INFECTION

Is SARS-CoV-2-induced testicular damage in hamsters relevant?

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The extent, duration and causes of alterations in the testes caused by SARS-CoV-2 are unclear. A new study has documented the effects of SARS-CoV-2 infection on the testes of a golden Syrian hamster model; however, the relevance of these findings to non-severe human infection is questionable.

Refers to Li, C. et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections by intranasal or testicular inoculation induces testicular damage preventable by vaccination in golden Syrian hamsters. *Clin. Infect. Dis.* https://doi.org/10.1093/cid/ciac142 (2022).

Since the beginning of the COVID-19 pandemic in 2020, SARS-CoV-2 has been suspected to infect the testes owing to the high expression of ACE2, the main receptor for the virus, in this organ[1]. Analysis of several extra-pulmonary sites of SARS-CoV-2 infection has demonstrated that SARS-CoV-2 can disseminate from the respiratory tract to other body parts. Alterations in semen parameters (such as sperm count and motility) and male hormones have been reported in patients with COVID-19 even with mild symptoms, whereas the presence of SARS-CoV-2 in semen seems to be a rare event[1]. The extent, duration and causes of these alterations are unclear. Dramatic morphological damage (such as germ cell degeneration) has been observed in the testes of patients who were severely ill with COVID-19 and died from the disease[1]. Whether the virus is able to replicate in the testes and directly affect testis functions, or whether these issues result from the effect of the generalized inflammation and/or fever and/or treatments in the male genital tract is unknown.

To investigate the effect of SARS-CoV-2 in the testes, Li et al.[2] intranasally challenged golden Syrian hamsters with SARS-CoV-2 and examined the acute and prolonged effect of the infection on sperm count, levels of serum testosterone (produced primarily by Leydig cells under the control of the gonadotropin luteinizing hormone (LH)) and inhibin B (produced by Sertoli cells under the control of follicle-stimulating hormone (FSH)), as well as testicular morphology. Their results show a significant ($P<0.05$) decrease in sperm count (from 1 day post-injection (dpi)) and male hormones (from 4 dpi) to 120 dpi, along with testis inflammation in about half of the acutely infected animals, and reduced testis weight at 120 dpi. The significant sperm decrease at 1 dpi ($P=0.0027$) is surprisingly early and cannot account for a testicular effect (it takes up to 15 days for the transit of testicular spermatozoa through the epididymis in hamsters, and 40 days for spermatogenesis). The epididymis at 4 dpi was not massively affected beyond a few infiltrates; thus, this result might reflect methodological issues, such as small animal number, and details as to how sperm count was undertaken are not provided. In the testes from acutely infected animals, immune cell infiltrates in the interstitial tissue and seminiferous tubules, along with Sertoli cell cytoplasm detached from the basal membrane, indicated a rupture of the testis–blood barrier constituted by the Sertoli cell tight junctions. This barrier protects the differentiating germ cells that appear after puberty from recognition as non-self by the adaptive immunity. The deposition of IgG and C3 complement in the interstitial tissue and tubules from 7 dpi further demonstrated the loss of immune privilege of the testes and the targeting of germ cells by antibodies. Unsurprisingly in the context of such severe inflammation, germ cells degenerated and spermatozoa were depleted. This pattern is similar to reports in men infected with mumps virus who, consecutively to orchitis, develop auto-immunity against their germ cells, leading to permanent sterility and testicular atrophy[3]. The damage in hamster testes is also, to some extent, reminiscent of morphological observations in patients who were severely ill with COVID-19 and died. Of note, the deterioration of testis function and morphology was much more rapid in SARS-CoV-2-infected hamsters than in immune-deficient mice infected with Zika virus (a virus that replicates to high levels in the testis and persists), in which alterations in male hormones and testis morphology only began at 14 dpi (REF.[4]). This observation suggests that different mechanisms underlie the testes damage in these rodent models following SARS-CoV-2 or Zika virus infection.

A key question is the cause of the strong testis inflammation in SARS-CoV-2-infected hamsters and its consecutive long-term damage. To attempt to answer this question, Li et al.[2] looked for evidence of SARS-CoV-2 infection in the testes. In contrast to the lung, SARS-CoV-2 RNA was almost undetectable in the testes after intranasal infection, and only very few cells harbored the virus N protein. Intratesticular injection of SARS-CoV-2 only induced a very transient and weak replication at 1 dpi in two animals. The pattern of pro-inflammatory cytokine upregulation in the testes after injection with SARS-CoV-2 versus influenza (used in this study[2] as a control) was similar overall. Altogether, although the authors concluded that SARS-CoV-2 can infect the testes, the data clearly demonstrate that hamster testes are not a major target for this virus and that the drastic testicular damage in infected hamsters is unlikely to result from viral replication. In support of a lack of direct effect of the virus, in another hamster study, SARS-CoV-2 RNA was transiently detected in the testes after intranasal inoculation (up to 28 days in some animals) but without histopathological changes[5]. Importantly, the tropism (or lack of) of SARS-CoV-2 and its consequences for human testes cannot be inferred from rodent models. For instance, the innate immune response to viral infections in the testes — an essential component of the host control of infection in the immune-privileged testes — differs massively between rodents and humans[3,6]. Rodents seem much better equipped than humans to fight a viral attack in the testes, as they exhibit a strong innate response to a range of pathogens including Zika virus and mumps virus, which contrasts with the weak response in human testes[6–8]. These differences in the testicular innate immune response could influence cell tropism and the outcome of the infection. In men, SARS-CoV-2 RNA and proteins have been occasionally detected in the testes of those who have died from COVID-19 after an extended period of time since acute infection[1]. In a subset of patients with COVID-19, elevated LH concentrations associated with low-to-normal testosterone levels (that is, compensated hypogonadism for the latter)

