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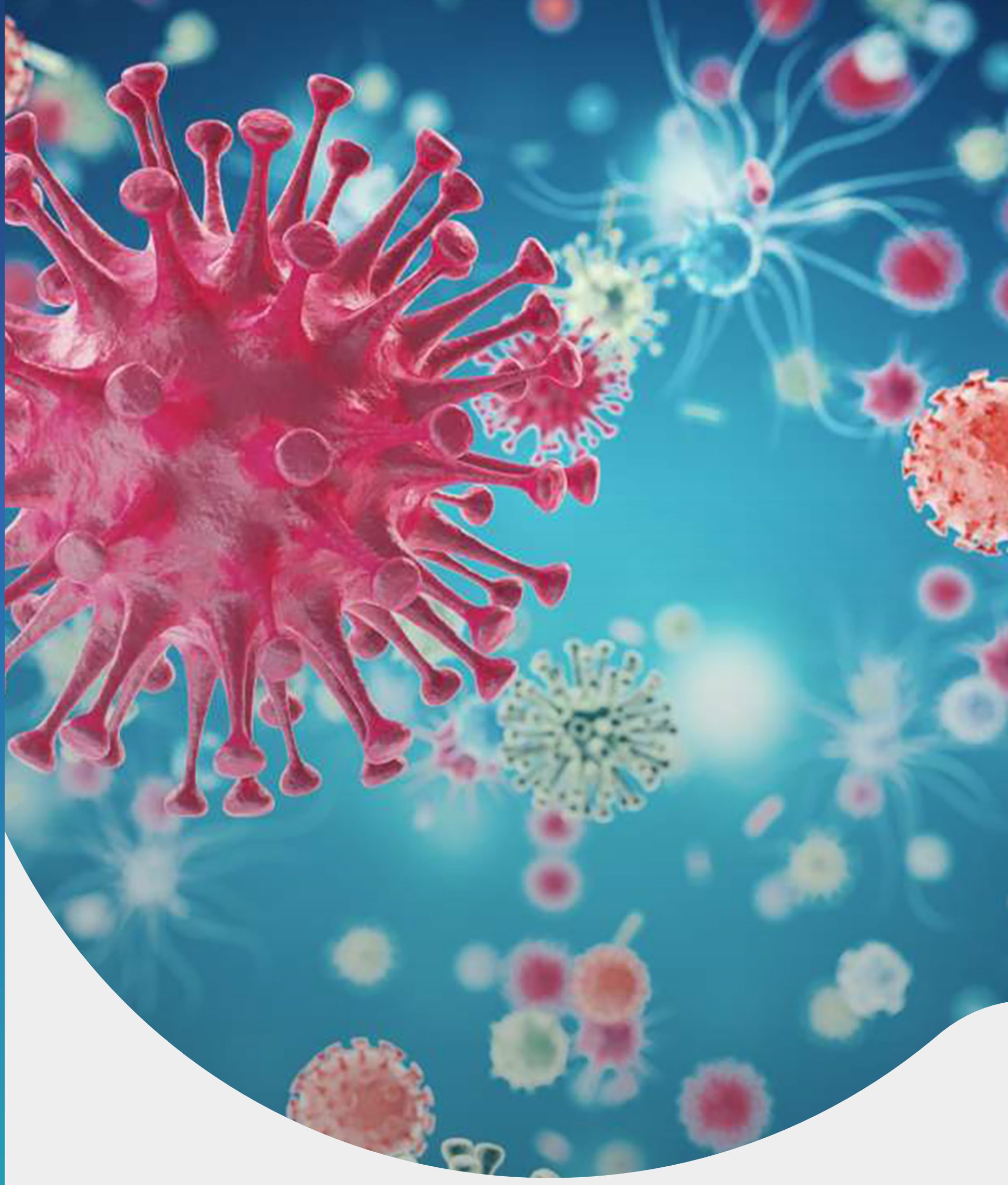

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Medically assisted reproduction in patients with a viral infection or disease

JULY 2021

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

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Introduction to the guideline

The guideline was developed according to a well-documented methodology, universal to ESHRE guidelines and described in the Manual for ESHRE guideline development (www.eshre.eu/guidelines). Details on the methodology of the current guideline are outlined in Annex 4.

The guideline development group (GDG) was composed of (previous) members of the SIG Safety and Quality in ART, Ethics and Law and the former task force on Viral Diseases, with addition of experts in the field that replied on a call for experts to the ESHRE audience. The members of the guideline development group are listed in Annex 1.

GUIDELINE SCOPE

The aim of this guideline is to provide professionals with evidence-based information on the different options for medically assisted reproduction in couples with a viral infection/disease (HBV, HCV, HIV, HPV, HTLV I/II, Zika, SARS-CoV-2). Techniques for medically assisted reproduction in this guideline refer to IUI, IVF and ICSI (Zegers-Hochschild et al., 2017).

The following issues were outside the scope of the current document: natural conception and other viral infections. Due to the complex and unique pathology of patients infected with more than one virus (such as HIV-HCV coinfection), the guideline group decided not to include coinfected patient populations in the guideline, with the exception of the question on semen processing.

TARGET USERS OF THE GUIDELINE

Treatment of active and chronic viral infections has evolved significantly over the last decade, resulting in improvements in mortality (life expectancy) and quality of life. For couples at a reproductive age, this may involve starting or growing a family. Therefore, this guideline aims to provide guidance for clinical and laboratory professionals on conditions that warrant medically assisted reproduction (MAR) and how to manage MAR in these couples. The target users include, but are not limited to, reproductive medicine specialists, obstetricians and gynaecologists, embryologists and andrologists, policy makers and regulators.

TERMINOLOGY

The current guideline applies the terms and definitions as described in the international glossary on Infertility and Fertility Care (Zegers-Hochschild, et al., 2017). Specifically, the term MAR refers to IUI, IVF and ICSI. A list of further abbreviations can be found in Annex 2.

Outcomes for this guideline

Outcomes for this guideline include:

- Safety:
  - risk of horizontal transmission to partner/family/healthcare providers
  - risk of vertical transmission to the infant
- Efficacy: implantation rates, pregnancy rates, live birth rates, miscarriage rates

REFERENCES

Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, Rienzi L, Sunde A, Schmidt L, Cooke ID *et al.* The International Glossary on Infertility and Fertility Care, 2017. *Human reproduction (Oxford, England)* 2017;32: 1786-1801.

List of all recommendations

Chapter	No.	Recommendation	Strength	Quality of evidence	Justification	Remarks
Hepatitis B virus						
Prevention of transmission before medically assisted reproduction						
A2	1	Partners of Hepatitis B virus (HBV)-positive individuals should be vaccinated.	Strong	⊕◯◯◯	The availability of highly effective vaccines outside and during pregnancy allows prevention of horizontal and vertical transmission.	
A2	2	Barrier contraception should be used until the completion of the HBV vaccination protocol.	Strong	⊕⊕◯◯	Providing a successful vaccination course, the risk of HBV horizontal transmission is eliminated during unprotected intercourse for spontaneous conception.	
A2	3	Medically Assisted Reproduction (MAR) services staff should be vaccinated against HBV.	GPP		Staff working in general healthcare are required to have HBV vaccination and to have a completed HBV vaccination schedule.	
A2	4	All patients with an active or chronic HBV-infection must be reviewed by an infection disease/ liver specialist before initiating any MAR treatment.	Strong	⊕◯◯◯	It has been reported that there is a direct correlation between maternal viral load and the risk of viral vertical transmission	
A2	5	Commencing with MAR treatments in patients positive for HBV should be a joint decision between the patient, their partner, the fertility doctor and the infectious disease/liver specialist.	Strong	⊕◯◯◯		
A2	6	In the case of the female testing positive for HBV, the possibility of viral vertical transmission, the availability of vaccination during pregnancy and newborn prophylaxis should all be discussed.	GPP			

Assisted reproduction techniques and impact on outcomes						
A3	7	The cause of infertility should dictate the specific technique (IUI/IVF/ICSI) used for MAR in couples where one or both partners test positive for HBV.	Strong	⊕◯◯◯	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.	
A3	8	Women testing positive for HBV should be informed that MAR does not eliminate the risk of vertical transmission.	GPP			
A3		HBV can be detected in sperm cells, oocytes, granulosa cells and embryos. This equates with a theoretical risk of vertical HBV transmission that remains to be proven.				Conclusion
A3		Existing evidence cannot clarify if the presence of HBV-infection in the male impacts the outcomes of MAR. Multiple studies showed no differences in reproductive outcomes following MAR when comparing seronegative with HBV-seropositive women.				Conclusion
Prevention/reduction of transmission during assisted reproduction						
A4	9	Men testing positive for HBV should be informed that no current semen preparation technique can select HBV DNA-free spermatozoa for use in MAR.	GPP			
A4	10	Routine semen processing according to the ESHRE guideline on good practice in the IVF laboratory should be used when performing MAR in men testing positive for HBV.	GPP			
A4	11	Based on the current evidence, HBV DNA testing on seminal fluid or sperm is not recommended.	Strong	⊕◯◯◯	Considering that we have recommended before that HBV negative women should be vaccinated, the measurement of HBV DNA in semen is not necessary.	
Reducing/avoiding vertical transmission						
A5	12	Caesarean delivery is not recommended on the basis of maternal HBV-positivity alone.	Strong	⊕⊕◯◯	There is no evidence that the risk of HBV transmission from mother to child after caesarean section is lower compared to that after vaginal delivery.	
A5	13	Breastfeeding is probably not contra-indicated in women testing positive for HBV.	Conditional	⊕⊕◯◯	There is no association between breastfeeding and the risk of HBV transmission from mother to child. Breastfeeding has significant health benefits.	

A5	14	All neonates born to HBV-positive couples should be vaccinated.	Strong	⊕⊕⊕◯	Current evidence shows that perinatal transmission of HBV, which is responsible for the majority of cases of chronic HBV infection, can be prevented by vaccination.
A5	15	Administration of Hepatitis B immunoglobulin (HBIG) in addition to vaccination is recommended for children born to mothers testing positive for HBV.	Strong	⊕⊕◯◯	
A5	16	HBIG administration should follow local or national guidelines.	GPP		

Hepatitis C virus

Prevention of transmission before medically assisted reproduction

B2	17	In a monogamous heterosexual relationship of more than 12 months, there is no indication for the use of barrier contraceptives to reduce the risk of Hepatitis C virus (HCV) transmission in a serodiscordant infected couple.	Conditional	⊕⊕◯◯	The large prospective studies show a very low transmission between sexual partners. The major transmission route of HCV is parenteral transmission and not sexual intercourse in the absence of STDs or medical comorbidities such as HIV or liver pathology.
B2	18	All patients with an active or chronic HCV-infection must be reviewed by an infectious disease/ liver specialist before initiating any medically assisted reproduction treatment (MAR).	GPP		
B2	19	Commencing with MAR treatments in patients positive for HCV should be a joint decision between the patient, their partner, the fertility doctor and the infectious disease/ liver specialist.	Strong	⊕◯◯◯	Vertical transmission has gained importance as the primary HCV transmission route among children once the blood products screening has been implemented
B2	20	In the case of the female testing positive for HCV, the possibility of viral vertical transmission should be discussed prior to MAR treatment.	GPP		

Assisted reproduction techniques and impact on outcomes

B3	21	The cause of infertility should dictate the specific technique (IUI/IVF/ICSI) used for MAR in couples where one or both partners test positive for HCV.	Strong	⊕◯◯◯	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.

B3	22	Women testing positive for HCV should be informed that MAR does not eliminate the risk of vertical transmission.	GPP			
B3		The possibility of HCV viral RNA presence in oocytes cannot be excluded. However, the risk of HCV transmission through the use of reproductive material remains to be proven.				Conclusion
B3		There are contradictory results evaluating effects of male HCV-infection on infertility treatments outcomes. Although the fertilization rate has been reported significantly lower in couples with HCV-RNA-positive men, other studies report that HCV-infection does not affect the IVF-ICSI cycle outcomes in these couples.				Conclusion
B3		There are contradictory results evaluating effects of female HCV infection on infertility treatments outcomes. Although some studies report significantly reduced implantation rates, higher cycle cancellations, and higher FSH use, in HCV positive women, other report no significant differences.				Conclusion
Prevention/reduction of transmission during assisted reproduction						
B4		There are no data regarding antiviral therapy in men or women with HCV without co-infections requiring MAR in order to reduce the risk of HCV transmission. None of the currently available HCV antiviral drugs are licensed for use in pregnancy.				Conclusion
Semen processing						
B5	23	A discontinuous gradient centrifugation followed by swim-up and washing is recommended for semen processing in patients testing positive for HCV.	Strong	⊕◯◯◯	Current evidence shows that semen can test positive for HCV after single continuous density centrifugation or after discontinuous density centrifugation without wash steps.	
B5	24	After advanced semen processing, PCR testing for HCV is not necessary.	Strong	⊕◯◯◯	All available evidence show that semen sample test PCR negative after advanced semen processing. In addition, the viral load in semen is low.	
B5	25	Good laboratory practice regarding semen processing should be applied irrespective of whether only the male or both partners are testing positive for HCV.	GPP			

B5		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.				Conclusion
Reducing/avoiding vertical transmission						
B6	26	Caesarean delivery is not recommended on the basis of maternal HCV-positivity alone.	Strong	⊕◯◯◯	There is no evidence that the risk of HCV transmission from mother to child after caesarean section is lower compared to that after vaginal delivery.	
B6	27	Breastfeeding is not contra-indicated in women testing positive for HCV.	Strong	⊕⊕◯◯	There is no association between breastfeeding and the risk of HCV transmission from mother to child. Breastfeeding has significant health benefits.	
Human Immunodeficiency Virus						
Prevention of transmission before medically assisted reproduction						
C2	28	Human immunodeficiency virus (HIV)-1-serodiscordant couples should be informed that there is a risk of sexual transmission of the virus to the unaffected partner. To reduce this risk, couples must be advised to use barrier contraception and seek active therapy to reduce viral load.	Strong	⊕⊕◯◯	The viral presence of HIV-1 cannot be eliminated to date, however, anti-retroviral therapy can reduce the HIV-1 viral load to undetectable levels, thereby eliminating the risk of horizontal transmission. Patients newly diagnosed with HIV infection, should get advice from an infectious disease specialist to discuss treatment options and start treatment to reduce viral load.	
C2	29	Individuals testing positive for HIV-1, antiretroviral therapy can suppress viral replication. These patients should remain on antiretroviral therapy and providing undetectable viral loads in serum can be achieved and sustained, the risk of horizontal transmission through unprotected intercourse is minimal in the absence of other sexually transmitted diseases.	Strong	⊕⊕◯◯		
C2	30	Commencing with medically assisted reproduction (MAR) treatments in patients testing positive for HIV-1 or 2 should be a joint decision between the patient, their partner, the fertility doctor and the infectious disease specialist.	Strong	⊕◯◯◯	The decision to commence MAR, including the medications to be used, should be a joint decision by the fertility specialist and the infectious disease specialist.	

C2	31	All patients testing positive for HIV, wishing to have a child should be counselled about the risk of horizontal and vertical transmission. In the case of the male testing positive for HIV, antiretroviral therapy can reduce the viral load in blood and semen to undetectable levels, allowing the possibility of natural conception. Reproductive counselling should include fertility and antiretroviral covariates.	GPP		
C2	32	In the case of the female testing positive for HIV-1 or 2, and even with undetectable viremia, the possibility of viral vertical transmission should be discussed prior to MAR treatment.	GPP		

Assisted reproduction techniques and impact on outcomes

C3	33	HIV infection status is not a reason to deny MAR treatment.	Strong	⊕◯◯◯	There are no studies published comparing different MAR techniques in similar patient populations testing positive for HIV-1. The current evidence shows that safety is equal with all MAR techniques after specific semen processing.
C3	34	The cause of infertility should dictate the specific technique (IUI/IVF/ICSI) used for MAR in couples where one or both partners test positive for HIV.	Strong	⊕⊕◯◯	
C3	35	Advanced semen processing should be used for male patients testing positive for HIV-1 to reduce the likelihood of viral presence.	Strong	⊕◯◯◯	Viral DNA and RNA can be detected in semen and spermatozoa of males testing positive for HIV, also co-incubation experiments show the presence of HIV viral-like particles in spermatozoa.
C3	36	No special laboratory techniques are needed for processing of oocytes from female patients testing positive for HIV.	Strong	⊕◯◯◯	Viral DNA and RNA cannot be detected in oocytes when co-incubation experiments with HIV are performed. It is unlikely that HIV-1 will bind to and infect oocytes.
C3	37	Serodiscordant couples with a male partner testing positive for HIV-1 should be informed that the efficacy of MAR is not impacted compared to HIV-seronegative couples.	Strong	⊕◯◯◯	MAR efficacy in HIV serodiscordant couples are not negatively impacted by the HIV-1 infection of the male partner. Therefore, couples requiring MAR may achieve comparable results as HIV seronegative couples.
C3	38	Serodiscordant couples with a female partner testing positive for HIV should be informed that the efficacy of IVF/ICSI could be reduced compared to HIV-seronegative couples.	Conditional	⊕◯◯◯	MAR efficacy in HIV serodiscordant couples is conflicted by the HIV infection of the female partner.

Semen processing					
C5	39	The technique recommended for processing ejaculated semen for males testing positive for HIV is to perform a density gradient centrifugation followed by 2 semen washing steps, followed by swim-up.	Strong	⊕⊕◯◯	The discontinuous density gradient, followed by 2 wash steps combined with a swim-up has been most described in literature and has been proven to be effective, the technique is not 100% failure proof.
C5	40	Regardless of the semen processing technique used, the post-preparation sample that is going to be used in MAR from males tested positive for HIV should be HIV PCR tested.	Strong	⊕⊕◯◯	Studies show that post-preparation semen samples can test HIV positive. Therefore, semen samples should be PCR tested before use.
C5	41	In serodiscordant couples with the male testing positive for HIV, only a HIV-negative tested sperm sample should be used for MAR.	Strong	⊕⊕◯◯	
C5	42	Good laboratory practice regarding semen processing should be applied irrespective of whether only the male or both partners are testing positive for HIV.	GPP		
C5	43	Advanced semen processing is recommended for male patients testing positive for HIV, regardless of the viral load in the serum and therapy status.	Strong	⊕◯◯◯	Although there are studies reporting on various correlation coefficients between HIV viral load in semen and blood, it is clear that there is no definite strong correlation between the parameters.
Reducing/avoiding vertical transmission					
C6	44	Caesarean section is recommended in women with detectable HIV viral loads.	Strong	⊕⊕◯◯	Published cohort data from the European countries have shown vertical transmission rates of <0.5% in women with plasma HIV RNA <50 HIV RNA copies/mL on antiretroviral therapy, irrespective of mode of delivery. The risk of transmission increases significantly with increased viral load.
C6	45	A female testing positive for HIV should refrain from breastfeeding when and where she has safe nutritional alternatives.	Strong	⊕⊕◯◯	In the Europe and other high-income settings, the safest way to feed infants born to women with HIV is with formula milk, as there is on-going risk of HIV exposure after birth.
C6	46	Combined neonatal prophylaxis (CNP) is recommended for neonates born to mothers testing positive for HIV.	Strong	⊕⊕⊕◯	There is no doubt that the introduction of antiretroviral prophylaxis of neonates has significantly reduced the rates of vertical transmission of HIV.

Human Papilloma Virus

Prevention of transmission before medically assisted reproduction

D2	47	The use of barrier contraception during sexual intercourse is advised to lower the risk of Human Papilloma virus (HPV) transmission.	GPP			
D2	48	All women starting medically assisted reproduction (MAR) should undergo testing to detect HPV-related cervical lesions.	GPP			
D2		There is no evidence that there is a specific HPV DNA copy number threshold below which (horizontal or vertical) transmission is unlikely.				Conclusion

Assisted reproduction techniques and impact on outcomes

D3	49	The cause of infertility should dictate the specific technique (IUI/IVF/ICSI) used for MAR in couples where one or both partners test positive for HPV.	Strong	⊕◯◯◯	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 HPV.	
D3	50	Women infected with HPV should be informed that MAR does not eliminate the risk of vertical transmission.	GPP			
D3	51	The possibility of HPV testing could be discussed with couples undergoing IUI.	Research only			
D3	52	Couples with a known positive HPV test should be advised that HPV is a transient infection, and postponing MAR treatment is an option depending on the individual circumstances.	GPP			

Prevention/reduction of transmission during assisted reproduction

D4		There is weak evidence that therapeutic HPV vaccination in HPV-positive men may increase pregnancy rates in natural conception and reduce miscarriage rates. However, more studies are necessary.				Conclusion

D4	53	HPV-positive males should be informed that no current semen preparation technique can eliminate the virus from the infected semen sample.	GPP			
Reducing/avoiding vertical transmission						
D5	54	Caesarean delivery is not recommended on the basis of maternal HPV-positivity alone.	Strong	⊕⊕◯◯	Current evidence does not support the use of caesarean section to lower the risk or prevent mother-to-infant transmission of HPV.	
D5	55	Breastfeeding is probably not contra-indicated in HPV-positive women.	Conditional	⊕◯◯◯	Transmission of HPV to the offspring by breastfeeding is very rare. To date there is no evidence of harm to the newborn by vertical transmission of HPV.	
Human T-cell lymphotrophic virus I/II						
Prevention of transmission before medically assisted reproduction						
E2	56	It is suggested to inform Human T-cell lymphotrophic virus (HTLV) I/II-serodiscordant couples that there is a risk of sexual transmission of the virus to the unaffected partner. To reduce this risk, couples could be advised to use barrier contraception and receive reproductive counselling if they want to conceive.	Conditional	⊕◯◯◯	There is a risk of sexual transmission of HTLV I/II. The risk appears to be higher from male to female.	
E2		Based on current evidence, we cannot define a threshold of HTLV I/II viral load below which horizontal or vertical transmission of HTLV I/II is not occurring.				Conclusion
Assisted reproduction techniques and impact on outcomes						
E3	57	The cause of infertility should dictate the specific technique (IUI/IVF/ICSI) used for medically assisted reproduction (MAR) in couples where one or both partners test positive for HTLV I/II.	Strong	⊕◯◯◯	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 HTLV I/II.	
E3	58	Women testing positive for HTLV I/II should be informed that MAR does not eliminate the risk of vertical transmission.	GPP			

E3		Studies on HTLV I/II viruses are dated and the technology to detect these viruses has changed a lot since. Therefore, the possibility of HTLV I/II presence in gametes or placenta cannot be confirmed or excluded. To date, the risk of HTLV I/II transmission through the use of infected semen or oocytes remains to be proven.				Conclusion
E3		The impact of female HTLV I-infection on MAR outcomes remains unknown.				Conclusion
Prevention/reduction of transmission during assisted reproduction						
E4		There are no techniques known for prevention/reduction of transmission of HTLV I/II during MAR.				Conclusion
Reducing/avoiding vertical transmission						
E5	59	Caesarean delivery is not recommended on the basis of maternal HTLV I/II-positivity alone.	Strong	⊕◯◯◯	There is only very limited and low quality evidence comparing the risk of vertical transmission between vaginal and caesarean delivery.	
E5	60	A female testing positive for HTLV I/II should refrain from breastfeeding when and where she has safe nutritional alternatives.	Strong	⊕◯◯◯	Current evidence indicates that breastfeeding is associated with an increased risk of vertical transmission of HTLV I. Therefore, avoiding breastfeeding should be considered in women testing positive for HTLV I/II when and where safe nutritional alternatives exist.	
Zika virus						
Prevention of transmission before medically assisted reproduction						
F2	61	A male diagnosed with ZIKV-infection or returning from a ZIKV endemic region should use barrier contraception with any partner, for 3 months.	GPP			
F2	62	A female diagnosed with ZIKV-infection or returning from a ZIKV endemic region should use barrier contraception and avoid pregnancy for 2 months.	GPP			

F2		There is no agreed threshold described in the literature below which transmission is unlikely. We advocate the use of barrier contraception to prevent horizontal transmission and avoiding pregnancy for 3 months after diagnosis or return from a ZIKV endemic area to reduce vertical transmission.				Conclusion
Assisted reproduction techniques and impact on outcomes						
F3	63	If a patient or partner has been diagnosed with ZIKV-infection or returning from a ZIKV endemic region in the last 3 months, medically assisted reproduction (MAR) treatment should be postponed.	GPP			
F3	64	In case of fertility preservation, the approach should be tailored to the individual situation.	GPP			
F3	65	In the case of fertility preservation, there is insufficient data on the risk of viral transmission using gametes potentially infected with ZIKV. An individual risk assessment is advised before using these gametes.	GPP		There is insufficient evidence on the association between Zika infection and gametes or potential of transmission to offspring in the absence of maternal infection	
F3	66	If ZIKV-infection is diagnosed in male or female during MAR treatment, the cycle should be stopped, and the couple should be advised to use barrier contraception for 3 months.	GPP			
Prevention/reduction of transmission during assisted reproduction						
F4		There are currently no semen processing techniques available that can completely remove ZIKV from semen.				Conclusion
F4	67	MAR is not advised even if male serum is free of ZIKV because of poor correlation between serum and semen viral load.	Strong	⊕◯◯◯	All infected patients, regardless of viral load, may be infectious through semen. The clearance of Zika virus is slower from semen compared to blood. Therefore, a negative test in plasma/serum does not offer 100% reassurance.	

Reducing/avoiding vertical transmission						
F5		ZIKV has been found in breast milk of women with confirmed ZIKV-infection. The possibility of transmission of ZIKV through breastfeeding has only been assessed in 12 mother-child pairs. This provides insufficient evidence to establish a recommendation.				Conclusion
Laboratory safety						
H	68	Since viruses can survive and be transmitted via liquid nitrogen (LN2), separate storage of reproductive cells according to viral positive and viral negative status is recommended.	GPP		There is a lot of variation in practice. Some clinics store all viral positive samples together, and some clinics have separate storage per type of virus.	
H	69	Emptied and dried cryo tanks and transport shippers should be disinfected according to local standard operating procedures to reduce the potential of cross-contamination.	GPP			
H	70	Individual clinics must risk assess to decide the number of cryo tanks needed.	GPP			
H	71	Separate cryopreservation dewars should be used to quarantine gametes and embryos from patients with unknown infectious status.	GPP		There is a lot of variation in practice. Some clinics store all viral positive samples together, and some clinics have separate storage per type of virus.	
H	72	Vapour phase cryopreservation could be considered over liquid nitrogen in terms of safety to reduce the risk of cross-contamination.	Conditional	⊕◯◯◯	No storage environment can guarantee 100% prevention of cross-contamination. Current evidence shows that the risk of cross-contamination is smaller with the use of vapour phase as compared to liquid nitrogen.	
H	73	Provided the cryomaterial is not compromised, cryodevices such as sealed semen straws/vials should be cleaned with a disinfectant wipe after removal from LN2 storage to mitigate risk of transmission of pathogens from the cryodevice surface.	GPP			

H	74	Hermetical sealing of cryovials with additional covers could reduce the risk of cross-contamination of stored material.	Conditional	⊕◯◯◯	LN2 infiltrates inner thread vials, therefore increasing the risk of cross-contamination or explosion of the vial upon warming. Sealing the vials, using a membrane resistant to liquid nitrogen can overcome this issue.
H	75	The use of high security straws in combination with thermal sealing is the preferred approach as it minimises the risk of cross-contamination.	Strong	⊕◯◯◯	Cryostorage devices should provide closure integrity and sample stability, without risk of infection from the cryo tank.
H	76	At the time of thawing, decontamination of the exterior of the straw and the single use of sterile scissors will reduce the risk of contaminating the stored contents with potential pathogens.	GPP		
H	77	Given that personal protective equipment (PPE), laboratory equipment and exposed surfaces can be contaminated even after good laboratory practice, disinfection and changing PPE between cases can prevent cross-contamination	GPP		
H	78	The recommended procurement, processing, release and storage procedures should be used for all samples, not only virally positive samples.	GPP		

Table 1: Summary of the available evidence on the topics included in the guideline.

	Type of infection	Vaccine available	Horizontal / sexual transmission	Horizontal transmission during MAR	Prevention of vertical transmission by CS	Vertical transmission via breastfeeding	Prophylaxis in neonate
HBV	Acute / Persistent	Yes	Yes	Yes → Vaccinate unaffected partner	Probably not	Probably not	Yes
HCV	Acute / Persistent	No	Limited	Limited	Probably not	Probably not	No
HIV	Acute / Persistent	No	Yes	Yes → Semen processing for males	If detectable viral load	Yes	Yes
HPV	Transient	Yes	Yes	Yes	Probably not	Probably not	No
HTLV I/II	Acute / Persistent	No	Yes	Yes	Unknown	Yes	No
ZIKV	Transient	No	Yes	Yes	Probably not	Unknown	No

Table 1 Continued.

	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
HPV	Yes	No data	Contradictory data	Yes	Yes	Unclear
HTLV I/II	No data	No data	No data	Yes	No data	Probably not
ZIKV	Yes	No data	Yes	Yes	No data	No data

** only viral-like particles the size of HIV have been detected in spermatozoa.*

PART A: Hepatitis B virus

A1. Prevalence and testing

NARRATIVE QUESTION: WHAT IS THE PREVALENCE OF HEPATITIS B VIRUS?

Hepatitis B virus (HBV) is an enveloped DNA virus, and a member of the family Hepadnaviridae hepatotropic DNA viruses. Hepatitis B virus causes both acute and chronic infection that can range from asymptomatic infection or mild disease to severe or fulminant hepatitis. In 2015, the global prevalence of HBV infection in the general population was 3.5%. Prevalence was the highest in the African (6.1%), Western Pacific regions (6.2%) and Eastern Mediterranean region (3.3%). In Europe, 30 member' states reported 26,907 cases of HBV infections for 2017, corresponding to a crude rate of 6.7 cases per 100 000 population. Twenty countries reported HBV chronic infections, leading to an overall notification rate of 7.2 cases per 100,000 population. The United Kingdom reported 62% of all chronic cases in 2017 (ECDC, 2017). Overall, about 257 million persons were living with HBV infection. However, rates of chronic Hepatitis B cases are dependent on local notification requirements. Many infected people remain undiagnosed. Among those born before the Hepatitis B vaccine became available, the proportion of persons living with chronic HBV infection remains high. Approximately 15–40 % of chronically infected patients will develop liver cirrhosis, liver failure, or hepatocellular carcinoma and 15–25 % will ultimately die as a result of their HBV infection. Mortality due to HBV infection is increasing and expected to further increase.

