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Expression of SARS-CoV2 Infectivity Machinery in the Male Reproductive Tract: Possible Outcomes on Fertility

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Abstract

Emergence of the COVID-19 pandemic continues to rage and rattles the entire world causing multifaceted hardships. Though initially thought to be a disease that primarily affects the lungs, latest evidence suggests its possible Long-term effects on multiple organ systems. SARS-Cov-2, the virus responsible for this disease infects the cells through ACE2 receptor and the serine protease TMPRSS2. In light of the fact that ACE2 expression is very high in the testis and the expression of TMPRSS2 in other reproductive organs, there has been growing interest to determine the effect of SARS-Cov-2 infection on the male reproductive system, especially on fertility. Through bioinformatics analyses, *in vitro* and cohort studies, the effects on SARS-Cov-2 infection at the molecular to physiological levels are proposed. Perturbations in hormonal levels, damage to the anatomical structure and inflammation in reproductive organs, decline in sperm count and sperm function have been reported. Thus, the significance of COVID-19 on global reproductive health has gained importance. In this article, we summarize the reported facts related to SARS-Cov-2 infectivity on male reproductive system. Such a comprehensive summation herein will help the researchers to have an up to date knowledge in this area of research and to coronavirus newer studies to address the effects of the COVID-19 pandemic on male reproduction, especially fertility.

Keywords: COVID-19, Fertility, Male Reproductive System, SARS-Cov-2

1. Introduction

The emergence of COVID-19 pandemic affected almost all the countries in a number of ways. As on 24th April, 2020, the pandemic caused 3,101,540 deaths and 146,312,705 infections worldwide to cause severe hardships in terms of health and economic crisis (https://www.worldometers.info/coronavirus/). COVID-19 is caused by the novel corona virus, SARS-Corona virus 2 (SARS-Cov-2), which primarily affects the respiratory system to cause severe lung disease[1]. The infections primarily originated from the Wuhan city of Hubei province, China and culminated to infect millions of people across the world. In the last one and half years after the first outbreak of this disease in December 2019, vast number of research studies reported the etiology and molecular mechanisms of this infection in the primarily affected organ i.e. the lung[2]. Further, the susceptibility of other organs and the risks of post infection were also reported[3,4]. Over a period of time it is emerging that this disease is not only fatal, but can cause High-risk mortality and morbidity in patients who have recovered from the infection. Considerable interest has also been generated to identify the effect of SARS-Cov-2 infections on the male reproductive tract. The gender bias for higher incidence of COVID-19 in men evinced interest to study the relation between the etiology of this disease and male reproductive physiology[5]. The purpose of this review is to consolidate the known aspects on the

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effects of COVID-19 on the male reproductive system. Facts presented in this review will help the researchers to have an up to date knowledge in this domain and to think critically to conduct further studies to decipher the relation between SARS-Cov-2 infectivity and male fertility.

2. SARS-Cov-2 Infection in Male Reproductive Tract

The primary basis for all the studies that report on the effects of SARS-Cov-2 infection on male reproductive system relies on the fact that there is an abundant expression of ACE2, the receptor for the virus, in the testes. Single-cell RNA-sequencing (scRNA-seq) data of adult human testis indicated ACE2 expression in Sertoli and germ cells, with a higher expression level in infertile men[6]. The role of ACE2 receptors to cause testicular dysfunction by promoting SARS-CoV-2-induced blood-testis/epididymal barrier infiltration is proposed[7]. On the other hand, co-expression of ACE2 and TMPRSS2 (a serine protease required for processing of viral protein) was not observed and the risk of SARS-Cov-2 infection in reproductive tract tissues was highly unlikely[8]. To understand the effects of SARS-CoV-2 on the pathology of male reproductive physiology, it is important to have an in-depth knowledge on the molecular mechanisms that govern the binding, and entry of this virus into host cells.

3. Molecular Aspects of SARS-CoV-2 Infectivity

The molecular mechanism by which SARS-Cov-2 infects the target cell was recently reported[9]. Entry and propagation are governed by binding of the viral spike protein (SARS-2-S) and ACE2; and the subsequent processing of the SARS-2-S protein by the cellular serine protease TMPRSS2. While the importance of TMPRSS2 in viral entry and propagation is highlighted for SARS-Cov-2, previous studies have demonstrated that other SARS-CoV viruses can also use Cathepsin B and L (CTLB/L) for the priming of their S protein (SARS-S) in cell lines[10] and that inhibition of both TMPRSS2 and CTLB/L is required to block viral entry[11]. Subsequent studies have shown that the activity of CALB/H is dispensable for the pathogenesis of the virus[12-15]. In a recent landmark study, Hoffman *et al.*[9] demonstrated that in TMPRSS2negative 293T cells, elevation of endosomal pH by ammonium chloride inhibited SARS-2-S and SARS-S mediated entry, suggesting a strong dependence on CATB/L[9]. In the TMPRSS2 positive CaCo cells, SARS-2-S mediated entry was partially blocked by ammonium chloride, suggesting that the entry was CATB/L dependent. Complete inhibition of SARS-2-S-mediated viral entry was achieved when TMPRSS2-positive cells were treated with camostat mesylate and E-64d, inhibitors of TMPRSS2 and CATB/L, respectively, indicating that SARS-2-S can use both TMPRSS2 and CATB/L for entry. Further, it is shown that camostat mesylate did not block SARS-2-S-mediated viral entry in TMPRSS2 negative 293T cells, suggesting that CATB/L is used in the absence of TMPRSS2. These results overall indicate that SARS-2-S-mediated SARS-Cov-2 entry is primarily dependent on TMPRSS2 for priming, whereas the same can be achieved by CATB/L when TMPRSS2 is not present[9].

