Koukila-Kähkölä P, Paulin L, Brander E, Jantzen E, Eho-Remes M, Katila ML. Characterisation of a new isolate of *Mycobacterium* shimoidei from Finland. J Med Microbiol. 2000;49:937–40. http://dx.doi.org/10.1099/0022-1317-49-10-937

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The Breadth of Viruses in Human Semen

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Zika virus RNA is frequently detected in the semen of men after Zika virus infection. To learn more about persistence of viruses in genital fluids, we searched PubMed for relevant articles. We found evidence that 27 viruses, across a broad range of virus families, can be found in human semen.

The finding by Atkinson et al. that Zika virus RNA is frequently detected in the semen of men after infection (I)highlights our knowledge gaps regarding the persistence of viruses in genital fluids, especially semen. Replicating Zika virus (2), like Ebola and Marburg viruses (3), has been isolated from semen and has been sexually transmitted. However, it is probable that many more viruses capable of causing viremia (presence of virus in the blood) can be found in semen. Seeding to the male reproductive tract may frequently occur in the context of viremia because the bloodtestes/deferens/epididymis barriers are imperfect barriers to viruses, especially in the presence of systemic or local inflammation (4). Virus may persist even if incapable of replicating within the male reproductive tract because the testes are immunologically privileged (4); that is, within the testes, the immune response is restricted to enable the survival of sperm, which are immunogenic. Virus may also be transmitted to semen as a result of survival and replication within the accessory glands (5).

To investigate the breadth of viruses in semen, we performed a PubMed search by using the terms "virus* AND semen OR sperm* OR seminal." We imposed no date or

language restrictions. This search returned 3,818 results. We screened the titles, abstracts, and full text articles for data that described detection of viruses in semen by nucleic acid amplification or detection, antigen detection, replication in cell culture, or replication in an animal system. We restricted the results to viruses capable of causing viremia. Where we found evidence for virus in semen, we then searched PubMed for evidence of sexual transmission by using the terms "(name of virus) AND sex* AND Transm*."

Our search revealed that 27 viruses that can result in viremia have been found in human semen (Table). For many of these, data on sexual transmission are lacking. Of these 27 viruses, many cause chronic or latent infection (e.g., HIV virus, cytomegalovirus). However, several cause acute infections, including Lassa fever, Rift Valley fever, and chikungunya viruses. Of those causing acute infections, only Zika and Ebola viruses have been systematically screened for in semen (i.e., in case series or cohort studies rather than case reports). These 27 viruses come from diverse families, suggesting that the presence of many viruses in semen is unlikely to be exclusively dependent on specific or conserved viral epitopes, ability of virus to replicate within the male reproductive tract, or common mechanisms of immune evasion. Other factors that may also influence whether viruses exist in semen are level of viremia, inflammatory mediators (altering bloodbarrier permeability), systemic immunosuppression, male reproductive tract immune responses, presence of sexually transmitted diseases, and virus structural stability. In mammals, numerous viruses are detectable in semen, including viruses that can cause disease in humans, such as Japanese encephalitis virus, foot and mouth disease virus, parainfluenza virus, and paravaccinia virus (6). Several other viruses that result in viremia can cause orchitis and have been detected in human testes, suggesting the possibility that these viruses may also be detectable in semen. These viruses include influenza virus, lymphocytic choriomeningitis virus, phlebotomus fever virus, cocksackie B virus, echovirus, dengue virus, systemic acute respiratory syndrome virus, parvovirus, smallpox virus, vaccinia virus, and rubella virus (7).

