

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

**Viruses in seminal fluid  
prevention and risk minimization  
in ART Laboratory**

# Which viruses are shed in semen?

**27 viruses** from diverse families were identified in semen.

Of these, **17 different viruses** showed epidemiological and/or molecular evidence of sexual transmission and/or replication-competent or infection-competent virus were isolated from semen.

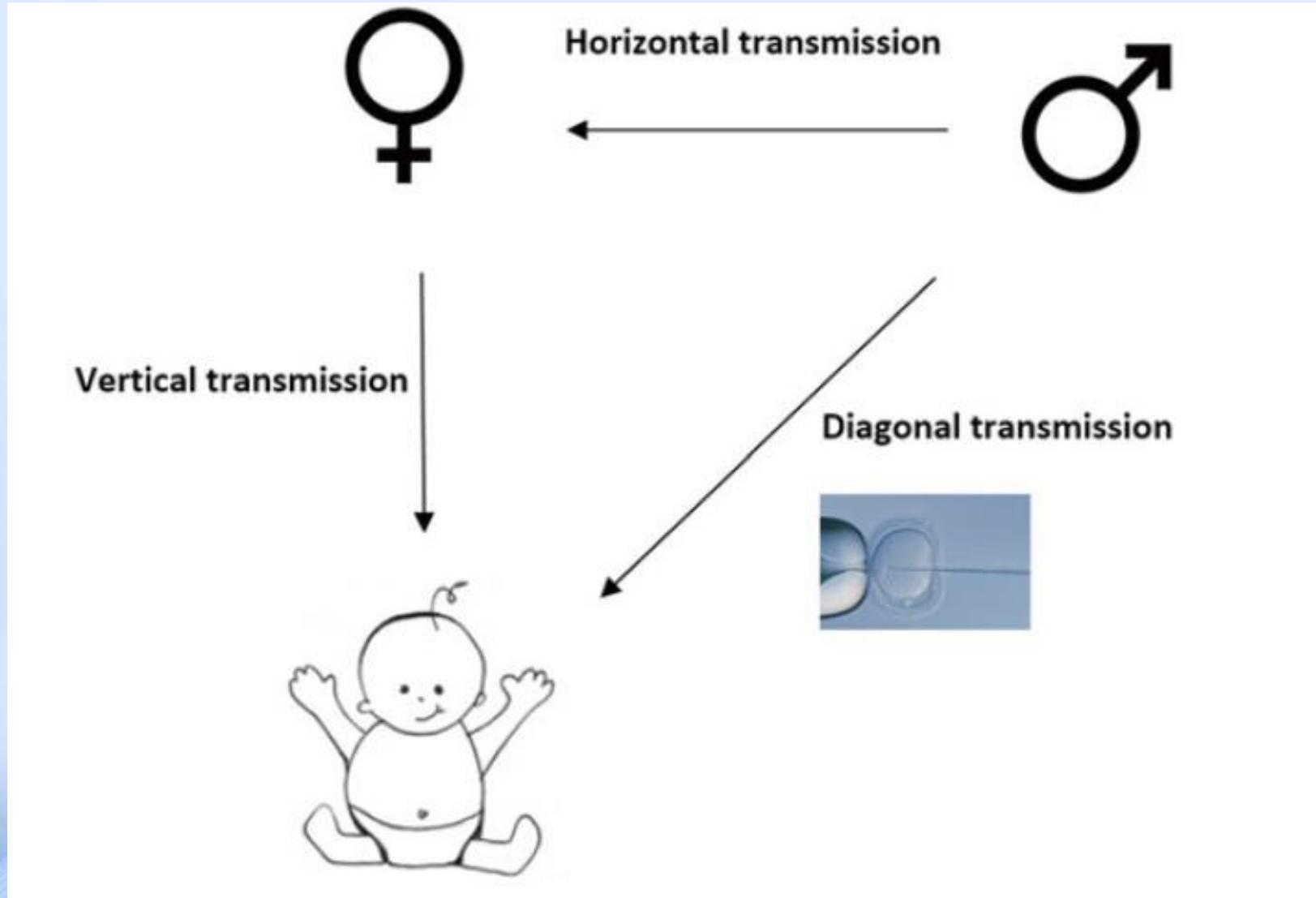
**Hepatitis B virus, hepatitis C virus, human immunodeficiency virus (HIV), human papilloma virus, cytomegalovirus (CMV), Epstein-Barr virus, human herpes virus 8, herpes simplex viruses type 1 and 2 (HSV-1 and HSV-2), mumps virus, adenovirus, adeno-associated virus, Ebola, Marburg, Lassa, GB virus C, Zika, and human T-cell lymphotropic virus type I**