In 2016, the World Health Assembly adopted the Global Health Sector Strategy (GHSS) on viral hepatitis, committing to eliminating viral hepatitis as a public health threat by 2030. The synergistic interventions for prevention, testing and treatment are at the core of an effective hepatitis response and are promoted through the GHSS on viral hepatitis. Assuming that women of reproductive age constitute 25.3% of the world's population (United Nations data), adults chronically infected may include 65 million women of childbearing age who can potentially transmit HBV to their babies (Lavanchy and Kane, 2016). Most of the burden of disease from HBV infection comes from infections acquired before the age of 5 years (ECDC, 2017). Therefore, prevention of HBV infection focuses on children under 5 years of age. In the 1990s, the World Health Assembly had already asked for the inclusion of Hepatitis B vaccine in routine infant immunization schedule. The low incidence of chronic HBV infection in children under 5 years of age at present can be attributed to the widespread use of Hepatitis B vaccine. Worldwide, in 2015, the estimated prevalence of HBV infection in this age group was about 1.3%, compared with about 4.7% in the pre-vaccination era (which, according to the year of introduction can range from the 1980s to the early 2000s). So, most of the people currently living with HBV infection are persons born before Hepatitis B vaccine was widely available and used in infancy. However, there are regional differences in coverage. The African, Eastern Mediterranean and European regions remain below the global average (WHO, 2017).

NARRATIVE QUESTION: HOW SHOULD TESTING OF HEPATITIS B STATUS PRIOR TO MEDICALLY ASSISTED REPRODUCTION BE PERFORMED?

Serological assays are typically used as the first line of the testing strategy to screen for exposure to a virus because of their relatively low cost (compared to nucleic acid testing (NAT)). Serological tests for the detection of Hepatitis B (HB) e-antigen and anti-HBe antibody may also aid in the management of the patient and are widely available. For the diagnosis of chronic HBV infection in adults, adolescents and children (>12 months of age), a serological assay (in either rapid diagnostic test (RDT) or laboratory-based immunoassay format (enzyme immunoassay or chemiluminiscence immunoassay) that meets minimum quality, safety and performance standards (with regard to both analytical and clinical sensitivity and specificity) is recommended to detect Hepatitis B surface antigen (HBsAg). In settings where existing laboratory testing is already available and accessible, laboratory-based enzyme immunoassays (EIAs) are recommended as the preferred assay format. A cut-off value, usually determined by the manufacturer of the assay, specifies the point at which the results are considered to be reactive, and therefore, EIA results are generally reported as optical density divided by the assay cut-off (OD/CO) values. These types of assays are best suited for and most cost–effective to perform in settings with a high throughput of specimens (in excess of 40 per day). In settings where there is limited access to laboratory testing and/or in populations where access to rapid testing would facilitate linkage to care and treatment, use of RDTs is recommended to improve access.

- In settings or populations with an HBsAg seroprevalence of ≥0.4%, a single serological assay for detection of HBsAg is recommended, prior to further evaluation for HBV DNA and staging of liver disease.
- In settings or populations with a low HBsAg seroprevalence of <0.4%, confirmation of HBsAg positivity on the same immunoassay with a neutralization step or a second different RDT assay for detection of HBsAg may be considered. Conditional recommendation, low quality of evidence

Directly following a positive HBsAg serological test, the use of quantitative or qualitative NAT for detection of HBV DNA is recommended as the preferred strategy and to guide who to treat or not treat if there is no evidence of cirrhosis, and to monitor for treatment response, based on existing recommendations from the 2015 WHO HBV management guidelines (WHO, 2015). These assays detect the presence of viral nucleic acid – DNA through targeting a specific segment of the virus, which is then amplified. The amplification step enables the detection of low levels of the virus in the original specimen, which might not otherwise have been detectable. Serum HBV DNA is measured in international units (IU)/mL as the recognized international standard or copies/ml by NAT.

HB core antibody (HBcAb) is a marker of past HBV exposure, the guideline review did not look for evidence on this specific antibody.

Conclusion

HBV testing is mandatory according to the European Tissues and Cells Directive as a preventative measure to reduce the risks of transmission to partners and offspring.

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A2. Prevention of transmission before medically assisted reproduction

PICO QUESTION: WHAT ARE THE RISKS OF HEPATITIS B VIRUS TRANSMISSION THROUGH VAGINAL/ANAL INTERCOURSE?

Evidence

A cross-sectional study including 203 participants testing positive for Hepatitis B virus (HBV) and their 138 sexual partners tested for HBV antigen (Ag) and antibody (Ab) to determine current and past HBV infections in sexual partners of HBV index cases. Of the 138 sexual partners, 28 (20.3%) were vaccinated for HBV, 20 (14.5%) tested positive for HBsAg and 36 (26.1%) had evidence of past and current HBV infection. Female sexual partners were significantly more likely to get infected compared to male partners (crude OR 2.31, 95% CI 1.01 to 5.29). Furthermore, partners who were cohabiting were more likely to get infected (OR 3.95, 95% CI 1.73 to 9.04) compared to sexual partners who were not cohabiting (Tufon et al., 2019).

A large cross-sectional study including 2590 individuals testing positive for HBsAg and their 1454 spouses (1003 females and 451 males) measured HBsAg on serum samples of all partners and reported the rate of HBV-exposure (HB core antigen (HBcAb) positivity) to be 48% (n=480) in female spouses, 62.9% (n=281) in male spouses. However, HBsAg was positive in only 2.3% (n=33) of the spouses (4.2% in husbands and 1.4% in wives) (Katoonizadeh et al., 2018).

A small cohort study included 5 index cases testing positive for HBV and their partners (married or engaged) and investigated the HBV sequence homology between spouses. For all five couples, the HBV-infected index subject and the spouse shared a 100% sequence homology for the cloned region (Huo et al., 1998).

An old study including 83 patients with acute HBV infection, investigated the sexual transmission in these patients and found that 18/24 sex partners of included patients tested positive for HBsAg and HBV DNA (Hou et al., 1993).

A cross-sectional study including 1368 females reported that heterosexual transmission was the only risk factor for disease acquisition in 27% of females with a positive HBV test. Furthermore, having anal intercourse and failure to use barrier contraceptives may facilitate transmission of HBV infection to women (Rosenblum et al., 1992).

A prospective cohort study, including 68 pregnant women testing positive for HBsAg and their husbands, investigated the sexual transmission between partners and reported that 30/68 husbands were positive (44.2%), 11.8% were Ag positive and 32.4% were Ab positive. Furthermore, they concluded that transmission occurs particularly if sexual contact takes place during or immediately after menstruation (Inaba et al., 1979).

Recommendation

Partners of Hepatitis B virus (HBV)-positive individuals should be vaccinated.	Strong	⊕◯◯◯
Barrier contraception should be used until the completion of the HBV vaccination protocol.	Strong	⊕⊕◯◯
Medically assisted reproduction (MAR) services staff should be vaccinated against HBV.	GPP	

Justification

Multiple studies have shown that sexual transmission of HBV may double the risk of horizontal and vertical transmission. The availability of highly effective vaccines outside and during pregnancy allows prevention of horizontal and vertical transmission.

Providing a successful vaccination course, the risk of HBV horizontal transmission is eliminated during unprotected intercourse for spontaneous conception.

Staff working in general healthcare are required to have HBV vaccination and to have a completed HBV vaccination schedule. For this reason, staff employed by MAR services should follow the same occupational health protocols.

PICO QUESTION: IS THERE A THRESHOLD BELOW WHICH TRANSMISSION OF HEPATITIS B VIRUS IS UNLIKELY?

Evidence

Horizontal transmission

No studies could be identified reporting a serum Hepatitis B virus (HBV) DNA load threshold below which horizontal transmission does not occur.

Mother-to-child transmission (MTCT)

No publications could be identified where maternal viral load was determined before pregnancy.

Father-to-child transmission (FTCT)

Only a single retrospective study including 398 couples (spontaneous pregnancy, no semen processing) of males testing positive for HBV with uninfected female partners. There was decreased HBV vertical transmission from father to infant with lower HBV DNA in paternal serum, whatever HBsAb levels in mother. HBV DNA was not detected when paternal serum HBV DNA was $<10^2$ IU/ml. However, tests

have been performed on blood cord and not after immunoprohylaxis and vaccination in infant (Cao et al., 2016).

There was no evidence of HBV vertical transmission through MAR in the circumstances of a HBV negative woman and a HBV positive male partner.

Conclusion

Based on the current evidence, in the circumstances of medically assisted reproduction, it is not possible to identify a pre-treatment HBV DNA load threshold below which vertical transmission is very unlikely.

Recommendation

All patients with an active or chronic HBV-infection must be reviewed by an infectious disease/ liver specialist before initiating any MAR treatment.	Strong	⊕○○○
Commencing with MAR treatments in patients positive for HBV should be a joint decision between the patient, their partner, the fertility doctor and the infectious disease/ liver specialist.	Strong	⊕○○○
In the case of the female testing positive for HBV, the possibility of viral vertical transmission, the availability of vaccination during pregnancy and newborn prophylaxis should all be discussed.	GPP	

Justification

Consulting a hepatologist is an important step before considering a pregnancy as it allows an assessment of both partners and prophylactic vaccination of the uninfected partner.

Most studies investigating the threshold of HBV transmission analysed viral load during pregnancy or at delivery as opposed to before pregnancy (pre-treatment). Any HBV-infected mother carries the risk of vertically transmitting the virus to the newborn (Liu et al., 2015, Lu et al., 2017, Sellier et al., 2015, Wiseman et al., 2009).

Therefore, it should be noted that maternal vaccination and neonate immunoprophylaxis significantly reduces but does not eliminate the risk of vertical HBV transmission.

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A3. Assisted reproduction techniques and impact on outcomes

PICO QUESTION: SHOULD IUI, IVF OR ICSI BE PREFERENTIALLY USED FOR MAR IN HEPATITIS B INFECTED COUPLES?

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.

Evidence

A prospective cohort study compared IVF with ICSI in 125 women testing positive for Hepatitis B virus (HBV). In total, 176 children were born in the assisted reproduction group, 145 by IVF and 31 by ICSI. When twins were considered as one, the rate of positive HBsAg in IVF children, 5.9% (6/102), was lower than that in ICSI children, 13% (3/23), although the difference was not statistically significant. When twins were considered as two, no difference was found in the rate of HBsAg-positive IVF children as compared with ICSI children (4.8% (7/145) vs. 12.9% (4/31)). All HBsAg-positive children received Hepatitis B Immune globulin (HBIG) treatment and seroconverted to negative at 9-15 months of age (Nie et al., 2019).

Conclusion

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.

Recommendation

The cause of infertility should dictate the specific technique (IUI/IVF/ICSI) used for MAR in couples where one or both partners test positive for HBV.	Strong	⊕○○○
Women testing positive for HBV should be informed that MAR does not eliminate the risk of vertical transmission.	GPP	

Justification

Only vaccination against HBV and maintenance of measurable antibody levels can protect a woman from HBV infection and her child from vertical transmission. In women testing positive for HBV, the HBV viral DNA level dictates the risk of vertical transmission. Even in the circumstances of infant prophylaxis, the risk of vertical transmission is not zero, yet very low (Boucheron et al., 2021).

PICO QUESTION: CAN HEPATITIS B VIRUS DNA BE DETECTED IN OOCYTES/ SPERM/ PLACENTA?

Evidence

DNA integration in sperm

In an experimental study, 233 sperm metaphase spreads from 9 males testing positive for HBV were analysed for DNA integration of HBV. Only one patient had HBV DNA integration in the sperm genetic material (Huang et al., 2003).

DNA integration in oocytes/embryos

A prospective cohort study including 72 HBV serodiscordant couples (31 male HBsAg positive, and 41 female HBsAg positive) investigated the relation between HBsAg positivity of oocytes and embryos and the risk of vertical transmission. Twelve infants were born to couples with HBV positive oocytes/embryos: 2 with HBV DNA positive oocytes/embryos, 7 with HBV RNA positive oocytes/embryos and 3 with HBsAg positive oocytes/embryos. Twenty children tested anti-HBs positive. At 6 months, only 1 infant was seropositive for anti-HBs, anti-HBc, and anti-HBe, however, this child had seroconverted by 9 months (Jin et al., 2016).

In the study by Kong et al. ovarian tissues from 50 patients with gynaecological disease and HBV positivity were used to investigate HBV expression and replication in ovum. HBcAg was detected in 12% of ovarian tissue samples (6/50). HBV DNA was detected in the interstitial cells, granulosa cells, and ova in ovarian tissues at a positive rate of 14% (7/50). Three samples were positive for HBV mRNA (3%). Positive signal of HBV mRNA was mainly distributed in the cytoplasm of the ova and the granulosa cells. Patients with detectable HBV markers in ovaries had a higher level of serum HBV DNA (Kong et al., 2016).

In the study by Hu et al. 250 oocytes and 578 embryos that were not used for IVF-ICSI from HBV positive couples were analysed for presence and expression of HBV. HBV DNA was found in 9.6% of oocytes (24/250) and in 14.4% of embryos (83/578). A significant increase in viral positivity in oocytes and embryos was found in women with a high serum HBV DNA level (Hu et al., 2011).

In a report by Quint et al., culture medium for embryo culture in IVF was contaminated with HBV infected serum. HBV DNA could not be demonstrated by PCR in any of the children of mothers exposed to HBV during IVF (Quint et al., 1994).

Placenta

Wei et al. investigated 155 placentae from women testing positive for HBsAg and reported that the total rate of placentae testing positive for HBsAg was 37.42% (58/155) by immunohistochemistry. Furthermore, the placental positivity for HBsAg was higher in mothers testing positive for HBeAg (OR 2.00; 95% CI 1.02 to 3.95). The risk of an HBsAg-positive placenta was higher with increasing maternal blood HBV DNA levels (the relative risk estimate OR was 3.24 to 3.85) (Wei et al., 2015).

Chen et al. investigated the role of placental HBV infection in vertical transmission of HBV to the newborn. Hereto, they collected 157 placental tissue samples from 171 pregnant women testing positive for HBV. The rate of placental HBsAg-positivity by immunohistochemistry (IHC) was 36.9% (58/157) and the rate of HBcAg was 31.8% (50/157). HBV DNA was detected in 42.7% of cases (67/157) by RT-PCR and in situ hybridisation (ISH) showed that the HBV infection rate was 55.4% (87/157) in

decidual cells, 51.0% (80/157) in trophoblastic cells,46.5% (73/157) in villous mesenchymal cells, and 29.9% (47/157) in villous capillary endothelial cells (Chen et al., 2013).

In a case-control study, placental tissue from 101 women testing positive for HBsAg was investigated. IHC and ISH showed that HBsAg was present in 33.7% of placental samples (34/101), HBxAg in 37.6% (38/101), HBcAg in 20.8% (21/101) and HBV DNA in 44.6% of placental samples (45/101). Furthermore, the HBV infection rates decreased gradually from the maternal side to the fetal side (Xu et al., 2002).

Conclusion

HBV can be detected in sperm cells, oocytes, granulosa cells and embryos. This equates with a theoretical risk of vertical HBV transmission that remains to be proven.

PICO QUESTION: DOES HEPATITIS B VIRUS AND/OR TREATMENT OF HEPATITIS B VIRUS BEFORE MAR IMPACT THE OUTCOME OF MAR?

MALE INFECTED

Evidence

Male infected

A retrospective cohort study, including 66 Hepatitis B (HBV)-serodiscordant couples and 68 controls, compared IVF-ICSI cycles outcomes and reported no significant differences between HBV serodiscordant couples and controls for implantation rate (34.5% (20/58) vs. 25.3 (25/99)), pregnancy rate per cycle (25.8% (17/66) vs. 30.9% (21/68)), miscarriage rate per cycle (17.6% (3/17) vs. 33.3% (7/21)) or live birth rate per cycle (21.2% (14/66) vs. 19.1% (13/68)) (Cito et al., 2019).

A retrospective cohort study included 92 serodiscordant couples with active HBV infection (HBV DNA+), 125 serodiscordant couples with convalescent infection (HBsAg+, HBeAb+, HBcAb+, HBV DNA negative) and 121 seronegative controls. In the couples where ejaculated sperm was used, there was no significant difference between couples with active or convalescent HBV infection or controls for implantation rate (28.3% (45/159) vs. 32.8% (39/122) vs. 23.0% (38/165)), clinical pregnancy rate (44.2% (34/77) vs. 50.8% (30/59) vs. 38.5% (30/78)), early miscarriage rate (8.8% (3/34) vs. 0% (0/30) vs. 6.7% (2/30)) or live birth rate (36.4% (28/77) vs. 49.2% (29/59) vs. 35.9% (28/78)). In the couples where surgically retrieved sperm was used, the early miscarriage rate was significantly higher in couples with convalescent HBV infection as compared to active HBV infection and controls (0% vs. 23.1% (3/13) vs. 5.0% (1/20)). However, there were no significant differences between active and convalescent HBV infection and control couples for implantation rate (31.1% (28/90) vs. 26.1% (18/69) vs. 25.3% (24/95)), clinical pregnancy rate (50% (22/44) vs. 39.4% (13/33) vs. 42.6% (20/47)) or live birth rate (50% (22/44) vs. 27.3% (9/33) vs. 36.2% (17/47)) (Zheng et al., 2016).

A retrospective cohort study including 136 males testing positive for HBsAg and 426 HBV-seronegative controls reported no significant differences for implantation rate (38.5% (104/270) vs. 37.7% (206/547)) or clinical pregnancy rate (58.1% (79/136) vs. 53.7% (146/272)) between groups (Shi et al., 2014).

A matched case-control study including 32 males testing positive for HBsAg and 64 controls reported no difference for implantation rate (13.5% vs. 20.0%), clinical pregnancy rate per cycle (18.8% (6/32) vs. 31.3% (20/64)) or live birth rate per cycle (15.6% (5/32) vs. 23.4% (15/64)) between groups (Oger et al., 2011).

A retrospective cohort study including 916 patients testing positive for HBV (457 HBsAg and 459 HBsAg negative) compared the reproductive outcomes of 1824 IVF-ICSI cycles and reported no significant differences in reproductive outcomes after IVF between HBV positive and negative men for implantation rate (24.9% (284/1140) vs. 26.7% (296/1108)) or clinical pregnancy rate (40.5% (217/535) vs. 40.3% (210/521)). However, ICSI in HBV positive males resulted in a significantly lower implantation rate (18.3% (126/688) vs. 24.2% (159/657)) and clinical pregnancy rate (31.2% (96/308) vs. 39.3% (118/300)) as compared to controls (Zhou et al., 2011).

A retrospective cohort study analysing IVF-ICSI reproductive outcomes in HBV-serodiscordant couples (n=161) reported no significant difference between seropositive and seronegative men in ongoing pregnancy rates per started cycle (30.4% vs. 29.9%) (Lee et al., 2010).

Female infected

A retrospective cohort study investigated IVF-ICSI reproductive outcomes in HBsAg+/HBeAg+ women (n=180) with HBsAg+/HBeAg- women (n=714) and seronegative controls (7565). The implantation rate was significantly lower in HBsAg+/HBeAg- women as compared to controls (35.7% (607/1701) vs. 38.7% (6950/17939)), but not in HBsAg+/HBeAg+ women as compared to controls (39.6% (158/399) vs. 38.7% (6950/17939)). There was no significant difference between HBsAg+/HBeAg+ women, HBsAg+/HBeAg- women and controls for clinical pregnancy rate (61.7% (111/180) vs. 57.6% (411/714) vs. 60.4% (4628/7656)), miscarriage rate (11.7% (13/111) vs. 10.0 (41/411) vs. 11.7% (541/4628)) or live birth rate (53.1% (93/175) vs. 51.1% (360/704) vs. 52.3% (3911/7480)) (Wang et al., 2019).

A retrospective case-control study comparing IVF-ICSI cycle outcomes from chronic HBV-serodiscordant couples (n=123 cycles) with matched HBV-negative couples (246 cycles) reported no significant differences between chronic HBV-infected couples and matched HBV-negative controls for implantation rate (30.52% (76/249) vs. 28.34% (142/501)), clinical pregnancy rate (44.72% (55/123) vs. 43.09% (106/246)) or live birth rate (42.28% (52/123) vs. 40.65% (100/246)) (Chen et al., 2014).

A retrospective cohort study including 136 males testing positive for HBsAg and 426 HBV seronegative controls reported no significant differences for implantation rate (36.0% (54/150) vs. 38.5% (117/304)) or clinical pregnancy rate (48.1% (37/77) vs. 50.6% (78/154)) between groups (Shi, et al., 2014).

A retrospective cohort study analysing IVF-ICSI reproductive outcomes in HBV-serodiscordant (n=131) couples reported no significant difference between seropositive and seronegative women in ongoing pregnancy rates per started cycle (26.7% versus 30.2%) (Lee, et al., 2010).

Conclusion

Existing evidence cannot clarify if the presence of HBV infection in the male impacts the outcomes of MAR. Multiple studies showed no differences in reproductive outcomes following MAR when comparing seronegative with HBV seropositive women.

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A4. Prevention/ reduction of transmission during assisted reproduction

PICO QUESTION: WHICH TECHNIQUES CAN BE USED TO PREVENT/ REDUCE HEPATITIS B TRANSMISSION DURING MAR?

VACCINATION

Evidence

No studies could be retrieved that compare vaccinating female partners of Hepatitis B virus (HBV) positive males versus not vaccinating.

Recommendation

Partners of HBV-positive individuals should be vaccinated.	Strong	⊕○○○

Justification

The availability of highly effective HBV vaccines allows prevention of horizontal and vertical transmission.

SEMEN PROCESSING

Evidence

A pilot experiment, including sperm samples from 4 males testing positive for Hepatitis B virus (HBV), hypothesized that a specific ICSI preparation technique (swim-up), separating spermatozoa based on differences in motility, can be used to isolate spermatozoa free from HBV DNA in order to perform ICSI in men with chronic HBV-infection. No HBV DNA was detected in the fraction containing immotile or progressive spermatozoa. In one nonprogressive spermatozoa fraction, HBV DNA was found, however, it was not quantifiable (<15 IU/L) (Condijts et al., 2020).

Recommendation

Men testing positive for HBV should be informed that no current semen preparation technique can select HBV DNA-free spermatozoa for use in MAR.	GPP

Routine semen processing according to the ESHRE guideline on good practice in the IVF laboratory should be used when performing MAR in men testing positive for HBV.	GPP

Justification

The study by Condijts is a pilot study and needs further confirmation (Condijts, et al., 2020). Currently there are no semen processing techniques that are able to select HBV DNA free spermatozoa.

Any further questions on semen processing will not be discussed.

PICO QUESTION: DOES THE PLASMA VIRAL LOAD CORRELATE WITH HEPATITIS B VIRUS DETECTION IN SEMEN?

Evidence

Four retrospective studies, including 211 HBsAg+ patients, suggest that HBV DNA can be observed in semen (Ayoola et al., 1981, Fei et al., 2015, Hadchouel et al., 1985, Qian et al., 2005) with lower titers of HBV DNA in semen as compared to serum (Hadchouel, et al., 1985, Qian, et al., 2005). Hepatitis serologic status could be correlated with HBV in semen, with the combination of serum HBV DNA and HBeAg as best predictors to identify those men with positive semen HBV DNA (Fei, et al., 2015).

Recommendation

Based on the current evidence, HBV DNA testing on seminal fluid or sperm is not recommended.	Strong	⊕◯◯◯

Justification

It is suggested that not all chronically HBV infected men harbour HBV DNA in sperm and that lower HBV DNA titers could be found in sperm as compared to serum. Considering that all female partners of HBV positive males should be immunised prior to MAR, the measurement of HBV DNA in semen is not necessary.

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A5. Reducing/ avoiding vertical transmission

PICO QUESTION: WHICH INTERVENTIONS CAN BE USED TO REDUCE/AVOID VERTICAL TRANSMISSION OF HEPATITIS B VIRUS TO THE NEWBORN?

ELECTIVE CAESAREAN SECTION

Evidence

A systematic review and meta-analysis investigated the mother-to-child transmission rate of Hepatitis B virus (HBV) after caesarean section (CS) and vaginal delivery (VD). When assessed at birth, 7.2% (151/1940) of newborns tested serological positive for HBV after CS compared to 16.6% (301/1813) after vaginal delivery (OR 0.269; 95% CI 0.139 to 0.520; 7 studies; 3904 mother-infant pairs). When assessed at 6 months of age, the difference between both groups was no longer present with 3.3% (132/4022) infants testing serological positive for HBV after CS compared to 4.1% (145/3520) after vaginal delivery (OR 0.790; 95% CI 0.614 to 1.016; 13 studies; 7542 mother-infant pairs) (Chen et al., 2019).

A prospective cohort study including 1384 pregnant women testing positive for HBV compared 867 CS with 517 vaginal deliveries and reported no significant difference in the proportion of neonates testing positive HBV DNA between the CS group and the vaginal delivery group (0.7% vs. 1.7%). However, after follow-up, 0.6% of infants (5/888) in the CS group compared to 1.7% of infants (9/519) in the vaginal delivery group were identified as having chronic Hepatitis B infection (Peng et al., 2018).

A prospective cohort study compared the rate of HBV transmission to infants after vaginal birth or CS. All infants received HBV vaccination and 239/447 infants also received Hepatitis B immunoglobulin (HBIG). Infants who received HBV vaccination alone had a similar rate of HBV infection whether delivered by CS or vaginally. In the infants who received HBV vaccine plus HBIG at birth, however, the HBV infection rate was significantly lower in those delivered by CS (3/53) than in those delivered vaginally (57/286) (Lee et al., 1988).

Recommendation

Caesarean delivery is not recommended on the basis of maternal HBV-positivity alone.	Strong	⊕⊕◯◯

Justification

There is no evidence that the risk of HBV transmission from mother to child after caesarean section is lower compared to that after vaginal delivery.

BREASTFEEDING

Evidence

A systematic review and meta-analysis investigated the effect of feeding practices on the vertical transmission of HBV and found that breastfeeding does not increase the risk of HBV infection in the infant (risk difference -0.8%, 95% CI -1.6% to 0.1%, 32 studies, 5650 infants) (Zheng et al., 2011).

In the study by Azzari et al., breast milk samples from all included women were tested for HBV markers, and reported that all samples tested positive for HBsAg, anti-HBcAg and anti-HBeAg. However, all samples tested negative for HBeAg and HBV DNA. No data on the seroconversion of the included infants were reported (Azzari et al., 1990).

A large cohort study by Zhang et al., including 1186 mothers testing positive for HBsAg and their infants, investigated HBV markers in the infants according to breast or bottle feeding. In mothers testing negative for HBeAg none of the infants were HBV infected no matter what immunization they received. In formula fed infants of mothers testing positive for HBeAg, there was no difference in HBV infection whether they received vaccination only, or vaccination with HBIG. In breastfed infants of mothers testing positive for HBeAg there were significant differences in rates of HBV transmission between the vaccination only group (7/26, 26.9%) and the vaccination with HBIG group (4/106, 3.8%) (Zhang et al., 2014a).

A retrospective cohort study, including 544 mothers testing positive for HBsAg and their 546 infants, investigated the effect of feeding practices on HBV transmission to the infant. Chronic HBV infection occurred in 1.5% (6/397) of breastfed children and 4.7% (7/149) of formula-fed children respectively. After adjusting for confounding factors such as maternal HBeAg status, breastfeeding was not associated with chronic HBV infection (adjusted OR 0.99, 95% CI 0.28 to 3.58) or with the self-resolved infection (adjusted OR 0.57, 95% CI 0.20 to 1.65) in the infants (Chen et al., 2013).

Recommendation

Breastfeeding is probably not contra-indicated in women testing positive for HBV.	Conditional ⊕⊕◯◯

Justification

There is no association between breastfeeding and the risk of HBV transmission from mother to child. Breastfeeding has significant health benefits.

This recommendation is supported by the guidelines of the European Association for the Study of the Liver (EASL) (Lampertico et al., 2017).

VACCINATION

Evidence

A systematic review and meta-analysis investigated efficacy of vaccination to prevent the mother-to-child transmission of HBV. For infants of mothers testing positive for HBsAg (including those who did

and did not receive Hepatitis B immunoglobulin (HBIG)), vaccine efficacy ranged from 79 to 98% (7 studies). The median seroprotection proportion across all studies including HBsAg-positive and HBsAg-negative mothers was 98% (range 52% to 100%) (Schillie and Murphy, 2013).

An older systematic review and meta-analysis also investigating the efficacy of vaccination to prevent HBV-infection in the infant reported that compared to placebo or no intervention, vaccination significantly decreased the risk of Hepatitis B occurrence (RR 0.28, 95% CI 0.20 to 0.40, 4 studies) (Lee et al., 2006).

In a randomized controlled trial, including 238 women testing positive for HBsAg, early vaccination (within 2 days of birth, at 1 and 2 months) was compared with delayed vaccination (at 3,4 and 5 months). None of the newborns tested positive for HBsAg at 3 months of age. Subclinical HBV infection developed in 2 infants from the early vaccination programme. Furthermore, the anti-HB concentrations were significantly higher with the delayed vaccination programme at 11 and 24 months of age (Schalm et al., 1989).

In a large prospective cohort study, including 863 mothers testing positive for HBsAg and their corresponding 871 infants, 2 dosages of HBV vaccine were compared. No immunoprophylaxis failure was observed with the higher vaccine dose (0/565), compared to 5.2% (16/306) with the lower dose. All seroconverted infants were born to mothers testing positive for HBeAg with a viral load over $4x10^7$ IU/ml (Wang et al., 2016).