Given these findings, the following questions need to be addressed: which viruses are shed and remain viable in semen, for how long, and at what concentrations? The answers to these questions have implications for risks for sexual transmission and, therefore, embryonic infection, congenital disease, miscarriage, and effects on epidemiologic and transmission models. The presence of virus in the male reproductive tract may increase the risk for acquisition of sexually transmitted infections and may reduce male fertility through spermatogonial stem cell infection or local inflammation. Infection of spermatozoa could result in transmission of virus-induced mutations to subsequent

Table. Viruses that are capable of causing viremia and found in human semen*

		Detection in	Isolation from	Evidence for
		semen, maximum	semen, maximum	sexual transmission
Virus	Family	detection time, d	detection time, d	within same cohort
Adenoviruses	Adenoviridae	AD	RCC	Unknown
Transfusion transmitted virus	Anelloviridae	NAA	No data found	Unknown
Lassa fever virus†	Arenaviridae	NAA, 103	RCC, 20	Unknown
Rift Valley fever virus†	Bunyaviridae	NAA, 117	No data found	Unknown
Ebola virus	Filoviridae	NAA, 531	RCC, 82	Epi + mol + sem
Marburg virus†	Filoviridae	AD, 83	RAS, 83	Epi + sem
GB virus C	Flaviviridae	NAA	No data found	Epi + mol
Hepatitis C virus	Flaviviridae	NAA; AD	No data found	Epi + mol
Zika virus	Flaviviridae	NAA, 188	RCC, 7	Epi + mol + sem
Hepatitis B virus	Hepadnaviridae	NAA; AD	RAS	Epi + mol
Cytomegalovirus	Herpesviridae	NAA	RCC	Epi + mol + sem
Epstein Barr virus	Herpesviridae	NAA	No data found	Epi and semen
Human herpes virus 8	Herpesviridae	NAA	RCC	Epi + mol
Human herpes virus 7	Herpesviridae	NAA	No data found	Unknown
Human herpes virus 6	Herpesviridae	NAA	No data found	Unknown
Human simplex viruses 1 and 2	Herpesviridae	NAA; AD	RCC	Epi + mol + sem
Varicella zoster virus	Herpesviridae	NAA	No data found	Unknown
Mumps virus†	Paramyxoviridae	NAA, 40	RCC, 14	Unknown
Adeno-associated virus	Parvoviridae	NAA	RCC	Unknown
BK virus	Polyomaviridae	NAA	No data found	Unknown
JC virus	Polyomaviridae	NAA	No data found	Unknown
Simian virus 40	Polyomaviridae	NAA	No data found	Unknown
HIV	Retroviridae	NAA; AD	RCC	Epi + mol + sem
Human T-cell lymphoma virus 1†	Retroviridae	No data found	RAS	Epi + mol
Simian foamy virus	Retroviridae	NAA	No data found	Unknown
Chikungunya virus†	Togaviridae	NAA, 30	No data found	Unknown

*Presence of nucleic acid or antigen in semen does not represent the presence of replication-competent or infection-competent virus, which can generally only be demonstrated by isolation and culture of virus. Maximum detection time refers to time from symptom onset (only in viruses that cause acute only, not chronic, infection). A complete table with references is provided in the online Technical Appendix (https://wwwnc.cdc.gov/EID/article/23/11/17-1049-Techapp1.pdf). AD, antigen detection; Epi, epidemiologic evidence of sexual transmission; mol, molecular/phylogenetic evidence of sexual transmission; NAA, nucleic acid amplification or detection; RAS, replication in animal system; RCC, replication in cell culture; sem, isolation from semen. †Data found only in the context of case reports and not case series, case control, or cohort studies.

generations, thereby elevating risks for cancer and other disorders. Indeed, when virus has been detected in human semen, the extent to which virus existence and replication occurs within spermatozoa is unclear (δ). Not all therapeutics will cross the male reproductive tract-blood barriers, and viruses may persist in semen despite systemic clearance of virus, highlighting the need to consider the male reproductive tract-blood barriers when choosing therapeutic agents in clinical trials. Virus within the male reproductive tract can also be genetically distinct from virus in other compartments, including blood (θ), which has implications for gene-based vaccines and therapeutics.

The presence of viruses in semen is probably more widespread than currently appreciated, and the absence of virus in genital secretions should not be assumed for traditionally non–sexually transmitted viruses. The investigation of virus detection and persistence in semen across a range of viruses is useful for clinical and public health reasons, in particular for viruses that lead to high mortality or morbidity rates or to epidemics.