# **Viral infection in seminal fluid**

**In semen, viruses can present in:**

- ❑ Semen**
  - ❑ Sperm or sperm precursor cells**
  - ❑ Seminal immune cells.**
- 
- ❑ Overcome an infertility problem.**
  - ❑ Decrease the risk of horizontal transmission.**

# Viral infection in seminal fluid



# HBV transmission

- ❑ HBV not only passes through **the blood–testis barrier**, it is also thought to **integrate into the genome** of germ cells and **human sperm**.
- ❑ It was revealed that **human sperms carrying HBV genes** can pass through the golden hamster oolemma and into the cytoplasm of oocytes and **complete fertilisation normally** and HBV genes were able **to replicate and** be expressed in **two-cell embryos**.

# HBV transmission

## Presence of hepatitis B virus in oocytes and embryos: a risk of hepatitis B virus transmission during in vitro fertilization

*Rui Nie, M.Med., Lei Jin, Ph.D., Hanwang Zhang, Ph.D., Bei Xu, Ph.D., Wen Chen, Ph.D., and Guijin Zhu, M.Med.*

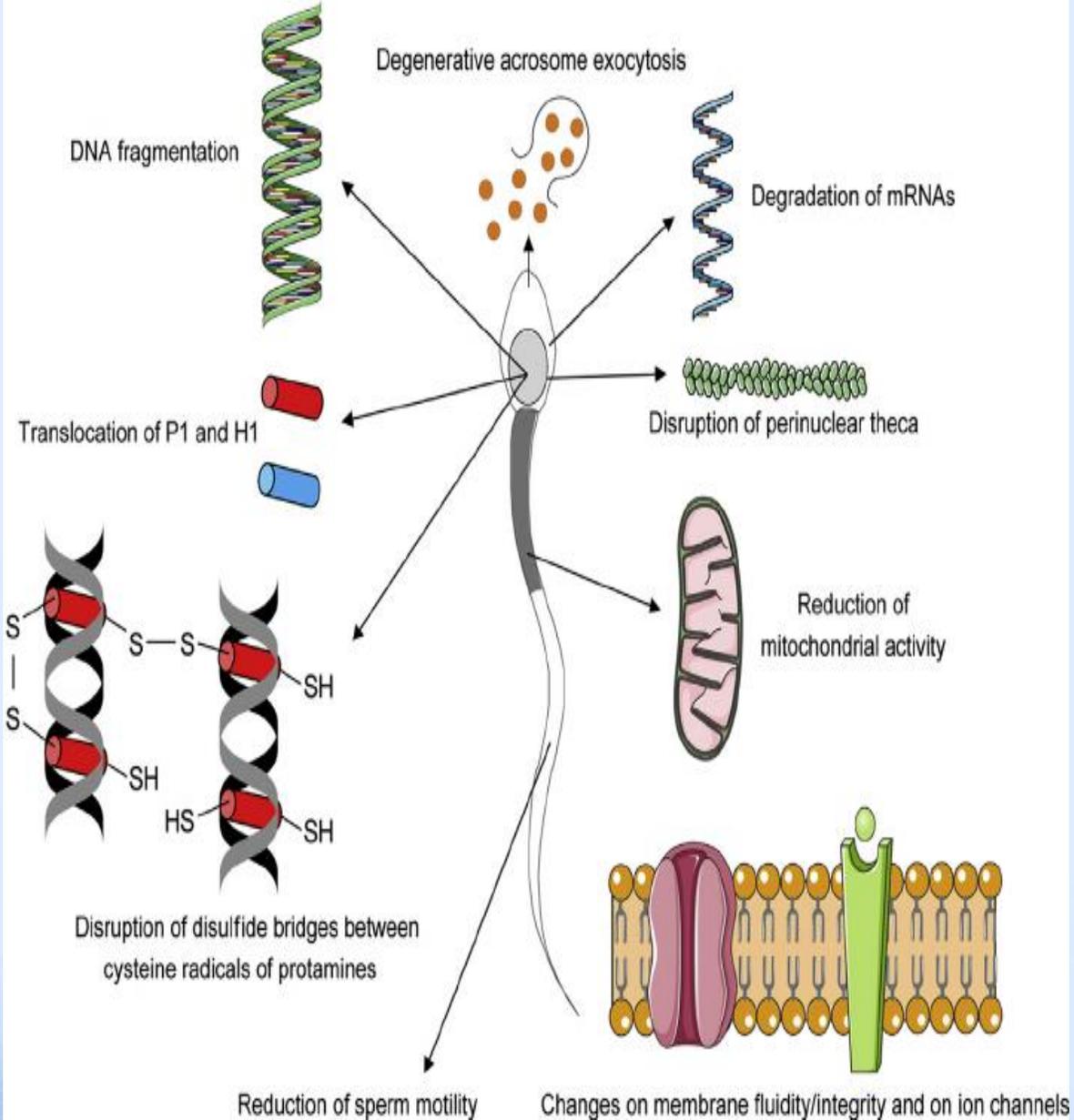
Reproductive Medicine Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People's Republic of China

