transcriptomic data sets that were present across the various phases of endometrial progression were utilized for assessing the molecularly the risk of transmission of SARS-CoV-2 at the time of this COVID pandemic. Henarejes-Castello et al. [43] aimed to find the vulnerability of the endometrium to infection by-and hence the probability of injury from-SARS-CoV-2. By trying to evaluate SARS-Cov-2 infection-related gene expression from endometrial transcriptomic data sets. In an Infertility research department that was attached with a public, they tried to evaluate Gene expression data from five studies in 112 patients with normal endometrium collected throughout the menstrual cycle. Basic idea was to find out the Gene expression and association among viral infectivity genes and age throughout the menstrual cycle. They observed that Gene expression was high for TMPRSS4, CTSL, CTSB, FURIN, MX1, and BSG; medium for TMPRSS2; and low for ACE2. ACE2, TMPRSS4, CTSB, CTSL, and MX1 expression escalated toward the window of implantation. TMPRSS4 expression was positively correlated with ACE2, CTSB, CTSL, MX1, and FURIN during several cycle phases; TMPRSS2 was not statistically significantly altered across the cycle. ACE2, TMPRSS4, CTSB, CTSL, BSG, and MX1 expression increased with age, especially in early phases of the cycle. Thus, concluding that Endometrial tissue is likely safe from SARS-CoV-2 cell entry based on ACE2 and TMPRSS2 expression, but susceptibility increases with age. Further, TMPRSS4, along with BSG-mediated viral entry into cells, could imply a susceptible environment for SARS-CoV-2 entry *via* different mechanisms. Additional studies are essential to determine the true risk of endometrial infection by SARS-CoV-2 and implications for fertility treatments (Figure 6,7) [43].

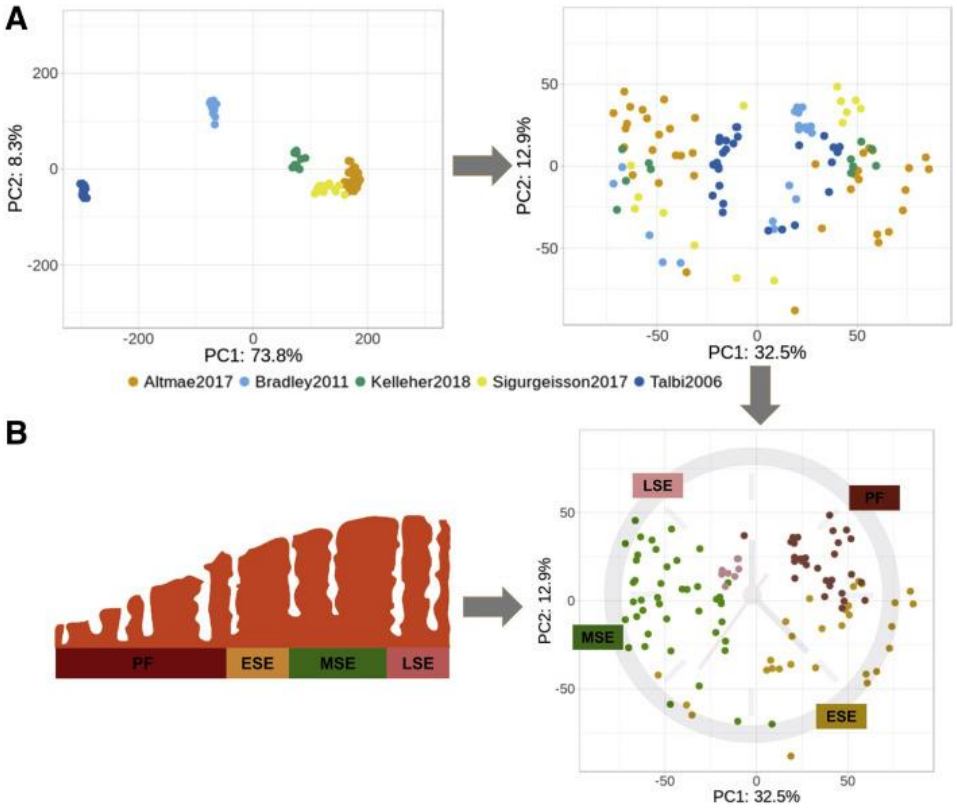
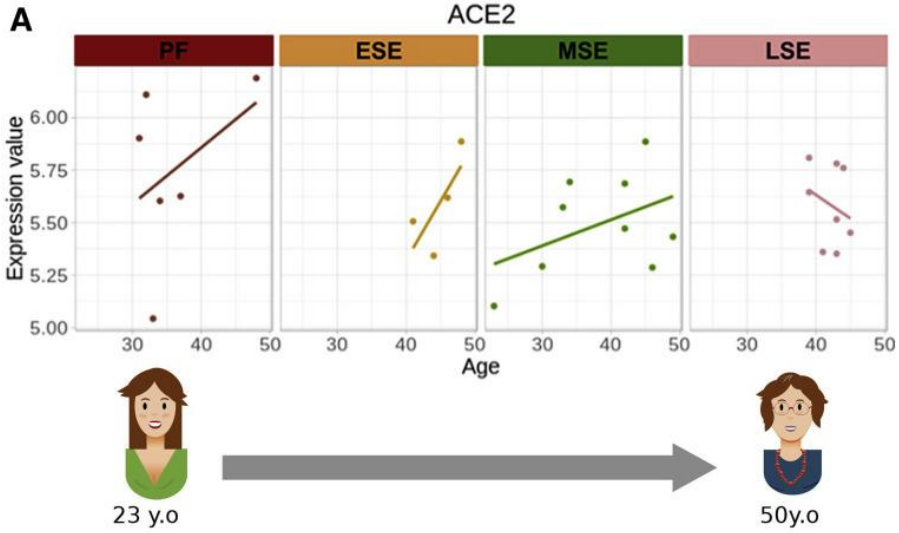