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References

- Atkinson B, Thorburn F, Petridou C, Bailey D, Hewson R, Simpson AJH, et al. Presence and persistence of Zika virus RNA in semen, United Kingdom, 2016. Emerg Infect Dis. 2017;23:611–5. http://dx.doi.org/10.3201/eid2304.161692
- Moreira J, Peixoto TM, Siqueira AM, Lamas CC. Sexually acquired Zika virus: a systematic review. Clin Microbiol Infect. 2017;23:296–305. http://dx.doi.org/10.1016/j.cmi.2016.12.027
- Brainard J, Pond K, Hooper L, Edmunds K, Hunter P. Presence and persistence of Ebola or Marburg virus in patients and survivors: a rapid systematic review. PLoS Negl Trop Dis. 2016;10:e0004475– 17. http://dx.doi.org/10.1371/journal.pntd.0004475
- Li N, Wang T, Han D. Structural, cellular and molecular aspects of immune privilege in the testis. Front Immunol. 2012;3:152. http://dx.doi.org/10.3389/fimmu.2012.00152
- Hirsch AJ, Smith JL, Haese NN, Broeckel RM, Parkins CJ, Kreklywich C, et al. Zika virus infection of rhesus macaques leads to viral persistence in multiple tissues. PLoS Pathog. 2017; 13:e1006219–23. http://dx.doi.org/10.1371/journal.ppat.1006219
- Kahrs RF, Gibbs EP, Larsen RE. The search for viruses in bovine semen, a review. Theriogenology. 1980;14:151–65. http://dx.doi.org/10.1016/0093-691X(80)90101-6

RESEARCH LETTERS

- Dejucq N, Jégou B. Viruses in the mammalian male genital tract and their effects on the reproductive system. Microbiol mol Biol Rev. 2001;65:208–31. http://dx.doi.org/10.1128/ MMBR.65.2.208-231.2001
- Mansuy JM, Suberbielle E, Chapuy-Regaud S, Mengelle C, Bujan L, Marchou B, et al. Zika virus in semen and spermatozoa. Lancet Infect Dis. 2016;16:1106–7. http://dx.doi.org/10.1016/ S1473-3099(16)30336-X
- Pillai SK, Good B, Pond SK, Wong JK, Strain MC, Richman DD, et al. Semen-specific genetic characteristics of human immunodeficiency virus type 1 env. J Virol. 2005;79:1734

 –42. http://dx.doi.org/10.1128/JVI.79.3.1734-1742.2005

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Legionella pneumophila Serogroup 1 in the Water Facilitites of a Tertiary Healthcare Center, India

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Proactive environmental surveillance for Legionella pneumophila in hospitals that treat immunocompromised patients is a useful strategy for preventing nosocomial Legionnaires' disease. We report the presence of L. pneumophila serogroup 1 in 15.2% of the water systems of our tertiary healthcare center, which should prompt health officials to formulate mitigation policies.

Legionnaires' disease (LD), is a bacterium omnipresent in aquatic environments and increasingly recognized as a major cause of community- and hospital-acquired pneumonia. L. pneumophila serogroup 1 (Lp1), the dominant serogroup, accounts for \approx 84% of human infections worldwide (1,2). Hospital-acquired LD has been reported globally, and routine use of environmental cultures is recommended as a useful strategy to prevent infections (3). Although proactive environmental surveillance of Legionella and regular

treatment of cooling tower installations are recommended in many countries, these practices are not routine in India, and limited studies have been conducted in this country for monitoring *Legionella* contamination in hospital water systems (4). We conducted a study to detect *L. pneumophila* and to identify *Lp1* in the water systems of a tertiary health-care center in northern India that has organ transplantation and cancer treatment facilities.