In a prospective cohort study, including mothers testing positive for HBsAg, 6 different prophylaxis regimes were compared. Over the years, there was a gradual decline in the proportion of high anti-HBs level in all groups. The HB antibody levels were significantly higher (45.6%) with regime B (2, 3, 8 months), compared to regime A (0, 1, 6 months) (30.9%) or regime C (0, 1, 2 months) (30.7%). Furthermore, the use of regime B offered higher protection from anti-HBc seroconversion after 2 years of age (Young et al., 2003).

A small cohort study, including 81 children from asymptomatic HBsAg carrier mothers, compared vaccination only with vaccination in combination with HBIG. After 5 years, no statistical difference was observed between the HB antibody titers in infants that received vaccination and HBIG and vaccination only (Gonzalez et al., 1993).

Recommendation

All neonates born to HBV-positive couples should be vaccinated.	Strong	⊕⊕⊕◯

Justification

Current evidence shows that perinatal transmission of HBV, which is responsible for the majority of cases of chronic HBV infection, can be prevented by vaccination. This recommendation is supported by the guidelines of the European Association for the Study of the Liver (EASL) (Lampertico, et al., 2017).

Hepatitis B Immune globulin (HBIG)

Evidence

A systematic review and meta-analysis compared newborns injected with HBV vaccine and Hepatitis B immune globulin (HBIG) to infants injected with HBV vaccine and maternal HBIG during pregnancy. The meta-RR comparing these 2 groups for newborn HBsAg infection rate was 0.66 (95% CI 0.52 to 0.84; 7 RCTs; 1061 infants) at birth, 0.54 (95% CI 0.42 to 0.69; 12 RCTs; 1453 infants) at 7-12 months of age and 0.54 (95% CI 0.42 to 0.69; 7 RCTs; 1214 infants) after 12 months of age. Newborns receiving vaccination and HBIG had a higher amount of protective antibodies at birth (RR 2.12; 95% CI 1.66 to 2.70; 4 RCT; 291 infants), and at 7-12 months of age (RR 1.12; 95% CI 1.03 to 1.22; 8 RCT; 566 infants) but not after 12 months of age (RR 1.06; 95% CI 0.96 to 1.16; 5 RCT) (Jin et al., 2014).

Another systematic review and meta-analysis compared HBV vaccination only with HBV vaccination and HBIG in infants from HBsAg+/HBeAg- mothers and reported no difference in seroprotection rate (OR 1.24; 95% CI 0.97 to 1.58; 4 studies; 1323 patients) (Machaira et al., 2015).

A randomised control trial, including 202 infants born to mothers testing positive for HBsAg, compared one dose of HBIG at birth with a 3-dose regime (at birth, 3 and 6 months) with placebo or saline. All infants but 2 in the placebo group tested positive for HBsAg at 3 months of age, compared to 50% in the group receiving one dose of HBIG and 23% in the 3-dose regimen group (Beasley et al., 1981).

In a prospective cohort study, including mothers testing positive for HBsAg, two dosages of HBIG (100 and 200 IU) were compared. All infants received a 3-dose vaccination as well. At 7 months, there was no significant difference in the number of perinatal infected infants (1.5% (8/545) vs. 1.9% (12/632)) (Wei et al., 2018).

A prospective cohort study, including 90 women testing positive for HBsAg and their infants, compared 3 HBIG regimes. In the first group, the infants received vaccination only. In the second group, the infants received vaccination and a dose of HBIG within 2 hours after birth. In the third group, the mothers received 3 dosages of HBIG during pregnancy and the infants were vaccinated and received a birth dose of HBIG. The number of infants testing positive for HBs antibodies was 24/30, 27/30 and 29/30 respectively (Gong and Liu, 2018).

A prospective cohort study, including 1150 women testing positive for HBsAg, compared vaccination only with vaccination plus HBIG for infant immunoprophylaxis and reported that immunoprophylaxis failure was significantly higher in the vaccination only group compared to vaccination plus HBIG (RR 0.371, 95 % CI 0.167 to 0.825) (Zhang et al., 2014b).

A prospective cohort study, including 324 women testing positive for HBsAg, compared HBIG for the mothers with HBIG for the infants, both mother and infant and no HBIG for either mother or infant. Compared with the no-HBIG group, HBIG for both the mother and infant group had the lowest HBsAg-positive rate (OR 0.14; 95% CI 0.02 to 0.90), whereas HBIG for the infant group had the lowest HBsAb-positive rate (OR 0.07; 95% CI 0.02 to 0.23). The HBsAg-positive rate of the no HBIG group was 14.3% (Guo et al., 2012).

A large prospective cohort study, including 1010 infants born to women testing positive for HBsAg, compared vaccination only (4 dosages) with vaccination plus a birth dose of HBIG. The antibody titer at 1 and 2 months of age was significantly higher in infants who had received HBIG at birth. However, at

6, 9, and 18 months old there was no statistically significant difference in antibody titer between the two groups (Wheeley et al., 1991).

A prospective cohort study, including infants of women testing positive for HB s- and e-antigen, compared 3 prophylaxis regimes. The first group received a dose of HBIG at birth and at 3 months of age, at which time vaccination was initiated. The second group received HBIG at birth and vaccination was initiated at 4-7 days old. The third group also received HBIG at birth but initiated vaccination at 1 month old. 9/159 (5.7%) infants treated with any of the HBIG vaccine schedules became chronic HBsAg carriers, of which 4 were already positive before vaccination was started. The HBsAg carrier rate was 2.0%, 6.0%, and 8.6% among infants in the three prophylaxis schedules; the differences were not statistically significant (Beasley et al., 1983).

A retrospective cohort study, including 298 mothers testing positive for HBsAg and their infants, reporting 11 infants (3.7%) testing positive for HBsAg, and 16 (5.4%) testing negative for HBsAg but anti-HBc positive, indicating past resolved infection. Of the 11 children infected with HBV, only one received timely administration of both HBIG and Hepatitis B vaccine, and 10 others did not receive HBIG or received delayed Hepatitis B vaccine. Of the 16 children with the resolved infections, 9 were not administered with HBIG and one was given the first dose of vaccine 40 days after birth (Hu et al., 2012)

A cross-sectional study, including 4112 women testing positive for HBsAg and their infants, reported that for infants receiving the birth dose of HBV vaccine between 12-24h after birth the adjusted odds of HBV transmission was 1.9 times higher than infants immunized within 12h after birth (2.4% vs. 0.6%; adjusted OR 2.9; 95% CI 1.4 to 6.3). Furthermore, they reported no significant association between HBV transmission and HBIG administration (Qiao et al., 2019).

Recommendation

Administration of Hepatitis B immunoglobulin (HBIG) in addition to vaccination is recommended for children born to mothers testing positive for HBV.	Strong	⊕⊕◯◯
HBIG administration should follow local or national guidelines.	GPP	

Justification

HBIG provides passively acquired anti-HBs and temporary protection. HBIG can augment protection until a response to vaccination is attained.

The Advisory Committee on Immunization Practices (ACIP) recommends administration of Hepatitis B vaccine and Hepatitis B immune globulin (HBIG) for infants born to HBV-infected women within 12 hours of birth, followed by completion of the vaccine series and postvaccination serologic testing (Schillie et al., 2018). Furthermore, this recommendation is supported by the guidelines of the European Association for the Study of the Liver (EASL) (Lampertico, et al., 2017).

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Summary

HBV	Male testing positive	Female testing positive	Couple testing positive
Before MAR	Vaccinate non-infected partner		
	Consult with infectious disease / liver disease specialist		
		Discuss: - Risk of viral vertical transmission (not eliminated by MAR) - Newborn prophylaxis	
During MAR	IUI, IVF or ICSI depending on infertility work-up		
	Routine semen processing		
After MAR	Caesarean section not recommended		
	Breastfeeding not contra-indicated		
	Vaccination of the neonate	Vaccination of the neonate + HBIG administration	

Figure 1: Summary of management of medically assisted reproduction in patients testing positive for Hepatitis B virus.

PART B: Hepatitis C Virus

B1. Prevalence and testing

NARRATIVE QUESTION: WHAT IS THE PREVALENCE OF HEPATITIS C VIRUS?

In 2015, 71 million persons were living with chronic Hepatitis C virus (HCV) infection with uneven global epidemic and differences across and within countries (WHO, 2017). The Eastern Mediterranean Region reporting the highest number of HCV RNA positive persons (±15 million), followed by the Western Pacific Region and European Region (both ± 14 million), the South-East Asia Region and African Region (both ± 10 million) and the Region of the Americas reporting the lowest number of HCV RNA positive persons (± 8 million).

The overall global prevalence of HCV infection in 2015 was 1%, with the highest estimated prevalence in the Eastern Mediterranean Region (2.3%), followed by the European Region (1.5%), the African Region (1.0%), the Region of the Americas and Western Pacific Region (both 0.7%) and the South-East Asian Region with the lowest estimated prevalence (0.5%).

In the European Union and Economic Area Countries the overall HCV prevalence in 2015 was estimated to be 1.1% (95% CI 0.9-1.4), equaling 5.6 million anti-HCV positive cases. The estimated prevalence of anti-HCV of the majority of countries was < 1%. Spain, Slovakia, Greece, Latvia, Romania and Italy had an estimated anti-HCV prevalence of 1.1%, 2.0%, 2.2%, 2.4%, 3.2% and 5.9% respectively (Hofstraat et al., 2017). In the Central European Region the estimated anti-HCV positive prevalence varies between 0.2% and 2.1% (Urbanek et al., 2016) and the estimated HCV RNA positive prevalence between 0.2% and 3.5% (Madalinski et al., 2015).

The global estimates of HCV prevalence appear to decrease in the last years (Cornberg et al., 2011, Lanini et al., 2016, WHO, 2017).

Unsafe health-care-practices and injection drug use remain the leading modes of transmission. In high income countries the prevalence of chronic HCV is generally below 2%. However, there is variability within countries with similar socio-economic conditions because of differences in size of high-risk population. Countries with the highest prevalence (>5%) are mainly low-middle income countries and include Egypt, Gabon, Uzbekistan, Cameroon, Mongolia, Pakistan, Nigeria, Georgia (Lanini, et al., 2016).

NARRATIVE QUESTION: HOW SHOULD TESTING OF HEPATITIS C STATUS PRIOR TO MEDICALLY ASSISTED REPRODUCTION BE PERFORMED?

Virological markers of Hepatitis C virus (HCV) infection are anti-HCV antibodies, HCV core antigen, HCV RNA and HCV genotype (Pawlotsky, 2002).

Screening for HCV is based on detection of total HCV antibodies (IgM and IgG). Enzyme immunoassay (EIA) and chemiluminescence immunoassay (CIA) are most commonly used for detection of anti HCV antibodies in serum. Whole blood or plasma also qualify. Serological tests have evolved over time yielding several generations of EIA's. Still anti-HCV assays have disadvantages (e.g. prolonged window of detection after infection, low positive predictive value) and therefore confirmation tests are widely used (Cobb et al., 2014, Saludes et al., 2014, Uliana et al., 2014).

In recent years rapid diagnostic tests (RDTs) enabled screening for HCV antibodies at a lower cost compared to classical EIAs (Chevaliez and Pawlotsky, 2018, Smith et al., 2012).

Performance of the different HCV antibody tests varies (Khuroo et al., 2015, Tang et al., 2017).

Confirmatory antibody testing can be done with recombinant immunoblot assays (RIBA) for individuals who have tested positive by EIA. A main problem of RIBA is the occurrence of intermediate results (Ponde, 2013).

Confirmation of an HCV infection and circulating viral genome is based on detection of HCV RNA. Nucleic Acid Technology (NAT) is the gold standard and most commonly used as confirmation test (Benjamin, 2001, Sarrazin, 2002, Stramer, 2002).

Polymerase chain reaction (PCR), in particular real time PCR, is the most relevant of NAT (Carithers et al., 2000, Colucci, 2012, Gullett and Nolte, 2015, Irshad et al., 2016, Kessler and Stelzl, 2017, Krajden, 2000, Pawlotsky, 2003, Schutten, 2008, Scott and Gretch, 2007, Singh et al., 2017). New technologies in the field of PCR are digital droplet PCR, next-generation sequencing, lab-on-a-chip, digital antigen assays (Roth, 2019).

Alternatively, transcription mediated amplification (TMA) and branched-DNA assays can be used for detecting HCV RNA (Al Olaby and Azzazy, 2011, Albertoni et al., 2014).

HCV core Antigen can be used as an indirect marker of HCV replication and assays have the potential to replace NAT (Freiman et al., 2016). They have the advantage of reduced costs compared to PCR and can be performed on the same diagnostic platforms as some EIA assays.

HCV genotyping can be performed by antibody tests and by a PCR technique, but is only essential in the treatment of HCV (Al Olaby and Azzazy, 2011, Podzorski, 2002, Wilson et al., 2017, Yang and Wei, 2018).

Conclusion

HCV testing is mandatory according to the European Tissues and Cells Directive as a preventative measure to reduce the risks of transmission to partners and offspring.

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B2. Prevention of transmission before medically assisted reproduction

PICO QUESTION: WHAT ARE THE RISKS OF HEPATITIS C VIRUS TRANSMISSION THROUGH VAGINAL/ANAL INTERCOURSE?

Evidence

A systematic review including 38 studies, of which 9 controlled studies among sexual contacts of non-haemodialysis, non-renal transplantation patients and 29 uncontrolled studies, resulting in a total of 4250 stable sexual heterosexual contacts reported 573/4250 testing positive for anti-HCV antibodies. Calculated pooled prevalence among the spouses was 13.48% (95% CI 12.48 to 14.55). In the 9 controlled studies, spouses of anti-HCV-positive patients in areas non-endemic for HCV have a significantly higher prevalence of anti-HCV than spouses of negative controls (15.2% vs. 0.9%, OR 20.57, 95% CI 6.05 to 84.08). Two studies reported prevalence of anti-HCV among female contacts of HCV-infected males and male contacts of female infected. Only in the group of male contacts of HCV-infected females was the prevalence of HCV infection significantly higher than in controls (OR 2.14, 95% CI 1.12 to 4.08) (Ackerman et al., 1998).

A cross-sectional study enrolled 500 heterosexual, monogamous patients chronically infected with HCV in a relationship ≥ 36 months and their partners and ≥ 3 sexual contacts in preceding 6 months. 20/500 (4.0%) partners tested anti-HCV-positive and of these 13/20 (65.0%) tested HCV-PCR-positive. (9/20 (45.0%) concordant, 8/20 (40.0%) discordant, 3/20 (15.0%) indeterminant). Prevalence potentially attributable to sex contact was 3/500 (0.6%; 95% CI 0.0% to 1.3%) resulting in an estimated incidence of minimum 3.6/10,000 person-years (95% CI 0.0 to 7.7) based on 3 confirmed couples and maximum 7.2/10,000 person-years (95% CI 1.3 to 13.0) based on 3 confirmed and 3 unknown couples. The estimated risk per sex act was minimum 1/380,000 (95% CI 1/600,000 to 1/280,000) and maximum 1/190,000 (95% CI 1/1.3 million to 1/100,000) (Terrault et al., 2013).

A case control study with 60 HCV-PCR-positive patients. Two spouses (3.39%) were anti-HCV-positive and one was HCV-PCR-positive. Relationship duration of 2 positive spouses with index patient was > 15 years (Hajiani et al., 2006).

A cross sectional study among 53 haemodialysis patients (of which 16 both anti-HCV and HCV-PCR-positive). None of spouses was anti-HCV-positive (0/16) (Fadil-Romao et al., 2006).

One study group conducted a retrospective cohort analysis among 600 chronically infected HCV patients (320 male + 280 female) and their partners as well as a prospective cohort analysis among 216 anti-HCV-negative partners from these chronically infected HCV patients. In the retrospective cohort 12/600 (2%) of partners (4/280 female and 8/320 male) were anti-HCV-positive and 11/600 (1.8%) were HCV-RNA-positive. All 11 couples were concordantly infected with the same genotype (type 1b). The rate of sexual intercourse was 1.73/week. In the prospective cohort analysis, the intercourse rate was 1.9/week. No partners were anti-HCV-positive (Tahan et al., 2005).

A prospective cohort analysis among 112 anti-HCV and HCV-RNA-positive patients (75 men and 37 women) and their anti-HCV negative spouses. One seroconversion (one female partner, genotype 1b) occurred after 20 months, resulting in a transmission risk of 2.33/1000 person-years (Kao et al., 2000).

In a cross-sectional study among 585 anti-HCV and HCV-RNA-positive patients and their 455 spouses HCV prevalence among spouses was 71/455 (15.6%). Of the infected patients, 19.8% had a relationship > 20 years vs. 8% had a relationship < 20 years (OR 2.8, 95% CI 1.5 to 5.3) (Caporaso et al., 1998).

A cross-sectional study analysed 121 patients with chronic liver disease (anti-HCV positive and 116 were also HCV-PCR-positive) and their spouses. 21/121 (17.4%) spouses were anti-HCV-positive of which 19 HCV-PCR-positive. 12/19 (63.2%) couples were concordant for genotype (Koda et al., 1996).

A cross-sectional study among 68 anti-HCV and HCV-PCR-positive patients with chronic hepatitis and their partners showed 4/68 (5.9%) of spouses were anti-HCV positive, of which 2 were HCV-PCR-positive (concordant type 1). Duration of relationship for HCV-positive vs. HCV-negative spouses was 25 years (15-30 years) vs. 10 years (2-43 years) (Tong et al., 1995).

A prospective cohort analysis among 895 heterosexual anti-HCV and HCV-PCR-positive patients and their anti-HCV-negative partners reported a mean frequency of intercourse 1.8/week (no anal intercourse, and no condom use). Three spouses HCV seroconverted (2 with concordant genotype for 1b and 2a, 1 discordant (had dental implant prior to test), resulting in a transmission rate of 0.37/1000 person-years. Transmission rate for concordant couples was 0.25/1000 person-years (Vandelli et al., 2004).

Recommendation

In a monogamous heterosexual relationship of more than 12 months, there is no indication for the use of barrier contraceptives to reduce the risk of Hepatitis C virus (HCV) transmission in a serodiscordant infected couple.	Conditional ⊕⊕◯◯

Justification

The presented large prospective studies show a very low transmission between sexual partners. The major transmission route of HCV is parenteral transmission and not sexual intercourse in the absence of STDs or medical comorbidities such as HIV or liver pathology. A correlation was found between risk of sexual HCV transmission and the number of partners.

Only one study reports in detail the sexual behaviour and shows that there is no significant difference in HCV transmission between vaginal and anal intercourse.

PICO QUESTION: IS THERE A PRE-TREATMENT (BEFORE MAR) THRESHOLD BELOW WHICH TRANSMISSION OF HEPATITIS C VIRUS IS UNLIKELY?

Evidence

Horizontal transmission

We identified no studies reporting a Hepatitis C virus (HCV) RNA serum threshold below which horizontal transmission does not occur.

Vertical transmission

No publications could be identified where maternal HCV viral load was measured before pregnancy.

Recommendation

All patients with an active or chronic HCV-infection must be reviewed by an infectious disease/ liver specialist before initiating any medically assisted reproduction treatment (MAR).	GPP	
Commencing with MAR treatments in patients positive for HCV should be a joint decision between the patient, their partner, the fertility doctor and the infectious disease/ liver specialist.	Strong	⊕◯◯◯
In the case of the female testing positive for HCV, the possibility of viral vertical transmission should be discussed prior to MAR treatment.	GPP	

Justification

All identified studies focus on measuring the HCV-RNA viral load during pregnancy, and therefore there is not enough evidence to indicate a specific cut-off point for viremia before MAR to avoid vertical transmission and HCV infection in the offspring.

Vertical transmission has gained importance as the primary HCV transmission route among children once the blood products screening has been implemented. The estimates of HCV vertical transmission from HCV positive/HIV negative women ranged from 1.1 to 10.7% (Benova et al., 2014). The pooled risk of vertical HCV infection is 5-8%.

Clinical cohort studies evaluating the correlation between pre-pregnancy HCV-RNA viral load and vertical transmission are required to find a threshold value beyond which neonatal infection is more likely.

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B3. Assisted reproduction techniques and impact on outcomes

PICO QUESTION: SHOULD IUI, IVF OR ICSI BE PREFERENTIALLY USED FOR MAR IN HEPATITIS C INFECTED COUPLES?

Evidence

In a prospective cohort study, 35 couples with viraemic and chronically Hepatitis C virus (HCV)-infected male partners underwent either ovarian stimulation and IUI (n=14) or ICSI (n=21) after semen processing (gradient centrifugation and swim-up). There was no case of horizontal or vertical transmission after IUI or ICSI (Savasi et al., 2013).

A cross-sectional observational study including 60 women testing positive for HCV (30 HCV+RNA- and 30 HCV+RNA+ women) investigated the risk of vertical transmission after ICSI. There was no vertical transmission in women who were RNA-, however, 1 infant born to an HCV+RNA+ women tested positive for HCV (Nesrine and Saleh, 2012).

A prospective cohort study, including 40 HCV-serodiscordant couples (male HCV positive) reported the results of ICSI treatment in these couples. None of the female partners seroconverted (Garrido et al., 2004).

Conclusion

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.

Recommendation

The cause of infertility should dictate the specific technique (IUI/IVF/ICSI) used for MAR in couples where one or both partners test positive for HCV.	Strong	⊕○○○
Women testing positive for HCV should be informed that MAR does not eliminate the risk of vertical transmission.	GPP	

PICO QUESTION: CAN HEPATITIS C VIRAL RNA BE DETECTED IN OOCYTES/ SPERM/ PLACENTA?

Evidence

RNA in sperm

No studies could be retrieved investigating the integration of HCV in sperm.

RNA in oocytes

The experimental study by Papaxanthos-Roche et al. investigated the susceptibility of human oocytes from Hepatitis C virus (HCV) RNA-positive women to HCV contamination during assisted reproductive technology (Papaxanthos-Roche et al., 2004). HCV RNA was detected in 17/24 unfertilized oocytes, 6/7 oocytes after an ICSI attempt and 11/17 after a conventional IVF attempt. HCV RNA was found in 19/20 (95%) follicular fluid samples. A weak correlation was found between plasma and follicular fluid HCV RNA loads (Papaxanthos-Roche, et al., 2004).

Placenta

An experimental study reported that primary human trophoblast cells and an extravillous trophoblast cell line (HTR8), from first and second trimester of pregnancy, express receptors relevant for HCV binding/entry and are permissive for HCV uptake (Giugliano et al., 2015).

Conclusion

> The possibility of Hepatitis C viral RNA presence in oocytes cannot be excluded. However, the risk of Hepatitis C transmission through the use of reproductive material remains to be proven.

PICO QUESTION: DOES HEPATITIS C VIRUS AND/OR TREATMENT OF HEPATITIS C VIRUS BEFORE MAR IMPACT THE OUTCOME OF MAR?

MALE INFECTED

Evidence

No articles in the literature search evaluating impact of antiviral treatment before MAR on reproductive outcomes.

A retrospective cohort study including 60 Hepatitis C virus (HCV)-serodiscordant and 69 non-infected couples investigated the reproductive outcomes of ICSI. Fertilization rate was significantly lower in HCV-serodiscordant couples as compared to controls (61% vs. 75%). However, there was no statistical difference in pregnancy rate per cycle (17.6% (18/102) vs. 20.2% (14/69)), miscarriages rate per cycle (11.1% (2/18) vs. 28.5% (4/14)), or live birth per cycle (15.7% (16/102) vs. 15.9% (11/69)) (Cito et al., 2019).

A retrospective cohort study including 78 HCV-serodiscordant and 1256 non-infected couples investigated reproductive outcomes of IVF. There was no statistical difference between HCV-serodiscordant couples and controls in fertilization rate (80.99±19.95 vs. 78.14±19.73), clinical

pregnancy rate (48.6% (36/74) vs. 55.0% (691/1256)) or miscarriage rate (18.9% (14/74) vs. 19.0% (239/1256)) (Yang et al., 2015).

A prospective case-control study including 28 HCV-serodiscordant couples and 46 non-infected controls compared reproductive outcomes of IVF-ICSI. Fertilization rate was significantly lower in HCV-serodiscordant couples as compared to controls (54.7% vs. 68.2%). However, there was no statistical difference between HCV-serodiscordant couples and controls in implantation rate (12.8% vs. 4.2%), clinical pregnancy rate per embryo transfer (17.5% vs. 7.0%) or the number of children born (8/28 vs. 2/46) (Prisant et al., 2010).

Another retrospective cohort study including 12 HCV-serodiscordant (9 male and 3 female testing positive for HCV) and 27 non-infected couples, investigated the reproductive outcomes of IVF-ICSI. There was no statistical difference in fertilization rate between HCV-serodiscordant and control couples (63.9% vs. 75.9%), however, there was a significant difference in clinical pregnancy rate per cycle (0% (0/12) vs. 41% (11/27)) (Pirwany et al., 2004).

Conclusion

There are contradictory results evaluating effects of male HCV infection on infertility treatments outcomes. Although the fertilization rate has been reported significantly lower in couples with HCV-RNA positive men, other studies report that HCV infection does not affect the IVF-ICSI cycle outcomes in these couples.

FEMALE INFECTED

Evidence

No articles in the literature search evaluating impact of antiviral treatment before MAR on reproductive outcomes.

A retrospective cohort study compared the clinical outcomes of 25 first IVF-ICSI cycles in Hepatitis C virus (HCV)-serodiscordant with 25 matched control cycles. There was a statistically significant reduction in fertilization rates (67% vs. 86%) and implantation rates (6% (3/47) vs. 23% (11/47)) between HCV-serodiscordant couples and controls. However, there was no statistical difference between HCV-serodiscordant couples and controls in clinical pregnancy rate (12% (3/25) vs. 36% (9/25)), miscarriage rate (4% 1/25 vs. 12% (3/25)) or the number of children born (2/25 vs. 7/25). A subgroup of 17 patients with confirmed active HCV replication also showed no implantation (0% (0/22) vs. 26% (6/23)) and no live birth occurred in HCV positive patients with active replication (Shaw-Jackson et al., 2017).

A retrospective cohort study including 90 HCV-serodiscordant and 1256 non-infected couples investigated reproductive outcomes of IVF. There was no statistical difference between HCV-serodiscordant couples and controls in fertilization rate (76.93±19.18 vs. 78.14±19.73), clinical pregnancy rate (45.6% (41/90) vs. 55.0% (691/1256)) or miscarriage rate (23.3% (21/90) vs. 19.0% (239/1256)) (Yang, et al., 2015).

A retrospective cohort study including 40 HCV RNA+, 40 HCV RNA- seropositive and 40 HCV PCR-seronegative women compared clinical outcomes of ICSI. Comparing HCV PCR+ and PCR – women and

controls, significantly more cycles were cancelled (52% vs. 30% vs. 5%) and fertilization rate was reduced (28% vs. 32% vs. 67%), respectively. In addition, pregnancy rates were significantly lower in HCV PCR+ women compared to HCV PCR- and controls (5% (2/40) vs. 32.5% (13/40) vs. 47.5% (19/40)). Furthermore, the investigators reported a negative correlation between number of oocytes and HCV viral load (Hanafi et al., 2011).

A prospective case-control study including 22 HCV-serodiscordant couples and 42 non-infected controls compared reproductive outcomes of IVF-ICSI. There was no statistical difference between HCV-serodiscordant couples and controls in fertilization rate (71.1% vs. 70.2%), implantation rate (5.1% vs. 9.6%), clinical pregnancy rate per embryo transfer (10.8% vs. 12.8%) or the number of children born (2/22 vs. 4/42) (Prisant, et al., 2010).

A retrospective cohort study compared the outcomes of IVF-ICSI cycles in 42 HCV-serodiscordant and 84 matched non-infected couples. There was no statistical difference between HCV-serodiscordant couples and controls in fertilization rate (56.4% vs. 59%), implantation rate (19% vs. 19.2%) and pregnancy rate per embryo transfer (28.5% (8/28) vs. 29.3% (22/75)). However, significantly more cycles were cancelled in the HCV-serodiscordant couples (Englert et al., 2007).

Another retrospective cohort study including 12 HCV-serodiscordant (9 male and 3 female HCV-infected) and 27 non-infected couples, investigated the reproductive outcomes of IVF-ICSI. There was no statistical difference in fertilization rate between HCV-serodiscordant and control couples (63.9% vs. 75.9%), however, there was a significant difference in clinical pregnancy rate per cycle (0% (0/12) vs. 41% (11/27)) (Pirwany, et al., 2004).

Conclusion

There are contradictory results evaluating effects of female HCV infection on infertility treatments outcomes. Although some studies report significantly reduced implantation rates, higher cycle cancellations, and higher FSH use in HCV positive women, other report no significant differences.

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B4. Prevention/ reduction of transmission during assisted reproduction

PICO QUESTION: WHICH TECHNIQUES CAN BE USED TO PREVENT/ REDUCE HEPATITIS C VIRUS TRANSMISSION DURING MAR?

SEMEN PROCESSING

The evidence on semen processing will be discussed in detail in the next section (B5).

ANTIVIRAL TREATMENT

No studies on antiviral treatment and MAR were identified.

Conclusion

There are no data regarding antiviral therapy in men or women with HCV without co-infections requiring MAR in order to reduce the risk of HCV transmission. None of the currently available HCV antiviral drugs are licensed for use in pregnancy.

B5. Semen processing

PICO QUESTION: WHAT IS THE BEST TECHNIQUE FOR SEMEN PROCESSING TO REDUCE HEPATITIS C VIRAL LOAD?

Evidence

In a sub group of 93 couples with HIV, HCV or HBV-positive men requiring ICSI, 23 couples were analysed with an HCV-positive male (incl. 1 HCV concordant couple). Semen processing by density gradient (80-40%) centrifugation and washing followed by real time PCR of HIV RNA, HCV RNA and HBV DNA before ICSI. No semen samples were positive for HCV RNA after washing. 48 ICSI cycles performed yielded 8 children and no HCV seroconversion detected in the women or their children (Molina et al., 2014).

A small cohort study, including 35 HCV-serodiscordant (male infected) couples reported no seroconversion after IUI and ICSI. Semen was processed by density gradient centrifugation and swim-up, however, was not PCR tested after processing (Savasi et al., 2013).