- ❑ These results suggest that human sperm cells might integrate the HBV DNA, acting as **vectors for** the transmission of **HBV genes during IVF as well as ICSI** procedures.

# HBV and sperm parameters

- ❑ Sperm concentration, motility, morphology, and viability were significantly impaired in HBV-seropositive patients.
- ❑ HBV induces **ROS generation** and **reduces antioxidant** capacity in sperm cells, resulting in **oxidative stress** and sperm dysfunction
- ❑ HBV genome can integrate into human sperm chromosomes and induce **chromosomal aberrations**.

# HBV and sperm parameters



# Prevention of transmission during ART

First International Journal of Andrology

## andrologia

ORIGINAL ARTICLE

### Swim-up as a strategy for isolation of spermatozoa without viral incorporation in men with chronic hepatitis B: A pilot study

Thomas Condijs  Liesl Bourdeaud'huy, Kelly Tilleman, Sylvie Lierman, Chantal Dewinter, Elizaveta Padalko

First published: 13 July 2020 | <https://doi.org/10.1111/and.13732> | Citations: 1

Condijs and Bourdeaud'huy contributed equally to this work.

Since no **HBV DNA was detected in progressive fractions**, this study suggests swim-up a successful strategy to select HBV-free spermatozoa.

# Prevention of transmission during ART

- ❑ Men testing positive for HBV should be informed that **no current semen preparation technique can select HBV DNA-free spermatozoa** for use in ART.
- ❑ Considering that all the female partners of HBV positive males should be **immunised prior** to ART, measurement of **HBV DNA in semen is not necessary**.