We collected 79 water samples (41 potable, 38 nonpotable) from the hospital and general areas of the healthcare center during an 18-month period (May 2015-October 2016). Of 79 samples, 27 were collected from patient areas (wards, intensive care units, outpatient departments, emergency units, and procedure rooms); 14 from residential areas; 15 from cooling towers; and 23 from other buildings (e.g., laboratory divisions, teaching departments, library, and recreational zones). We followed guidelines issued by the US Centers for Disease Control and Prevention regarding isolation of Legionella (5). In brief, we concentrated 500 mL of water samples and decontaminated 1 part by using heat treatment (in water bath at 50°C for 30 min) and 1 part by acid (in equal volume of HCl-KCl acid buffer [pH 2.2]). We then inoculated 0.1-mL samples onto buffered charcoal yeast extract agar (Becton Dickinson, Sparks, MD, USA) supplemented with glycine, vancomycin, polymyxin B, and cycloheximide (Oxoid, Basingstoke, UK). We presumptively identified colonies growing only on buffered charcoal yeast extract but not on blood agar as Legionella species and confirmed the presence of L. pneumophila by amplification of a 375-bp region of the mip gene using previously published primers (6). We identified Lp1 by using a real-time PCR (rPCR) assay targeting the wzm gene (7). We used genomic DNA isolated from L. pneumophila strain Philadelphia (ATCC 33152) for standardization of PCR and rPCR and L. pneumophila strain Knoxville (ATCC 33153) for standardization of culture.

We identified *Legionella* spp. in 21 (26.6%) of 79 water samples (10 potable and 11 nonpotable) by culture. We obtained a collection of 28 isolates from the 79 samples and identified all of them as *L. pneumophila* by PCR. Among these 28 isolates, 18 (64.3%) tested positive for *Lp*1 by rPCR, indicating the presence of this pathogenic serogroup in 12 (15.2%) of the 79 water samples (5 potable and 7 nonpotable).

We repeatedly isolated *L. pneumophila* (>4 times) from 2 high-risk sites: a drinking water unit and a cooling tower situated inside the hospital campus. Four water samples collected from patient areas tested positive for *L. pneumophila*, posing a risk for nosocomial infection. We isolated *L. pneumophila* from water bodies with temperatures ranging from 12°C to 57°C but most frequently (11 times) from those with temperatures of 25°C–50°C. We summarized the isolation of *L. pneumophila* with

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Technical Appendix

Technical Appendix Table. Viruses that are capable of causing viremia and found in human semen*

••	•	Detection in semen,	Isolation from semen	Evidence for sexual
		maximum detection	maximum detection	transmission within same
Virus	Family	time, d	time, d	cohort
Adenoviruses	Adenoviridae	AD (1)	RCC	Unknown
Transfusion transmitted virus	Anelloviridae	NAA (2)	No data found	Unknown
Lassa fever virus†	Arenaviridae	NAA, 103 (3)	RCC, 20 (3)	Unknown
Rift Valley fever virus†	Bunyaviridae	NAA, 117 (<i>4</i>)	No data found	Unknown
Ebola virus	Filoviridae	NAA, 531 (<i>5</i>)	RCC, 82 (6)	Epi + mol + sem (7)
Marburg virus†	Filoviridae	AD, 83 (8)	RAS, 83 (8)	Epi + sem (9)
GB virus C	Flaviviridae	NAA (10)	No data found	Epi + mol (11)
Hepatitis C virus	Flaviviridae	NAA (12); AD (13)	No data found	Epi + mol (14)
Zika virus	Flaviviridae	NAA, 188 (<i>15</i>)	RCC, 7 (16)	Epi + mol + sem (17)
Hepatitis B virus	Hepadnaviridae	NAA (18); AD (19)	RAS (20)	Epi + mol (21)
Cytomegalovirus	Herpesviridae	NAA (22)	RCC (23)	Epi + mol + sem (24)
Epstein Barr virus	Herpesviridae	NAA (22)	No data found	Epi and semen (25)
Human herpes virus 8	Herpesviridae	NAA (26)	RCC (27)	Epi + mol (28)
Human herpes virus 7	Herpesviridae	NAA (29)	No data found	Unknown
Human herpes virus 6	Herpesviridae	NAA (22)	No data found	Unknown
Human simplex viruses 1 and 2	Herpesviridae	NAA (22); AD (1)	RCC (1)	Epi + mol + sem (30)
Varicella zoster virus	Herpesviridae	NAA (22)	No data found	Unknown
Mumps virus†	Paramyxoviridae	NAA, 40 (31)	RCC, 14 (31)	Unknown
Adeno-associated virus	Parvoviridae	NAA (32)	RCC (33)	Unknown
BK virus	Polyomaviridae	NAA (<i>34</i>)	No data found	Unknown
JC virus	Polyomaviridae	NAA (<i>34</i>)	No data found	Unknown
Simian virus 40	Polyomaviridae	NAA (35)	No data found	Unknown
HIV	Retroviridae	NAA (36), AD (37)	RCC (38)	Epi + mol + sem (39)
Human T-cell lymphoma virus	Retroviridae	No data found	RAS (<i>40</i>)	Epi + mol (41)
1†				
Simian foamy virus	Retroviridae	NAA (42)	No data found	Unknown
Chikungunya virus†	Togaviridae	NAA, 30 (<i>43</i>)	No data found	Unknown