4. Expression of SARS-Cov-2 Infection-Related Proteins in Male Reproductive Tract

From the studies reported recently, it is evident that ACE2, TMPRSS2 and CATB/L play a pivotal role in the entry of SARS-CoV-2 into the host cells. Analyses of the expression of these proteins in the male reproductive tract will provide ample evidence on the possibility of this organ system's vulnerability to COVID-19. Though *in silico* (bioinformatics and computational predictions) have provided information on the expression pattern of the effectors of SARS-Cov-2 entry into the host cells, it is surprising to find very little information from the *in vitro* or *in vivo* systems. The expression pattern of major players in SARS-Cov-2 entry in the male reproductive tract tissues was obtained from The Human Protein Atlas (https://www.proteinatlas.org/) and the same presented below.

4.1 ACE2

According to the Human Protein Atlas, ACE2 mRNA expression is indicated in all the male reproductive tract tissues, with the highest in the testis. The protein is abundantly expressed in the testis and to a relatively lower extent in the seminal vesicle (Figure 1). As per the Consensus dataset prediction, among the tissues that are enriched with ACE2 mRNA, testis stands at the 5th

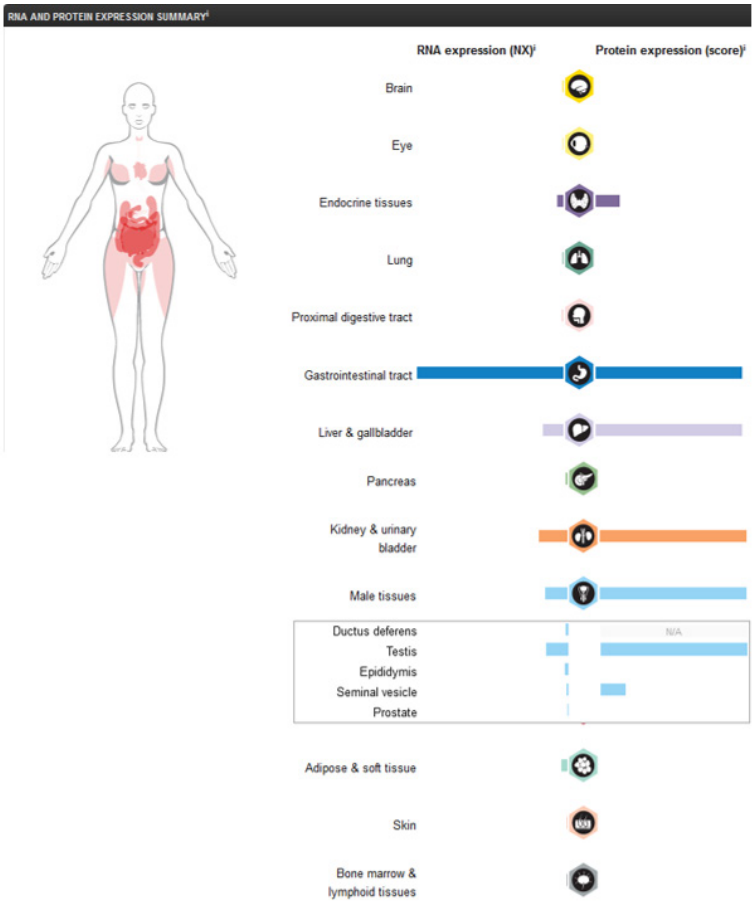

Figure 1. Representation of the RNA and protein expression profiles of ACE2 in human tissues in The Human Protein Atlas.

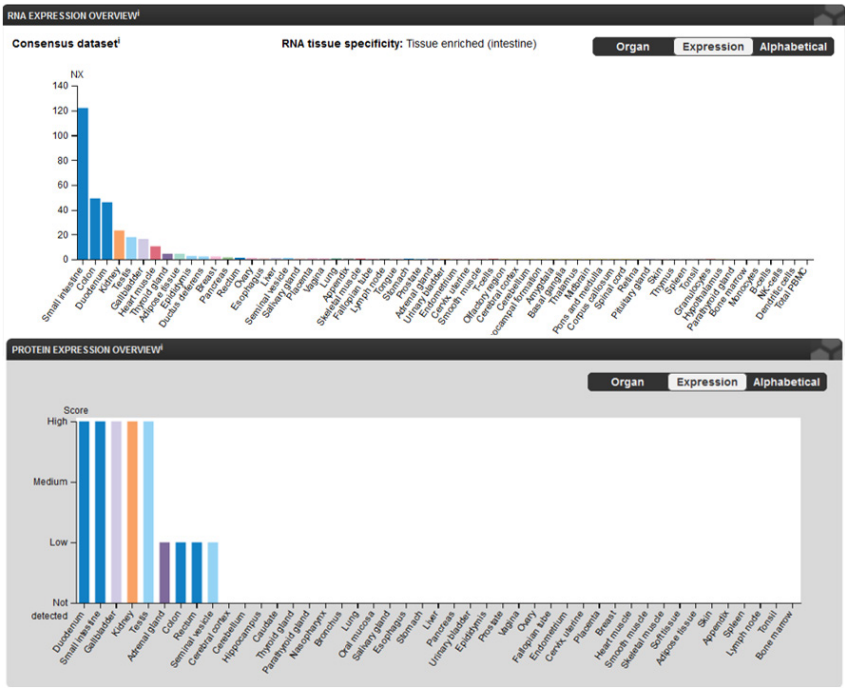

Figure 2. RNA and protein expression overview of ACE2 in various tissues as per the consensus data sets available in The Human Protein Atlas.