In a small cohort study of 16 males testing positive for HCV/HIV-1 requiring MAR, processing of semen was performed by density gradient centrifugation (80-40%), washing and swim-up. HCV RNA was found in seminal plasma and non-sperm cells (31.5%) after density gradient alone. All washed motile sperm samples before and after swim-up were HCV RNA negative (Savasi et al., 2010).

A prospective cohort study included 86 HCV-serodiscordant couples (76 men HCV positive and 10 men HCV/HIV positive). Semen was processed by density gradient (90-70-50%) centrifugation and swim-up. 20.4% of seminal plasma samples were positive for HCV RNA. All sperm fractions were HCV RNA negative. The infants were tested for HCV 3 months after birth, women were not tested. In 135 MAR cycles (10 IVF, 78 ICSI, 12 frozen embryo transfer, 35 IUI- cycles) 36 pregnancies occurred, 28 live births and none of the babies tested positive for HCV (Bourlet et al., 2009).

In a small cohort study of 7 males testing positive for HCV (6 HIV/HCV-positive and 1 HCV-positive), sperm was processed by 1:1 (vol/vol) dilution and centrifugation followed twice by 1:1 dilution of pellet and centrifugation, no swim-up was performed. 7 spermatozoa samples were HCV negative and 1 data of HCV PCR was not available (Garrido et al., 2006).

In another study, semen samples from a small cohort of 20 males testing positive for HIV/HCV not requiring MAR were divided into two aliquots. One total sperm and one for density gradient (90-45%) centrifugation. Following centrifugation three semen fractions originated (seminal plasma, non-motile cells and motile spermatozoa). 100uL of the three fractions for virological analysis and 900µL of the three fractions were washed 3 times and motile sperm fraction was submitted to swim-up. 5% (1/20) of total sperm tested HCV-positive. 0% of three pre-wash semen fractions HCV-positive. 0% of post-wash semen fractions HCV-positive (Canto et al., 2006).

In a cohort study of 32 males testing positive for HCV requiring MAR (HIV and HBV-negative), 11 MAR cycles were performed with proven HCV-negative sperm after triple layer density gradient centrifugation. 5 women became pregnant and 9 babies were born, none were HCV positive at birth or at 6 months of age. Women were not tested for HCV (Bourlet et al., 2002).

A cohort study of 35 infertile couples requiring MAR with a male partner testing positive for HCV without HBV or HIV co-infection where 50 frozen thawed semen samples were used after semen processing with density gradient (90-45%) centrifugation. Only 90% density gradient fraction used for IVF-cycles (n=26) and ICSI-cycles (n=24). 14% of total semen was HCV RNA positive. 2% of the 45% fraction was HCV-RNA-positive and 0% of the 90% fraction was HCV-RNA-positive. HCV viral load in all HCV-positive total semen samples measured below 600 IU/ml. No women or children tested positive for HCV after IVF or ICSI (Cassuto et al., 2002).

A prospective controlled trial included 34 men testing positive for HIV of which 21 also testing positive for HCV. Semen samples were processed by a triple density gradient centrifugation followed by swim up. 12.2% of samples were HIV-positive and 23.8% HCV positive with nested PCR-test versus 0% of samples HIV-positive and 0% HCV-positive with one round PCR-test (Meseguer et al., 2002).

Surgically retrieved sperm

One small cohort study comprising 4 males testing positive for HCV with obstructive azoospermia, anejaculation and cryptozoospermia. Two men underwent testicular sperm extraction (TESE) and microsurgical epididymal sperm aspiration (MESA), two underwent TESE. Epididymal spermatozoa preparation by density gradient (90-45%) centrifugation followed by resuspension of sperm pellet and centrifugation. Testicular spermatozoa preparation by centrifugation of spermatozoa/medium suspension over one layer (45%) gradient followed by resuspension of sperm pellet and centrifugation. No HCV was detected in the final sperm samples after MESA and TESE. No transmission of HCV to female partner after ICSI and 1 healthy child born (Leruez-Ville et al., 2013).

Recommendation

A discontinuous gradient centrifugation followed by swim-up and washing is recommended for semen processing in patients testing positive for HCV.	Strong	⊕○○○

Justification

No studies were identified comparing routine semen preparation with advanced semen processing (such as gradient centrifugation and swim-up) in male testing positive for HCV without co-infections.

Current evidence shows that semen can test positive for HCV after single continuous density centrifugation or after discontinuous density centrifugation without wash steps.

PICO QUESTION: IS THERE A NEED FOR PCR TESTING OF POST-WASHED SPERM?

Evidence

In a sub group of 93 couples with HIV, HCV or HBV-positive men requiring ICSI, 23 couples were analysed with an HCV-positive male (incl. 1 HCV-concordant couple). Semen processing by density gradient (80-40%) centrifugation and washing followed by real time PCR of HIV RNA, HCV RNA and HBV DNA before ICSI. No semen samples were positive for HCV RNA after washing (Molina, et al., 2014).

In a small cohort study of 16 HCV/HIV1-positive men requiring MAR, processing of semen was performed by density gradient centrifugation (80-40%), washing and swim-up. HCV RNA was found in seminal plasma and non-sperm cells (31.5%) after density gradient alone. All washed motile sperm samples before and after swim-up were HCV RNA negative (Savasi, et al., 2010).

A prospective cohort study included 86 HCV-serodiscordant couples (76 men HCV-positive and 10 men HCV/HIV-positive). Semen was processed by density gradient (90-70-50%) centrifugation and swim-up. 20.4% of seminal plasma samples were positive for HCV RNA. All sperm fractions were HCV RNA negative (Bourlet, et al., 2009).

A small cohort study of 7 males testing positive for HCV (6 HIV/HCV-positive and 1 HCV-positive) processed the sperm by 1:1 (vol/vol) dilution and centrifugation followed twice by 1:1 dilution of pellet and centrifugation, no swim-up was performed. 7 spermatozoa samples were HCV negative and for 1 sample the PCR result was not available (Garrido, et al., 2006).

Semen samples from a small cohort of 20 HIV/HCV-positive men not requiring MAR were divided into two aliquots. One total sperm and one for density gradient (90-45%) centrifugation. Following centrifugation three semen fractions originated (seminal plasma, non-motile cells and motile spermatozoa). 100uL of the fractions were washed 3 times with RPMI and motile sperm fraction was submitted to swim-up. 5% (1/20) of total sperm tested HCV positive. 0% of three pre-wash semen fractions HCV positive. 0% of post-wash semen fractions HCV positive (Canto, et al., 2006).

A cohort study of 35 infertile couples requiring MAR with an HCV-positive male partner without HBV or HIV co-infection. 50 frozen semen samples after semen processing with density gradient (90-45%) centrifugation. Only 90% density gradient fraction used for IVF-cycles (n=26) and ICSI-cycles (n=24). 14% of total semen was HCV RNA positive. 2% of the 45% fraction was HCV RNA positive and 0% of the 90% fraction was HCV RNA positive. HCV viral load in all HCV positive total semen samples < 600 IU/ml (Cassuto, et al., 2002).

One small cohort study comprising 4 males testing positive for HCV with obstructive azoospermia, anejaculation and cryptozoospermia. Two men underwent TESE and MESA, two underwent TESE. Epididymal spermatozoa preparation by density gradient (90-45%) centrifugation followed by resuspension of sperm pellet and centrifugation. Testicular spermatozoa preparation by centrifugation of spermatozoa/medium suspension over one layer (45%) gradient followed by resuspension of sperm pellet and centrifugation. No HCV was detected in the final sperm samples after MESA and TESE (Leruez-Ville, et al., 2013).

Recommendation

After advanced semen processing, PCR testing for HCV is not necessary.	Strong	⊕◯◯◯

Justification

All available evidence show that semen sample test PCR negative after advanced semen processing. In addition, the viral load in semen is low. Thus, no PCR testing for HCV is necessary after advanced processing.

PICO QUESTION: IS THERE A NEED FOR SEMEN PROCESSING WHEN BOTH THE MALE AND FEMALE ARE INFECTED?

Evidence

No studies could be found investigating this PICO question.

Recommendation

Good laboratory practice regarding semen processing should be applied irrespective of whether only the male or both partners are testing positive for HCV.	GPP

Recommendation

There is no evidence that semen processing might make a difference when both partners test positive for HCV. Practices on semen processing in these cases may differ between centers, however, especially for cryopreservation and storage purposes, it might be justified to perform semen processing.

The fact that both partners are infected does not mean it is justified to lower sperm processing standards. Good laboratory practice is required also when both partners are testing positive for HCV.

PICO QUESTION: DOES THE PLASMA VIRAL LOAD CORRELATE WITH HEPATITIS C VIRUS DETECTION IN SEMEN?

Evidence

A small cohort study including 32 HCV+RNA+ (26 chronic and 4 acute HCV-infected) men investigated the association between serum and semen HCV viral load. The mean blood viral load was significantly higher in patients positive for HCV RNA in semen (n=4) than in those negative for HCV RNA in semen (n=28) (6.52 ± 0.55 vs. 5.88 ± 0.46 log copies/ml) (Bourlet, et al., 2002).

In a sub group analysis, including 30 HCV-positive HIV-negative men, Bradshaw et al. reported that the median blood HCV RNA levels in those with detectable HCV RNA was higher than that in those with undetectable HCV RNA in semen (6.2 log IU/mL; interquartile range (IQR) 5.7 to 6.7 log IU/mL vs. 6.0 log IU/mL; (IQR 5.3 to 6.2 log IU/mL), however the difference was not statistically significant (Bradshaw et al., 2015).

Conclusion

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.

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B6. Reducing/ avoiding vertical transmission

PICO QUESTION: WHICH INTERVENTIONS CAN BE USED TO REDUCE/AVOID VERTICAL TRANSMISSION OF HEPATITIS C VIRUS TO THE NEWBORN?

Elective Caesarean section

Evidence

A systematic review and meta-analysis including 8 cohort studies, compared HCV seroconversion rates in children after vaginal delivery and caesarean section (CS) and reported 7% seroconversion after vaginal delivery (36/510) versus 6.1% (8/131) after CS (OR 1.1, 95% CI 0.45 to 2.67). CS does not decrease the risk of HCV transmission to the infant (Ghamar Chehreh et al., 2011).

Recommendation

Caesarean delivery is not recommended on the basis of maternal HCV-positivity alone.	Strong	⊕○○○

Justification

There is no evidence that the risk of HCV transmission from mother to child after caesarean section is lower compared to that after vaginal delivery.

Breastfeeding

Evidence

A systematic review and meta-analysis including 14 cohort studies (2971 mother–infant pairs), investigated the risk of HCV transmission to the infant by breastfeeding and reported no association between breastfeeding and risk of transmission to the infant (Cottrell et al., 2013). Methodologic shortcomings in the poor quality studies included failure to perform statistical adjustment on potential confounders and insufficient information to determine comparability of groups at baseline stratified by breastfeeding status (Cottrell, et al., 2013).

Recommendation

Breastfeeding is not contra-indicated in women testing positive for HCV.	Strong	⊕⊕○○

Justification

We identified no association between breastfeeding and the risk of HCV transmission from mother to child. Breastfeeding has significant health benefits. This recommendation is supported by the guidelines of the European Association for the Study of the Liver (EASL) (Pawlotsky et al., 2020).

There are 4 studies included in the systematic review by Cottrell et al. (2013) possibly including HCV mothers co-infected with HIV which may influence the reported results (Cottrell, et al., 2013).

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Summary

HCV	Male testing positive	Female testing positive	Couple testing positive
Before MAR	Consult with infectious disease / liver disease specialist		
		Discuss: - Risk of viral horizontal transmission (not eliminated by MAR) - Risk of viral vertical transmission (not eliminated by MAR)	
During MAR	IUI, IVF or ICSI depending on infertility work-up		
	Specific semen processing*	Standard oocyte processing	Specific semen processing*
After MAR	Caesarean section not recommended		
	Breastfeeding not contra-indicated		

*Density gradient centrifugation followed by washing and swim-up

Figure 2: Summary of management of medically assisted reproduction in patients testing positive for Hepatitis C virus.

PART C: Human Immunodeficiency Virus

C1. Prevalence and testing

NARRATIVE QUESTION: WHAT IS THE PREVALENCE OF HUMAN IMMUNODEFICIENCY VIRUS?

Human Immunodeficiency virus (HIV) continues to be a major global public health issue, having claimed almost 33 million lives so far. There were an estimated 38 million people living with HIV at the end of 2019. However, with increasing access to effective HIV prevention, diagnosis, treatment and care, including for opportunistic infections, HIV infection has become a manageable chronic health condition, enabling people with HIV to lead long and healthy lives. In 2019, 68% of adults and 53% of children living with HIV globally were receiving lifelong antiretroviral therapy.

Over two thirds of all people living with HIV live in the WHO African Region (25.7 million). While HIV is prevalent among the general population in this region, an increasing number of new infections occur among key population groups (WHO, 2018).

Since the late 1990s, the progressive availability and success of combination antiretroviral therapy has reduced the risk of opportunistic infections and malignancies in people living with HIV, remarkably decreasing morbidity and mortality (UNAIDS, 2015).

Although HIV infection is preventable, significant HIV transmission continues across the WHO European Region. In 2019, 136 449 newly diagnosed HIV infections were reported in 47 of the 53 Member States in the Region, including 24 801 from countries of the European Union/European Economic Area (EU/EEA). This corresponds to a crude rate of 15.6 newly diagnosed infections per 100 000 population (ECDC and WHO, 2020).

In 2014, the Joint United Nations Program on HIV and AIDS (UNAIDS) and partners set the '90-90-90 targets'; aiming by 2020 to (a) diagnose 90% of all HIV positive people; (b) provide antiretroviral therapy for 90% of those diagnosed; and achieve viral suppression for 90% of those treated (UNAIDS, 2015).

The first lentivirus, SIVmac was identified in 1984 in rhesus macaques and HIV was transmitted from chimpanzees to humans in the beginning of the 20th century. The mature HIV virion consists of 2 copies of single-stranded RNA surrounded by structural proteins, a matrix shell, and lipid envelope.

HIV targets the immune system and weakens people's defence against many infections and some types of cancer, which people with a healthy immune system can fight off. As the virus destroys and impairs the function of immune cells, infected individuals gradually become immunodeficient. Immune function is typically measured by CD4 cell count. The most advanced stage of HIV infection is acquired immunodeficiency syndrome (AIDS), which can take many years to develop if not treated, depending

on the individual. AIDS is defined by the development of certain cancers, infections or other severe long-term clinical manifestations (WHO, 2018).

There are two major viral species of HIV known, HIV-1 and 2. The HIV-1 strain was identified first, is the more virulent, the most prevalent and most researched strain. There are a number of distinct HIV-1 lineages (or genetic subtypes), designated by letters. This genetic variability is caused by the reverse transcriptase enzyme, which is error-prone, resulting in mutations and recombinations. A recombination can occur when a person is infected with 2 or more virus strains. According to internationally defined nomenclature, 106 distinct circulating recombinant forms are known thus far. This diversification of the HIV virus has significant consequences for diagnosis, screening and management (for instance drug resistance), but also for vaccine development (Hemelaar et al., 2020).

The HIV-2 strain was first isolated in 1986, and has a serologic profile more closely related to the SIVmac than HIV-1. Like HIV-1, 9 distinct lineages (A-I) have been identified. Contrary to HIV-1, only two recombinant forms have been described so far. Epidemiological data for HIV-2 are very limited and rather outdated. HIV-2 is mainly restricted to West Africa where it infected up to 1–2 million people. In Europe, the two countries with the highest prevalence are Portugal and France. According to data from 2008, 4.5% of AIDS diagnosis in Portugal and 1-2% in France are due to HIV-2. HIV-2 transmission is slower than for HIV-1 due to lower viral loads with HIV-2 infections. The lower plasmatic viral load of HIV-2 is also reflected in semen, resulting in a 4 times lower likelihood of sexual transmission compared to HIV-1. Treatment of HIV-2 is challenging, since it displays a high level of intrinsic resistance against antiretroviral drugs developed for HIV-1 therapy. (Visseaux et al., 2016).

NARRATIVE QUESTION: HOW SHOULD TESTING OF HUMAN IMMUNODEFICIENCY VIRUS STATUS PRIOR TO MEDICALLY ASSISTED REPRODUCTION BE PERFORMED?

After human Immunodeficiency virus (HIV)-1 infection, HIV-1-specific markers appear in the blood in a chronologic order: HIV-1 RNA, p24 antigen, HIV-1 IgM antibody, and HIV-1 IgG antibody. Time from HIV acquisition to reactivity for an assay depends on which target is being detected, when that target can be detected after infection, concentration of the target in the specimen, the volume of specimen tested, and the test's analytical sensitivity (Branson, 2019, Hurt et al., 2017).

The standard of care test for diagnosing HIV in a clinical setting is the serum immunoassay test (EIA), known as the HIV fourth-generation test, which is a combination antibody (Ab) and antigen (Ag) test. With this test, antibodies against both HIV-1 and HIV-2 are detected, as well as the p24 antigen, allowing for earlier HIV detection after exposure. If there is a strong suspicion of a very early HIV infection (less than 14 days), a nucleic acid test (NAT) can be performed to detect HIV RNA (as early as 5-10 days after the transmission, depending on the sensitivity of the assay). NAT should also be performed if the fourth-generation test is inconclusive. The false-positive rates of both the third and fourth generations are very low. Data show a false positive rate of third-generation testing (with confirmatory western blot) to be as low as 0.0004% to 0.0007%. (Branson, 2019, Huynh and Kahwaji, 2020).

HIV self-testing and rapid tests are some of the new strategies to encourage HIV diagnosis. HIV self-testing is a process whereby a person who wants to know his or her HIV status collects a specimen, performs a test, and interprets the test results in private or with someone they trust. HIV self-testing does not provide a definitive HIV-positive diagnosis, but it could be used as an initial test to be followed

by confirmatory testing (such as NAT or serum western blot) by a health worker. Many countries are now using innovative approaches to develop and support HIV self-testing using digital platforms and online support for help with the testing procedure and linkage to services (WHO, 2018). Rapid tests (mostly oral swab tests) are primarily ELISA tests, and provide the result in 20-30 minutes. The advantage of rapid tests is that they can be performed outside of a clinical setting. However, the results need to be confirmed by a confirmatory test (Huynh and Kahwaji, 2020, Parekh et al., 2019). In resource limited settings, HIV testing algorithms include a combination of two or three rapid tests, usually in a serial algorithm, depending on HIV prevalence; this has produced suitable sensitivities and specificities and improved the accuracy of testing results in point of care settings (Parekh, et al., 2019).

The sexual partners and drug-injecting partners of people diagnosed with HIV infection have an increased risk of also being HIV-positive. WHO recommends voluntary assisted HIV partner notification services as a simple and effective way to reach these partners – many of whom are undiagnosed and unaware of their HIV exposure and may welcome support and an opportunity to be tested for HIV (WHO, 2018).

Conclusion

HIV testing is mandatory according to the European Tissues and Cells Directive as a preventative measure to reduce the risks of transmission to partners and offspring.

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C2. Prevention of transmission before medically assisted reproduction

PICO QUESTION: WHAT ARE THE RISKS OF HUMAN IMMUNODEFICIENCY VIRUS TRANSMISSION THROUGH VAGINAL/ANAL INTERCOURSE?

Evidence

A systematic review and meta-analysis reported that among heterosexual sex partners, with the index case on antiretroviral therapy (with varying levels of viral load) 23 linked transmissions were identified over 9922 person-years (pooled incidence 0.23 transmissions/100 person-years, 95% CI 0.15 to 0.35, 10 studies). Among couples where the index case had suppressed viral load, no transmissions over 1327 person-years were identified (pooled incidence 0.00 transmissions/100 person-years, 95% CI 0.00 to 0.28, 2 studies). Among serodiscordant couples who reported "always" using condoms, there were 1.14 HIV transmissions per 100 person-years (95% CI 0.56 to 2.04) (LeMessurier et al., 2018).

A systematic review and meta-analysis, including 15 studies, calculated that the summary estimate for HIV infectiousness of anal intercourse per act is 1.8% (95% CI 0.3 to 3.2) (Baggaley et al., 2010).

A further 8 prospective observational studies, not included in the systematic reviews, reported HIV seroconversion rates in HIV-serodiscordant couples. Ma et al., including 231 HIV-serodiscordant couples, reported 45 seroconversion before the start of the study and 2 during the follow-up (seroconversion rate of 0.39 per 100 person-years) (Ma et al., 2019). In the study by Colombe et al., 14 unaffected partners HIV-1 seroconverted in 105 serodiscordant couples (Colombe et al., 2019). In the study by Rodgers et al., including 888 HIV-1-serodiscordant couples (548 heterosexual and 340 male homosexual) contributing to 1238 eligible couple-years of follow-up, 11 HIV-1 unaffected partners seroconverted (Rodger et al., 2016). In the study by Quinn et al., 90 HIV-1 unaffected partners seroconverted in 415 HIV-1-serodiscordant couples during 30 months of follow-up (Quinn et al., 2000). In the study by Ragni et al., 39 partnered HIV-infected haemophilic men not on antiretroviral therapy were included. Five out of 39 transmitted HIV to their unaffected partner (Ragni et al., 1998). In the study by Operskalski et al., including 18 HIV-1-serodiscordant couples, 2 unaffected partners already seroconverted before the study and 4 seroconverted during 23 person-years of observation (Operskalski et al., 1997). In the study by Deschamps et al., 135 HIV-serodiscordant couples were included, of which 19 unaffected partners seroconverted during follow-up (Deschamps et al., 1996). De Vincenzi et al. reported that 12 unaffected partners out of 256 couples HIV seroconverted (2.3/100 person-years (95% CI 1.2 to 4.0)) (de Vincenzi, 1994).

In the study by Ma et al., the seroconversions occurred in couples where the index person did not immediately receive antiretroviral therapy after HIV diagnosis (Ma, et al., 2019). Similarly, Rodgers et al. reported that the estimated rate for transmission through any unprotected intercourse with the HIV-1-positive partner on antiretroviral therapy with HIV-1 load less than 200 copies/mL was zero, with an upper 95% confidence limit of 0.30 per 100 couple-years of follow-up (Rodger, et al., 2016). Also, in the study by Quinn et al. the rate of HIV-1 transmission was zero among the 51 couples in which the HIV-1–positive partner had undetectable serum levels of HIV-1 RNA or less than 1500 copies/ml (Quinn, et

al., 2000). In contrast, in the study by Zheng et al., compared with those who were on antiretroviral therapy, the OR for HIV acquisition in the antiretroviral therapy-naïve group was 1.14 (95% CI 0.91 to 1.43), there was no significant difference between the antiretroviral therapy and antiretroviral therapy naïve group (Zheng et al., 2018).

Deschamps et al. reported that the incidence of HIV infection was 1.0 per 100 person-years for persons who always used condoms and 6.8 per 100 person-years for persons who used condoms irregularly or not at all (Deschamps, et al., 1996). Similarly, De Vincenzi et al. reported no seroconversions in couples with consistent condom use (de Vincenzi, 1994).

Two retrospective cohort studies reported respectively 53 seroconversions within 5218 person-years of follow-up in 4481 HIV-serodiscordant couples (incidence rate of 1.02 (95%CI 0.76±1.33) per 100 person-years) and 72 seroconversions in 239 monogamous couples (index partner not on antiretroviral therapy) (rate of HIV-1 transmission per coital act was 0.0012 (95% CI 0.0009 to 0.0015)) (Tang et al., 2016, Wawer et al., 2005).

Recommendation

Human immunodeficiency virus (HIV)-1-serodiscordant couples should be informed that there is a risk of sexual transmission of the virus to the unaffected partner. To reduce this risk, couples must be advised to use barrier contraception and seek active therapy to reduce viral load.	Strong	⊕⊕◯◯

In individuals testing positive for HIV-1, antiretroviral therapy can suppress viral replication. These patients should remain on antiretroviral therapy and providing undetectable viral loads in serum can be achieved and sustained, the risk of horizontal transmission through unprotected intercourse is minimal in the absence of other sexually transmitted diseases.	Strong	⊕⊕◯◯

Justification

The HIV-1 viral presence cannot be eliminated to date, however, anti-retroviral therapy can reduce the HIV-1 viral load to undetectable levels, thereby minimizing the risk of horizontal transmission.

PICO QUESTION: IS THERE A THRESHOLD BELOW WHICH TRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS IS UNLIKELY?

Evidence

Horizontal transmission

A systematic review and meta-analysis including 11 cohorts reporting on 5021 couples and 461 human Immunodeficiency virus (HIV) transmission events, reported that the HIV transmission rate from people not on antiretroviral therapy was 0.16 (95% CI 0.02 to 1.13 per 100 person-years, based on one episode of HIV, 6 studies). Furthermore, no HIV transmission events occurred in discordant heterosexual couples if the partner testing positive for HIV was treated with antiretroviral therapy and had a viral load below 400 copies/ml (Attia et al., 2009).

Two prospective observational studies, not included in the systematic review, reported on the risk of transmission in relation to the HIV-1 viral load. In the study by Rodger et al., the estimated rate of HIV-1 transmission through unprotected intercourse was zero when the partner testing positive for HIV-1 was on antiretroviral therapy with a viral load <200 copies/ml (upper 95% confidence limit of 0.30 per 100 couple-years of follow-up) (Rodger, et al., 2016). Pedraza et al. reported that HIV-1 transmitters had a higher viral load than non-transmitters (21.139 vs. 5.484 RNA copies/ml) (Pedraza et al., 1999)

Vertical transmission

We were unable to retrieve studies that investigated the maternal viral load before MAR and the risk of vertical transmission to the newborn.

Recommendation

Commencing with medically assisted reproduction (MAR) treatments in patients positive for HIV-1 or 2 should be a joint decision between the patient, their partner, the fertility doctor and the infectious disease specialist.	Strong	⊕◯◯◯

All patients testing positive for HIV, wishing to have a child should be counselled about the risk of horizontal and vertical transmission. In the case of the male testing positive for HIV, antiretroviral therapy can reduce the viral load in blood and semen to undetectable levels, allowing the possibility of natural conception. Reproductive counselling should include fertility and antiretroviral covariates.	GPP

In the case of the female testing positive for HIV-1 or 2, and even with undetectable viremia, the possibility of viral vertical transmission should be discussed prior to MAR treatment.	GPP

Justification

Patients newly diagnosed with HIV infection, should get advice from an infectious disease specialist to discuss treatment options and start treatment to reduce viral load. Engaging with a HIV specialist when planning fertility treatment makes it possible to have a multi-disciplinary pre-conception counselling which could include the optimization of health status, including reviewing antiretroviral agents and reviewing blood viral load.

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C3. Assisted reproduction techniques and impact on outcomes

PICO QUESTION: SHOULD IUI, IVF OR ICSI BE PREFERENTIALLY USED FOR MAR IN HUMAN IMMUNODEFICIENCY VIRUS INFECTED COUPLES?

Evidence

A systematic review and meta-analysis included 14 studies reporting on the outcomes of IUI in couples testing positive for human Immunodeficiency virus (HIV)-1. In the 14 studies on IUI, 12 studies reported on serodiscordant couples with a male patient testing positive for HIV-1 and 4 with a female patient testing positive for HIV-1. Results on IUI in males testing positive for HIV-1 comprising of 2393 patients, showed a cumulative pregnancy rate of 17% (95 CI 15% to 20%) and 14% in female patients testing positive for HIV-1 (n=28) (95 CI 25% to 35%). The miscarriage rate for IUI in serodiscordant couples with male patients testing positive for HIV-1 (n=2393) was 19% (95 CI 14% to 25%) and for female patients testing positive for HIV-1 (n=25) was 13% (95 CI 1% to 34%). No HIV-1 transmission was observed in the seronegative partner of a total of 8212 IUI cycles (Barnes et al., 2014). The meta-analysis also included 15 studies on the outcomes of IVF/ICSI in HIV-1 infected couples. In these 15 studies, 12 studies reported on serodiscordant couples with a male partner testing positive for HIV-1 and 7 with a female partner testing positive for HIV-1. The clinical pregnancy rate was 30% (95 CI 25% to 35%) in couples with male partners testing positive for HIV-1 (n=780) and 16% (95 CI 13% to 20%) for couples where the female partner tested positive for HIV-1 (n= 253). No HIV-1 transmission was observed in 1254 IVF/ICSI cycles in serodiscordant couple with a male partner testing positive for HIV-1 (Barnes, et al., 2014).

A systematic review and meta-analysis included 11 studies reporting on the outcome of 3900 IUI cycles in 1184 couples and 738 IVF/ICSI cycles performed in 579 serodiscordant couples with a male partner testing positive for HIV-1 (Vitorino et al., 2011). This meta-analysis does include 4 studies that were also included in the structured review of Barnes et al. The median clinical pregnancy rate was 18% (range from 14.5% to 23%) for IUI and 38% (range 24.8% to 46.2%) for IVF/ICSI. There was no HIV-1 seroconversion in the female partners of couples where the male partner tested positive for HIV-1 and no vertical transmission was found in children at birth and 3-6 months after delivery (Vitorino, et al., 2011).

Recommendation

HIV infection status is not a reason to deny MAR treatment.	Strong	⊕○○○

The cause of infertility should dictate the specific technique (IUI/IVF/ICSI) used for MAR in couples where one or both partners test positive for HIV.	Strong	⊕⊕◯◯

Justification

There are no studies published comparing different MAR techniques in similar patient populations testing positive for HIV-1. Evidence from 2 structured reviews, although having overlapping studies in their results, showed that the pregnancy outcomes in IUI are lower compared to IVF/ICSI cycles as is the case in patients testing negative for HIV-1. The decision on which type of MAR treatment is chosen, is to be solely based on the fertility status of the couples.