^{*}Presence of nucleic acid or antigen in semen does not represent the presence of replication-competent or infection-competent virus, which can generally only be demonstration by isolation and culture of virus. Maximum detection time refers to time from symptom onset (only in viruses that cause acute only, not chronic, infection). AD, antigen detection; Epi, Epidemiological evidence of sexual transmission; mol, molecular/phylogenetic evidence of sexual transmission; NAA, nucleic acid amplification or detection; RAS, replication in animal system; RCC, replication in cell culture; sem, isolation from semen. †Data found only in the context of case reports, and not case series, case control, or cohort studies.

References

- Kulcsár G, Csata S, Nász I. Investigations into virus carriership in human semen and mouse testicular cells. Acta Microbiol Hung. 1991;38:127–32. <u>PubMed</u>
- Matsubara H, Michitaka K, Horiike N, Yano M, Akbar SM, Torisu M, et al. Existence of TT virus DNA in extracellular body fluids from normal healthy Japanese subjects. Intervirology. 2000;43:16–9. PubMed
- 3. Raabe VN, Kann G, Ribner BS, Morales A, Varkey JB, Mehta AK, et al. Favipiravir and ribavirin treatment of epidemiologically linked cases of lassa fever. Clin Infect Dis. 2017;65:1–5.

- 4. Haneche F, Leparc-Goffart I, Simon F, Hentzien M, Martinez-Pourcher V, Caumes E, et al. Rift Valley fever in kidney transplant recipient returning from Mali with viral RNA detected in semen up to four months from symptom onset, France, autumn 2015. Euro Surveill. 2016;21:30222–4.
 PubMed
- 5. Diallo B, Sissoko D, Loman NJ, Bah HA, Bah H, Worrell MC, et al. Resurgence of Ebola virus disease in guinea linked to a survivor with virus persistence in seminal fluid for more than 500 days. Clin Infect Dis. 2016;63:1353–6. PubMed
- Rodriguez LL, De Roo A, Guimard Y, Trappier SG, Sanchez A, Bressler D, Et al. Persistence and genetic stability of Ebola virus during the outbreak in Kikwit, Democratic Republic of the Congo, 1995. J Infect Dis. 1999 Feb;179 Suppl 1(S1):S170–6.
- 7. Mate SE, Kugelman JR, Nyenswah TG, Ladner JT, Wiley MR, Cordier-Lassalle T, et al. Molecular evidence of sexual transmission of Ebola virus. N Engl J Med. 2015;373:2448–54. PubMed
- 8. Siegert R, Shu HL, Slenczka W. Nachweis des "Marburg-Virus" beim Patienten [in German]. Dtsch Med Wochenschr. 1968;93:616–9. PubMed
- 9. Martini GA, Schmidt HA. Spermatogenic transmission of the "Marburg virus". (Causes of "Marburg simian disease") [in German]. Klin Wochenschr. 1968;46:398–400. PubMed
- 10. Semprini AE, Persico T, Thiers V, Oneta M, Tuveri R, Serafini P, et al. Absence of hepatitis C virus and detection of hepatitis G virus/GB virus C RNA sequences in the semen of infected men. J Infect Dis. 1998;177:848–54. PubMed
- 11. Sarrazin C, Roth WK, Zeuzem S. Heterosexual transmission of GB virus-C/hepatitis G virus infection. Eur J Gastroenterol Hepatol. 1997;9:1117–20. PubMed
- 12. Leruez-Ville M, Kunstmann J-M, De Almeida M, Rouzioux C, Chaix M-L. Detection of hepatitis C virus in the semen of infected men. Lancet. 2000;356:42–3. PubMed
- 13. Kotwal GJ, Rustgi VK, Baroudy BM. Detection of hepatitis C virus-specific antigens in semen from non-A, non-B hepatitis patients. Dig Dis Sci. 1992;37:641–4. <u>PubMed</u>
- 14. Terrault NA, Dodge JL, Murphy EL, Tavis JE, Kiss A, Levin TR, et al. Sexual transmission of hepatitis C virus among monogamous heterosexual couples: the HCV partners study. Hepatology. 2013;57:881–9. PubMed
- 15. Nicastri E, Castilletti C, Liuzzi G, Iannetta M, Capobianchi MR, Ippolito G. Persistent detection of Zika virus RNA in semen for six months after symptom onset in a traveller returning from Haiti to Italy, February 2016. Euro Surveill. 2016;21:30314–4. PubMed