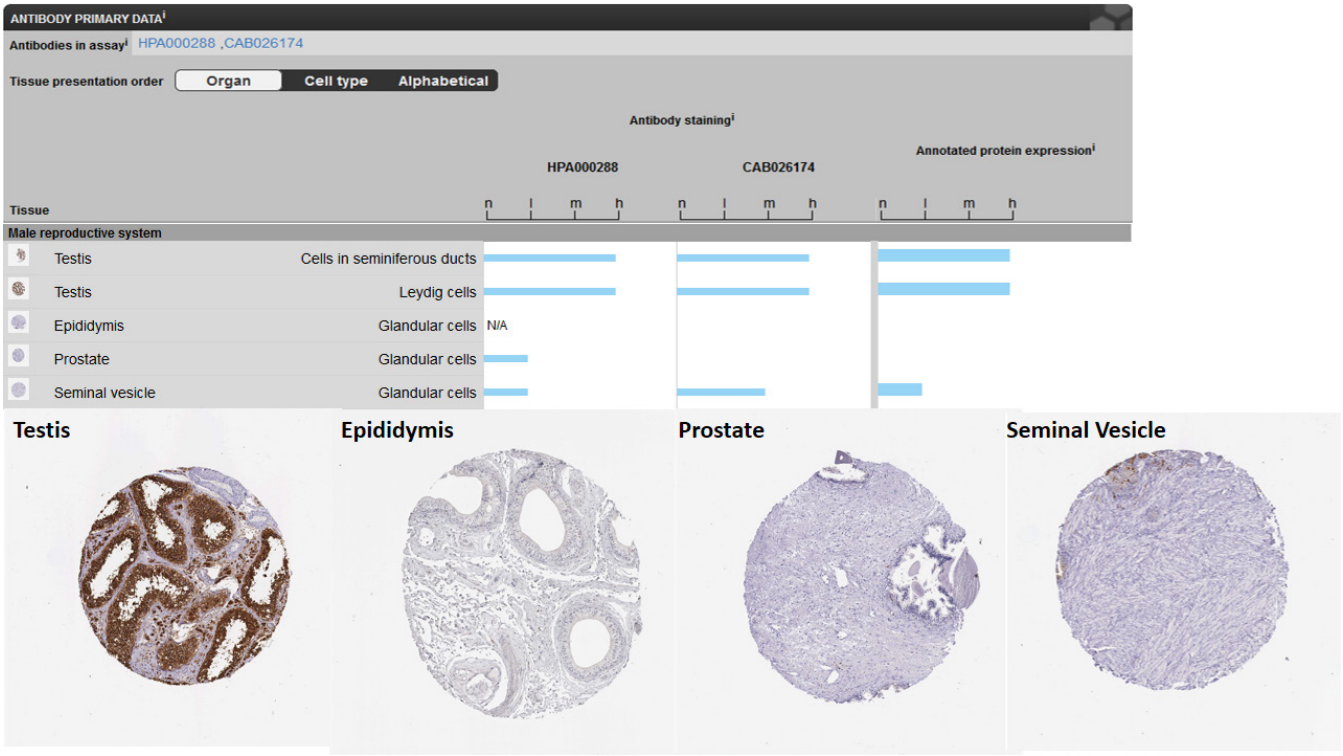

Figure 3. Immunohistochemical expression profile of ACE2 protein in the male reproductive tract tissues provided in The Human Protein Atlas.

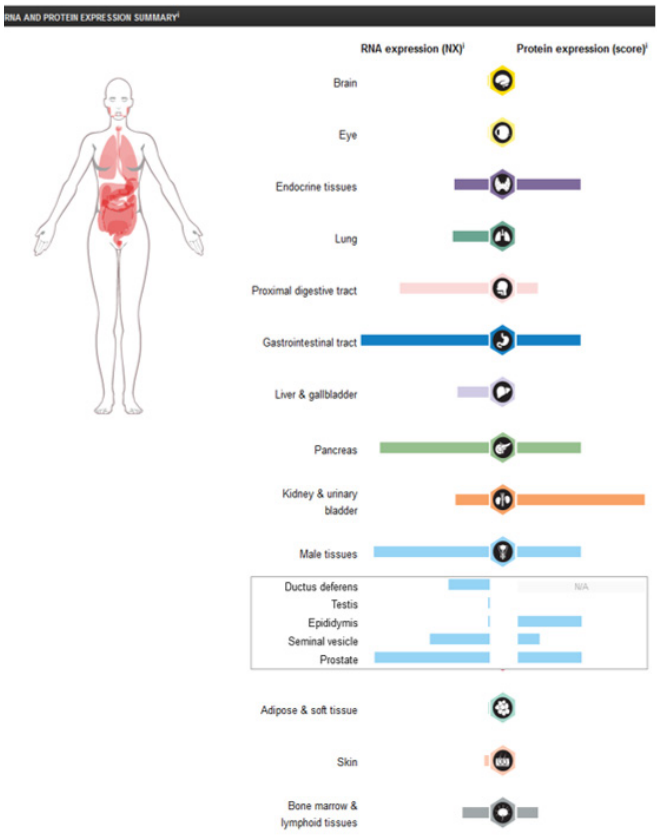

Figure 4. Representation of the RNA and protein expression profile of TMPRSS22 in human tissues in The Human Protein Atlas.