Figure 6: Courtesy ref no: 43-Gene expression of viral infection-related genes throughout the menstrual cycle. (A) Landscape of expression changes. Genes were located depending on their relative expression against the whole set. Low, medium, and high expression thresholds correspond to 1% to 10%, 11% to 50%, and 51% to 100% categories of gene expression values of the entire integrated data set, respectively. Analysis of variance results for overall change of expression during cycle are shown for each gene. *P<.05; ***P<.0001.

(B) Molecular scheme of SARS-CoV-2 endometrial infection. ACE2, TMPRSS4, FURIN, and BSG are shown in plasma membrane of an endometrial cell (lower left figure). CTSL and CTSB are represented outside the cell. MX1 is shown in cytoplasm. Expression of viral genes in comparison to whole transcriptomic set is represented as arrows next to their names: up = highly expressed; down = lowly expressed. Viral genes are positioned in their schematized cell locations as stablished by GeneCards database > Localization section (release 4.14) [56]. Only maximum confidence levels (5 and 4) for compartments-derived cell locations were used. Proteins were grouped considering the highest coactivation values between pairs of viral genes during the menstrual cycle, which are shown in the lower right table in the figure. Discontinuous arrow shows less evidence according to our results, given that only FURIN showed high activation with BSG and that further studies are needed to understand BSG-related mechanisms of SARS-CoV-2 entry. ESE = Early Secretory Endometrium; LSE = Late Secretory Endometrium; MSE = Midsecretory Endometrium; PF = Proliferative Phase.