- 16. Jang H-C, Park WB, Kim UJ, Chun JY, Choi S-J, Choe PG, et al. First imported case of Zika virus infection into Korea. J Korean Med Sci. 2016;31:1173–7. PubMed
- 17. D'Ortenzio E, Matheron S, Yazdanpanah Y, de Lamballerie X, Hubert B, Piorkowski G, et al. Evidence of sexual transmission of Zika virus. N Engl J Med. 2016;374:2195–8. <u>PubMed</u>
- 18. Fei QJ, Yang XD, Ni WH, Pan CS, Huang XF. Can hepatitis B virus DNA in semen be predicted by serum levels of hepatitis B virus DNA, HBeAg, And HBsAg In chronically infected men from infertile couples? Andrology. 2015;3:506–11.
- 19. Mansour W, Lemoine M, Neri Pinto F, Llabador de Royer MA, Le Gal F, Yazbeck C, et al. Markers of hepatitis delta virus infection can be detected in follicular fluid and semen. J Clin Virol. 2014;61:279–81. PubMed
- 20. Scott RM, Snitbhan R, Bancroft WH, Alter HJ, Tingpalapong M. Experimental transmission of hepatitis B virus by semen and saliva. J Infect Dis. 1980;142:67–71. PubMed
- 21. Huo TI, Wu JC, Huang YH, Yang UC, Sheen IJ, Chang FY, et al. Evidence of transmission of hepatitis B virus to spouses from sequence analysis of the viral genome. J Gastroenterol Hepatol. 1998;13:1138–42. PubMed
- 22. Neofytou E, Sourvinos G, Asmarianaki M, Spandidos DA, Makrigiannakis A. Prevalence of human herpes virus types 1-7 in the semen of men attending an infertility clinic and correlation with semen parameters. Fertil Steril. 2009;91:2487–94. PubMed
- 23. Bresson JL, Clavequin MC, Mazeron MC, Mengelle C, Scieux C, Segondy M, et al.; Fédération Française des CECOS. Risk of cytomegalovirus transmission by cryopreserved semen: a study of 635 semen samples from 231 donors. Hum Reprod. 2003;18:1881–6. PubMed
- 24. Handsfield HH, Chandler SH, Caine VA, Meyers JD, Corey L, Medeiros E, et al. Cytomegalovirus infection in sex partners: evidence for sexual transmission. J Infect Dis. 1985;151:344–8.
 PubMed
- 25. Thomas R, Macsween KF, McAulay K, Clutterbuck D, Anderson R, Reid S, et al. Evidence of shared Epstein-Barr viral isolates between sexual partners, and low level EBV in genital secretions. J Med Virol. 2006;78:1204–9. <u>PubMed</u>
- 26. Viviano E, Vitale F, Ajello F, Perna AM, Villafrate MR, Bonura F, et al. Human herpesvirus type 8

 DNA sequences in biological samples of HIV-positive and negative individuals in Sicily. AIDS.
 1997;11:607–12. PubMed