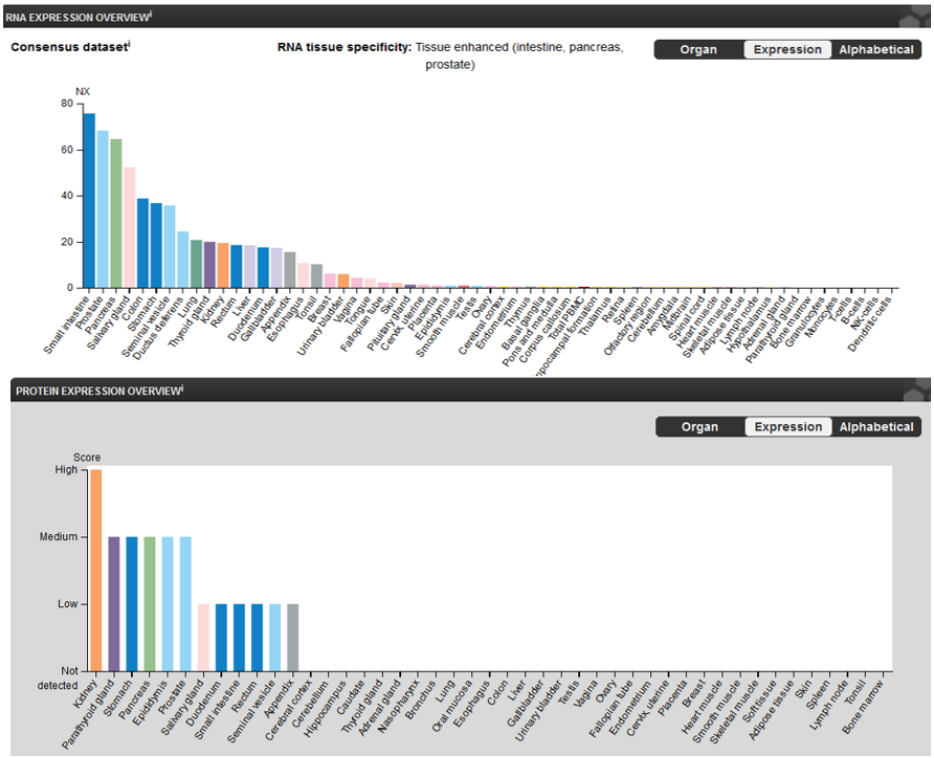

Figure 5. RNA and protein expression overview of TMPRSS2 in various tissues as per the consensus data sets available in The Human Protein Atlas.

position, while epididymis, ductus deferens, seminal vesicles and prostate are at 10th, 11th, 18th and 29th positions, respectively (Figure 2). Protein expression summary analyses project the expression of ACE2 in the testes and seminal vesicles as "high" and "low" levels, respectively (Figure 2). The intensity of expression is corroborated by the immunohistochemical staining provided at the Human Protein Atlas database (Figure 3).

4.2 TMPRSS2

Among the male reproductive tract tissues, the mRNA expression appears to be highest in the prostate, followed by seminal vesicles and ductus deferens (Figure 4). TMPRSS2 protein expression is abundant in the epididymis and prostate, with lower expression in the seminal vesicles (Figure 4). The mRNA enrichment analyses as per consensus dataset available on Human Protein Atlas indicate prostate at the 2nd position whereas seminal vesicles, ductus deferens, epididymis and testis are placed at 7th, 8th, 28th and 30th position, respectively (Figure 5). However, the protein expression overview analysis rates the expression as "medium" for epididymis and prostate and "low" in the seminal vesicles (Figure 5). Tissue staining for TMPRSS2 in human samples indicate similar expression pattern (Figure 6).

4.3 CTSL

Human Protein Atlas indicates the expression of CTSL mRNA in all the male reproductive tract tissues, while the protein is restricted to epididymis, seminal vesicles and prostate (Figure 7). In the consensus dataset for mRNA tissue specificity analyses, seminal vesicles, epididymis, prostate, testis and ductus deferens are placed at 19th, 23rd, 27th, 41st and 42nd positions, respectively (Figure 8). The expression of CTSL is indicated as "low" in the epididymis, seminal vesicles and prostate (Figure 8). Further evidence on the protein expression is provided by the immunohistochemical analyses, which is in agreement with the consensus analyses (Figure 9).