B

	PF	ESE	MSE	LSE
ACE2	0.4	0.73	0.44	-0.26
TMPRSS2	-0.08	0.12	-0.58	-0.5
TMPRSS4	0.61	0.75	0.2	-0.38
CTSL	0.65	-0.15	-0.26	-0.01
CTSB	0.9	0.56	-0.32	-0.13
BSG	-0.29	-0.87	-0.08	0.82
FURIN	0.07	0.11	-0.77	-0.43
MX1	-0.18	0.77	-0.42	0.19

Figure 7: Courtesy ref no: 43-Impact of age on viral-related infectivity gene expression throughout the menstrual cycle. (A) Effect of age on ACE2 expression. Gene expression is represented for ACE2 in each phase of the cycle according to the age of the sample analyzed. The range of age from patients involved in this study was 23 to 50 years. (B) Effect of age on viral gene expression. Pearson correlation R2 values are shown for each gene studied through of the phases of the menstrual cycle. Gray scale represents the magnitude of the correlation of increase or decrease in expression with age. High values are colored darker, and low values are colored lighter. ESE = Early Secretory Endometrium; LSE = Late Secretory Endometrium; MSE = Midsecretory Endometrium; PF = Proliferative Phase.

General Recommendations

Besides the organizations like the Italian Society, European Society of Human Reproduction and Embryology (ESHRE) gave the

scientific recommendations, as far as Assisted Reproductive Technology (ART) was concerned. They have advised to cancel fertility therapies, other than in poor responds, who are allowed to undergo therapy, though this might put more stress for couples who have been very keen to have babies fast. Further suspension of new therapies has been recommended or utilize freeze all protocols in cases where HCG has already been initiated. In patients who need urgent fertility preservation cryopreservation of gametes is advocated [44,45], although the correlation among SARS-CoV-2 as well as pregnancy secondary to ART therapies are not certain as proven by European Society of Human Reproduction and Embryology (ESHRE) along with recommendations from American Society of Reproductive Medicine (ASRM). The influence of virus in the markedly initial stages of embryo formation meaning fertilization to blastocyst as well as from implantation till the 1st trimester is not clear. On the basis of proof as far as pregnancy results that include abortions as well as Live Birth Rates (LBR) is not clear. Knowledge regarding seropositive in women having SARS-CoV-2 would aid although might never be available for ART. Despite the stoppage of all medical techniques associated with infertility therapy did not have any proof behind, the decision has generated a worldwide challenge in health care systems as well as cancellation of all fertility therapies will besides ensuring stoppage of transmission of virus, further prevent extra stress added to all these systems. Till now the statements given by ESHRE websites announcements have been made regarding minimal probability of gametes /embryos getting contaminated *via* SARS-CoV-2, since steps involving washing, culture as well as freezing protocols seem to have decreased the probability of potentially spreading the viral load although not totally removed. With the probable lack of SARS-CoV-2 receptors on the gametes (like spermatozoa as well as oocytes) as well as embryos would corroborate this presumption robustly, whereas the existence of Zona Pellucid (ZP) in the oocytes as well as embryos till the 6th day of formation might not negate our presumption that the virus might have a negative influence of In vitro Fertilization (IVF) therapies. Usually, the health care personnel tend to promote postponement of fertility therapies, if one of the partners is exhibiting symptomatology of or had recent recovering out of any viral infection simulating a flu. The explanation is easy as any viral infection like say Zika virus might result in infertility [46]. The present world experience has exemplified how SARS-CoV-2 results in much more severe illness as compared to any other viral infection like influenza as well as exaggerated morbidity as well as mortality. Considering the cellular level common influenza viruses aid in oxidant -sensitive pathways, resulting in inactivation of the pathogenic modes which the Oxidative Stress (OS) might lead to [47,48]. Escalated Oxidative Stress (OS) has been pointed to be responsible for male fertility [49-51], *via* a decrease of propagational motility as well as concomitant reduction in motility of spermatozoa as well as enhanced DNA fragmentation [52,53]. With these results, one can presume that SARS-CoV-2 *via* getting activated by pathogenic pathways might escalate DNA fragmentation that might influence the fertilizing capacity. In the same way SARS-CoV-2 might influence the oocytes working *via* modes which enhance the OS. Actually, OS correlates with changes in DNA methylation [54], whereas in association with In Vitro Fertilization (IVF) procedure it might have a negative influence on the DNA methylation circle, with poor neonatal results [55]. Understanding that SARS-CoV-2, works *via* the ACE2 receptor [56], a probable direct action of this particular virus on follicles /oocytes as well as spermatozoa can be ruled out. However, ACE2 receptors have been demonstrated on human leydig cells [57] that points to a probable direct action of the virus on the male reproductive system. However, two reports, one by Pan et al. (Figure 8) [58,59] reviewed by us earlier in ref 4, along with Song et al. 2020], pointed that the virus was not present in the semen of the patients who had recently come out of COVID-19 infection. However, one more report came that ACE seems to be key for the initial stages of embryo formation [60] since, ACE has been observed to have an important part in sperm function [61]. Additionally, ACE has been posited to cause decreased or total absence of fertilization at the time of conventional IVF [62,63]. Regarding oocyte the part of ACE was emphasized in 2013 by Pan et al. [64] as well as that this enzyme exists in preovulatory follicles, at least in case of rats [65] Even in human ovaries ACE2 receptors were documented to exist [66], whereas angiotensin [1-7] has been