- 27. Bagasra O, Patel D, Bobroski L, Abbasi JA, Bagasra AU, Baidouri H, et al. Localization of human herpesvirus type 8 in human sperms by in situ PCR. J Mol Histol. 2005;36:401–12. PubMed
- 28. Kouri V, Marini A, Doroudi R, Nambiar S, Rodriguez ME, Capo V, et al. Molecular epidemiology of Kaposi's sarcoma herpesvirus (KSHV) in Cuban and German patients with Kaposi's sarcoma (KS) and asymptomatic sexual contacts. Virology. 2005;337:297–303. PubMed
- 29. Michou V, Liarmakopoulou S, Thomas D, Tsimaratou K, Makarounis K, Constantoulakis P, et al. Herpes virus infected spermatozoa following density gradient centrifugation for IVF purposes. Andrologia. 2012;44:174–80. PubMed
- 30. Moore DE, Ashley RL, Zarutskie PW, Coombs RW, Soules MR, Corey L. Transmission of genital herpes by donor insemination. JAMA. 1989;261:3441–3. <u>PubMed</u>
- 31. Jalal H, Bahadur G, Knowles W, Jin L, Brink N. Mumps epididymo-orchitis with prolonged detection of virus in semen and the development of anti-sperm antibodies. J Med Virol. 2004;73:147–50.

 PubMed
- 32. Erles K, Rohde V, Thaele M, Roth S, Edler L, Schlehofer JR. DNA of adeno-associated virus (AAV) in testicular tissue and in abnormal semen samples. Hum Reprod. 2001;16:2333–7. <u>PubMed</u>
- 33. Schlehofer JR. Adeno-vssociated Virus (AAV) in semen and testis: a role in infertility? An overview. Andrologia. 2004;14:317–9.
- 34. Comar M, Zanotta N, Croci E, Murru I, Marci R, Pancaldi C, et al. Association between the JC polyomavirus infection and male infertility. PLoS One. 2012;7:e42880. PubMed
- 35. Martini F, Iaccheri L, Lazzarin L, Carinci P, Corallini A, Gerosa M, et al. SV40 early region and large T antigen in human brain tumors, peripheral blood cells, and sperm fluids from healthy individuals. Cancer Res. 1996;56:4820–5. PubMed
- 36. Mermin JH, Holodniy M, Katzenstein DA, Merigan TC. Detection of human immunodeficiency virus DNA and RNA in semen by the polymerase chain reaction. J Infect Dis. 1991;164:769–72.

 PubMed
- 37. Homsy J, Thomson-Honnebier GA, Cheng-Mayer C, Levy JA. Detection of human immunodeficiency virus (HIV) in serum and body fluids by sequential competition ELISA. J Virol Methods. 1988;19:43–56. PubMed
- 38. Van Voorhis BJ, Martinez A, Mayer K, Anderson DJ. Detection of human immunodeficiency virus type 1 in semen from seropositive men using culture and polymerase chain reaction deoxyribonucleic acid amplification techniques. Fertil Steril. 1991;55:588–94. PubMed

- 39. Butler DM, Delport W, Kosakovsky Pond SL, Lakdawala MK, Cheng PM, Little SJ, et al. The origins of sexually transmitted HIV among men who have sex with men. Sci Transl Med. 2010 Feb 10;2(18):18re1–18re1.
- 40. Iwahara Y, Takehara N, Kataoka R, Sawada T, Ohtsuki Y, Nakachi H, et al. Transmission of HTLV-I to rabbits via semen and breast milk from seropositive healthy persons. Int J Cancer. 1990;45:980–3. PubMed
- 41. Iga M, Okayama A, Stuver S, Matsuoka M, Mueller N, Aoki M, et al. Genetic evidence of transmission of human T cell lymphotropic virus type 1 between spouses. J Infect Dis. 2002;185:691–5. PubMed
- 42. Boneva RS, Switzer WM, Spira TJ, Bhullar VB, Shanmugam V, Cong ME, et al. Clinical and virological characterization of persistent human infection with simian foamy viruses. AIDS Res Hum Retroviruses. 2007;23:1330–7. PubMed
- 43. Bandeira AC, Campos GS, Rocha VFD, Souza BS, Soares MBP, Oliveira AA, et al. Prolonged shedding of Chikungunya virus in semen and urine: A new perspective for diagnosis and implications for transmission. IDCases. 2016;6:100–3. PubMed