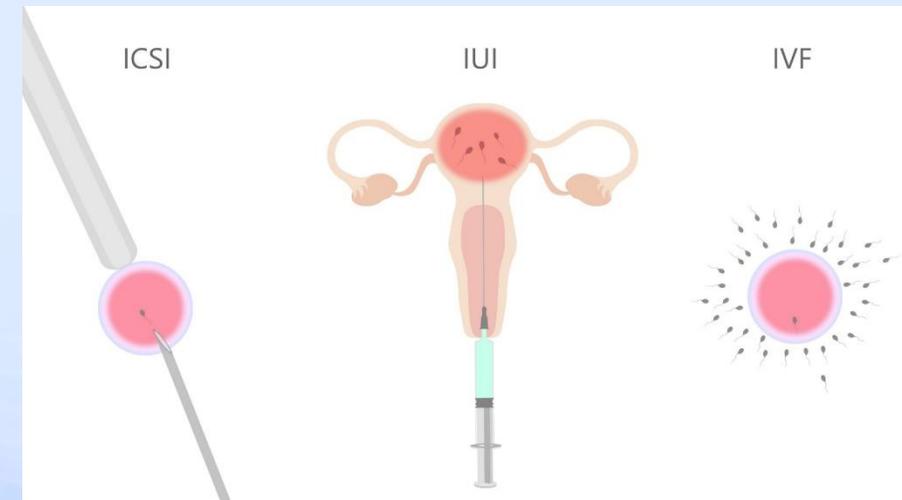
**Should IUI, IVF or ICSI be  
preferentially used for ART in HBV  
infected couples?**

# ART techniques and impact on outcomes

- ❑ No studies could be found that compare the **efficacy in terms of pregnancy rate and safety in terms of risk** of vertical transmission between different medically assisted reproductive techniques.
- ❑ Some claim that **ICSI should be applied with caution in hepatitis B**, because of the fear of the virus integrating into the developing embryo and resulting in a transfected embryo.
- ❑ From the perspective of **horizontal and vertical transmission**, there is currently **not enough evidence to recommend** one technique (IUI/IVF/ICSI) over another in patients infected with Hepatitis B.

# ART techniques and impact on outcomes

- ❑ **The cause of infertility** should dictate the specific technique (IUI/IVF/ICSI) used for ART in couples where one or both partners test positive for HBV.
  - ❑ Indication for ART is subfertility
- ❑ Some hospitals have an upper **bound limit of viral load** in which they accept patients for ICSI because of a fear of transfected embryos. **Patients with higher HBV loads** are then referred to their treating liver specialist for (temporary) treatment before ICSI is applied.



# HCV transmission

- ❑ The majority of the papers published in the literature reported a prevalence of seminal **HCV RNA varying from 0 to 30 %** using different PCR techniques .
- ❑ **High plasma HCV viral load is likely to be predictive** of the presence of HCV RNA in semen. Strong evidence for the correlation of HCV viral load between serum and semen is currently lacking.
- ❑ **No studies** could be retrieved investigating the **integration of HCV in sperm.**

# HCV and sperm parameters

- ❑ In general, **semen parameters** in **hepatitis C** are **normal** according to WHO criteria; in some studies, progressive motility was decreased.
- ❑ In a study that selected HCV positive men with no history of infertility, the duration of HCV infection correlated negatively with **semen volume and semen motility**.
- ❑ It is speculated that HCV **might stimulate oxidative stress**, which causes impaired spermatogenesis in general and impaired motility in particular.

# HCV and sperm parameters

*Eur J Intern Med.* 2012 Jan;23(1):e19-24. doi: 10.1016/j.ejim.2011.08.011. Epub 2011 Sep 3.

## **Sperm DNA damage in patients with chronic viral C hepatitis.**

La Vignera S<sup>1</sup>, Condorelli RA, Vicari E, D'Agata R, Calogero AE.

### ⊕ Author information

#### **Abstract**

**INTRODUCTION:** The aim of this study was to evaluate the conventional and biofunctional parameters of sperm in young infertile patients with Hepatitis C (HCV) infection.

**METHODS:** Forty HCV patients with primary infertility, aged 27 to 42 years (mean 36.4 years) and twenty HCV patients with secondary infertility aged 28 to 45 years (mean 35.0±2.8 years), underwent hormonal and sperm analysis in addition to the determination of reactive oxygen species (ROS) concentrations in the sperm and flow-cytometric evaluation. The following biofunctional sperm parameters were evaluated by flow cytometry: DNA fragmentation, mitochondrial membrane potential, chromatin condensation, and the rate of early apoptosis.