indicate primary testicular failure[1,9]. Thus, a direct effect of the virus on the human testis cannot be excluded.

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Nevertheless, in addition to the findings in this hamster model, other lines of evidence suggest that the drastic deterioration in testis morphology and function in men who are severely ill with COVID-19 occurs mostly as a secondary effect of the infection. Thus, low testosterone levels have been associated with disease severity and elevated markers of systemic inflammation in infected men[9]. In 75% of a cohort of 39 patients with severe infection, low testosteronaemia was concomitant with low levels of LH, suggesting secondary hypogonadism (that is, decreased production of gonadotropins by the pituitary gland)[9]. Unfortunately, the levels of gonadotropins and inflammation markers, which would have helped to decipher the origin of the altered testis functions, were not measured in the study by Li and colleagues[2]. Besides mentioning a self-limiting pulmonary infection, the authors provided no detail regarding the general level of inflammation, a potential driver of testis failure, after SARS-CoV-2 infection in their animals. The authors infected some animals with influenza as a control and showed a lack of effect on the testis despite pulmonary infection, but this observation does not rule out a systemic aetiology for testis damage nor prove a specific effect of SARS-CoV-2 as influenza in hamsters induces a milder infection than SARS-CoV-2 (REF.[10]) and the levels of systemic inflammation were not compared. Of note, cases of orchitis linked with elevated inflammation have been reported in men with severe influenza infection[3]. An interesting finding not discussed in the study by Li et al.[2] is the expansion of the interstitial space in the testes, an indicator of an elevated volume of testis interstitial fluid owing to increased testis capillary permeability, of SARS-CoV-2-infected animals at 1 dpi. Thus, the vasculopathy associated with SARS-CoV-2 infection, together with the high level of systemic inflammation, might be the cause of acute testis inflammation and subsequent chronic damage. Despite self-limiting pulmonary infection, the hamster model does not reflect a mild or moderate infection in men when considering the dramatic effect on testis morphology. Infection with Omicron or Delta variants of SARS-CoV-2 had overall similar effects to the original Alpha strain, at least during the acute stage of infection and in the small number of animals tested. That vaccination prevented SARS-CoV-2 testicular damages in this model is reassuring.

In summary, the results from the study by Li et al.[2] on the testes of SARS-CoV-2-infected hamsters suggest that this animal model is not appropriate for the study of the consequences of SARS-CoV-2 on testicular function and its aetiology in patients with mild-to-moderate disease. Further studies in other models are urgently needed to decipher the effect of SARS-CoV-2 infection on the testicular endocrine and exocrine functions in these patients.

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1. He, Y. et al. Effect of COVID-19 on male reproductive system–a systematic review. *Front. Endocrinol.* **12**, 677701 (2021).
2. Li, C. et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections by intranasal or testicular inoculation induces testicular damage preventable by vaccination in golden Syrian hamsters. *Clin. Infect. Dis.* https://doi.org/10.1093/cid/ciac142 (2022).
3. Le Tortorec, A. et al. From ancient to emerging infections: the odyssey of viruses in the male genital tract. *Physiol. Rev.* **100**, 1349–1414 (2020).
4. Govero, J. et al. Zika virus infection damages the testes in mice. *Nature* **540**, 438–442 (2016).
5. Campos, R. K. et al. SARS-CoV-2 infects hamster testes. *Microorganisms* **9**, 1318 (2021).
6. Matusali, G. et al. Zika virus infects human testicular tissue and germ cells. *J. Clin. Invest.* **128**, 4697–4710 (2018).
7. Le Tortorec, A. et al. Antiviral responses of human Leydig cells to mumps virus infection or poly I: C stimulation. *Hum. Reprod.* **23**, 2095–2103 (2008).
8. Wu, H. et al. Mumps virus-induced innate immune responses in mouse Sertoli and Leydig cells. *Sci. Rep.* **6**, 19507 (2016).
9. Schroeder, M. et al. High estradiol and low testosterone levels are associated with critical illness in male but not in female COVID-19 patients: a retrospective cohort study. *Emerg. Microbes Infect.* **10**, 1807–1818 (2021).
10. Zhang, A. J. et al. Coinfection by severe acute respiratory syndrome coronavirus 2 and influenza A(H1N1)pdm09 virus enhances the severity of pneumonia in golden Syrian hamsters. *Clin. Infect. Dis.* **72**, e978–e992 (2021).

Competing interests
The author declares no competing interests.