INFECTION

A new threat on the horizon — Zika virus and male fertility

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Amongst the many causes of male-factor infertility, a diagnosed viral cause is a rather infrequent aetiological factor[1]. However, a recent study has illustrated that Zika virus infections affect not only developing fetuses in pregnant women, but are also a threat to fertility in men. Whether this threat could be managed or mitigated remains uncertain.

Refers to Govero, J. *et al.* Zika virus infection damages the testis in mice. *Nature* http://dx.doi.org/10.1038/nature20556 (2016)

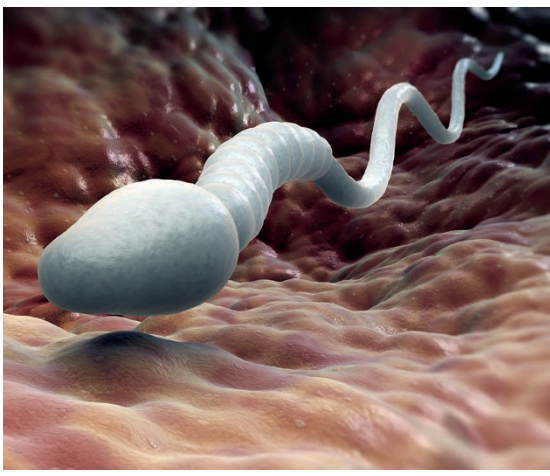
Infertility affects approximately one in 20 men. Many different factors have been shown to affect fertility in men and in animal models, including viral infection, with mumps virus being the classic example. However, the recent Zika virus (ZIKV) epidemic has brought viral aetiologies of infertility back into the limelight. A recent article from Govero and colleagues[2] adds important information to the field, in which the testis has received comparably more attention than the epididymis. Thus, it is reassuring that the authors included assessments of both the testis and the epididymis in their study. For a long time, mumps infection has been considered the most relevant viral threat to male fertility; however, broad-scale immunization schemes make the contemporary presentation of men with mumps orchitis a rare occurrence in urology or andrology outpatient clinics, at least in developed countries.

> “…testicular damage might be mediated by both the infection itself and the host’s adaptive immune response…”

Now Zika virus (ZIKV) enters the stage. Prompted by previous studies in mice, in which the highest ZIKV load was found in the testes[3], the detection of ZIKV in semen, and data that show male-to-female and male-to-male transmission in humans, Govero *et al.*[2] were the first group to study in depth the possible damage to the testis caused by ZIKV infection. Using a mouse-adapted African ZIKV strain (Dakar 41519) in a longitudinal study, initial damage to spermatogenesis was observed just 14 days after infection, with germ cell loss and impairment of Sertoli cell function indicated by decreased levels of serum inhibin B, a Sertoli cell functional marker. If the virus persisted, progressive damage was observed until, at day 21, spermatogenesis was almost completely eradicated, and most germ cells were lost with only the robust Sertoli cells persisting in the tubules. Although ZIKV is found mainly in Sertoli cells, mitotic cells, and midmeiotic germ cells in the testis, the viral load in epididymal spermatozoa is already substantial 7 days after infection[2]. This observation provides a clear indication that ZIKV can infect spermatozoa directly and efficiently in the epididymal lumen, as the kinetics of spermatogenesis would not have enabled a timely transit of spermatozoa developing from ZIKV-infected meiotic cells to appear in the epididymis.

The testicular interstitial cells — consisting mostly of the steroidogenic Leydig cells and leukocytes — are virus-free up to day 21 post-infection, but Sertoli cells seem to be particularly susceptible to ZIKV infection. Although the Sertoli cells are fairly robust, impairment of their function can quickly accelerate and magnify the extent of spermatogenic damage, as each Sertoli cell sustains dozens of germ cells at different stages of their development.