**RESULTS:** Overall, patients with HCV showed significantly worse median values of conventional and biofunctional sperm parameters than control subjects, including sperm density (31.7 vs. 80.4 million/ml), forward motility (9.4 vs. 25%), normal forms (15.4 vs. 24.8%), DNA fragmentation (6.6 vs. 2.2%), low MMP (45.5 vs. 8%), an early apoptosis rate (5 vs. 2.7%), and abnormal chromatin (18.9 vs. 13.9%). Finally, HCV patients had significantly higher basal (250 vs. 75×10<sup>3</sup>/cpm) and stimulated (550 vs. 120×10<sup>3</sup>/cpm) ROS levels in semen compared to control subjects. None of the examined parameters (sperm, hormonal, biofunctional and assessment of oxidative status in the semen) was significantly different between HCV patients with primary and secondary infertilities.

**DISCUSSION:** These results confirm that HCV infection has a negative impact on sperm parameters. The overlap of the results observed in the two groups of HCV patients supports the hypothesis that HCV infection may cause to alterations in sperm parameters.

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- ❑ To our knowledge, there are **no studies** that describe the effects of the newer **antivirals on semen quality.**

# Prevention of transmission during ART

- ❑ After semen-washing, **HCV was no longer detectable**, whereas HCV was detected in 20.4 % of 153 samples before semen-washing
- ❑ A discontinuous **gradient** centrifugation followed by **swim-up** and **washing** is recommended for semen processing in patients testing positive for HCV.
- ❑ Current evidence shows that semen can test **positive for HCV after single continuous density centrifugation** or after discontinuous density centrifugation without wash steps.
- ❑ After advanced semen processing, **PCR testing for HCV is not necessary.**

# ART techniques and impact on outcomes

- ❑ From the perspective of **horizontal and vertical transmission**, there is currently **not enough evidence to recommend one technique (IUI/IVF/ICSI)** over another in patients infected with Hepatitis C.
- ❑ In theory, **the diagonal transmission of HCV is possible via ICSI**. In HCV-positive men semen-washing procedures are performed with ICSI to diminish the chance of horizontal and diagonal transmission.

# ART techniques and impact on outcomes

- ❑ **Most researchers believe** that a sequential preparation with density–gradient centrifugation–washing–swim-up is recommended for HCV-positive men, similar to HIV semen preparation. **Other authors do not consider HCV** to be a sexually transmitted disease and for this reason believe that it is **unnecessary to perform sperm-washing**.
- ❑ **Should fertile HCV-discordant couples be treated?**

Some researchers believe that **it is not necessary** if they do not need reproductive assistance.

- ❑ **Indication for ART are prevent of transmission and subfertility**

# Human Immunodeficiency Virus (HIV)

- ❑ Until recently, these couples were directed to **sperm donation, adoption or abandoning** their plans of having children
- ❑ **ASRM** has stated that fertility services **cannot be withheld ethically** from individuals with chronic HIV infections, if a center has the resources to provide care.
- ❑ **Centers that do not have the resources** or facilities to provide care should assist in referral to a center with protocols in place to manage such patients.
- ❑ Thanks to the use of sperm-washing and ARTs, a **noticeably large number of births of offspring free from HIV fathered by infected males** has been reported, which is the proof that this theory is correct.

# HIV transmission

- ❑ The HIV is present in the semen of asymptomatic men as cell-free HIV-RNA particles **in seminal plasma and as a cell-associated** virus in non-spermatozoal cells, such as **lymphocytes and macrophages**.
- ❑ **Studies in the earlier days** of HIV claim that HIV-1 DNA might be present in spermatozoa and spermatogonial stem cells, **but later** studies have contradicted these findings.
- ❑ **HIV co-receptors** which are necessary for the cellular entry of HIV-1, have not been demonstrated on the spermatozoal surface.