4.4 CTSB

As per the general expression summary provided in Human Protein Atlas, CTSB mRNA is indicated in all the male reproductive tract tissues, while the protein expression is restricted to testis, epididymis, seminal

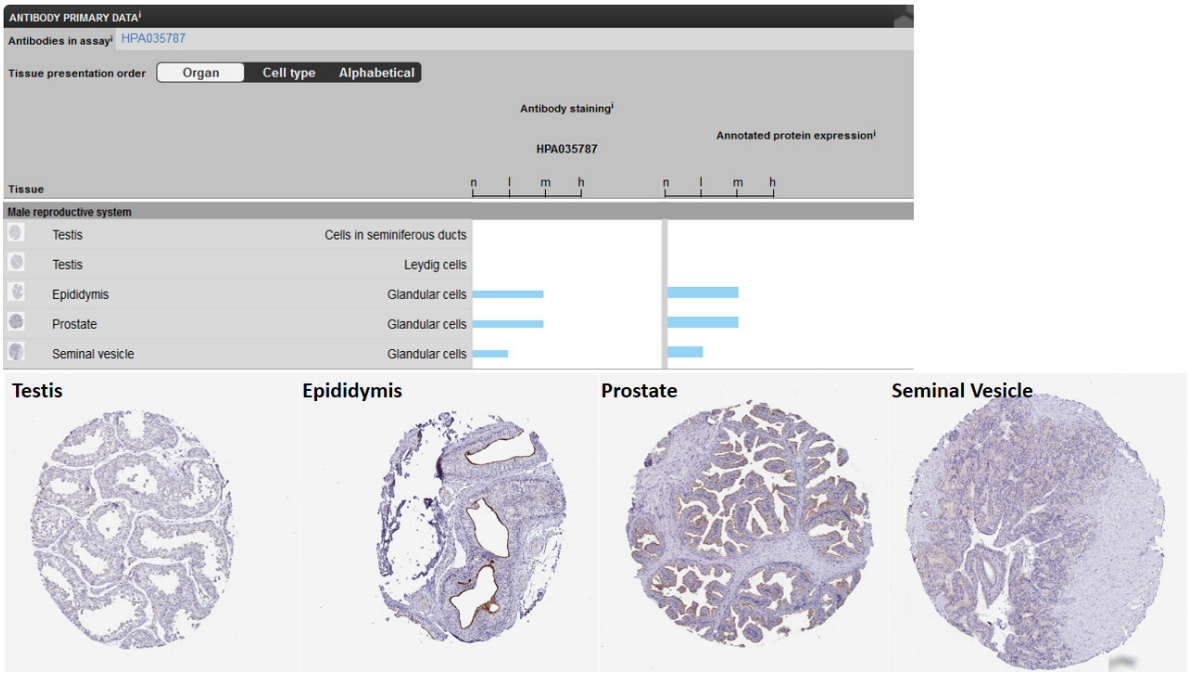

Figure 6. Immunohistochemical expression profile of TMPRSS2 protein in the male reproductive tract tissues provided in The Human Protein Atlas.

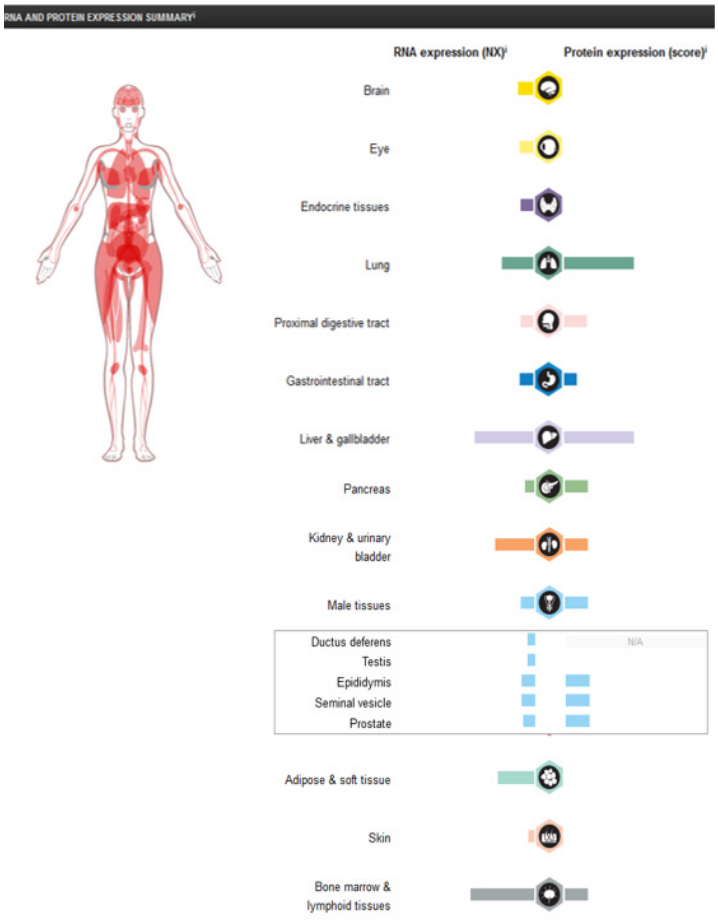

Figure 7. Representation of the RNA and protein expression profile of CTSL in human tissues in The Human Protein Atlas.