found in follicular fluid that can get measured. Thus, a probable crosstalk among the oocyte as well as somatic cells can't get excluded. As per the embryo it has been determined that human germ cells as well as early embryos express high amounts of ACE2 [67], along with recent finding of escalated ACE2 *via* SARS-CoV-2 infection [68]. In combination these observations suggest that a direct action of SARS-CoV-2 on follicles/oocytes as well as spermatozoa, however greater cellular evaluations are required for proof. Regarding probable contamination of the embryology laboratories as well as the embryology staff by SARS-CoV-2 is highly likely, with the usual precautions taken at the time of Oocyte Pick Up (OPU) or gamete/embryo manipulation would get altered during IVF therapy with a virus positive asymptomatic case. 1^{ST} A different laboratory will be required. Utilization of physical barriers like glass windows can be utilized for screening the places surrounding registration desks present within fertility clinics along with embryology laboratories, for avoiding spaces as well as staff that is getting exposure of the COVID-19 virus. Further the spaces that are already getting utilized (like oocyte recovery area, sperm collection area) needs cleaning as well as disinfected with particular products following each case. The whole staff needs to have training with regards to particular protocols to manage those infected *via* SARS-CoV-2 as well as require to wear particular filtering face masks. While physicians presence is not essential, utilization of telemedicine in case of any fertility queries, that includes embryological outcomes, will aid in reducing the transmission of the virus to the least. The routine workup uniform needs to get replaced by one similar to or identical to the uniform with which they work for SARS-CoV-2 infection, like protective cover all shoes, along with isolation clothes that is combined using a head cover. This aids in forming a physical barrier for avoidance of any probable contamination. Gloves as well as face masks need to be worn all the times on a single utilization basis (meaning besides in SARS-CoV-2 positive asymptomatic cases). This personal protective equipment, that include masks with respirators for health care personnel in fertility clinics is probably needed to be added to the everyday condition. In case of clinics having lots of stimulation cycles, it would be advisable to limit the numbers of the staff that come in close association with COVID-19 cases. Contamination of the embryology laboratories appears to be a realistic probability, in spite of negative pressure which is present in maximum laboratories. Since the virus survives for various hrs. in the air as well as temperature >220°C (the common temperature in maximum laboratories), it can readily be posited that incubators could get contaminated. Apparently, SARS-CoV-2 appears not to follow the seasonal patterns, as observed in maximum flu outbreaks, and thus the virus might still possess the capacity to contaminate at temperatures over 250°C. Hence it might be of use to reexamine the temperature of laboratories along with pressures, to ensure that the virus is not there in the air or does not get a possibility of survival. Keeping different incubators, for the infected patients with COVID-19 infection might be utilized to prevent any cross contamination potentially as SARS-CoV-2 might still contaminate plastics for about 72 hrs [69,70], implicating that non embryo toxic dishes might not be free off the virus. In the coming yrs., it might be that quality control of culture dishes with need SARS-CoV-2 free sheets to be sure that exposable dishes as well as all associated equipment are free of the virus. Although this SARS-CoV-2 virus has not been examined for its resistance to cooling rates at the time of vitrification, the resistance of rest of the viruses to cryogenic liquid nitrogen temperatures was documented 2 yrs back, as reported a couple of years ago by Merrilet et al. [71]. In view of the resemblance to other common viruses it seems it would be safer to utilize different liquid nitrogen cryostorage for SARS-CoV-2 positive women. Further another different tank for storage for COVID-19 positive sperm samples might be a good decision. In the same fashion, all donors will require to show the results of SARS-CoV-2 tests. All liquids need to be examined for the virus, as well as all suppliers need to give a statement regarding the ingredients being free of the virus. In spite of early details with lack of proper scientific proof, one can conclude that this SARS-CoV-2 explosion has presented separate challenges to the world reproductive healthcare community, with probable harmful repercussions for the couples looking for infertility therapy. In the coming