Conflicting results in the outcome can be the origin of different types of subfertility in couples, certainly in studies describing smaller patient cohorts, moreover the patients are sometimes co-infected with other infectious diseases like HCV and/or HBV in several studies, and the infertility aetiology is also not always clearly described in the studies. The systematic review of Barnes (Barnes, et al., 2014), showed that in women on antiretroviral therapy and testing positive for HIV-1, 32%-75% suffered tubal factor infertility (5 studies), 8%-12.5% ovarian factor infertility (2 studies), 2% were diagnosed with endometriosis (1 study), 10%-37% had partners with male infertility (5 studies) and 12%-20% were 'other' or unknown factors (2 studies). It was clear that for HIV-1 infected women, tubal factor infertility was more prevalent than in the general infertility population where isolated tubal infertility is about 8% (CDC et al., 2011).

There is no preferred MAR method of choice based on infection status of the patient or the couple. The current evidence shows that safety is equal in all MAR techniques after specific semen processing. Reassuringly, evidence in the reported papers, showed no seroconversion in female partners of serodiscordant couples where the male tested positive for HIV-1 and no vertical transmission has been shown in the babies born from MAR in couples testing positive for HIV-1. It could be argued that MAR in couples where the male is HIV-1 positive is a therapeutic intervention that reduces the risk of horizontal transmission.

PICO QUESTION: CAN HUMAN IMMUNODEFICIENCY VIRUS DNA BE DETECTED IN OOCYTES/ SPERM/ PLACENTA?

Evidence

DNA integration in sperm

An original research paper using semen samples from sperm donors testing negative for human Immunodeficiency virus (HIV)-1, co-incubated with HIV, reported a dose-dependent binding of HIV to spermatozoa. This binding occurred with low affinity and superficially on the sperm surface (Young et al., 2019).

An observational cohort study in 10 males tested positive for HIV, on antiviral therapy for 6 months undergoing bilateral orchiectomy for gender confirmation treatment, reported HIV persistence in the testis. The study analysing HIV diversity and genetic compartmentalization, reported dynamics of

proliferation of latently HIV-infected cells to be different between blood and testes. The authors noticed marked differences in the quantity and distribution of identical HIV sequences between patients and between anatomical sites within each patient, which may complicate the complete eradication on HIV in patients (Miller et al., 2019).

An original basic research study showed the presence of HIV-1 protein and viral RNA in seminal vesicles in testicular tissue section of 7/9 male patients testing positive for HIV-1. Viral integrated cells were observed either in the stroma close to the epithelium or in the lumen of the seminal vesicles (Deleage et al., 2011).

An observational study, including semen of 22 male patients testing positive for HIV-1 compared to 12 males testing negative for HIV-1, observed that in 75% of all semen samples tested; T cells were most commonly infected with HIV-1, followed by macrophages in 38% of all samples. Viral DNA was, in this study, never detected in motile spermatozoa (Quayle et al., 1997).

An original basic research study showed sperm-associated virus-like particles in the spermatozoa of 15 male patients testing positive for HIV-1. In contrast, 15 male patients testing negative for HIV-1 did not exhibit sperm-associated virus-like particles, however spermatozoa derived from patients testing negative for HIV-1 co-incubated with HIV-1 showed sperm-associated virus-like particles in the spermatozoa. This study described the presence of the putative HIV-1 receptor (GalAAG) on spermatozoa and found virus-like particles in an 8-cell stage embryo after IVF using sperm of a male patient testing positive for HIV-1 (Baccetti et al., 1994).

A basic research study where semen samples of 17 male persons testing negative with HIV-1 were co-incubated with HIV-1, observed the binding of HIV-1 to the plasma membrane of the spermatozoa and the presence of virus-like particles in vacuoles in the apical nuclear region of the spermatozoa (Dussaix et al., 1993).

DNA integration in oocytes

An original basic research study using 41 oocytes of a female donor testing negative for HIV-1, injected 28 of these oocytes with very high amounts of HIV-1 ($4x10^4$ cop/ml) and observed in 3/28 oocytes (11%), HIV-1 viral integration (Steenvoorden et al., 2012).

A series of 4 cases described by Bertrand et al. investigating 24 follicular fluid samples, 15 follicular flushing samples and 1 cumulus cells samples obtained from 4 female patients testing positive for HIV-1 of which 3 had an undetectable viral load in plasma, did not detect HIV-1 RNA in any of the samples analysed. The patient with a detectable viral load showed HIV-1 RNA in 1 follicular fluid sample and 1 follicular flushing sample. The other 2 flushing samples of this patient were HIV-1 RNA negative (Bertrand et al., 2004).

An original basic research study where 100 oocytes from 15 female patients testing negative for HIV-1 were co-incubated with HIV-1, found no cell associated HIV-1 antigen neither virus-like particles in any of the experiments. The putative HIV-1 receptor (GalAAG) could not be detected on the oocytes (Baccetti et al., 1999).

Placenta

In a prospective cohort study, including 37 women testing positive for HIV-1 (39 pregnancies), HIV-1 was detected in 12/37 placentas with immunohistochemistry (IHC), staining syncytiotrophoblast and

villous mesenchymal cells. In addition, 3/18 placentas tested positive for HIV-1 by PCR. However, only for 1 placenta, the tests were concordant (Dictor et al., 2001).

Another cohort study, investigating the placentas 75 women testing positive for HIV-1 reported that no HIV-1 protein positive cells were found in the frozen sections of the placentas by IHC. Furthermore, in-situ hybridisation (ISH) also showed no HIV-1 proteins, regardless of the clinical status of the mother (Peuchmaur et al., 1991).

A third cohort study investigated 27 placentas (19 from women testing positive for HIV, 4 women testing negative for HIV and 4 from untested women considered low risk). P24/25 antigen was identified in 5 (26%) of the 19 placentas from seropositive pregnancies and in none of the 8 placentas of seronegative or untested, low-risk pregnancies. HIV was isolated from 3 (27%) of the 11 placentas from HIV-seropositive pregnancies and from none of the 3 placentas of HIV-seronegative pregnancies. Two of the 3 HIV culture positive placentas also had p24/55 antigen detected by immunoperoxidase staining and 1 was negative (Mattern et al., 1992).

Conclusion

There is no evidence of HIV nucleic acid integration in the genome in human spermatozoa. Semen can be prepared to collect spermatozoa free from HIV RNA and DNA and used for MAR. Similarly, HIV RNA/DNA has not been detected in human oocytes.

Advanced semen processing should be used for male patients testing positive for HIV-1 to reduce the likelihood of viral presence.	Strong	⊕○○○

No special laboratory techniques are needed for processing of oocytes from female patients testing positive for HIV.	Strong	⊕○○○

Justification

DNA integration in sperm

Viral DNA and RNA can be detected in semen and on spermatozoa of males testing positive for HIV, also co-incubation experiments show the presence of HIV viral-like particles in spermatozoa, albeit it has never been proven that these are infectious HIV virions.

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 (Baccetti, et al., 1994).

Seminal vesicles, macrophages and T-cells are most probably the carriers of HIV particles in a semen sample. Semen processing techniques are therefore necessary to eliminate these contaminating cells

(Quayle, et al., 1997). Although there is a theoretical risk for introducing HIV particles through ICSI, the probability is very small with processed semen as shown in the studies presented.

DNA integration in oocytes

There are very few studies reporting on the RNA/DNA detection in oocytes from female patients testing positive for HIV. Viral DNA and RNA cannot be detected in oocytes when co-incubation experiments with HIV are performed. No observation of virus-like particles in the oocytes were found, whereas similar experiments using sperm, did detect virus-like particles in the spermatozoa (Baccetti, et al., 1999). HIV receptors could not be detected on oocytes (not found on granulosa cells nor the zona pellucida) (Baccetti, et al., 1999). It is therefore unlikely that HIV-1 will bind to and infect oocytes.

Oocytes could get infected with HIV upon performing ICSI, however the study of Steenvoorden et al. showed that this risk, although theoretically possible, is highly unlikely to occur when using a processed semen sample of a patient testing positive for HIV (Steenvoorden, et al., 2012). The viral integration shown in this study was <2% when 40 copies of HIV were directly injected into an oocyte. The detection limit for HIV PCR testing at the moment is 10-40 cop/ml. Processed semen samples testing negative by PCR exhibit thus an even lower risk for injecting a viral particle in an oocyte. The theoretical chance of viral integration of human oocytes through ICSI using processed semen was calculated to be 0.00002% (Steenvoorden, et al., 2012).

There is 1 study describing the presence of HIV-1 RNA in follicular fluid and follicular flushes of a female patient testing positive for HIV with a detectable viral load, whereas the samples from the 3 HIV-infected women with an undetectable viral load, did not show viral RNA or DNA (Bertrand, et al., 2004).

PICO QUESTION: DOES HUMAN IMMUNODEFICIENCY VIRUS AND/OR TREATMENT OF HUMAN IMMUNODEFICIENCY VIRUS BEFORE MAR IMPACT THE OUTCOME OF MAR?

MALE INFECTED

Evidence

Male infected

A case control study reported on the outcomes of ICSI cycles in 24 serodiscordant couples with a male partner testing positive for Human Immunodeficiency virus (HIV) compared to 69 couples testing negative for HIV. No statistical differences were observed in the following outcome measures all calculated per ET: clinical pregnancy rate (21.7% vs. 20.3%), rate of pregnancy loss (20% vs. 28.5%) and live birth rate (17.4% vs. 15.9%) between the serodiscordant and control couples, respectively (Cito et al., 2019).

A case control study reported on the outcomes of 44 IVF/ICSI day 2 ET cycles in 28 serodiscordant couples with a male partner testing positive for HIV-1 matched to a control group of 41 couples testing negative for HIV-1, having the same age, aetiology of infertility, rank of oocyte retrieval and type of MAR. The authors reported lower but not statistically significant differences in clinical pregnancy rate per ET (10.8% vs. 22.2%) or live birth rate per ET (6 vs. 4) between serodiscordant couples and control group (Prisant et al., 2010).

A case-control study including data from 84 HIV-1-serodiscordant couples compared IUI outcomes (294 cycles) with 90 heterosexual couples undergoing donor insemination (320 cycles) due to male sterility in the same timeframe. There was no difference in baby take home rate (52.4% vs. 41.1%), clinical pregnancy rate per IUI cycle (expressed as HCG+ >2000 mIU) (18.0% vs. 14.7%) or miscarriage rate per IUI cycle (17% vs. 21.3%) between HIV-1+ serodiscordant couples and couples testing negative for HIV-1. This study reported zero seroconversions in the seronegative female partners (Bujan et al., 2007).

An age-matched control study compared outcomes from 43 serodiscordant couples with a male partner testing positive for HIV-1 (55 ICSI cycles) with 50 age-matched couples testing negative for HIV-1 (55 ICSI cycles). There was no significant difference in clinical pregnancy rate per ET (45% vs. 40%) or miscarriage rate per ET between serodiscordant couples and the control group. No seroconversions were observed in the female partners and all 17 babies born from serodiscordant couples were HIV-1 negative, 3 months after their birth (Sauer and Chang, 2002).

Female infected

A systematic review and meta-analysis summarized publications on outcomes of IVF/ICSI from 10 studies. Results were compiled from in total 342 serodiscordant couples with an HIV+ female partner and 516 IVF/ICSI cycles. The clinical pregnancy rate per ET ranged from 9.1% to 63% for HIV+ females. In this review, 6/10 studies comprised a matched control study (2 studies), a lower pregnancy rate was observed for HIV+ women and in 4 case control studies, there was no significant difference in pregnancy outcome for HIV+ women compared to seronegative control couples, although lower values were observed in 3 studies and a higher value in 1 study (Marques et al., 2015).

Recommendation

Serodiscordant couples with a male partner testing positive for HIV-1 should be informed that the efficacy of MAR is not impacted compared to HIV-seronegative couples.	Strong	⊕○○○

Serodiscordant couples with a female partner testing positive for HIV should be informed that the efficacy of IVF/ICSI could be reduced compared to HIV-seronegative couples.	Conditional	⊕○○○

Justification

MAR outcomes in this section are described in female or male patients testing positive for HIV. Studies where patients testing positive for HIV and other infectious diseases like HBV or HCV were excluded from this analysis except for the structured review of Marques et al. where individual studies did not always report the co-infectious status of the women involved (Marques, et al., 2015). MAR efficacy in HIV serodiscordant couples is conflicted by the HIV infection of the female partner. This outcome is probably not related to the co-infectious status in these studies, as the proportion of females testing positive for HIV and other co-infections is most likely lower than 10%.

MAR efficacy in HIV serodiscordant couples is not negatively impacted by the HIV-1 infection of the male partner.

When performing MAR in serodiscordant couples with a male partner testing positive for HIV-1, there is zero seroconversion in the seronegative female partner. The follow-up of the children born from serodiscordant couples show that there are no vertical transmissions reported, whether it be that the male partner is testing positive for HIV, or the female partner. There is no harm in performing MAR in serodiscordant couples and above all the outcome in MAR for couples with a male testing positive for HIV is similar to couples where both partners test negative for HIV.

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C4. Prevention/ reduction of transmission during assisted reproduction

PICO QUESTION: WHICH TECHNIQUES CAN BE USED TO PREVENT/ REDUCE HUMAN IMMUNODEFICIENCY VIRUS TRANSMISSION DURING MAR?

SEMEN PROCESSING

Evidence

The evidence on semen processing will be discussed in detail in the next section (C5).

PRE-EXPOSITION PROPHYLAXIS (PREP)

Evidence

We could not identify any studies investigating the effect of PreP during MAR on the risk of vertical transmission of human Immunodeficiency virus.

C5. Semen processing

PICO QUESTION: WHAT IS THE BEST TECHNIQUE FOR SEMEN PROCESSING TO REDUCE HUMAN IMMUNODEFICIENCY VIRUS VIRAL LOAD?

Evidence

A systematic review and meta-analysis included 40 studies on 4257 Human Immunodeficiency virus (HIV)-serodiscordant couples with a male partner testing positive for HIV and 11,915 MAR cycles. This review summarized the semen processing techniques of 38 studies, where 29 studies performed a density gradient combined with a swim-up procedure. In 8 studies, only a density gradient centrifugation was performed and only 1 study reported on the HIV status of the post-wash semen: 2.9% of the semen samples were HIV+ (DNA). There was 1 study that reported on a double swim-up technique. In all these studies, 93.8% of the female partners were tested for HIV and no (0/3994 women) seroconversions in 11,585 MAR cycles were reported. There was no vertical transmission in the 1026 babies born (Zafer et al., 2016).

In the cohort study of Zamora et al., 269 semen processing procedures were performed in 183 serodiscordant couples with a male partner testing positive for HIV-1. The semen was used in 234 completed ICSI cycles. 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 (Zamora et al., 2016).

The study of Persico et al. including 55 male patients testing positive for HIV-1 where the semen was prepared through a density gradient (90-47%) followed by swim-up. HIV-1 RNA was found in 2% of the samples after density gradient and all samples tested negative for HIV-1 after the swim-up procedure (Persico et al., 2006).

In the cohort study of Leruez-Ville et al., semen processing through density gradient was performed in 125 men testing positive for HIV-1. Semen was processed using a density gradient (90-45%) followed by 2 semen washes. HIV-1 RNA and DNA were detected in the seminal fraction of 40.7% of the samples, HIV-1 RNA was found in 6.4% and HIV-1 DNA in 1.6% of the spermatozoa fraction (Leruez-Ville et al., 2002).

In the cohort study of Pasquier et al. including 32 men testing positive for HIV-1 (co-infected with HCV) reported HIV-1 RNA positivity in the seminal plasma of 30% of the 51 semen samples. In the spermatozoa fraction, 0% of the samples tested positive for HIV-1. The semen was prepared via triple gradient (90-70-50%) centrifugation followed by swim-up. The study reported that the 50% fraction of the gradient tested positive for HIV-1 whereas none of the pellet fractions tested positive for HIV-1. After swim-up, none of the semen samples tested positive for HIV-1 (Pasquier et al., 2000).

Two studies described the use of an insert device for semen preparation of HIV-1 positive samples. The descriptive cohort study of Fourie et al. including 95 men testing positive for HIV-1 and the preparation of 186 semen samples reported 1.9% of samples being HIV-1+ after density gradient (80-40%) centrifugation using an insert tube (Fourie et al., 2015). The descriptive cohort study of Inoue et al. including 129 serodiscordant couples with a male partner testing positive for HIV-1 and reporting on the outcomes of the semen processing procedure of 183 ejaculates showed 2.2% samples HIV-1+ after

semen processing. The semen procedure consisted of a continuous gradient (80-0%) centrifugation followed by a swim-up procedure where the sample was introduced via an insert tube. This study also described the outcomes of 334 MAR procedures where no seroconversion was observed in the female partner up to 3 months after embryo transfer. In pregnant women, HIV-1 tests were negative at 36 weeks of gestation, at delivery and 6 months after birth. There were no obvious malformations at birth and 100% of the 91 live births tested negative for HIV-1 (Inoue et al., 2017).

Surgically retrieved sperm

There are only a few case reports on the preparation of surgically retrieved sperm.

Nicopoullos et al. reported on a case of a 40-year old male patient testing positive for HIV and diagnosed with congenital bilateral absence of vas deference (CBAVD) experiencing obstructive azoospermia in need of an ICSI treatment. Microsurgical epididymal sperm aspiration (MESA) was performed, but the sperm quality was not suitable for cryopreservation. Hence, MESA was performed on the day of oocyte retrieval. The MESA sample was processed through semen preparation via discontinuous gradient (90-45%) centrifugation and 3 subsequent washes followed by a swim-up (Nicopoullos et al., 2004). The post-wash sample tested negative for HIV-1. Successful fertilization was obtained in this case report however, the embryo transfer did not result in a pregnancy.

Garrido et al. reported on 1 case of a couple with a man testing positive for HIV (without coinfections) undergoing testicular sperm extraction (TESE) owing to the absence of sperm in two consecutive sperm analyses. The TESE fragments were minced mechanically with sterile slides. The suspension was transferred to a tube and centrifuged at 600g for 5 minutes. The pellet was suspended in bicarbonate buffered medium and incubated at 37°C, 5% CO^2 for 1h and samples were frozen. Upon thawing, the samples were washed with bicarbonate buffer at 600g for 5 min. The supernatant was carefully removed and the sample was suspended in bicarbonate medium and washed 2 times and processed to a final volume of 0.5-1 ml. Half of the sample was processed for HIV proviral DNA testing. All samples tested HIV-negative after processing. The TESE samples were used in MAR, but did not results in any pregnancies in the HIV serodiscordant couple. The female partner was tested and found HIV negative (Garrido et al., 2009).

Leruez-Ville et al. reported the outcome of MAR treatment in 2 non-obstructive azoospermic males testing positive for HIV-1 but with undetectable plasmatic HIV-1 viral load. The testicular tissue pieces were washed to eliminate blood contamination. Sterile needles were used for seminiferous tubules dilacerations. The suspension was processed through density centrifugation (1 ml gradient 45%) at 300g for 20 min. The sperm pellet was collected and resuspended in 5 ml medium and centrifuges for 10 min. at 600g. After 2 washes, the pellet was resuspended and used for ICSI in only 1 couple as the TESE samples of the other male patient did not contain any spermatozoa. The final processed samples of both patients were negative for HIV-1 RNA testing. Although no pregnancies were obtained, the female partner tested negative for HIV after the ICSI cycle (Leruez-Ville et al., 2013).

Recommendation

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.	Strong	⊕⊕◯◯

Justification

Current evidence is in support of a thorough semen preparation. Most papers cite a method where sperm is prepared at minimum through a density gradient (80-40% or 90-(50)45%) centrifugation (400 g (10-20 min)) after which the pellet is washed 2 times by adding 5 ml of HEPES buffered medium the first time, 2.5 ml the second time. After these 2 washes, the pellet can be resuspended 1:1 in HEPES buffered medium and swim-up can be performed (1h, angle of 45° at 37°C). Before the density gradient is performed, it is possible to dilute the sperm sample 1:1 in HEPES buffered medium pelleting of the sperm by centrifugation. This pellet is then suspended and used for the density gradient centrifugation. This optional first step is used to remove the excess of seminal plasma immediately before sperm is processed, hence removing the largest amount of virus particles. Although the discontinuous density gradient, followed by 2 wash steps combined with a swim-up has been most described in literature, the technique is not 100% failure proof. Indeed, studies show that post-preparation semen samples can test positive for HIV RNA and/or DNA.

The detection limit for the PCR testing is less sensitive in earlier studies then the detection limit of the PCR testing in more recent studies. This may affect the results of post-preparation PCR testing.

The study of Politch et al. reported on a comparison of a double tube insert and gradient density centrifugation compared to sperm processing in a standard tube using semen samples of men testing negative for HIV-1 and artificially spiked with HIV. A gradient (90-47%) centrifugation with or without a tube insert was used followed by a swim-up procedure. The methods were compared to direct swim-up procedures of samples that were washed 2 times by centrifugation to a pellet and resuspending. All samples were spiked with the same viral load. HIV-1 RNA was detected in the motile fraction of all techniques. This study showed that a direct swim-up procedure with no prior preparation steps is less suitable for removal of HIV particles. At first, a discontinuous gradient centrifugation is needed to remove the majority of the HIV particles. The use of an insert device of a double tube which made it possible to obtain the pellet fraction without contamination of the upper layers, showed superiority in removing of HIV particles (Politch et al., 2004).

The reported procedures with or without a tube insert, are easy to implement in an IVF laboratory and there is a small extra cost compared to a single sperm preparation step. An extra PCR test also needs to be taken into account. This extra cost is small compared to the benefits of performing a safe MAR procedure. When multiple steps are used in semen processing, viable spermatozoa can be lost along the way. Hence, male patients testing positive with HIV and with poor semen quality might benefit from a shorter semen processing procedure and 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. The intervention of a multi-step semen processing procedure for obtaining negative HIV test on the post-preparation semen sample is justified and accepted.

PICO QUESTION: IS THERE A NEED FOR PCR TESTING OF POST-WASHED SPERM?

Evidence

The systematic review and meta-analysis by Zafer et al. mentioned before reported that in 4/29 studies, there were post-wash semen samples that tested Human Immunodeficiency virus (HIV) positive by PCR. The range for positive HIV post-preparation semen samples in these studies ranged from 1.3% to 7.7% (Zafer, et al., 2016).

The descriptive cohort study of Inoue et al. including 129 serodiscordant couples with a male partner testing positive for HIV-1 reported a positive HIV PCR test in 2.2% of the 183 semen samples after continuous gradient (80-0%) centrifugation followed by a swim-up procedure where the sample was introduced via an insert tube (Inoue, et al., 2017).

In the cohort study of Zamora et al., 269 semen processing procedures were performed in 183 serodiscordant couples with a male partner testing positive for HIV-1. The semen was processed via triple density gradient (90-70-45%) centrifugation followed by swim-up and 1.86% of the resulting samples tested positive for HIV-1 (Zamora, et al., 2016).

The study of Persico et al. including 55 male patients testing positive for HIV-1 where the semen was prepared via density gradient (90-47%) centrifugation followed by swim-up. HIV-1 RNA was found in 2% of the samples after density gradient and all samples tested negative for HIV-1 after the swim-up procedure (Persico, et al., 2006).

The study of Fiore et al. investigated 8 semen samples obtained from 8 men testing negative for HIV-1, spiked with different concentration of plasma of a person testing positive with HIV-1. In semen samples containing $1x10^3$ to $5x\ 10^4$ HIV-1 RNA copies/ml, no virus was detected after density gradient. In semen samples containing $1x10^5$ to $5x\ 10^5$ HIV-1 RNA copies/ml, complete removal of the virus was only observed after swim-up (Fiore et al., 2005).

In the cohort study of Leruez-Ville et al., semen processing in 125 males testing positive for HIV-1 via density gradient (90-45%) centrifugation followed by 2 semen washes, showed the detection of HIV-1 RNA and DNA in the seminal fraction in 40.7% of the samples; HIV RNA was found in 6.4% and HIV-1 DNA in 1.6% of the spermatozoa fraction (Leruez-Ville, et al., 2002).

In the cohort study of Pasquier et al. including 32 men testing positive for HIV-1 (co-infected with HCV) reported HIV-1 RNA positivity in the seminal plasma of 30% of the 51 semen samples after processing via triple gradient (90-70-50%) centrifugation followed by swim-up. In the spermatozoa fraction, 0% of the samples tested positive for HIV-1 (Pasquier, et al., 2000).

The study of Kuji et al investigated the buoyant density of HIV-1 and showed that the density of HIV-1 was approximately 1.042 in isopyknic centrifugation. After centrifugation at 1600g for 40 minutes, almost all HIV particles remained in the superficial fractions, where the density was near or less than 1.042 which means that the sedimentation velocity of HIV is very low in the continuous gradients (80-0% or 90-0%) used in this paper. However, a very small amount of virus was found in be clustered at the bottom of the tube. It is thus very important that washing procedures are PCR tested for effectiveness in removing HIV particles from semen (Kuji et al., 2008).

Recommendation

Regardless of the semen processing technique used, the post-preparation sample that is going to be used in MAR from males tested positive for HIV should be HIV PCR tested.	Strong	⊕⊕○○

In serodiscordant couples with the male testing positive for HIV, only a HIV negative tested sperm sample should be used for MAR.	Strong	⊕⊕○○

Justification

Evidence shows that post-prepared semen is not 100% virus-free. There are variations between the studies published. It is thus important to PCR test the semen after preparation and this sample must be negative to be used in MAR. Fluctuations in these data can be the origin of semen samples originating from males with different HIV disease status, using anti-viral drugs or not exerting variable viral loads. Moreover, the technical execution of a semen processing procedure for semen originating from males testing positive for HIV should be performed by a well-trained embryologist. Technical variability in the execution of the procedures can also lead to variability in the post-processing HIV PCR result.

There is very strong evidence in literature that if PCR-tested HIV-negative semen samples are used in MAR, there is minimal risk of transmission.

Under strict conditions, national policies may allow the use of processed semen samples of HIV-infected males without confirmatory PCR testing.

PICO QUESTION: IS THERE A NEED FOR SEMEN PROCESSING WHEN BOTH THE MALE AND FEMALE ARE INFECTED?

Evidence

No studies could be found investigating this PICO question.

Recommendation

Good laboratory practice regarding semen processing should be applied irrespective of whether only the male or both partners are testing positive for HIV.	GPP	

Justification

Most studies excluded couples where both partners tested positive for HIV. Only two studies were found where these couples were included (Stora et al., 2016) and (Santulli et al., 2011). The latter study

included 17 couples were both partners tested HIV positive. In this study, conception in these couples had been sought by unprotected intercourse.

There is no evidence that semen processing might make a difference when both partners test positive for HIV. Practices on semen processing in these cases may differ between centers, however, especially for cryopreservation and storage purposes, consideration should be given to performing semen processing and post-processing HIV PCR testing.

The fact that both partners are infected does not mean it is justified to lower sperm processing standards. Good laboratory practice is required also when both partners are HIV-infected.

PICO QUESTION: DOES THE PLASMA VIRAL LOAD CORRELATE WITH HUMAN IMMUNODEFICIENCY VIRUS DETECTION IN SEMEN?

Evidence

The structured review and meta-analysis of Kalichman et al. reported 19 empirical studies on Human Immunodeficiency virus (HIV) RNA load in blood and semen samples. The review included in total 1226 patients (both on highly active antiretroviral therapy and therapy-naïve patients). An overall moderate mean correlation coefficient was observed between HIV-1 RNA viral load in semen and blood of 0.45 that ranged from 0.07 (very weak in certain studies) to 0.64 (strong in others) (Kalichman et al., 2008).

The study of Kariuki et al. including 43 anti-retroviral therapy naive males testing positive for HIV-1 with a median detectable blood viral load of 4.10 log10 cop/ml found that viral loads in semen correlated moderately with viral loads in blood (Kariuki et al., 2020).

The study of Pasquier et al. on 1396 paired semen and blood samples from 362 males testing positive for HIV-1 (299/362 on antiretroviral therapy) reported HIV-1 shedding in semen in 13% (46/362) of the patients (Pasquier et al., 2017).

The study of Cheret et al. including 19 males tested positive for HIV-1 (on combined anti-retroviral therapy). At the start of the combined anti-retroviral therapy, all patients had a detectable viral load in plasma and 10/19 patients tested positive for HIV-1 DNA in semen. After 2 years of combined therapy, all patients had an undetectable plasmatic viral load and an undetectable HIV-1 RNA load in semen. Semen HIV-1 RNA load correlated well with plasmic viral load in patients with acute infection, but not in patients with a recent infection (Cheret et al., 2017).

The study of Du et al. including 19 men testing positive for HIV-1 (on antiretroviral therapy for 6 months) of which paired semen and blood samples were analysed for HIV-1 RNA reported that HIV-1 RNA was undetectable in 17/19 persons and that seminal HIV-1 RNA was detected in 16/19 persons (Du et al., 2016).

The cross-sectional cohort study of Politch et al. including 52 men testing positive for HIV (on highly active antiretroviral therapy for at least 3 months with undetectable viral load in the blood), observed HIV RNA in semen of 19% (10/52) of the patients ranging from 59 to 800 cop/ml (Politch et al., 2016).

The study of Ferraretto et al., including 88 male patients testing positive for HIV-1 (on antiretroviral therapy with undetectable viral load for more than 6 months), reported that HIV-1 RNA was detected in 7.5% (23/306) of 306 semen samples analysed (Ferraretto et al., 2014).

The study of Lambert-Niclot et al. including 304 males testing positive for HIV-1 (on highly active antiretroviral therapy with an undetectable blood viral load) providing in total 628 paired blood and semen samples reported that 6.6% of the patients exhibited at least one semen sample testing positive for HIV-1 RNA although the blood viral load was undetectable. The HIV-1 RNA viral load in semen ranged from 135 to 2365 cop/ml (Lambert-Niclot et al., 2012).

The study of Politch et al. including 101 HIV-1 positive tested men (80% were on highly active antiretroviral therapy for > 1 year), reported an HIV-1 RNA viral load in blood in 18% of the men and of 30% (RNA and/or DNA) in the compared semen sample (Politch et al., 2012).