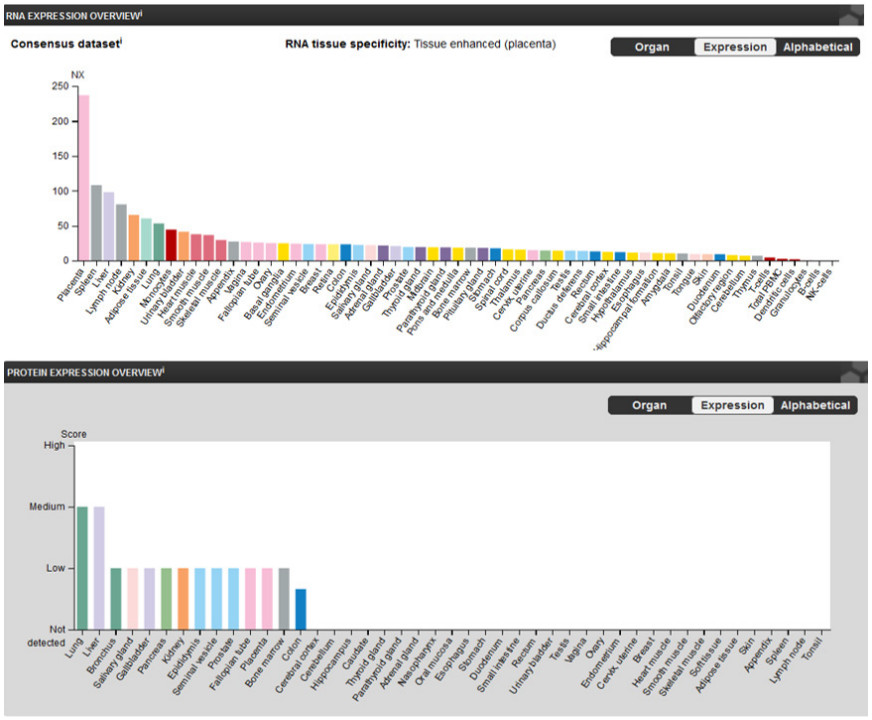

Figure 8. RNA and protein expression overview of CTSL in various tissues as per the consensus data sets available in The Human Protein Atlas.

Figure 9. Immunohistochemical expression profile of CTSL protein in the male reproductive tract tissues provided in The Human Protein Atlas.

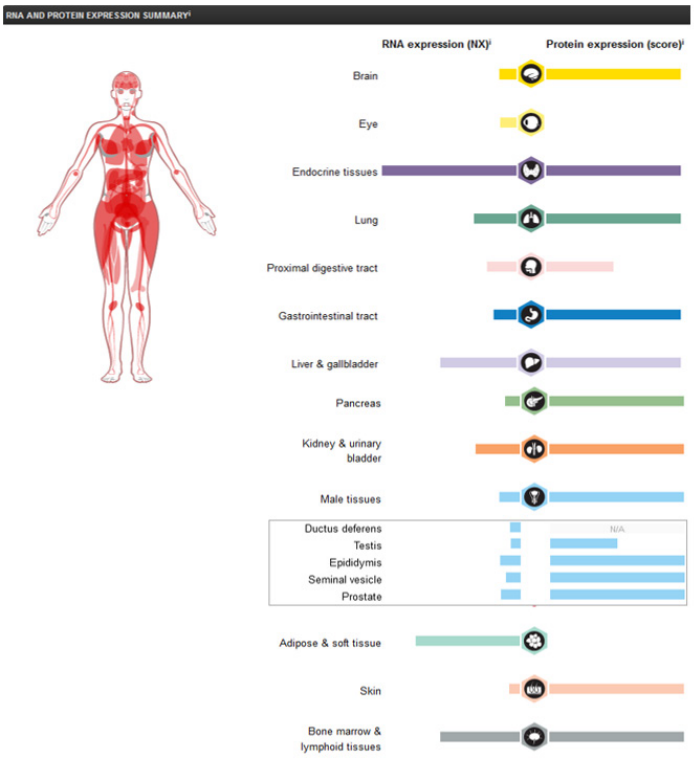

Figure 10. Representation of the RNA and protein expression profile of CTSB in human tissues in The Human Protein Atlas.