few yrs, we might witness decreased fertilization, implantation as well as LBR, whereas at the same time innovative challenges will come for embryology laboratories regarding neutralization of any viruses that are present while conducting out any delicate procedures.

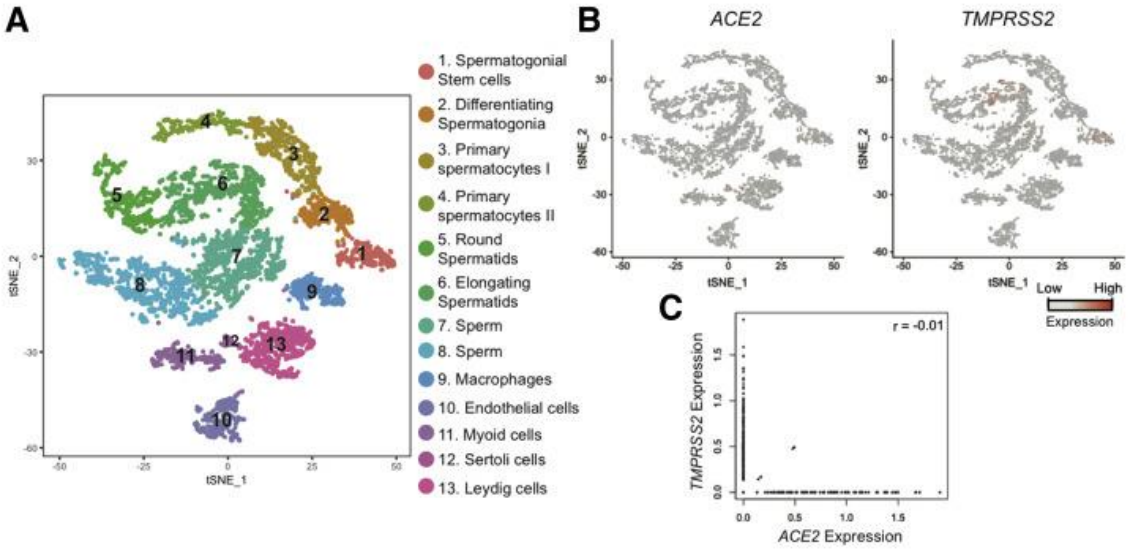


Figure 8: Male Reproduction.