The cross-sectional cohort study of Sheth et al. including 25 untreated HIV-1 positive tested men where in total 116 paired semen and blood samples were collected over a period of 24 weeks of the start of anti-retroviral therapy, reported that semen shedding was present during 16.4% of the study visits (19/116) with a compared blood samples presenting an undetectable viral load. No association was found between the isolated semen shedding and specific antiretroviral agents or classes (Sheth et al., 2009).

The study of Bujan et al. reported on the HIV-1 RNA viral load of 281 paired blood and semen samples 94 males testing positive for HIV-1 (78/94 were on antiretroviral therapy). HIV-1 RNA was detected in the blood of 72.2% of the patients corresponding to 53.7% of all the blood samples tested with a range of 3 to 130,000 cop/ml. HIV-1 RNA was detected in 38 semen samples ranging from 5 to 277,500 cop/ml. HIV-1 RNA in blood and seminal plasma were not correlated in this study. When blood HIV-1 RNA was detected, 19.4% of semen samples were positive for HIV-1 RNA. When blood HIV-1 RNA was undetectable, 7.9% of seminal plasma tested positive for HIV-1 RNA. HIV-1 DNA was detected in 8.7% of native semen samples (Bujan et al., 2004).

The cross-sectional cohort study of Leruez-Ville et al. reported on the HIV-1 viral loads in paired semen and blood samples of 125 males testing positive for HIV-1 (mixed treatment regimens). In the total population, HIV-1 RNA was detected in the blood of 40% (50/125) of the patients and in the semen of 6.4% (8/125) of the patients, 2 patients (1.6%) tested HIV-1-DNA-positive in their semen sample. This study showed a strong correlation between HIV-1 RNA loads in blood and in seminal plasma (Leruez-Ville, et al., 2002).

The cross-sectional study of Gupta et al. included 18 asymptomatic males testing positive for HIV-1, with a median CD4 cell count of 434 cells/mm^3 (range: 117 to 935 cells/mm^3) (no protease inhibitor therapy during the study period). Paired semen and blood samples were collected over a period of 10 weeks. This study showed 3 patterns of viral shedding in semen: (i) the intermittent shedder where the plasma viral load remained stable of the 10 weeks, where the semen viral load fluctuated, (ii) the persistent shedder where the plasma HIV-1 viral load and the viral load in semen showed some association and (iii) the non-shedder where the viral load in blood was low to median and there was no viral detection in semen. The data in this study shows that shedding of HIV-1 in semen is different and can be complex in males testing positive for HIV-1 (Gupta et al., 2000).

The cross-sectional cohort study of Ball et al. including 34 male patients testing positive for HIV-1 (no anti-viral therapy in the preceding 3 months of the study), reported on a moderate correlation between blood and semen viral RNA ($r=0.5156$, $p<0.005$). Blood proviral DNA was found in 100% of the samples tested, with a median of 496 cop/ml ranging from 9 to 5678 cop/ml. Proviral HIV-1 DNA was found in 47% of the semen samples tested with a median of <6 cop/ml ranging from <6 to 2171 cop/ml. HIV-1

viral RNA was detected in 76% of the blood samples analysed with a median of 18,600 cop/ml ranging from <2000 to 977,600 cop/ml. Viral RNA was detected in 63% of the semen samples with a median of 5600 cop/ml ranging from <2000 to 667,800 cop/ml. Proviral DNA in semen was associated with concomitant viral RNA in semen ($p<0.05$) and proviral DNA and viral RNA were significantly higher in blood compared to the corresponding semen (Ball et al., 1999).

The study of Xu et al. including 74 untreated men tested positive for HIV-1 reported on HIV-1 DNA detection in 100% of the blood samples ranging from 20 to 2500 cop/ml and in 65% of the semen samples ranging from <10 to 5000 cop/ml. There was a weak correlation ($r=0.35$, $p<0.05$) between HIV DNA detected in blood compared to semen (Xu et al., 1997).

The cross-sectional study of Liuzzi et al. including 23 male patients tested HIV-1 positive (no antiretroviral therapy) reported that there was no correlation between HIV-1 RNA levels in blood and semen (Liuzzi et al., 1996).

Recommendation

Advanced semen processing is recommended for male patients testing positive for HIV, regardless of the viral load in the serum and therapy status.	Strong	⊕○○○

Justification

Although there are studies reporting on various correlation coefficients between HIV viral load in semen and blood, it is clear that there is no definite strong correlation between the parameters. There is strong evidence showing that there is a variability in the correlation between the viral load in semen and blood and there is only very weak evidence showing a strong correlation between viral load in blood and semen. There is variability in patient parameters in the studies where patients were on highly active antiretroviral therapy, others had not been treated yet. Even in studies with patients on highly active antiretroviral therapy and having a stable undetectable viral load in blood, there was a small percentage of semen samples testing positive for HIV.

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C6. Reducing/ avoiding vertical transmission

PICO QUESTION: WHICH INTERVENTIONS CAN BE USED TO REDUCE/AVOID VERTICAL TRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS TO THE NEWBORN?

ELECTIVE CAESAREAN SECTION

Evidence

A systematic review and meta-analysis investigated the association between mode of delivery and the risk of vertical Human Immunodeficiency virus (HIV) transmission. One RCT was included, which reported significantly fewer HIV infections among infants delivered by elective caesarean section (ECS) (1.7%) versus vaginal delivery (10.6%) (OR 0.2; 95% CI 0.0 to 0.5; 1 RCT; 385 infants). The OR was nonsignificant for women who received zidovudine in pregnancy (OR 0.4; 95% CI 0 to 1.4) compared with the OR for women who received no zidovudine in pregnancy (OR 0.2; 95% CI 0 to 0.8). Meta-analysis of all observational studies showed that ECS was also associated with a decreased risk of infant HIV infection with ECS compared to vaginal delivery (OR 0.43, 95% CI 0.30 to 0.63, 13 studies, 16204 infants). Stratifying to patients receiving combined antiretroviral therapy, the association between ECS and lower infant HIV infection was no longer statistically significant (OR 0.82, 95% CI 0.47 to 1.43, 4 studies, 8823 infants) (Kennedy et al., 2017).

A large cohort study, including 2297 women testing positive for HIV-1, compared vaginal delivery with elective and non-elective CS. Last maternal HIV-1 viral load before delivery of ≤ 400 copies/ml was 93% in the vaginal delivery group, 73% in the ECS group and 79% in the non-elective CS group. In mothers with viral load ≤ 400 copies/ml, HIV-1 transmission to the infant occurred in 4 (0.4%) in the vaginal delivery group, 1 (0.1%) in the ECS group and none in the non-elective CS group. In mothers with viral load > 400 copies/ml, HIV-1 transmission to the infant occurred in 2 (0.2%) %) in the vaginal delivery group, 3 (0.4%) in the ECS group and 2 (0.5%) in the non-elective CS group (Livingston et al., 2016).

Edathodu et al. compared 11 vaginal deliveries with 28 elective CS in women testing positive for HIV-1 (on antiretroviral therapy during pregnancy and delivery) and reported no perinatal transmission of HIV-1 at 18 months of age (Edathodu et al., 2010).

A prospective cohort study including 259 infants born to women testing positive for HIV-1, reported the risk of transmission by mode of delivery in 2 periods of time (with and without standard maternal zidovudine treatment). In period 1, without maternal zidovudine treatment, 105 women delivered vaginally and 27 by CS. The risk of transmission was 20.6% (28/136; 95% CI 14.1 to 28.4%). In period 2, with maternal zidovudine treatment, 101 women delivered vaginally and 24 by CS. The risk of transmission of HIV was 9.8% (12/123; 95% CI 5.1 to 16.4%). There was no difference between vaginal and CS delivery in HIV transmission over both periods (14.8% (30/203) vs. 14.9 (7/47)) (Simpson et al., 1997).

A prospective cohort study, including 848 women testing positive for HIV-1 and their infants, investigated the vertical transmission rate by mode of delivery. 723/848 infants were delivered

vaginally and 121/848 by CS. At 18 months of age, 171/848 infants were infected with HIV-1. However, the risk of transmission did not differ according to the type of delivery (RR 1.0; 95% CI 0.7 to 1.4) (Mayaux et al., 1995)

In a retrospective cohort study, including 580 pregnancies of women testing positive for HIV, and reported 142 (24.5%) vaginal deliveries, 323 (55.7%) CS and 115 (19.8%) non-elective CS. Vertical transmission occurred in none of the vaginal deliveries (0/139), 0.3% (1/316) of CS and 0.9% (1/113) of non-elective CS (Tibaldi et al., 2019).

In a retrospective case comparison study, including 389 pregnancies of women testing positive for HIV-1 (all on antiretroviral therapy at delivery), the cut-off for vaginal delivery was a viral load ≤1000 copies/ml. 130/389 (33.4%) deliveries were vaginal, 158/389 (40.6%) ECS and 101/389 emergency CS (26%). No perinatal HIV-1 infection occurred (Orbaek et al., 2017).

In a retrospective cohort study, including 8237 samples from infants born to mothers testing positive for HIV were tested for HIV. There was no significant difference in HIV transmission rates between vaginal and CS delivery if both mother and infant received PMTCT interventions (Torpey et al., 2012).

A registry study from Finland, including 212 women testing positive for HIV, reported 7.8% CS (21/264) because of poorly controlled HIV between 1999 and 2013. The overall rate of vaginal delivery in this period was 74.5%. No perinatal HIV transmission occurred (Aho et al., 2018).

Recommendation

Caesarean section is recommended in women with detectable HIV viral loads.	Strong	⊕⊕◯◯

Justification

Published cohort data from the European countries have shown vertical transmission rates of <0.5% in women with plasma HIV RNA <50 HIV RNA copies/mL on antiretroviral therapy, irrespective of mode of delivery. These studies support the practice of recommending planned vaginal delivery for women on antiretroviral therapy with plasma viral load <50 HIV RNA cop/mL. The risk of transmission increases significantly with increased viral load.

BREASTFEEDING

Evidence

A randomised controlled trial, including 425 women testing positive for HIV-1, assigned 213 women to the formula feeding arm and 212 women to the breastfeeding arm. Ninety-two infants acquired HIV-1 infection during the study, 21% (31/204) in the formula feeding arm and 37% (61/197) in the breastfeeding arm. There was no significant difference in mortality rates at 2 years of age between formula-fed and breastfed infants (Mbori-Ngacha et al., 2002).

A prospective cohort study, including 318 women testing positive for HIV-1 and their infants, investigated the risk of HIV-1 infection in their infants with breastfeeding (with vs. without antiretroviral

therapy) and not breastfeeding. The rate of perinatal HIV-1 infection significantly higher in breastfed infants compared with non-breastfed infants (OR 7.08; 95% CI 3.27 to 13.30). The use of antiretroviral therapy during breastfeeding was not associated with post-natal HIV-1 infection among infants (OR 0.02; 95% CI 0.004 to 0.091) compared to not on antiretroviral therapy during breastfeeding (OR 54.94; 95% CI 10.94 to 276.00) (Imade et al., 2010)

A prospective cohort study, including 562 women testing positive for HIV-1, investigated the risk of HIV-1 transmission by breastfeeding versus formula feeding. All included women received highly active antiretroviral therapy during pregnancy and all infants received immunoprophylaxis for one week irrespective of the feeding choice. 240 women (42.7%) preferred to breastfeed under highly active antiretroviral therapy and 322 women (57.3%) chose to bottle feed. At 6 weeks of age, the cumulative probability of HIV-1 transmission was 1.3 (95% CI 0.4 to 4.1) with breastfeeding versus 1 (95% CI, 0.3 to 3.0) with formula feeding. At 9 months of age, the cumulative probability of HIV-1 transmission was 1.8 (95% CI 0.7 to 4.8) with breastfeeding versus 1 (95% CI 0.3 to 3.0) with formula feeding (Peltier et al., 2009).

A prospective cohort study including 182 infants born to women testing positive for HIV, compared the mortality and HIV-free survival of infants by feeding practices. All women and infants received antiretroviral therapy or immunoprophylaxis if indicated. 59% of mothers chose to breastfeed and 41% chose to formula-feed. At one month of age, 13.0% (12/92) of the breastfed infants tested positive for HIV compared to 4.4% (3/69) of the formula-fed infants. There was no statistically significant difference in HIV-free survival with formula feeding compared to breastfeeding (86% vs. 96%; adjusted HR 2.8, 95% CI 0.67 to 11.7) (Kagaayi et al., 2008).

A prospective cohort study, including 306 infants born to women testing positive for HIV, compared HIV transmission rates between exclusive breastfeeding, exclusive formula feeding and mixed feeding. At 6 weeks of age, the HIV transmission rate was 11.2% (17/152) with exclusive breastfeeding, 3.4% (4/117) with exclusive formula feeding and 17.1% (6/35) with mixed feeding. At 6 months of age, HIV transmission rates were 16.0% (19/119) with exclusive breastfeeding, 3.7% (4/108) with exclusive formula feeding and 20.4% (10/49) with mixed feeding (Magoni et al., 2005).

A prospective cohort study including 203 infants born to women testing positive for HIV-1 investigated risk of vertical transmission by feeding practices. At 3 months of age, 59.1% (120/203) of infants were exclusively breastfed, while 39.9% (81/203) were mixed fed and 1.0% (2/203) were formula fed. HIV-1 incidence was 8.33 and 8.64 per 100 child months for breastfed only and mixed fed infants, respectively. There was no formula-only fed infant diagnosed as HIV-1 infected at 3 months of age (Olayinka et al., 2000).

A prospective cohort study, including 549 women testing positive for HIV-1 and their infants, investigated the HIV-1 transmission rate by feeding practices (exclusive breastfeeding, formula feeding or mixed feeding). At 3 months of age, the HIV-1 transmission rate in the formula feeding group was 18.8%, compared to 24.1% in the mixed feeding group and 14.6% in the breastfeeding group (Coutsoudis, 2000).

A cohort study including 961 infants born to women testing positive for HIV-1 (part of the subjects were enrolled prospectively and part retrospectively) assessed the effect of breastfeeding on HIV-1 transmission to the infants. 168 infants were breastfed and 793 infants were bottle-fed. The estimated

adjusted infection ratio for one day of breastfeeding versus bottle feeding was 1.19 (95% CI 1.10 to 1.28) (De Martino et al., 1992).

A large retrospective cohort study, including 1086 infants born to mothers testing positive for HIV-1, compared exclusive breastfeeding with exclusive formula feeding and mixed feeding. At 3 months of age, 61.1% (663/1086) were on exclusive formula feeding, 37.6% (408/1086) were on exclusive breastfeeding and 1.4% (15/1086) were mixed-fed. Vertical transmission was 3.8% with exclusive formula feeding, 2.7% with exclusive breastfeeding and 21.4% with mixed feeding. Stratified according to maternal antiretroviral therapy, HIV vertical transmission rates were 1.7% (10/566) with combined antiretroviral therapy, 1.9% (8/411) with zidovudine, and 19.2% (21/109) without antiretroviral therapy (Njom Nlend et al., 2018).

A retrospective cohort study, including 857 infants born to women testing positive for HIV, investigated the HIV-free survival of the infants by feeding practices. Immunoprophylaxis was provided to 77.2% of infants in the breastfeeding group and 75.0% of infants in the formula feeding group. The cumulative probability of HIV-free survival of infants in the breastfeeding group was 95% and 93% at 180 and 360 days respectively. However, in the formula-fed group, this cumulative probability was 97% (Assefa et al., 2017).

In a retrospective cohort study, data from 432 infants born to women testing positive for HIV-1 (naïve for zidovudine) were assessed for the risk of transmission by feeding practices. Infants who were breastfed had a significantly higher risk of being infected with HIV-1 than those who were never breastfed (21% vs. 13%). No clear pattern in risk of transmission by duration of breastfeeding could be observed (Tess et al., 1998).

Recommendation

A female testing positive for HIV should refrain from breastfeeding when and where she has safe nutritional alternatives.	Strong	⊕⊕◯◯

Justification

Suppressive maternal antiretroviral therapy significantly reduces, but does not eliminate, the risk of vertical transmission of HIV through breastfeeding.

In Europe and other high-income settings, the safest way to feed infants born to women with HIV is with formula milk, as there is on-going risk of HIV exposure after birth. We recommend that women testing positive for HIV feed their babies with formula milk. Current WHO advice on breastfeeding for women with HIV is aimed at low- and middle-income countries where there is a high risk of infant morbidity and mortality from diarrhoea and other infections, and where formula feeding is not available for many families (WHO, 2016).

COMBINED NEONATAL PROPHYLAXIS (CNP)

Evidence

A systematic review and meta-analysis, compared infant prophylaxis with a single drug with a multidrug regimen. Transmission rates of HIV for infants receiving single-drug prophylaxis ranged from 2% (95% CI 0.3% to 5.2%) to 4.8% (95% CI 3.2% to 7.1%). In the multidrug arm HIV transmission rates were 2.2% (95% CI 1.2% to 3.9%) in the 2-drug arm, and ranging from 0.4% (95% CI 0.1% to 1.4%) to 2.4% (95% CI 1.4% to 4.3%) in the 3 drug arm. In the EPPICC study, however, transmission rates were higher in the multidrug group (6.3% vs. 3.4%; OR 1.41; 95% CI 0.97 to 2.05). The higher transmission rate in this study, and in contrast with other studies, is likely the result of severe confounding by indication (Beste et al., 2018).

A systematic review and meta-analysis, including 4459 children from HIV-infected women, reported that mother-to-child transmission (MTCT) rates were 3.4% (95% CI 2.7 to 4.0), 6.3% (95% CI 4.8 to 7.6) and 17.7% (95% CI 13.9 to 21.5) for one-drug neonatal prophylaxis, combined neonatal prophylaxis (CNP) and no neonatal prophylaxis, respectively. Crude MTCT rates were 1.8% (39/2140) and 4.2% (29/681; adjusted OR 1.97; 95% CI 1.14 to 3.39) in one drug and CNP groups, respectively, among infants whose mothers received antenatal antiretroviral therapy; 7.0% (18/257) and 5.9% (8/134; adjusted OR 0.86; 95% CI 0.28 to 2.64) in those whose mothers received no antenatal or intrapartum antiretroviral prophylaxis; 8.0% (42/523) and 13.7% (27/198) (adjusted OR 1.57; 95% CI 0.81 to 3.08) among those whose mothers received only intrapartum prophylaxis (Chiappini et al., 2013).

A systematic review and meta-analysis, including 10 studies, reported that the combined transmission rate for arms that used antiretroviral therapy (both in mothers to reduce viral load as in neonates as prophylaxis) is 10.6% (95% CI 8.6 to 13.1), while the combined transmission rate for arms that used placebo is 21.0% (95% CI 15.5 to 27.7). Using the combined transmission rates above, the efficacy of using antiretroviral therapy to reduce MTCT is approximately 50% (1–10.6/21.0) (Chigwedere et al., 2008).

An RCT including 1522 infants born to women testing positive for HIV-1 (29% on antiretroviral therapy in each treatment arm), compared extending the infant Nevirapine (NVP) prophylaxis treatment beyond 6 weeks until 6 months of age with not extending prophylaxis. All infants had received NVP prophylaxis until 6 weeks of age. At 6 months of age, HIV-1 infection was significant lower in the NVP arm (1.1%; 95% CI 0.3 to 1.8%) compared to 2.4% (95% CI 1.3 to 3.6%) in the placebo arm. However, at 18 months of age, the HIV-1 infection rates were no longer significantly different (Fowler et al., 2014).

An RCT including 407 infants born to women testing positive for HIV-1, compared two prophylaxis regimes (6 months NVP vs. 6 weeks NVP) with placebo. In the placebo arm, 7.2% (7/97) infants tested positive for HIV-1 compared to 2.7% (4/146) with 6 months NVP treatment and 5.3% (3/57) with 6 weeks of NVP treatment (Aizire et al., 2012).

An RCT including 1829 women testing positive for HIV and their infants, compared a maternal antiretroviral therapy regime (according to the ruling regulations) with an infant NVP regime (during breastfeeding) and a control group (no intervention after the initial 7 days prophylaxis). At 48 weeks of age, the cumulative risk of HIV-1 transmission was significantly higher in the control group (7%; 95% CI 5 to 9) compared to the maternal regime (4%; 3 to 6) or the infant regime group (4%; 2 to 5) (Jamieson et al., 2012).

An RCT, including 1890 infants born to women testing positive for HIV-1, compared a single dose of NVP during labour for the mother and a single dose after birth for the infant with a single dose for the mother during labour and an extended 6-weeks regimen for the infant (during breastfeeding). HIV transmission was 8.9% in the extended-dose group compared to 10.4% in the single-dose group, but the difference was not significant (RR 0.87, 95% CI 0.65 to 1.15) (Omer, 2011).

An RCT including 3126 infants born to women testing positive for HIV-1, reporting the follow-up results at 9 months of age of those infants not infected at birth. The control group received a single dose of NVP at birth combined with one week of ZDV. Group A received the control regimen combined with extended NVP treatment until 14 weeks of age. Group B received the control regimen combined with NVP and ZDV until 14 weeks of age. At 9 months of age, significantly more HIV infections had occurred in the control arm (11.1%; 95% CI 9.3 to 13.3) compared to 5.0% (95% CI 3.8 to 6.6) in the extended NVP arm and 6.0% (95% CI 4.7 to 7.7) of the extended NVP/ZDV combination arm. At 2 years of age, significantly more HIV infections had occurred in the control arm (15.6%) compared to the extended NVP arm and the extended NVP/ZDV combination arm (10.8% and 11.2% respectively) (Taha et al., 2011).

An RCT including 2369 mothers testing positive for HIV-1 and their infants, compared providing prophylaxis during pregnancy with providing prophylaxis to the infant (during breastfeeding) with a control group (no extended postnatal antiretroviral therapy). At 2 weeks of age, there was no significant different in risk of HIV-1 transmission between the control group (5.4%; 95% CI 3.9 to 7.4), the maternal regimen group (5.5%; 95% CI 4.1 to 7.2) and the infant regimen group (4.4%; 95% CI 3.2 to 6.0). Among infants who were HIV-1–negative at 2 weeks of age, the estimated risk of HIV-1 infection by 28 weeks of age was 5.7% in the control group, 2.9% in the maternal regimen group, and 1.7% in the infant regimen group (Chasela et al., 2010).

An RCT including 1200 women testing positive for HIV-1, compared 1 month of ZDV prophylaxis in the infant with or without a birth-dose of NVP or placebo (formula feeding group) with 6 months of ZDV prophylaxis in the infant (breastfeeding group). Late HIV-1 transmission (after 1 month of age) occurred in 4.4% (24/547) of infants in the breastfeeding group, compared to 2 infants in the formula-fed group (Shapiro et al., 2009).

Recommendation

Combined neonatal prophylaxis (CNP) is recommended for neonates born to mothers testing positive for HIV.	Strong	⊕⊕⊕○

Justification

There is no doubt that the introduction of antiretroviral prophylaxis of neonates has significantly reduced the rates of vertical transmission of HIV. Due to the complexity of available regimens coupled with the challenges of neonatal pharmacokinetics, the GDG advises to consult national and International guidelines for more details.

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Summary

HIV	Male testing positive		Female testing positive		Couple testing positive	
Before MAR	Consult with infectious disease specialist					
	Undetectable viral load	HIV detected in blood	Undetectable viral load	HIV detected in blood	Undetectable viral load (female)	HIV detected in blood
		Risk of HT	Risk of VT	Risk of VT + HT	Risk of VT	Risk of VT + HT
During MAR	IUI, IVF or ICSI depending on infertility work-up					
	Specific semen processing* and semen HIV PCR testing recommended		Standard oocyte processing		Specific semen processing* and semen HIV PCR testing recommended	
After MAR			Caesarean section recommended if detectable HIV viral load			
	Breastfeeding = option		Breastfeeding not recommended			
			CNP			

*Density gradient centrifugation followed by 2 semen washing steps, followed by swim-up

Figure 3: Summary of management of medically assisted reproduction in patients testing positive for human immunodeficiency virus.

PART D: Human Papilloma virus

D1. Prevalence and testing

NARRATIVE QUESTION: WHAT IS THE PREVALENCE OF HUMAN PAPILLOMA VIRUS?

Human papillomavirus (HPV) is the most common sexually transmitted infection (Ciavattini et al., 2020).

HPV infection is generally asymptomatic, with the worldwide rate in women with normal cytology of 11.4% (Bruni et al., 2018). Globally, prevalence of genital HPV infection in men is higher than in women (Serrano et al., 2018). HPV is more prevalent among young women, with a rate of 24% in <25 years old women (Serrano, et al., 2018). As reviewed by Serrano et al., among men, HPV prevalence is highest at the penis and lowest at the urethra; among women, HPV prevalence is highest at the cervix and vagina and lower at the vulvar epithelium (Serrano, et al., 2018). About 90% of HPV infections cause no symptoms and resolve spontaneously within two years (Baseman and Koutsky, 2005).

Considerable regional differences and substantial variations between studies have been documented on the prevalence of HPV (Serrano, et al., 2018). A high rate of HPV infection has been found in Africa and Oceania (Forman et al., 2012). Epidemiological studies also indicate that nearly 80% of HPV-positive women acquire genital HPV by age 50 (Coscia et al., 2015). No differences in the HPV type distribution between different geographical areas has been reported (Ciavattini, et al., 2020). The most common types of HPV worldwide are HPV16 (3.2%), HPV18 (1.4%), HPV52 (0.9%), HPV31 (0.8%), and HPV58 (0.7%).

In the natural history of HPV infections, the HPV virions can induce two different pathways, namely the clonal transforming pathway and the infectious virion producing pathway. When the detected viral HPV DNA originates from a dividing cell, this DNA is never infectious (dividing cells do not support virion production) and does not affect fertility since it cannot interact with HPV receptors (syndecan-1) present on spermatozoa or endometrial cells (Depuydt FVV 2016a). However, the viral DNA can transform the dividing cell which could in time lead to pre-cancer and cancer (Garolla et al., 2013). HPV infection has been identified to be involved in cervical, oropharyngeal, vulvar, vaginal and anal cancers. High-risk, oncogenic HPV types, such as HPV16/18 are cumulatively associated with over 99% of all cervical cancers (Smith et al., 2007). Only the infectious HPV virions can bind cells that influence fertility in both men (spermatozoa) and women (endometrial cells) via the Syndecan-1 receptor. The bulk of the detected HPV DNA whether in men or women is infectious from origin. In women with HPV-induced cancer, the detected viral HPV DNA is inside dividing cells and (Depuydt et al., 2016a).

The role of HPV in infertility remains to be elucidated. Determining the origin of HPV DNA is the key to predict the impact of the HPV infection (Depuydt, et al., 2016a). A growing number of studies demonstrated a correlation between HPV sperm infection and unexplained asthenozoospermia and unexplained infertility (Foresta et al., 2010, Foresta et al., 2011, Garolla et al., 2012, Garolla, et al., 2013, Gizzo et al., 2014, Lee et al., 2002).

Although in the majority of cases only one of the two HPV induced pathways is present, in almost one fifth of HPV infected women (18%) both the virion producing and HPV transforming pathway occurs at the same time. In most cases multiple HPV types are present but it is also possible that only one HPV type is detected (Depuydt et al., 2016b).

Independent of the number of HPV types detected, serial measurement of type specific viral load allows to identify the origin of the detected HPV DNA and makes it possible to assess the impact of the HPV infection for cancer screening or fertility.

Although HPV is not a lytic virus, harbouring its DNA will ultimately lead to cell death or immortality depending on the cell type.

NARRATIVE QUESTION: HOW SHOULD TESTING OF HUMAN PAPILLOMA VIRUS STATUS PRIOR TO MEDICALLY ASSISTED REPRODUCTION BE PERFORMED?

Human papillomaviruses (HPVs) are small DNA viruses that mainly infect mucosal epithelia of anogenital and upper respiratory tracts (Tommasino, 2019).

HPVs can be divided into high-risk and low risk groups according to their ability to induce cellular transformation and carcinogenesis. Despite the well-known role of HPV in cervical carcinogenesis, the current standard screening test for cervical cancer and CIN lesions is a cytological staining based technique, known as the pap-smear test. A more sensitive technique, which is the HPV-specific qualitative PCR, has been adopted only in certain circumstances (Berkowitz, 2013). So far, cytology-based screening programs have reduced cervical cancer incidence/mortality. However, the highest impact of this screening protocol has already been reached, in terms of cervical cancer incidence (Berkowitz, 2013).

Efforts to detect HPV, both DNA and antibodies, have increased significantly in recent years. The actual gold standard for HPV detection is Nucleic Acid Tests (NAT), that allow also for the genotyping of the virus. The NAT currently uses PCR techniques, such as qPCR and ddPCR (droplet digital PCR) as well as blotting tests, such as: (i) Line blot assay, (ii) Linear Array and (iii) Dot-blot hybridization.

Quantitative real-time PCR (qPCR) has been widely employed due to (i) its use in HPV DNA load quantification, its broad detection-range of target molecules and multiplexing potential (Malagutti et al., 2020). However, qPCR has several intrinsic limitations, including: (i) low sensitivity in quantifying the amount of viral DNA when present in a low-copy number (Caraguel et al., 2011); (ii) lack of precision in estimating small differences in copy number among samples (Hindson et al., 2011); (iii) the need for calibration curves, represented by plasmid vectors carrying viral DNAs, thereby increasing the risk of false-positive results. Previous studies also reported on HPV DNA detection/quantification employing ddPCR methods (Biron et al., 2016, Jeannot et al., 2016, Lillsunde Larsson and Helenius, 2017). These studies are addressed to the identification of a single specific HPV type. Consequently, a large number of experimental runs is required, as HPV type specific primer sets and TaqMan probes are employed in each experiment.

A recent study also reported the implementation of the HPV-specific ddPCR method ensuring the simultaneous detection of different human papillomavirus types and quantification of their viral DNA load in clinical specimens (Rotondo et al., 2020).

The assays to detect HPV antibody response in serum/plasma/blood can be divided in (i) Neutralization assays; (ii) Competitive immunoassay; (iii) Enzyme Immunoassays (EIA). The first include Pseudovirion-based neutralization assay and PsV encapsidating. The second are based on Competitive luminex immunoassay (cLIA) and 9-plex competitive Luminex Immuno Assay (9-plex cLIA). The third are mainly Direct/Indirect Enzyme linked immunosorbent assay (ELISA), enzyme multiplied immunoassay technique (EMIT), Bio-Plex and digital ELISA tests.