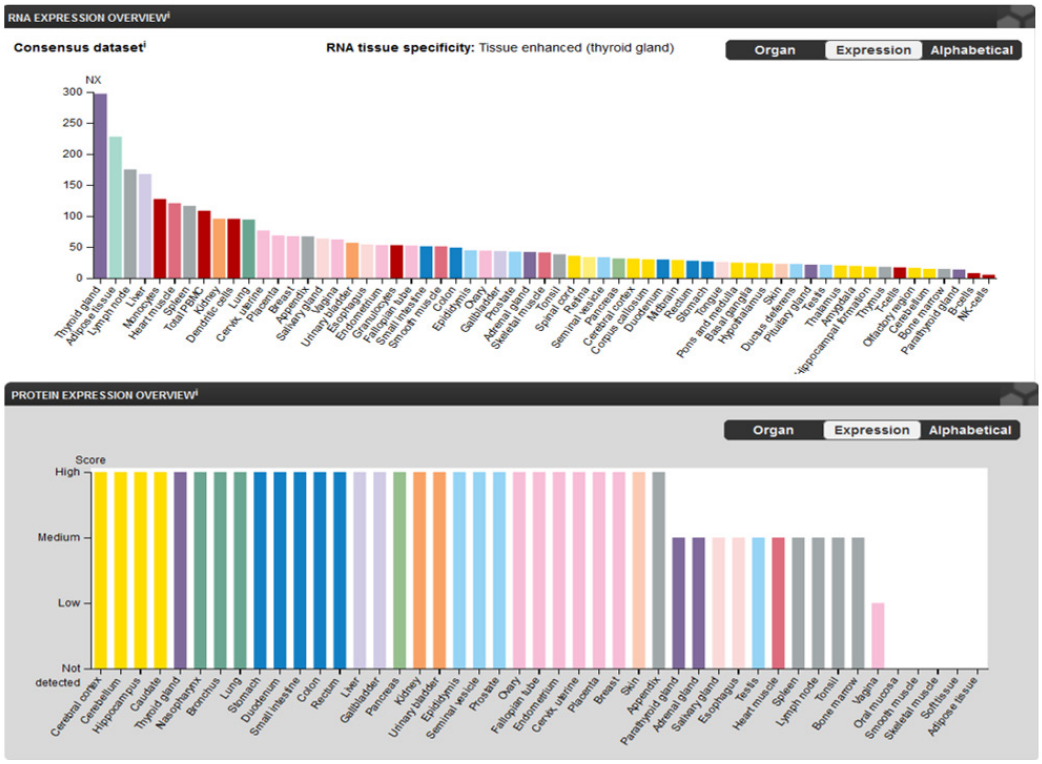

Figure 11. RNA and protein expression overview of CTSB in various tissues as per the consensus data sets available in The Human Protein Atlas.

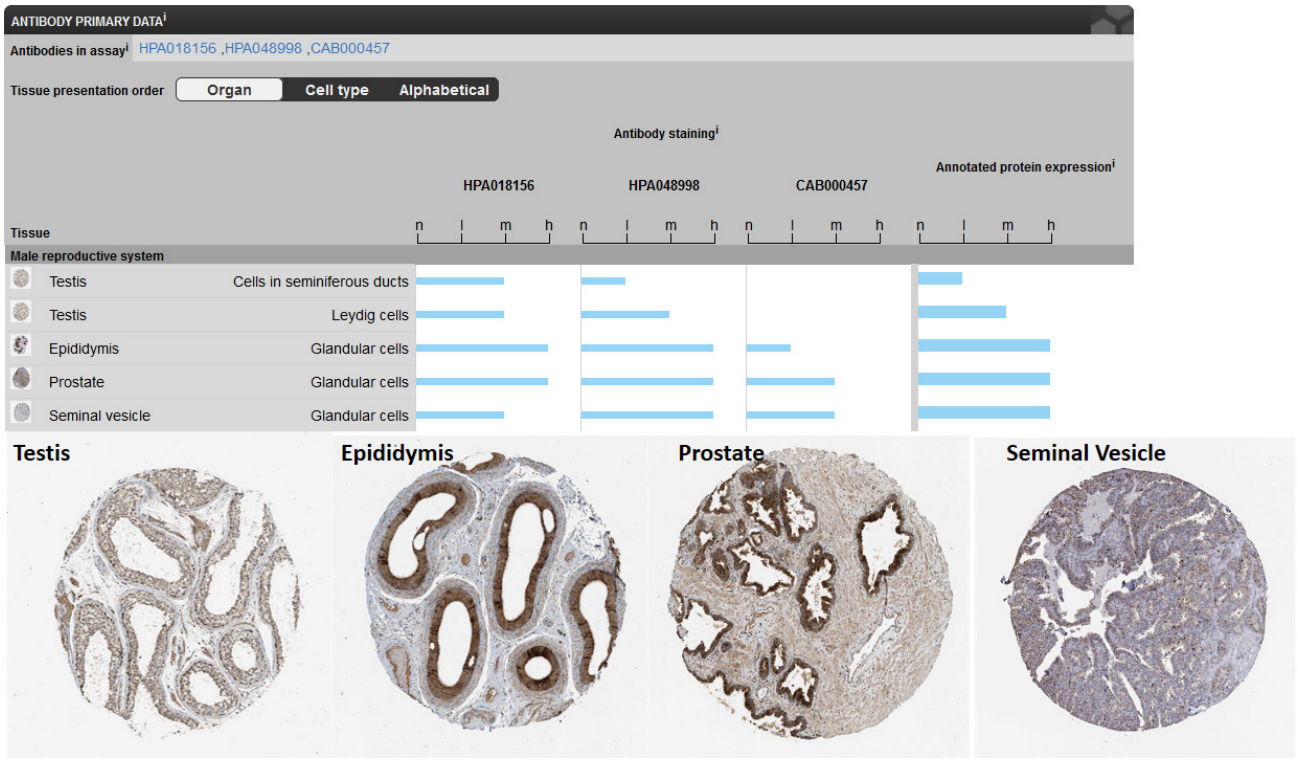

Figure 12. Immunohistochemical expression profile of CTSB protein in the male reproductive tract tissues provided in The Human Protein Atlas.

vesicles and prostate (Figure 10). Consensus dataset for mRNA specificity among tissues place epididymis, prostate, seminal vesicles, ductus deferens and testis at 26th, 29th, 35th, 48th and 50th positions, respectively (Figure 11). The expression of CTSB protein in the epididymis, seminal vesicles and prostate is rated as "high", whereas it is "medium" for testis (Figure 11). Tissue and cellular localization of CTSB in the male reproductive tract tissues is evidenced by immunohistochemical analyses (Figure 12).