Conclusion

As compared to influenza virus infectiosn, 118 pregnant women in Wuhan, China, presenting with COVID-19 infection did not show any escalated chances with regards to complications or severe disease in contrast to non-pregnant women presenting at same age as well as infection [72]. Neonatal throat swabs examination of 8 newborns for SARS-CoV2, were negative, just like milk samples obtained *via* 3 patients [72]. In view of the most centres cancelling all ART procedures that included medical therapy in view of uncertainty, considering the reducing success rates in ART/IVF in case, various countries have reintroduced, with a lot more considering starting these therapies once again, to start with in women over 39 yrs and subsequently in younger women as well. Thus, it has become essential to have insight if SARS-CoV2, might infect gametes as well as embryos, understanding the probable effects on natural conception, along with pregnancies secondary to ART/IVF outcome.

More recently in a documentation *via* Wuhan University hospital from China, neither of the throat swabs of the 6 swabs of newborns of 6 labouring women with corroborated COVID-19 showed SARS-CoV2 as per reverse -transcription Polymerase Chain Reaction (PCR) examination [73]. However, their neonatal umbilical blood actually showed virus-particular antibodies [73]. Five infants possessed enhanced IgG amounts, while two newborns displayed IgM antibodies [73]. Contrary to IgG, the greater macromolecular IgM mostly is not anticipated mostly *via* placenta from maternal compartment to fetus [73]. Another study where mothers presenting with SARS, aberrant weights as well as pathology were seen in the placentas of 2 cases infected with SARS-CoV2 in the 3rd trimester [73]. It has been posited that the IgM found in the newborns might have evolved secondary to aberrant or injured placentae or, besides the probably might have been formed by the neonates themselves, responding to transplacental viral infection [73]. In view of this Stanley et al. [29] study is markedly significant. They did not find co-expression of ACE2 as well as TMPRSS2 in the sperm or other testicular cells. Nevertheless, they observed the expression of ACE2 as well as TMPRSS2 in a sub-population of oocytes in non-human primate's varian tissue, although this co-expression was not seen in ovarian somatic cells. Further they analyzed the expression of the receptor basigin (BSG, CD147) that probably manipulates viral entry as well as the Cysteine Protein Cathepsin L (CTSL) that probably cleaves the viral

S protein. They observed that BSG had greater broad expression across testicular cell types as compared to ACE2 as well as was co-expressed in CTSL in early as well as late primary spermatocytes (78.7% as well as 90.8% of cell having mRNA transcripts, respectively). In the same line BSG as well as a CTSL transcripts were found in all of the 18 examined human cumulus cell samples. Yet, none or low expression of TMPRSS2 in the cumulus cell samples was observed.

On the basis of these outcomes, Stanley et al., concluded that SARS-CoV2 infection is not likely to exert long time influence on male as well as female reproductive function, pointing that the risks of ART/IVF has not changed with the COVID-19 pandemic. They might be correct. Still despite the reassurance we have to keep watching, alert as well as living with doubts. This is in view of SARS-CoV2 being observed in different liberations like saliva, stool, urine as well as Gastrointestinal Tract (GIT) secretions [74]. Hence the query if the virus gets transferred in semen needs to be revealed. While the blood testicular barrier is not perfect, SARS-CoV2 might infect the male reproductive tract, mainly in the existence of inflammation [74]. Till date 27viruses have been observed in semen correlated with viraemia [74]. It has been posited that the viral existence in semen might be more prevalent than we usually appreciate along with traditional Non sexually transmitted viruses might be existing in the genital liberations [74]. Actually Li et al. [74] recently isolated SARS-CoV2 in 6/38positive patients (15.8%), that included 4/15 patients (26.7%) in the acute stage of infection [4]. Moreover 2/23 recovering cases (8.7%) also tested positive for SARS-CoV2 in their semen, with no variations in days since clinically recovering, pointing that semen might be having the capacity to transmit virus, besides in the acute phase of illness even later on. As there was no variation among the positive as well as negative outcomes, it is still not clear that for how much time semen might be contagious, raises concern.