# HIV transmission

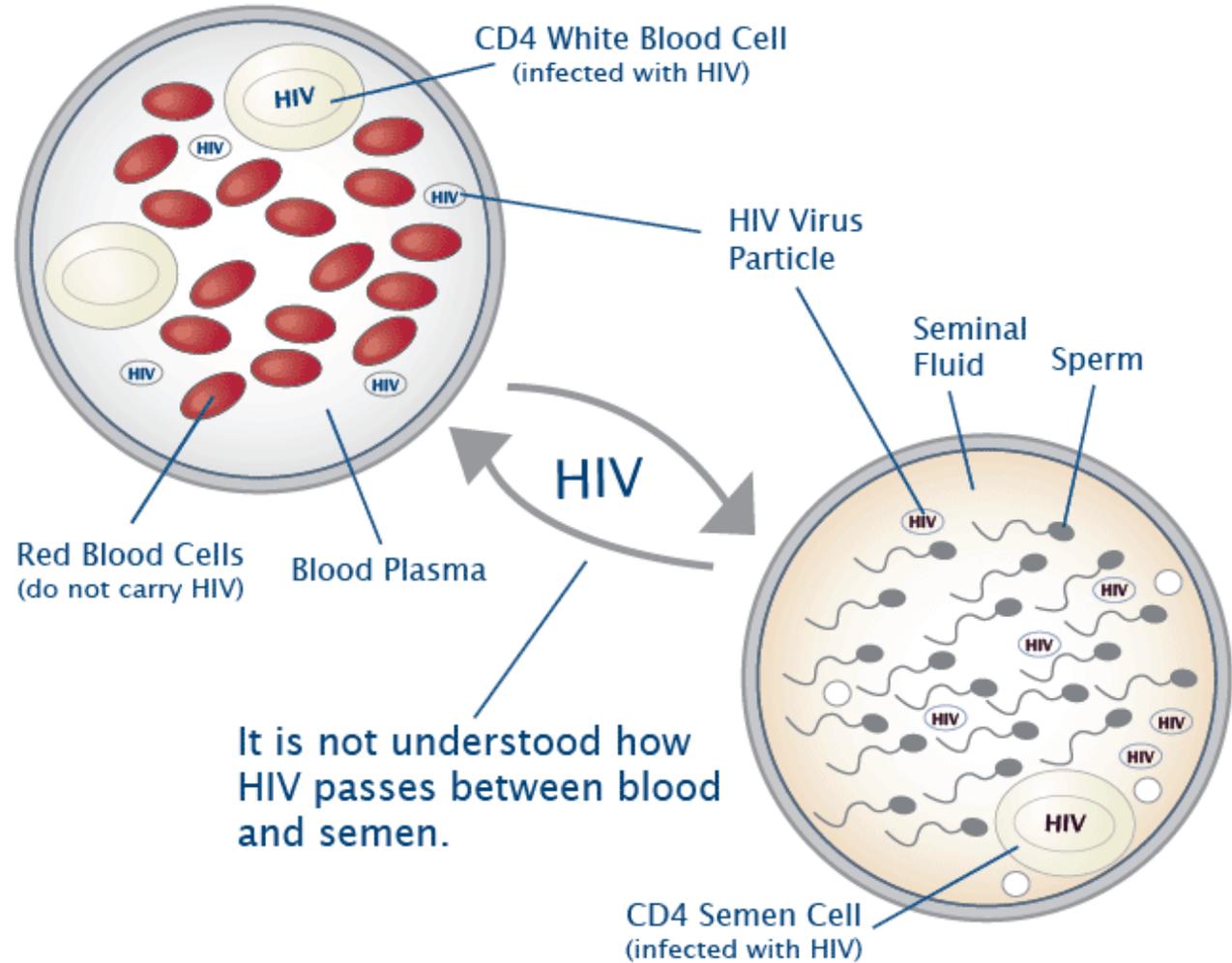
- **The virus particles** were found between the **plasma membrane and the outer acrosomal membrane** in the sperm head, the neck or in the mitochondrial districts. The particles did have the diameter of a virus particle, but **they never showed a nucleoid-like structure**, hence the authors concluded that viral particles were found in the sperm cytoplasm and these represented infecting but **not replicating virions**.