5. Impacts of COVID-19 on Male Reproduction

While it is clearly evident that the molecular machinery that is required for SARS-Cov-2 infection is expressed in the male reproductive tract tissues, the extent to which the infection can affect this organ system is still speculative. A PubMed search with the keywords "SARS-Cov-2 and male reproductive" yielded471 results as on 24th April, 2021, suggesting the growing evidence on the possible effects of COVID-19 on male reproductive physiology. Interestingly, many of these published articles are reviews and perspectives basing on already published or publicly available resources. Basing on the expression pattern of the receptors important for the viral infection and by analyzing reproductive parameters in COVID-19 patients, a number of theories have been proposed that the testis is highly vulnerable and thereby male fertility[16]. Transcriptome analyses of mediators of SARS-Cov-2 infection in male embryo Primordial Germ Cells (PGCs) and normal testis cells revealed high expression of these molecules[17,18]. The effect of COVID-19 pandemic on partner relationships, reproductive health and male fertility were systematically reviewed[19-22]. It appears that SARS-Cov-2 infection can lead to testicular orchitis, perturbations in the reproductive hormone profile and gamatetogenesis[23]. The risks in conception by *in vitro* fertilization using semen samples of COVI-19 patients are highlighted[24]. The possible causes for reduction in fertility and/or infertility due to corona viral infection were reviewed[25,26]. Immunomodulation and anti-inflammatory actions of the steroids 17β-estradiol (E2) and progesterone (P4) are proposed to mitigate COVID-19 morbidity and mortality[27]. The possibility of sexual transmission of the virus because of its presence in the

semen was discussed[28]. In contrast, in a cohort of patients with an infection of COVID-19, the virus was not detected in the testes and semen indicating the unlikelihood of sexual transmission[29-31]. On a different note, the effect of COVID-19 pandemic on the possible psychological status of couples seeking assisted reproduction technologies (ART) is reported[32]. A multicenter study with 69 COVID-19 patients indicated that this disease negatively affects many semen parameters[33]. Interestingly, reports suggest that the levels of testosterone (high and low) act as a double-edged sword in the progression of this disease[34]. Histological changes in the testis akin to orchitis, possible effects on fertility despite the absence of the virus in the semen and thus no transmissibility through intercourse is reported after a systematic review of SARS-Cov-2 effect on male sex organs and the existence of a sexual transmission path[35,36]. Similarly, the virus was not detected in the prostatic and seminal secretions of COVID-19-positive men also in those who recovered from COVID-19[29,31,37]. Perturbations in the production of pituitary and testicular hormones, oxidative stress and sperm DNA fragmentation are reported to be possible causes of decline in fertility[38,39]. Epididymal orchitis, reduced sperm count and motility are evident when urinary male reproductive tract parameters were systematically reviewed in 479 COVID-19 patients[40]. Interstitial edema, presence of RBC, $CD3^+$ and $CD68^+$ cells and apoptotic cells in testis and epididymides, reduced sperm concentration was observed in a single center study that involved 23 COVID-19 patients[41]. Higher levels of ACE2 activity, pro-inflammatory and oxidative stress molecules in seminal plasma, while testosterone deficiency and elevated LH levels were evident in COVID-19 patients studied under a prospective and longitudinal cohort studies[42-44]. On the other hand, lower levels of serum LH, FSH and testosterone in a cross-sectional pilot study of COVID-19 men[45]. Differences in monocyte counts and $CD4^+$ T cell and $CD8^+$ T cell proportions were evident among the male and female COVID-19 patients; and the significance was more in males[46]. Postmortem testis biopsies of COVID-19 patients damage to the anatomical architecture and impaired spermatogenesis[47]. The possible breaching of blood-testis barrier during SARS-Cov-2 infection via ACE2 has been thoroughly reviewed[7]. It is hypothesized that since ACE2 regulates autophagy, the same could be affected in testis since this organ is known to express a high level of the receptor[48].

6. Conclusions and Future Perspectives

From the studies generated during the past one year and a half, reviewed herein, it is evident that the male reproductive system is a potential target of SARS-Cov-2 infection, because of the high expression of molecules that are involved in the viral infectivity. Because of the disturbances in the hormonal profile and damage to the testes, epididymis and prostate in COVID-19 patients, effect on fertility appears to be imminent. However, the long-term implications of SARS-Cov-2 infection on the male reproductive system, especially on fertility, remains to be evaluated, since it is too early to arrive at a conclusion based on the available data. If the altered fertility parameters return to normal over a period of recovery is not yet reported. It appears that the virus is not detected in the semen and this observation indicates that the transmission does not happen by intercourse. The fact that the virus is not present in the semen (though the major organs of the male reproduction are infected) elicits interesting questions as to whether there are any specific factors in the seminal or prostatic secretions that may possibly prevent presence of the virus in the semen. Besides the effect on male fertility, long-term studies on men recovered from COVID-19 that determine the sexual behavior, their social and interpersonal relations in the family and the possible effects on the children born to such men are to be conducted. Since oxidative stress and apoptotic processes are affected in the male reproductive system during SARS-Cov-2 infection, the possibility of an increase in the incidence of cancers in this organ system needs in-depth investigations.

7. References

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