Anticipating that most patient's positive for SARS-CoV2 might refrain from coitus during the acute phase of illness, in view of weakness, erectile problems, fear of transmission of virus to their opposite number, or other reasons this might not hold true for recovering cases. Various other queries need to be immediately answered regarding the general public-

I. For how much time they need to observe abstinence.

II. Are condoms offering adequate protection?

The medical laboratory personnel in contact with infertile patients' semen, for Intra Uterine Insemination (IUI) or rest of ART/IVF, are at any risk of getting the viral infection? In case the semen might be infectious, produced embryos as well as female partners have a risk of catching SARS-CoV2? The late probable influence on the future infants? These are many queries that raise alarm with little answers. It was pointed recently that of the monoclonal antibodies that target SARS-CoV2 protein isolated from memory B cells of a person who got infected *via* SARS-CoV in 2003, had the ability to neutralize SARS-CoV2 [75]. This antibody known as S309, bound with the S RBD as well as recalled a glycan-possessing epitope without any competition with the receptor binding. Pinto et al., pointed that this antibody as well as S309 possessing antibody cocktails might be utilized either for prophylaxis in high-risk persons or as post exposure treatment to abrogate the disease severity. In the same way about a hundred potential vaccines are getting evaluated, with some of them undergoing human clinical trials for effectiveness as well as safety like cervarex in India, projected for release on august 15. More recently a pharmaceutical laboratory that was located in Massachusets has formed a corona virus's vaccine known as mRNA-1273 that has been evaluated in human volunteers and had apparent efficacy. One hopes that these initial reports that seem to give incentive get corroborated and turn out to be reliable. Hence it has been pointed that that one has to take into account immunizing infertile couples with the same vaccines prior to ART/IVF following proved effectiveness as well as safety, prior to globally immunizing public.

Further overall, although the study of Henarejos-Castello et al. [43] gives assurance, pointing to low risk of endometrial infection by SARS-CoV-2. The group used public transcriptomic datasets to evaluate the risk of endometrial SARS-CoV-2infection. Gene

expression data from 5 studies that included 112 women having normal endometrial pathology was utilized to characterize receptor expression throughout the menstrual cycle. Their study population comprised of 29 samples in the proliferative phase, 29 samples in early secretory phase, 43 in the medium secretory phase, and 8 in the late secretory phase. A relative expression value of low, medium, and high expression was established. The thresholds were equivalent to 1% - 10%, 11% - 50% and 51% - 100% categories of gene expression values of the total integrated dataset. However, low expression of ACE2, the major cell surface receptor used by SARS Cov-2 does not rule out the risk of endometrial infection since other proteases like TMPRSS4, CTSL, CTSB and FURIN which show high expression during certain periods of the menstrual cycle. However, it is important to note that none of the highly expressed proteases reported in this study are known to initiate SARS-CoV-2 infection.

The authors themselves refer to various limitations in the study. Because the data is extracted from existing datasets, the inclusion criteria for the selected studies may not represent the population contemplating conception or fertility treatment, and the dependence of the reported results is based on the quality of the original studies. In addition, the genetic profile of individuals may vary on the basis of age, ethnicity, medicines the patients have been utilizing as well as the associated and medical conditions such like endometriosis responsible for infertility. The outcomes of this study might not be generalized for all the patients who think of infertility therapy.

As pointed by Henarejos-Castello et al. [43] the endometrial genetic profile changes right through the menstrual cycle, actual risk of infection might basically be secondary to protein expression at the period of window of implantation. Furthermore, they pointed that ACE2 expression escalates with age has not been quantified clinically. Question arises how much escalation occurs? Is there any correlation of this escalation of risk clinically? Further as accepted by the authors the sample size utilized for the age-associated evaluation has to be considered [76].

All the medical specialties have got challenged, that includes our field of reproductive medicine. Since many clinical queries remain not totally answered or controversial, health care providers have to be conscious, keeping on adjusting the modalities of therapy as per the altering scenario of published experience on the way this new as well as unknown disease behaves [77].

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