Sertoli cells are also regarded as important cells in the establishment and maintenance of testicular immune privilege, a term describing a special ‘tolerant’ immune environment that is unique to a small set of organs. Immune-privileged organs include the testis, placenta, brain, and anterior chamber of the eye. Immune privilege in the testis is mandatory when neoantigens appear on the meiotic and postmeiotic germ cells at puberty, long after the establishment of self-tolerance around birth. In immune privilege, the function of Sertoli cells extends beyond effectively restricting the access of leukocytes to the developing germ cells by the blood–testis barrier; this is illustrated by studies showing that pancreatic islets survive much longer when cotransplanted with Sertoli cells[4,5]. These data clearly indicate that the role of Sertoli cells in preserving immune privilege goes way beyond a simple barrier function and probably relies on secreted and surface immunosuppressive or immunoregulatory factors[4]. In the case of an infection, this function could prove to be a double-edged sword: what was previously beneficial in protecting the sensitive germ cells by the provision of a tightly controlled anti-inflammatory immune environment under normal conditions can render these cells particularly prone to infection by microbes. Similarly, this phenomenon could be happening in spermatozoa, a transcriptionally quiescent cell that barely has any means of active protection against microbial (in particular viral) infection.



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Comparison of the damage observed in ZIKV and other forms of viral infection (such as mumps virus, herpes simplex virus in mice, Sendai virus in rats, myxoma virus and simian immunodeficiency (SIV) infection in rabbits, and cytomegalovirus in mice and humans), demonstrates that different viruses target different cells. In reports of a number of cases of virus-elicited orchitis, support of a direct tropism for testicular cells is lacking. The viral effect on germ cells varies and one virus can elicit different patterns of impairment of spermatogenesis[6,7]. In severe cases, all germ cells are lost irreversibly and the Sertoli cells remain as the only surviving cell in the seminiferous epithelium, a symptom termed Sertoli-cell-only-syndrome (SCO). In ZIKV infection, complete SCO is visible at day 41 after ZIKV infection[2]. Leukocytic infiltration of the testicular interstitium is prominent in ZIKV infection, but, as in most cases of testicular infection and/or inflammation where leukocytes populate the testis, they are excluded from the seminiferous epithelium, obviously still effectively restricted from entry by the Sertoli cells.

When considering clinical intervention to preserve fertility in infected men and the limited available options to combat the viral infection, assisted reproductive technologies (ART) seem to offer little hope to ZIKV-infected men, should they wish to father children now or in the future. Although spermatogenesis in ZIKV-infected men seems to be less affected than in mice[8], Govero and colleagues' data[2] regarding the negative effect on murine spermatogenesis should act as a warning and, in particular, the early and massive infection of epididymal spermatozoa suggests that only infected spermatozoa would be left for ART by the time the infection is diagnosed. Whether 'sperm washing' in an ART setting would prove as effective for ZIKV as it has been in serodiscordant couples where the male partner is HIV-positive[9], remains to be tested. Bearing in mind the congenital malformations such as microcephaly that are seen in the progeny of ZIKV-infected women, the risk is very high, particularly as any means of detecting the virus in individual spermatozoa used for ICSI would ultimately destroy the gamete.

In contrast to the epididymis, where the contribution of the immune response to the damage caused by ZIKV is substantial[10], the effect of the immune response on testicular damage is less clear. Here, the work of Govero *et al.*[1] breaks new ground. Their data provide valuable insight indicating that the testicular damage might be mediated by both the infection itself and the host's adaptive immune response, as indicated by their studies using ZIKV-infected *Rag*1$^{-/-}$ mice, which lack mature B and T lymphocytes[2]. Whether anti-inflammatory treatment could possibly attenuate the testicular damage elicited by ZIKV and, therefore, maintain some ongoing spermatogenesis as a fertility reserve needs to be elucidated.

Overall, ZIKV infection has been identified as a previously unknown threat to male fertility in mice. In mice, infection of spermatozoa is fast and extensive, and spermatogenesis eventually ceases. In men, data indicate a high infectious viral load in semen and established sexual transmission from men to women. However, the presence of sperm in the ejaculate indicates a different, probably less radical, pattern of spermatogenic damage in humans compared with mice[8]. Thus, fertility in men could be at risk predominantly owing to transmission of the disease by ZIKV-infected spermatozoa during natural conception or ART, whereas infertility by nonobstructive azoospermia — as seen in mice — could be less of a problem in humans.

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Competing interests statement
The author declares no competing interests.