In conclusion, a test characterized by optimized clinical sensitivity and specificity that may be used in the clinic for routine testing is needed. To this aim, the work of Rotondo et al. (2020) is proposed (Rotondo, et al., 2020).

Conclusion

HPV testing of couples pursuing MAR treatment is not included in the European Tissue and Cells Directive, however, this is a rapidly developing area and focussed research is urgently needed. Testing for HPV is challenging and its importance still needs to be clarified in the field of medically assisted reproduction.

In couples attempting IUI, it may be beneficial to test for the presence of HPV. The results of the testing could be informative for the cause of unexplained infertility since male HPV positivity contributes to the risk of male infertility. The role of HPV infection in females undergoing assisted reproduction is still controversial.

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D2. Prevention of transmission before medically assisted reproduction

PICO QUESTION: WHAT ARE THE RISKS OF HUMAN PAPILLOMA VIRUS TRANSMISSION THROUGH VAGINAL/ANAL INTERCOURSE?

Evidence

A prospective cohort study investigating transmission of human papilloma virus (HPV) between partners included 25 couples who were in a monogamous heterosexual relationship for at least 25 months. At each visit, the transmission rate from female to male was higher than from male to female. The overall transmission rate for female anogenital (genital and anal sites combined) to male anogenital areas between visit 1 and the other visits was 21.35 per 100 person-months, and the overall transmission rate for male-to-female transmission was 9.23 per 100 person-months (Widdice et al., 2013).

In the study by Burchell et al., 263 couples were screened for HPV (types 6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, and 89) in genital samples: vaginal swabs for women, epithelial cells from the penis (the glans up to and including the external opening of the meatus, coronal sulcus, penile shaft and foreskin) for men. HPV was detected in 56% of women and men. Prevalence was higher among persons with infected partners (85%) than in those whose partners were negative (19%). Type-specific detection was substantially higher among women (OR 55.2; 95% CI 38.0 to 80.1) and men (OR 58.7; 95% CI 39.8 to 86.3) if their partner harboured the type under consideration. Furthermore, the prevalence among women and men with 10 or more lifetime partners was 15.4 (95% CI 5.9 to 40.2) and 9.5 (95% CI 4.4 to 19.8) times higher than among those with 1 partner (Burchell et al., 2010).

A prospective cohort study, including 25 heterosexual, non-pregnant, monogamous couples, investigated the HPV transmission between partners. A total of 53 heterosexual transmission events were observed among 16 couples (14 male-to-female and 39 female-to male). All infections transmitted from male to female partners originated from the penis with or without additional involvement of the scrotum. Transmission from female to male partners originated from the cervix and/or urine to the male genitalia (Hernandez et al., 2008).

In the study by Kjaer et al., a cohort of 11,088 women was screened for HPV 16 in cervical swabs, Pap smears and blood samples. All of the virgins who stayed virginal throughout the study continued to be HPV DNA negative at follow-up. Risk factors for acquisitions of HPV DNA were the number of sex partners. women with ≥3 partners having a 9.1 times increased risk (95% CI 1.8 to 48.5) for acquiring HPV DNA when compared to women with one partner during follow-up. Likewise, the number of sex partners was also a risk factor for being HPV DNA-positive at follow-up among women who had one partner at enrolment in the study. Women who at follow-up reported to have had three or more partners had a 9.4 times higher risk (95% CI 2.1 to 41.3) of being HPV positive than women with one partner (Kjaer et al., 2001).

Dillner et al. reported that the proportion of HPV-16-seropositive subjects increased linearly at approximately 4% per partner ($P < 0.001$), from 4% among those with 1 lifetime partner to 35% among

those with >5 lifetime partners. The seroprevalence of HPV-33 and HPV-18 were also linearly dependent on the number of partners, with respectively 4% and 3% increase per partner (Dillner et al., 1996).

Conclusion

HPV transmission occurs during vaginal intercourse and the risk of transmission increases with the number of partners.

The use of condoms does not completely prevent HPV infection as it can be transmitted via skin-to-skin contact, however, it can lower the risk of HPV transmission.

Recommendation

The use of barrier contraception during sexual intercourse is advised to lower the risk of Human Papilloma virus (HPV) transmission.	GPP

Justification

The HPV virus can be found on scrotal skin, thus the use of condoms does not prevent HPV infection (Weaver et al., 2004). However, it can lower the chance of transmitting HPV.

PICO QUESTION: IS THERE A THRESHOLD BELOW WHICH TRANSMISSION OF HUMAN PAPILLOMA VIRUS IS UNLIKELY?

Evidence

Vertical transmission

A small study including 15 pregnant women by Kaye et al. reported that transmitters had a significant higher Human Papilloma virus (HPV)-16 viral load compared to non-transmitters (mean 2 standard deviation; 4.35 ± 2.84 U/PCR sample vs. 1.83 ± 1.12 U). The viral copy number was also significantly higher in transmitters versus non-transmitters (35 to 5 x 106 cop/PCR sample (629,886 ± 1,765,883) vs. between 17-195 copies (70.8 ±65.25 copies) $P < 0.05$) (Kaye et al., 1994).

A cohort study by Hahn et al. reported a statistical non-significant association of higher maternal HPV load with infection in neonates. There was no difference in maternal HPV copy number per cervical cell between HPV positive and HPV negative neonates (Hahn et al., 2013).

Conclusion

There is no evidence that there is a specific HPV DNA copy number threshold below which (horizontal or vertical) transmission is unlikely.

All women starting medically assisted reproduction (MAR) should undergo testing to detect HPV-related cervical lesions.	GPP

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D3. Assisted reproduction techniques and impact on outcomes

PICO QUESTION: SHOULD IUI, IVF OR ICSI BE PREFERENTIALLY USED FOR MAR IN HUMAN PAPILLOMA VIRUS INFECTED COUPLES?

We identified no studies that have compared different techniques for MAR in couples where one partner is infected with human papilloma virus (HPV) in terms of risk of transmission.

Recommendation

The cause of infertility should dictate the specific technique (IUI/IVF/ICSI) used for MAR in couples where one or both partners test positive for HPV.	Strong	⊕○○○
Women infected with HPV should be informed that MAR does not eliminate the risk of vertical transmission.	GPP	

Justification

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 HPV.

PICO QUESTION: CAN HUMAN PAPILLOMA VIRUS DNA BE DETECTED IN OOCYTES/ SPERM/ PLACENTA?

Evidence

DNA integration

Capra et al. investigated the presence of HPV in total semen and semen fractions after washing and found viral HPV DNA in total sperm, cell fraction and seminal plasma. However, no HPV DNA was found in sperm heads recovered after swim-up and lysis procedures (Capra et al., 2019).

Specimens of semen were collected from 308 male partners of couples undergoing IVF to detect the presence of human papilloma virus DNA. HPV DNA was found in 24/308 semen samples, and in-situ hybridisation (ISH) showed a clear localization of HPV at the equatorial region of the sperm head in infected samples (Schillaci et al., 2013).

The presence of 35 types of HPV was examined on DNA from semen samples of 188 Danish sperm donors using a sensitive HPV array. Characteristic protrusions at or near the equatorial segment of the sperm head were found (Kaspersen et al., 2011).

Similar findings were reported by Foresta et al. In sperm cells of a HPV-16 positive male, FISH analysis showed that HPV localizes at the equatorial region of the sperm head. Furthermore, they showed that sperm exposed to HPV can transfer the virus into oocytes (Foresta et al., 2011).

An older study by Lai et al. included 24 randomly selected males and investigated the possible presence and expression of HPV in sperm cells. HPV-16 DNA was found in 2 seminal plasma samples and in 6 sperm cell samples. HPV-16 RNA was only detected in 2 sperm cell specimens, not in seminal plasma samples. HPV-18 DNA was detected in 8 seminal plasma and 11 sperm cell samples. HPV-18 RNA was found in 2 seminal plasma and 5 sperm cell samples (Lai et al., 1996).

Placenta

A prospective cohort study, including 72 pregnant HPV positive women, reported a vertical transmission rate of 20.8% (15 neonates). However, all placenta and cord blood samples were negative for HPV by both PCR and Immunohistochemistry (IHC) (Hahn et al., 2013).

A prospective cohort study investigated the vertical transmission of HPV in 329 pregnant women and their newborns. HPV DNA was detected in 4.2% (13/306) of placental samples and in 3.5% (11/311) of cord blood samples. Oral HPV carriage in newborns was most significantly associated with the detection of HPV in the placenta (OR 14.0; 95% CI 3.7 to 52.2) (Koskimaa et al., 2012).

Another prospective cohort study investigated the transplacental transmission of HPV and included 49 HPV positive pregnant women at delivery. Twelve out of 49 placentas (24.5%) tested positive for HPV DNA, of which 5 on the fetal side of the placenta, 2 on the maternal side and 5 on both sides of the placenta. Eleven newborns tested positive for HPV; in 5 cases (10.2%) there was HPV type-specific agreement between genital/placenta/newborn samples (Rombaldi et al., 2008).

Conclusion

The association between HPV and male and female infertility is one of the research priorities at this point.

PICO QUESTION: DOES HUMAN PAPILLOMA VIRUS IMPACT THE OUTCOME OF MAR?

Evidence

Male infected

In a prospective multi-centre study, Depuydt et al. investigated the clinical pregnancy rate of 732 couples undergoing 1713 IUI cycles. The clinical pregnancy rate was significantly lower in women inseminated with HPV positive semen (2.9 % per cycle) versus IUI with HPV negative semen (11.1 % per cycle). Above a ratio of 0.66 HPV virions/spermatozoon no pregnancies occurred (Depuydt et al., 2019).

In an observational prospective cohort study of 226 infertile couples the reproductive outcome after IUI or ICSI was studied in HPV positive and HPV negative men. In the IUI treated group the clinical pregnancy rate was 20 % (12/60) for HPV negative and 9.5 % (2/21) for HPV positive men. In the ICSI treated group, the clinical pregnancy rate was 40/98 (40.8%) for HPV negative and 6/33 (18.2%) for the HPV positive patients. The cumulative pregnancy rate (IUI and ICSI) for HPV positive men was 14.2 %

(5/54) compared to 38.4 % (66/172) for HPV-negative men while the miscarriage rate was significantly higher in HPV-positive versus HPV-negative men (62.5 % vs. 16.7 %) (Garolla et al., 2016).

In another prospective study 199 infertile couples were treated with IVF (n=33, 16.6 %) or ICSI (n=166, 83.4 %). HPV-positivity was documented in 9.5 % of men (19/199) and 17.5 % (35/199) of women. Both partners were HPV-positive in 4.5 % (9/199). The pregnancy rate was not different in couples with HPV+ and HPV- males (31.6% vs. 33.3%) while all pregnancies in HPV-positive couples resulted in miscarriage, whereas there was a 15.9% overall miscarriage rate in HPV-negative couples (P<.001) (Perino et al., 2011).

514 donor sperm samples from 3 different sperm banks were retrospectively examined for 18 different HPV types. Overall, 3.9% (20/514) of tested donor sperm was positive for HPV, with different prevalence among the 3 different sperm banks (3.6% bank A, 3.1% bank B and 16.7% bank C). Also, the HPV virion per spermatozoon ratio in donor samples was similar across the different sperm banks (95% CI 0.01 to 1.07 HPV virions/spermatozoon). When HPV-positive donor sperm was used, no clinical pregnancies resulted, whereas when HPV-negative donor sperm was used the clinical pregnancy rate was 14.6% (Depuydt et al., 2018).

Female infected

A systematic review and meta-analysis including 7 cohort studies (4 prospective and 3 retrospective) with a total of 1390 participants, investigated the impact of HPV infection on the risk of clinical pregnancy rate after MAR. The pooled results indicated no significant association between HPV infection and clinical pregnancy rate in MAR, with a pooled RR of 1.04 (95% CI 0.64 to 1.70). Three studies reported infection with a high-risk HPV alone with a pooled RR of 1.66 (95% CI 0.29 to 9.63). The other four studies reported mixed-type infection with high risk/low risk-HPV with a pooled RR of 0.85 (95% CI 0.66 to 1.09). The risk ratio of spontaneous abortion in MAR pregnancies was 1.47 (95% CI 0.86 to 2.50) (Xiong et al., 2018).

In a cohort study of 1044 Chinese women undergoing IVF for tubal infertility or male subfertility, no association was found between IVF-ET outcome and cervical infection, cytopathologic result, HPV detection, or result from the colposcopy or biopsy (Wang et al., 2008).

Recommendation

The possibility of HPV testing could be discussed with couples undergoing IUI.	Research only

Couples with a known positive HPV test should be advised that HPV is a transient infection, and postponing MAR treatment is an option depending on the individual circumstances.	GPP

Justification

There are over 200 HPV subtypes and most people will test positive for a HPV type at some point during life. It is currently not clear which types of HPV are responsible for the observed reproductive effects, making it impossible to recommend routine testing of HPV outside research settings. Furthermore, HPV is a transient infection which most often clears spontaneously. It is unknown how fast infectious HPV is cleared in males and females.

Emerging evidence indicates that HPV infection in males affects sperm parameters and may cause reduced pregnancy and increase miscarriage rates. For this reason and in the case of appropriate research settings, couples may be advised to proceed with HPV testing prior to IUI.

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D4. Prevention/ reduction of transmission during assisted reproduction

PICO QUESTION: WHICH TECHNIQUES CAN BE USED TO PREVENT/ REDUCE HUMAN PAPILLOMA VIRUS TRANSMISSION DURING MAR?

VACCINATION

Evidence

No studies investigating the association between adjuvant human papilloma virus (HPV) vaccination in HPV+ males or females in MAR are published yet.

We found no studies that investigate the effect of vaccination during MAR on the risk of transmission to partner/infant.

We identified 2 studies investigating the effect of vaccination on HPV viral load in semen.

Foresta et al. studied the effect of prophylactic vaccination in males to improve the clearance of semen HPV infection (Foresta et al., 2015). 179 out of 619 infertile patients, showing HPV-DNA detection in semen by FISH analysis, were enrolled in the study. Subjects were split into 91 vaccine-sensitive (VSPs) and 88 non-vaccine-sensitive patients (NVSPs) by INNO-LiPA. 19 VSPs showed vaccine-type specific seroconversion at recruitment. Compared to seronegative patients, VSP seroconverted at recruitment showed a reduced prevalence of HPV semen infection at 12 ($p=0.039$), 18 ($p=0.034$) and 24 months ($p=0.034$) of follow-up. Vaccinated VSP showed improved healing ($p=0.001$ at 6 months and $p=0.001$ at 12 months vs. seroconverted VSP), achieving clearance in 12 months (Foresta, et al., 2015).

A retrospective analysis was performed on 151 infertile couples with detection of HPV in semen. Patients were counselled to receive adjuvant HPV vaccination. Seventy-nine accepted vaccination (vaccine group) whilst 72 did not (control group). Forty-one spontaneous pregnancies, 11 in the control group and 30 in the vaccine group, were recorded (respectively 15% and 38.9%, $p<0,05$) and resulted into 4 deliveries and 7 miscarriages (control group) and 29 deliveries and one miscarriage (vaccine group, $p<0.05$ vs. control) (Garolla et al., 2018).

Conclusion

There is weak evidence that therapeutic HPV vaccination in HPV-positive men may increase pregnancy rates in natural conception and reduce miscarriage rates. However, more studies are necessary.

Justification

The effect of systematic HPV vaccination upon the reproductive potential of young adolescents (male and female) in some countries has to be evaluated in the future.

SEMEN PROCESSING

Evidence

An observational study was performed on a small series of 15 clinically HPV-positive men. Gradient separation followed by swim-up from the washed pellet was used. HPV DNA was tested in all fractions including seminal plasma, cell pellet, round cells, non-motile spermatozoa and motile spermatozoa. Of the 15 subjects, 67% were positive in at least one of the seminal fractions. HPV was never found in the fraction of motile spermatozoa after sperm-wash. The sperm-washing technique, which was previously successfully used to remove HIV, can efficiently remove HPV from spermatozoa (Fenizia et al., 2020).

HPV testing was performed on semen samples from infected patients (n= 22), control subjects (n= 13) and on pooled control sperm samples incubated with HPV16-L1 (HPV capsid), before and after direct swim-up and modified swim-up (with added Heparinase-III). Direct swim-up reduces the number of HPV-infected sperm by approximately 24% (P< 0.01), while modified swim-up is able to remove completely HPV DNA both from naturally and artificially infected sperm (Garolla et al., 2012).

In a cross-sectional clinical study a group of 32 infertile patients positive for HPV in semen were studied. Semen analysis and in-situ hybridization for HPV detection were performed before and after semen processing, discontinuous gradient centrifugation, and swim-up protocols. Sperm washing centrifugation showed no changes in the number of infected samples and in the percentage of infected cells. Density gradient centrifugation and swim-up protocols induced a slight reduction in the number of infected samples (30 and 26, respectively) (Foresta et al., 2011).

Semen samples from 85 volunteers were studied, 45 with historical or clinical evidence of genital HPV infection (study group) and 40 were healthy, clinically HPV-negative semen donors. PCR detected HPV in 21 of 32 subjects (66%) with identifiable lesions and six of 53 (11%) without them (P <.001). Swim-up washings of all 27 prewash sperm cells with HPV reduced cellular HPV DNA below detectable levels in only two cases. Simple semen processing does not clear HPV in sperm (Olatunbosun et al., 2001).

In one study prewashed sperm were equally divided and sperm in one portion were exposed to L1 HPV DNA fragments for 30 min at 37 degrees C. Untreated washed sperm served as the control. After transfection, the sperm were washed by either centrifuge, two-layer Isolate colloid wash, or test-yolk buffer procedures. The objective was to compare three types of semen processing procedures for their capacity to remove exogenous HPV DNA from infected sperm. The obtained data showed that washing would not remove exogenous HPV DNA from sperm cells (Brossfield et al., 1999).

Recommendation

HPV-positive males should be informed that no current semen preparation technique can eliminate the virus from the infected semen sample.	GPP

Justification

Current techniques for semen processing in medical assisted reproduction laboratories are not effective in removing HPV which remains adherent to the sperm cells.

More studies are needed to recommend any kind of sperm preparation technique to remove HPV DNA from washed spermatozoa.

Any further question on semen processing will not be discussed.

PICO QUESTION: DOES THE PLASMA VIRAL LOAD CORRELATE WITH HUMAN PAPILLOMA VIRUS DETECTION IN SEMEN?

Evidence

We identified no publications investigating the correlation between plasma and semen human papilloma virus (HPV) load.

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D5. Reducing/ avoiding vertical transmission

PICO QUESTION: WHICH INTERVENTIONS CAN BE USED TO REDUCE/AVOID VERTICAL TRANSMISSION OF HUMAN PAPILLOMA VIRUS TO THE NEWBORN?

ELECTIVE CAESAREAN SECTION

Evidence

A systematic review and meta-analysis including 9 studies investigated the risk of transmission between caesarean and vaginal delivery. There was no statistically significant difference in risk of vertical transmission between caesarean and vaginal delivery (RR 0.912; 95% CI 0.226 to 3.674; n= 421 mother-infant pairs) (Zouridis et al., 2018).

An older systematic review and meta-analysis including 8 cohort studies, also investigated the prevalence of HPV in the offspring of HPV-infected women in association to their mode of delivery. They concluded that caesarean section is associated with significantly lower rates of HPV transmission than vaginal birth (14.9% vs. 28.2%; RR 0.515; 95% CI 0.34 to 0.78; n=446 mother-infant pairs). The number of caesarean sections needed to prevent one case of perinatal infection (number needed to treat) would be 7.5 (Chatzistamatiou et al., 2016).

A pilot study including 268 healthy infants and children investigated the association between the detection of HPV in the oral cavity and the method of delivery and reported that they found no statistically significant association (Summersgill et al., 2001).

A prospective cohort study, including 36 HPV infected women and 37 controls, also investigated the risk of vertical transmission and reported a fetal transmission rate of 50% (7/14) with vaginal delivery as compared to 33.3% (4/12) with caesarean section (Wang et al., 1998).

Recommendation

Caesarean delivery is not recommended on the basis of maternal HPV-positivity alone.	Strong	⊕⊕◯◯

Justification

Current evidence does not support the use of caesarean section to lower the risk or prevent mother-to-infant transmission of HPV.

The CDC guidelines on HPV state that caesarean section is indicated for women with anogenital warts if the pelvic outlet is obstructed or if vaginal delivery would result in excessive bleeding (Workowski and Bolan, 2015).

BREASTFEEDING

Evidence

Louvante et al. performed a study including 308 breastfeeding mothers. HPV DNA was found in breast milk in 10.1% (31/308), 20.1% (39/194) and 28.8% (17/59) of samples at day 3, months 2 and 6, respectively. Breast milk HPV persisted among 5.5% (9/164) of the lactating mothers. No significant associations were detected between the persistent breast milk HPV and the offspring's oral incidence of HPV infection. The authors concluded that HPV in breast milk is prevalent among lactating mothers and HPV can also persist in breast milk. According to their results breast milk is a potential vehicle for HPV transmission to oral mucosa of the spouse but not of the offspring (Louvanto et al., 2017).

High-risk HPV was identified in milk samples of 6 of 40 (15%) from normal Australian lactating women. The presence of high-risk HPV in human milk suggests the possibility of milk transmission of these viruses. Given the rarity of viral associated malignancies in young people, it is possible but unlikely, that such transmission is associated with breast or other cancers (Glenn et al., 2012).

Yoshida et al. analysed maternal milk samples (n=80) for high-risk HPV DNA. High-risk HPV-16 was detected in two of 80 samples (2.5%), and in these two cases, high-risk HPV was not detected in the uterine cervix or oral cavity of the child. They concluded that the infection of HPV in maternal milk is rare (2/80); vertical transmission through maternal milk was not detected in this study (0/80) (Yoshida et al., 2011).

Conclusion

Breastfeeding is probably not contra-indicated in HPV-positive women.	Conditional ⊕◯◯◯

Justification

Transmission of HPV to the offspring by breastfeeding is very rare. To date there is no evidence of harm to the newborn by vertical transmission of HPV.

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Summary

HPV	Male testing positive	Female testing positive	Couple testing positive
Before MAR	Discuss: - Possibility of postponing MAR (transient infection) - Risk of viral horizontal transmission (not eliminated by MAR)		
During MAR	IUI, IVF or ICSI depending on infertility work-up		
	Routine semen processing		
After MAR	Caesarean section not recommended		
	Breastfeeding not contra-indicated		

Figure 4: Summary of management of medically assisted reproduction in patients testing positive for human papilloma virus.

PART E: Human T-lymphotropic virus I/II

E1. Prevalence and testing

NARRATIVE QUESTION: WHAT IS THE PREVALENCE OF HUMAN T-LYMPHOTROPIC VIRUS I/II?

The human T-lymphotropic virus, human T-cell lymphotropic virus, or human T-cell leukemia-lymphoma virus (HTLV) family of viruses are a group of human retroviruses. These are known to cause a type of cancer called adult T-cell leukemia/lymphoma and a demyelinating disease called HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP).

The HTLVs belong to a larger group of primate T-lymphotropic viruses (PTLVs). Members of this family that infect humans are called HTLVs, and the ones that infect Old World monkeys are called Simian T-lymphotropic viruses (STLVs). To date, four types of HTLVs (HTLV-I, HTLV-II, HTLV-III, and HTLV-IV) and four types of STLVs (STLV-1, STLV-2, STLV-3, and STLV-5) have been identified. HTLV types HTLV-I and HTLV-II viruses are the first retroviruses which were discovered. Human T-lymphotropic virus types I and II are two closely related retroviruses belonging to the Retrovirus family and the Deltaretrovirus genus. While HTLV-I and II are retroviruses, they differ from the Lentivirus genus to which the more common human immunodeficiency virus types 1 and 2 (HIV-1 and 2) belong.

HTLV-I and HTLV-II are both involved in actively spreading epidemics, affecting 15-20 million people worldwide. HTLV-I is the most clinically significant of the two: at least 500,000 of the individuals infected with HTLV-I eventually develop an often rapidly fatal leukemia, while others will develop a debilitative myelopathy, and yet others will experience uveitis, infectious dermatitis, or another inflammatory disorder. HTLV-II is associated with milder neurologic disorders and chronic pulmonary infections. In the United States, HTLV-I/II seroprevalence rates among volunteer blood donors average 0.016 percent.

Recently, two new genetically distinct, but closely related viruses have been described from Africa (named HTLV-III and IV), but their epidemiology and disease-causing properties are as yet unknown. Therefore, and since they seem very rare, they are not included in this risk assessment. HTLV-I and II are RNA viruses which can reverse transcribe their genome into DNA and integrate into their host T-lymphocytes. Infection with both viruses is chronic and lifelong and only a fraction of those infected eventually develop disease (see below). No vaccine exists against infection by either of the viruses (European Centre for Disease Prevention and Control, 2012).

HTLV-1 has six reported subtypes (subtypes A to F). The great majority of infections are caused by the cosmopolitan subtype A. HTLV was discovered by Robert Gallo and colleagues in 1980. Between 1 in 20 and 1 in 25 infected people are thought to develop cancer as a result of the virus. HTLV-I infection is thought to spread only through dividing cells since reverse transcriptase generates proviral DNA from

genomic viral RNA, and the provirus is integrated into the host genome by viral integrase after transmission. Quantification of provirus reflects the number of HTLV-I-infected cells. An increase in numbers of HTLV-I-infected cell division, promoted by the activity of accessory viral genes, especially Tax, may provide an enhancement of infectivity. Tax expression induces proliferation, inhibits the apoptosis of HTLV-I-infected cells and, conversely, evokes the host immune response, including cytotoxic T cells, to kill virus-infected cells (Li et al., 2014).

HTLV-II is prevalent among injecting drug users in the United States and in Europe; more than 80% of HTLV-I/II seropositivity in drug users in the United States is due to HTLV-II infection. HTLV-II also appears to be endemic in American Indian populations, including the Guaymi Indians in Panama and North American Indians in Florida and New Mexico. Approximately half of U.S. volunteer blood donors seropositive for HTLV-I/II are infected with HTLV-II. HTLV-II-infected blood donors most often report either a history of injecting drug use or a history of sexual contact with an injecting drug user. A smaller percentage report a history of blood transfusion (CDC, 1993).

HTLV-I/II is endemic in Southern Japan, the Caribbean Islands and parts of central Africa. Transmission is by sexual contact, intravenous drug abuse, from an infected mother to her child, mainly via breast milk and by non-leukoreduced blood transfusion. HTLV-I causes Adult T-Cell Leukaemia (ATL) in 2-4% of infected individuals and typically after long latency periods. Once diagnosed with ATL life expectancy is typically less than a year. HTLV-I Associated Myelopathy (HAM) / Tropical Spastic Paraparesis (TSP) occurs in approximately 0.25-4% of HTLV-I infected individuals usually after a latency period of up to 20 years, although HAM/TSP may occur after a few months when HTLV-I infection is acquired through a blood transfusion. Patients with HAM/TSP may live with significant disability for 20-30 years post HAM/TSP diagnosis. HTLV-II infrequently causes HAM/TSP, increased incidence of pneumonia and bronchitis and perhaps higher all-cause and cancer mortality (Vermeulen et al., 2019).

NARRATIVE QUESTION: HOW SHOULD TESTING OF HUMAN T-LYMPHOTROPIC VIRUS I/II STATUS PRIOR TO MEDICALLY ASSISTED REPRODUCTION BE PERFORMED?

At present, the initial diagnosis of human T-lymphotropic virus (HTLV) I/II-infection is based mainly on screening for antibodies in bloodserum by Enzyme Linked Immuno Sorbent Assay (ELISA/EIA), Line immunoassays [LIA] or Particle Agglutination (PA). ELISA includes both HTLV I and II antigens and therefore detects antibodies for both viral types but cannot discriminate. Since the introduction of HTLV I/II assays in the mid 1980's, considerable progress has been made over the last decades in the field of HTLV I/II-antibody testing enabling discrimination between HTLV I and II, development of next generation EIA's (Berini et al., 2008, Caterino-de-Araujo, 2009, da Silva Brito et al., 2018, Jacob et al., 2009, Jacob et al., 2007, Verdonck et al., 2009), Chemiluminescent Microparticle Immunoassay [CLIA] (Malm et al., 2010, Tosswill and Taylor, 2018), Electrochemiluminescence Immunoassay [ECLIA] (Kapprell et al., 2010, Laperche et al., 2017, Qiu et al., 2008, Yun et al., 2019), Chemiluminescent Enzyme Immunoassay (Ishihara et al., 2014), Luciferase Immunoprecipitation System [LIPS] (Furuta et al., 2015).

A positive HTLV I/II antibody test needs to be confirmed. The techniques most commonly applied are Immuno Blot (IB) and Western Blot (WB) (Miller, 2016). Polymerase chain reaction (PCR) is commonly

used for confirm the diagnosis and can be used to of the presence as well as quantify HTLV proviral DNA.

A WHO international standard of Nucleic Acid Amplification Techniques (NAT's) for HTLV has not been established and no gold standard PCR test method for HTLV I or HTLV II has been identified. Moreover, a commercial kit is not available which is why numerous in-house quantitative PCR assays have been developed to quantify HTLV I or HTLV II.