# HIV transmission

Research suggests that virus replication is compartmentalized between blood and semen.

**An undetectable viral burden in blood may not indicate an undetectable burden in semen.**

Treating HIV in blood may not treat HIV in semen.

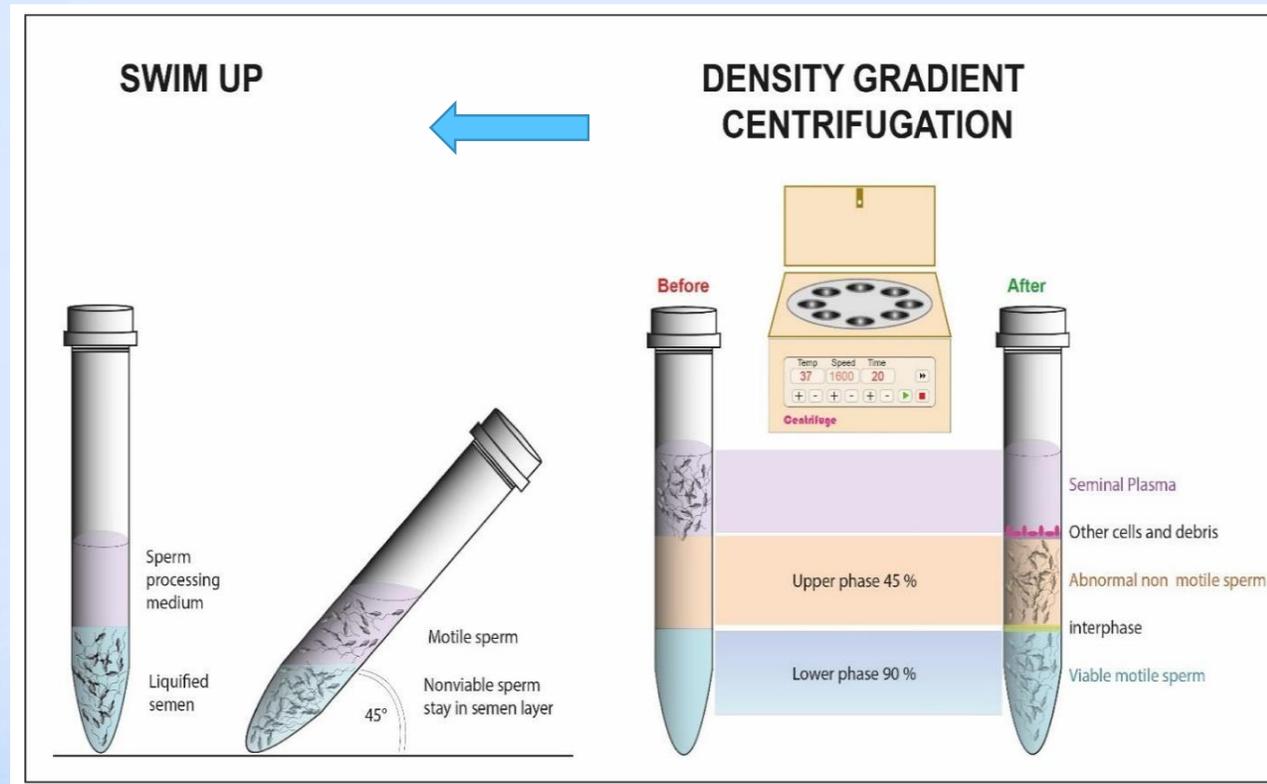


# HIV and sperm parameters

- ❑ It appears that, **semen parameters are not impaired** by asymptomatic HIV infection, although some studies reported that some semen parameters, i.e. **concentration, volume and progressive motility**, are impaired compared with HIV-negative men.
- ❑ It is not known at present if **some cART regimes** are more harmful to spermatozoa than others. **Most antiretrovirals penetrate** well into the male genital tract.

# Prevention of transmission during ART

- ❑ The technique recommended for processing ejaculated semen for males testing positive for HIV is to perform a **discontinuous density gradient centrifugation** followed by 2 **semen washing steps**, followed by **swim-up**.



# Prevention of transmission during ART

Reproductive BioMedicine Online (2016) ■■■, ■■■-■■■



www.sciencedirect.com  
www.rbmonline.com



ELSEVIER

ARTICLE

## Semen residual viral load and reproductive outcomes in HIV-infected men undergoing ICSI after extended semen preparation

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\* Corresponding author. E-mail address: rvassena@eugin.es (R Vassena).

- ❑ Semen was processed **via triple density gradient (90-70-45%)** centrifugation followed **by swim-up**. Even after this preparation, **1.86%** of the samples tested positive for HIV-1.

**Is there a need for PCR testing of  
Post-Washing sperm?**

# Prevention of transmission during ART

- ❑ Regardless of the semen processing technique used, the post-preparation sample that is going to be used in ART from males tested positive for HIV **should be HIV PCR tested.**
- ❑ In serodiscordant couples with the male testing positive for HIV, **only a HIV negative tested sperm sample should be used for ART.**
- ❑ There are some case reports describing preparation techniques for surgical retrieved sperm in males tested positive for HIV **obtaining HIV-negative samples which can be used for ICSI.**

# ART techniques and impact on outcomes

- ❑ The cause of infertility should dictate the specific technique **(IUI/IVF/ICSI)** used for ART in couples where one or both partners test positive for HIV.
- ❑ The current evidence shows that **safety is equal** in all ART techniques after specific semen processing.

# Summary of the available evidence

	Type of infection	Vaccine available	Horizontal / sexual transmission	Horizontal transmission during MAR
HBV	Acute / Persistent	Yes	Yes	Yes → Vaccinate unaffected partner
HCV	Acute / Persistent	No	Limited	Limited
HIV	Acute / Persistent	No	Yes	Yes → Semen processing for males

# Cross-contamination

- ❑ Cross-contamination between **infected and uninfected** patients and samples can potentially occur during clinical procedures and during subsequent laboratory procedures such as insemination, injection, incubation and cryopreservation.
- ❑ In addition, **HBV and HCV can remain viable** in dried blood on environmental surfaces at room temperature.
- ❑ Two cases of **nosocomial transmission of HCV** in patients attending the ART centre for ICSI/FIV have been reported. In both cases the contaminated patient had had follicular puncture immediately after the HCV infected patient puncture one.

# Infection Risk Related to Cryopreservation

- ❑ **Bacteria, fungi and viruses** have been shown to survive in liquid nitrogen (LN2) cryobanks. Viruses have been detected after storage at -70°C for HIV (9.1 years), HBV (4 years) and HCV (9 years).
- ❑ In fact, these viruses are well known to have the **ability to survive, and retain their virulence, in liquid nitrogen.**
- ❑ One study showed **viral transmission through LN2**, related to **physical damage** of stored material. In this study, transmission of HBV via damage to infected stored bone marrow was shown, which led to six patients becoming infected with the virus.

# Laboratory safety

- ❑ Therefore, the laboratory used for ART should be a “viral risk” area separated from the laboratory used for couples negative for HIV, HBV, HCV.

Andrea Borini  
Valeria Savasi *Editors*

# Assisted Reproductive Technologies and Infectious Diseases A Guide to Management

European Society of Human  
Reproduction and Embryology



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## Medically assisted reproduction in patients with a viral infection or disease

JULY 2021  
ESHRE guideline group for MAR in patients with viral infection/disease



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**ویراستار**  
بیبا پور

۹۷

**با تشکر از توجه شما**

# Summary of the available evidence

	Virus detected in sperm	Virus detected in oocytes	Virus detected in placenta	Virus detected in breastmilk	Impact on MAR outcome	
HBV	Yes	Yes	Yes	Yes (HBsAg)	 Contradictory data	 No effect
HCV	Probably not	Probably not	Probably not	Probably not	 Contradictory data	 Contradictory data
HIV	No*	No	Contradictory data	Yes	 No	 Yes