These include:

- quantitative real time PCR (qPCR, rtPCR) for HTLV I (Altamirano et al., 2010, Castro et al., 2013, Davidson et al., 2006, Dehee et al., 2002, Ishihara, et al., 2014, Kamihira et al., 2003, Kamihira et al., 2010, Kuramitsu et al., 2019, Miley et al., 2000, Naderi et al., 2012, Rosadas et al., 2013, Vitone et al., 2006)
- quantitative nested PCR (nested-PCR) for HTLV I (Dezzutti et al., 2003, Ishihara, et al., 2014)
- non-radioisotope PCR for HTLV I (Costa et al., 2006)
- quantitative real time PCR (qPCR, rtPCR) for HTLV I and HTLV II (Andrade et al., 2010, Estes and Sevall, 2003, Kuramitsu, et al., 2019, Lee et al., 2004, Waters et al., 2011)
- quantitative nested PCR (nested-PCR) for HTLV I and HTLV II (Gallego et al., 2004)
- digital droplet PCR (ddPCR) for HTLV I and HTLV II (Thulin Hedberg et al., 2018)
- quantitative real time PCR (qPCR, rtPCR) for HTLV I and HTLV II and HTLV III (Besson and Kazanji, 2009, Moens et al., 2009)

A diagnostic test strategy can be of use in the screening and detection of HTLV I/II (Costa et al., 2011, Stramer et al., 2018, Thorstensson et al., 2002).

Conclusion

> HTLV-I antibody testing must be performed for people living in, or originating from, high-incidence areas or with sexual partners or parents originate from those areas.

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E2. Prevention of transmission before medically assisted reproduction

PICO QUESTION: WHAT ARE THE RISKS OF HUMAN T-LYMPHOTROPIC VIRUS I/II TRANSMISSION THROUGH VAGINAL/ANAL INTERCOURSE?

Evidence

A prospective cohort study, including 85 couples, investigated sexual transmission of human T-lymphotropic virus (HTLV)-I and II. During the 13 years of follow-up, only 2 seroconversions per virus occurred, for both viruses one from male to female and one from female to male. The incidence rate for sexual transmission of HTLV-I was calculated to be 0.9/100 person-years (95% CI 0.1 to 3.3). The incidence rate for sexual transmission of HTLV-II was calculated to be 0.5 (95% CI 0.06 to 1.8) (Roucoux et al., 2005).

A prospective cohort study, including 342 married couples (97 HTLV-I-discordant, 95 HTLV-I-concordant, 342 negative concordant) investigated sexual transmission of HTLV-1. A man was 6.8 times more likely to be seropositive if the wife was positive than if she was negative (59.7% vs. 8.8%). Similarly, a woman was 4.7 times more likely to be seropositive if the husband was positive than if he were negative (74.2% vs. 15.8) (Stuver et al., 1993).

Recommendation

It is suggested to inform Human T-cell lymphotropic virus (HTLV I/II)-serodiscordant couples that there is a risk of sexual transmission of the virus to the unaffected partner. To reduce this risk, couples could be advised to use barrier contraception and receive reproductive counselling if they want to conceive.	Conditional ⊕◯◯◯

Justification

There is a risk of sexual transmission of HTLV I/II. The risk appears to be higher from male to female.

PICO QUESTION: IS THERE A THRESHOLD BELOW WHICH TRANSMISSION OF HUMAN T-LYMPHOTROPIC VIRUS I/II IS UNLIKELY?

Evidence

Horizontal transmission

A prospective cohort study including 19 seroconcordant and 37 serodiscordant human T-lymphotropic virus (HTLV)-I-infected couples investigated the risk of sexual transmission and reported that HTLV-I

pro-viral load was higher among seroconcordant couples than among serodiscordant couples (mean 363±433 vs. 145±145 copies/104 PBMC; by real-time PCR) (Paiva et al., 2017).

A prospective cohort study including 125 seropositive donors (40 men and 85 women) who brought their partners to enrol in the study, investigated the sexual transmission of HTLV-I and II. HTLV transmitter men had been in their relationships longer (mean 225 months vs. 122 months) and had higher viral loads (mean 257,549 vs. 2,945 copies/300,000 cells for HTLV-I; 5,541 vs.118 copies/300,000 cells for HTLV-II; by qPCR) than non-transmitters (Kaplan et al., 1996).

A prospective cohort study, including 342 married couples (97 HTLV-I-discordant, 95 HTLV-I-concordant, 342 negative concordant) investigated sexual transmission of HTLV-1. Four carrier husbands whose wives seroconverted had HTLV-I titers ≥1:1024 (by passive particle agglutination assay) and were anti-Tax positive (Stuver, et al., 1993).

Vertical transmission

We were unable to retrieve studies that investigated the maternal viral load before MAR and the risk of vertical transmission to the newborn.

Conclusion

Based on current evidence, we cannot define a threshold of HTLV I/II viral load below which horizontal or vertical transmission of HTLV I/II is not occurring.

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E3. Assisted reproduction techniques and impact on outcomes

PICO QUESTION: SHOULD IUI, IVF OR ICSI BE PREFERENTIALLY USED FOR MAR IN HUMAN T-LYMPHOTROPIC VIRUS I/II INFECTED COUPLES?

Evidence

No studies have compared different techniques for MAR in couples where one partner is infected with human T-lymphotropic virus (HTLV) I/II.

Recommendation

The cause of infertility should dictate the specific technique (IUI/IVF/ICSI) used for medically assisted reproduction (MAR) in couples where one or both partners test positive for HTLV I/II.	Strong	⊕○○○

Women testing positive for HTLV I/II should be informed that MAR does not eliminate the risk of vertical transmission.	GPP

Justification

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 HTLV I/II.

PICO QUESTION: CAN HUMAN T-LYMPHOTROPIC VIRUS I/II VIRUS DNA BE DETECTED IN OOCYTES/ SPERM/ PLACENTA?

Evidence

Sperm

One very old study, including 3 adult T-cell leukemia virus positive males, found that about 1% of mononuclear cells in semen express ATLA (Nakano et al., 1984).

Placenta

In a case report the placental villi from 9 HTLV-I-positive and 3 HTLV-I seronegative pregnant women at term were obtained. Histochemical double stain revealed that the placental epithelial cells were positive for HTLV-I. Six out of nine placentas from HTLV-I seropositive mothers were infected by HTLV-I (Fujino et al., 1992).

Conclusion

Studies on HTLV I/II viruses are dated and the technology to detect these viruses has changed a lot since. Therefore, the possibility of HTLV I/II presence in gametes or placenta cannot be confirmed or excluded. To date, the risk of HTLV I/II transmission through the use of infected semen or oocytes remains to be proven.

PICO QUESTION: DOES HUMAN T-LYMPHOTROPIC VIRUS I/II AND/OR TREATMENT OF HTLV I/II BEFORE MAR IMPACT THE OUTCOME OF MAR?

Evidence

One cohort study was identified comparing ICSI in 32 human T-lymphotropic virus (HTLV)-I-infected women and 62 non-infected (historic) controls (Mansouri Torshizi et al., 2014). There was no difference in pregnancy rates between HTLV-I-infected and control groups (46% (15/32) vs. 45% (28/62)). No data were reported regarding vertical transmission or safety (Mansouri Torshizi, et al., 2014).

Conclusion

The impact of female HTLV I-infection on MAR outcomes remains unknown.

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E4. Prevention/ reduction of transmission during assisted reproduction

PICO QUESTION: WHICH TECHNIQUES CAN BE USED TO PREVENT/ REDUCE HUMAN T-LYMPHOTROPIC VIRUS I/II TRANSMISSION DURING MAR?

SEMEN PROCESSING

Evidence

No studies were identified comparing routine semen preparation with advanced semen processing in males testing positive for HTLV I/II.

Conclusion

There are no techniques known for prevention/reduction of transmission of HTLV I/II during MAR

Any further questions on semen processing will not be discussed.

PICO QUESTION: DOES THE PLASMA VIRAL LOAD CORRELATE WITH HUMAN T-LYMPHOTROPIC VIRUS I/II DETECTION IN SEMEN?

Evidence

We found no studies investigating the correlation between viral load in semen and serum in human T-lymphotropic virus (HTLV) I/II-infected patients.

E5. Reducing/ avoiding vertical transmission

PICO QUESTION: WHICH INTERVENTIONS CAN BE USED TO REDUCE/AVOID VERTICAL TRANSMISSION OF HUMAN T-LYMPHOTROPIC VIRUS I/II TO THE NEWBORN?

ELECTIVE CAESAREAN SECTION

Evidence

A cross-sectional study including 134 mothers and their 288 children who were tested for human T-lymphotropic virus (HTLV)-I infection investigated the effect of mode of delivery. Out of 121 vaginal deliveries, 22 children tested positive for HTLV, versus 7 out of 74 caesarean deliveries. Protection from vertical transmission of HTLV-I by caesarean section did not reach statistical significance (OR 0.47; 95% CI 0.19 to 1.16) (Paiva et al., 2018).

Recommendation

Caesarean delivery is not recommended on the basis of maternal HTLV I/II-positivity alone.	Strong	⊕◯◯◯

Justification

There is only very limited and low quality evidence comparing the risk of vertical transmission between vaginal and caesarean delivery. Caesarean section is a major surgery with higher risks of complications and a longer recovery process as compared to vaginal delivery. Therefore, we cannot recommend caesarean section for the sole purpose of reducing the risk of vertical transmission of HTLV I/II.

BREASTFEEDING

Evidence

A systematic review and meta-analysis including 7 cohort studies investigated the effect of infant feeding practices on vertical transmission of HTLV-I. Pooled OR for breastfeeding versus bottle feeding was 3.48 (95% CI 1.58 to 7.64). Subgroup analysis according to the duration of breastfeeding showed a pooled OR of 0.912 (95% CI 0.45 to 1.80) for breastfeeding up to 6 months versus bottle feeding. In contrast, a pooled OR of 3.83 (95% CI 1.80 to 8.10) was calculated for breastfeeding beyond 6 months (Boostani et al., 2018).

A prospective cohort study including 150 HTLV-I positive mothers and their 154 children presented data on risk of HLTV-I transmission via breastfeeding with at least 18 months of follow-up. Compared to non-HTLV-I-infected children, OR for breastfeeding ≤6 months was 10.8 (95% CI 2.0 to 57.8). The risk of transmission was 4.4 fold higher among children breastfed for 6-12 months and 10.2 fold higher among

children breastfed for >12 months as compared to children breastfed for ≤6 months (Hisada et al., 2002).

A prospective cohort study, including 34 index children from mothers testing positive for HTLV-I investigated the risk of HLTV-I transmission through breastfeeding. In the group of mothers who breastfed for more than 6 months, 4/19 children tested positive for HTLV-I. In contrast, in the group of mothers who breastfed for 6 months or less, only 1 child was infected with HTLV-I (RR 3.2; 95% CI 0.4 to 22.1) (Wiktor et al., 1993).

In the study by Ando et al., 35 women testing positive for HTLV-I were included. Twenty four infants were breastfed and 11 infants were bottle fed. Twelve months after birth, 11/24 breastfed infants tested positive for HTLV 1 as compared to 1/11 in the bottle fed group (Ando et al., 1987).

A cross-sectional study including 134 mothers with their 288 children who were tested for HTLV-I infection. Out of the 288 included children, 253 were breastfed, and 41 tested positive for HTLV-1. Breastfeeding for 12 months or more was associated with an increased risk of transmission (OR 6.15; 95%CI 2.62 to 14.41) (Paiva, et al., 2018).

Recommendation

A female testing positive for HTLV I/II should refrain from breastfeeding when and where she has safe nutritional alternatives.	Strong	⊕○○○

Justification

Current evidence indicates that breastfeeding is associated with an increased risk of vertical transmission of HTLV I. Therefore, avoiding breastfeeding should be considered in women testing positive for HTLV I/II when and where safe nutritional alternatives exist.

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Summary

HTLV I/II	Male testing positive	Female testing positive	Couple testing positive
Before MAR	Consult with infectious disease specialist		
		Discuss: - Risk of viral horizontal transmission (not eliminated by MAR) - Risk of viral vertical transmission (not eliminated by MAR)	
During MAR	IUI, IVF or ICSI depending on infertility work-up		
	Routine semen processing		
After MAR	Caesarean section not recommended		
		Breastfeeding not recommended	

Figure 5: Summary of management of medically assisted reproduction in patients testing positive for human T-lymphotropic virus.

PART F: Zika virus

F1. Prevalence and testing

NARRATIVE QUESTION: WHAT IS THE PREVALENCE OF ZIKA VIRUS?

Zika virus (ZIKV) is a positive sense, single stranded RNA arthropod-borne flavivirus first isolated in 1947 in a febrile sentinel Rhesus macaque in Uganda (Dick et al., 1952). Other similar arboviral infections include dengue, chikungunya and yellow fever. Recent outbreaks of ZIKV have predominantly, but not exclusively, been in South America, particularly Brazil in 2015, and have been linked to adverse pregnancy outcome. Earlier outbreaks were reported in the Yap Islands in 2007 and French Polynesia in 2013 (Nugent et al., 2017). By early 2018, over 85 countries have reported evidence of ZIKV globally, with 500,000 suspected cases reported in Latin America and the Caribbean (WHO, 2018). With an increase in overseas travel, a fair proportion of European patients may be at risk of contracting ZIKV just prior to having medically assisted reproduction (MAR). Within the European Union, as of March 2017, 20 of 1737 cases with a known route of transmission were acquired through sexual transmission (WHO, 2018), hence awareness of ZIKV is important for fertility specialists (Ioos et al., 2014).

Two major lineages of ZIKV, known as the Asian and African lineages, have been identified. The Asian lineage was first identified in Asia and subsequently spread to the Pacific Islands and then to the Americas. The 2015-16 epidemic in the Americas was caused by a strain of the Asian lineage commonly referred to as the American strain. The 2018 ZIKV outbreak in India was due to the Asian lineage-Asian strain, demonstrating the epidemic potential of this older Asian strain. Accurate and up-to-date epidemiologic data on ZIKV are limited in many areas of the world. The majority of ZIKV infections are asymptomatic, and when disease occurs, symptoms are generally mild and non-specific, and therefore may not be detected or reported. Many countries lack or have limited systems for routine surveillance, case detection and reporting. Although numerous cases of travel associated ZIKV infections have been reported in European travellers, no countries in the region have reported autochthonous, mosquito borne transmission of ZIKV (WHO, 2018).

A recent systematic review looking at the prevalence of ZIKV in blood donations, estimated that the population prevalence of ZIKV might be 0.53% (Eick et al., 2019), with up to 75% of infections being asymptomatic, making a lack of viraemic symptoms unreliable (Flamand et al., 2019). This figure is higher at 0.88% for pregnant women in endemic regions (Borges et al., 2017). This will likely differ between countries and be higher in endemic areas at times of outbreak. This heterogeneity has led others to postulate that pooled prevalence estimates are probably not robust (Haby et al., 2018).

If ZIKV is contracted in the first trimester of pregnancy, 10% of infections are likely to result in neurological damage such as microcephaly, resulting in lifelong developmental issues (Reynolds et al., 2017). Given that ZIKV infection is a worldwide problem, new global alliances are needed to enable the combination of efforts and resources more effectively, to combine interventions known to be effective against multiple arboviral diseases (Wilder-Smith et al., 2017). The US 'Centers for Disease Control and Prevention' (CDC), has established a registry for ZIKV pregnancy and infancy, to further learning and

make recommendations for affected families and healthcare providers (https://www.cdc.gov/pregnancy/zika/research/registry.html).

NARRATIVE QUESTION: HOW SHOULD TESTING OF ZIKA VIRUS STATUS PRIOR TO MEDICALLY ASSISTED REPRODUCTION BE PERFORMED?

As the clearance of ZIKV is usually fairly quick and testing not conclusive, waiting the advised time period before MAR is the better and more reliable option.

Should testing be considered necessary, it can include antigen-antibody (Ag/Ab), PCR and viral culture (Paz-Bailey et al., 2018). More recent studies have suggested that being PCR positive may not necessarily indicate infectiousness by viral culture but publications reporting this are limited to small case series (Counotte et al., 2018). Arsuaga at al. conducted a review of viral culture studies on 22 men in 11 reports and concluded that median duration in semen was 9.5 days (95% CI 1.2-20.3), with a maximum of 69 days. Intermittent shedding is possible, so a single negative RT-PCR may not indicate definitive clearance (Arsuaga et al., 2016, Barzon et al., 2016).

WHO recommends nucleic acid testing (NAT) in patients presenting with onset of symptoms < 7 days. RT-PCR, primer and probe sets for Zika virus-specific assays have been published (Charrel et al., 2016). When using NAT, negative results should be interpreted with caution. This does not rule out infection, as viraemia drops rapidly 7 days after onset of symptoms and may not be detected by the test at the lower end of sensitivity. For patients presenting with onset of symptoms ≥ 7 days, serology is the preferred testing method. Recommended serological assays include enzyme immunoassays (EIAs) and immunofluorescence assays (IFA) detecting IgM antibodies using viral lysate, cell culture supernatant or recombinant proteins as well as neutralization assays such as plaque-reduction neutralization tests (PRNT). In general, a reactive result for Zika virus IgM in the absence of IgM to Dengue or other flaviviruses suggests recent exposure to Zika virus (WHO, 2016).

Zika IgM antibodies can persist for months to years following infection. Therefore, detecting Zika IgM antibodies might not indicate a recent infection. There is notable cross-reactivity between Dengue IgM and Zika IgM antibodies in serologic tests. Antibodies generated by a recent Dengue virus infection can cause the Zika IgM to be falsely positive. Asymptomatic non-pregnant patients should not routinely be tested for Dengue or Zika viruses or testing performed as part of preconception screening in non-endemic areas. For symptomatic non pregnant patients, Dengue and Zika virus NAT should be performed on serum collected ≤7 days after symptom onset. Dengue and Zika virus IgM antibody testing should be performed on NAT-negative serum specimens or serum collected >7 days after onset of symptoms. For symptomatic pregnant women, serum and urine specimens should be collected as soon as possible within 12 weeks of symptom onset for concurrent Dengue and Zika virus NAT and IgM antibody testing. Positive IgM antibody test results with negative NAT results should be confirmed by neutralizing antibody tests when clinically or epidemiologically indicated, including for all pregnant women. (CDC; https://www.cdc.gov/zika/hc-providers/testing-guidance.html; (2020).

Viraemia levels below which a 'safe' level is considered have not been fully established, however Paz-Bailey et al., (2018), when validating the Trioplex RT-PCR assay in semen considered a test to be positive if target amplification was detected within 38 threshold cycles (Paz-Bailey, et al., 2018).

Conclusion

Zika virus testing of couples pursuing MAR treatment is not included in the European Tissue and Cells Directive. Patients that have visited an endemic Zika region should be managed according to regional policies depending on risk and prevalence.

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F2. Prevention of transmission before medically assisted reproduction

PICO QUESTION: WHAT ARE THE RISKS OF ZIKA VIRUS TRANSMISSION THROUGH VAGINAL/ANAL INTERCOURSE?

Evidence

A systematic review was identified including 36 cases where transmission is presumed to have occurred sexually. In most cases transmission was from male to female (34/36), where it was not clear vaginal or anal, one case was female to male and one case male to male (Counotte et al., 2018).

In a retrospective cohort study, all cases in Rio de Janeiro were collected between 2013 and 2015. The regression results indicated that the incidence of Zika infection was significantly higher for sexually active women (1.8; 95% CI 0.500 to 3.053). However, being sexually active alone was not a significant predictor of Zika incidence (0.2; 95% CI 1.207 to 0.783) (Coelho et al., 2016).

A small prospective cohort study including 11 index cases (5 women, 6 men) and 6 sexual contacts, reported no sexual transmission of ZIKV (Sánchez-Montalvá et al., 2018).

Another small cohort study including 16 symptomatic index cases (9 women, 7 men) with confirmed Zika virus infection. One sexual partner of a male index case got infected (Sokal et al., 2016).

A case series including 5 male travellers reported that the sexual partners of 3/5 index cases were symptomatic. One of the sexual partners tested positive for ZIKV RNA, one tested positive on serology (not tested for ZIKV RNA) and one tested negative for ZIKV RNA (García-Bujalance et al., 2017).

MAR related, a case report described a pregnant woman (after frozen embryo transfer) and her husband. The husband travelled back and forth to an endemic Zika region. The couples had sexual relations in early pregnancy. Placental tissue tested positive for ZIKV RNA after birth. Serology on the couple was not possible due to it being too far removed from the date of suspected infection (Yarrington et al., 2019).

Recommendation

A male diagnosed with ZIKV-infection or returning from a ZIKV endemic region should use barrier contraception with any partner, for 3 months.	GPP

A female diagnosed with ZIKV-infection or returning from a ZIKV endemic region should use barrier contraception and avoid pregnancy for 2 months.	GPP

Justification

Quality of the evidence is very low, as there are only case reports and case series. Currently, the risk of transmission cannot be quantified based on the available evidence.

The recommendation to wait 3 months for men and 2 months for females before attempting conception, and use barrier contraception, is in line with the WHO recommendations.

PICO QUESTION: IS THERE A THRESHOLD BELOW WHICH TRANSMISSION OF ZIKA VIRUS IS UNLIKELY?

Evidence

We identified no studies investigating maternal ZIKV viral load and the risk of vertical transmission or ZIKV viral load in partner and risk of horizontal transmission.

Conclusion

There is no agreed threshold described in the literature below which transmission of ZIKV is unlikely. We advocate the use of barrier contraception to prevent horizontal transmission and avoiding pregnancy for 3 months after diagnosis or return from a ZIKV endemic area to reduce vertical transmission.

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F3. Assisted reproduction techniques and impact on outcomes

PICO QUESTION: SHOULD IUI, IVF OR ICSI BE PREFERENTIALLY USED FOR MAR IN ZIKA VIRUS INFECTED COUPLES?

Evidence

No studies have proven that MAR is safe in couples where one partner is infected with Zika virus. All current guidance advises against active therapy.

Recommendation

If a patient or partner has been diagnosed with ZIKV-infection or returning from a ZIKV endemic region in the last 3 months, medically assisted reproduction (MAR) treatment should be postponed.	GPP

In case of fertility preservation, the approach should be tailored to the individual situation.	GPP

PICO QUESTION: CAN ZIKA VIRUS RNA BE DETECTED IN OOCYTES/ SPERM/ PLACENTA?

This question was formulated to identify Zika-related specific risks of vertical transmission through MAR.

Evidence

Sperm

One study reported ZIKV-RNA-positivity in different semen factions after serial swim-up tests. After submitting the samples to a swim-up method to isolate motile spermatozoa, 7/11 samples tested ZIKV-RNA-positive (Joguet et al., 2017).

Oocytes

One case report from a ZIKV-positive woman (male tested negative) undergoing MAR showed that 2/7 retrieved oocytes tested positive for ZIKV RNA. However, follicular fluid and cumulus cells were negative for ZIKV RNA (Filho et al., 2019).

Placenta

A retrospective cohort study including 68 women with available amniotic fluid samples reported ZIKV-RNA-positivity in 39/68 samples. In 15/68 patients, ZIKV RNA was identified in amniotic fluid only. For 12 patients with concurrent serum and amniotic fluid samples 9/12 tested ZIKV-RNA-positive in amniotic fluid only and 3/12 in both (Reyes et al., 2020).

Similarly, a retrospective case control study analysed 291 fetal samples/placentas from ZIKV-infected women and reported that among infected placentas from infants with congenital ZIKV-infection, positive RT-PCR at birth were found in 51/58 (87.9; 95% CI 76.7 to 95.0) of placentas tested. They also reported that among these infected placentas, 27/43 (62.8%; 95% CI 48.3 to 77.2) demonstrated pathological anomalies (Pomar et al., 2019).

In a prospective case series including 8 cases, 3/8 cases placenta tested positive for ZIKV RNA, in 5/8 placenta tested negative despite detection of ZIKV in amniotic fluid (Schaub et al., 2017).

A case series of 49 pregnant women with ZIKV-infection, of which 17 placentas were available for sampling reported ZIKV-positivity in 14/17 placentas. No correlation was observed between the dissemination of ZIKV in the placenta and the presence of fetal findings (Venceslau et al., 2020).

A retrospective case series including 4 twins from infected women, 2 placentas discordant for ZIKV PCR testing, 1 both placentas infected, 1 both placentas negative (Sobhani et al., 2019).

A case series including 52 women with suspected ZIKV-infection during pregnancy reported placental tissue positivity from 32/52 (62%) case patients. In 12 of the 17 case-patients with adverse pregnancy outcomes, ZIKV RNA was detected by RT-PCR in placentas/umbilical cord/fetal tissues; all had symptom onset during the first trimester. The relative levels of ZIKV RNA in the first trimester placentas (13.10; 95% CI 1.718 to 99.87 cop/cell) were 25-fold higher than those in the second or third trimester or full-term placentas. The time frame from maternal symptom onset to detection of ZIKV RNA by RT-PCR in placentas was 15 to 210 (mean 81) days (Bhatnagar et al., 2017).

A case series including 24 women who contracted ZIKV-infection in different stages of pregnancy reported that placental Hofbauer cells showed immunostaining of flavivirus and anti-ZIKV antibodies, regardless of the gestational age when ZIKV occurred (de Noronha et al., 2018).

Case series including 3 mothers with ZIKV-infection at different stages during pregnancy reported that immunofluorescence staining for ZIKV protein co-localized with Hofbauer cells, indicating infection of the placenta, regardless of the pregnancy trimester in which ZIKV-infection occurred. The ZIKV proteins were present in the placenta up to delivery, without causing any physical harm to the newborn infant (Lum et al., 2019).

A case series including 5 cases of maternal ZIKV-infection during various stages of pregnancy. In 3/5 cases there was placental tissue available for testing. In all cases the placental tissue tested positive for ZIKV RNA by RT-PCR (de Noronha et al., 2016).

In a case report immunohistochemistry showed ZIKV viral particles in the decidua, fibroblasts and chorion and in the circulating cells in the intravascular compartment (Santos et al., 2020).

Recommendation

In the case of fertility preservation, there is insufficient data on the risk of viral transmission using gametes potentially infected with ZIKV. An individual risk assessment is advised before using these gametes.	GPP

Conclusion

Zika virus has been found on the surface of sperm and placental tissue, however, it does not contain the retrotranscription activity or enzymes to allow DNA-integration into gametes. The possible contamination of the oocytes with maternal blood, as a cause of positivity, has not been ruled out.

There is insufficient evidence on the association between ZIKV-infection and gametes or potential of transmission to offspring in the absence of maternal infection

PICO QUESTION: DOES ZIKA VIRUS IMPACT THE OUTCOME OF MAR?

Evidence

There were no studies investigating the effect of Zika virus on the outcome of medically assisted reproduction.

If ZIKV-infection is diagnosed in male or female during MAR treatment, cycle should be stopped, and the couple should be advised to use barrier contraception for 3 months.	GPP

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F4. Prevention/ reduction of transmission during assisted reproduction

PICO QUESTION: WHICH TECHNIQUES CAN BE USED TO PREVENT/ REDUCE ZIKA VIRUS TRANSMISSION DURING MAR?

SEMEN PROCESSING

Evidence

No studies were found comparing different techniques of semen processing in males with Zika virus infection.

One study reported ZIKV-RNA-positivity in different semen fractions after serial swim-up tests. After submitting the samples to a swim-up method to isolate motile spermatozoa, 7/11 samples tested ZIKV-RNA-positive (Joguet et al., 2017).

A more recent case report showed that ZIKV RNA was detected in the ejaculate by RT-PCR, but not in the prepared sperm after a bilayer gradient centrifugation (42 days after onset of symptoms). However, ZIKV RNA was detected in the prepared sperm after repeat testing 8 and 13 days after the initial test (Cassuto et al., 2018).

Conclusion

There are currently no semen processing techniques available that can completely remove ZIKV from semen.

Justification

Currently there is limited evidence that sperm preparation techniques can clear ZIKV from sperm. Semen processing will only reduce ZIKV RNA in 75-80% of cases of known active disease (based on a very small study of 14 patients) and so cannot be considered curative. Semen processing could be considered as risk reducing but not an effective intervention to eliminate risk.

Furthermore, ZIKV-infection is transient (Biava et al., 2018, Kurscheidt et al., 2019, Oliveira Souto et al., 2018). Therefore, it is recommended to postpone medically assisted reproduction for 3 months after diagnosis or on return from a Zika virus endemic area.

Any further questions on semen processing will not be discussed.

PICO QUESTION: DOES THE PLASMA VIRAL LOAD CORRELATE WITH ZIKA VIRUS DETECTION IN SEMEN?

Evidence

One prospective cohort study, including 15 men with acute ZIKV-infection, identified 3 different patterns of viral shedding in seminal plasma: a) non-shedding patients, with consistently negative ZIKV

RNA detection in seminal plasma during follow-up (4/15); b) seminal shedders with concomitant sera and/or urine shedding (6/15); c) persistent seminal shedders after virus clearance in sera and urine, i.e. discordant shedding patients (5/15) (Joguet, et al., 2017).

A prospective cohort study investigated ZIKV shedding in serum and semen in 55 men, and viral shedding in vaginal secretions in 50 women. Only 1/50 women had ZIKV RNA in vaginal secretions. ZIKV RNA was present in at least one semen sample of 31/55 male participants. Chance-corrected agreement of RNA detection was low in paired samples of semen and serum (Paz-Bailey et al., 2018).

In another prospective cohort study investigating viral shedding in semen samples, ZIKV RNA was demonstrated in 5/10 male patients. There was no significant association between viremia and detection of ZIKV in semen (Barzon et al., 2018).

A case series reported ZIKV-RNA-positivity in 5/17 semen samples. Plasma, collected at the same time as positive semen, tested negative for ZIKV RNA in 6/8 collections (Musso et al., 2017).

One prospective study compared the clearance of ZIKV in urine and semen. Out of 184 men participating, 60 had at least one PCR-positive semen sample. From the samples submitted within 30 days of disease onset, 61% tested positive for ZIKV. In contrast, only 1% of urine samples was positive for ZIKV RNA and the viral load was lower than in semen (Mead et al., 2018).

Recommendation

MAR is not advised even if serum is free of ZIKV because of poor correlation between serum and semen viral load.	Strong	⊕○○○

Justification

All infected patients, regardless of serum viral load, may be infectious through semen. The clearance of Zika virus is slower from semen compared to blood. Therefore, a negative test in plasma/serum does not offer 100% reassurance.

It was not specified in the methods of the papers whether seminal plasma or sperm cells were tested.

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F5. Reducing/ avoiding vertical transmission

PICO QUESTION: WHICH INTERVENTIONS CAN BE USED TO REDUCE/AVOID VERTICAL TRANSMISSION OF ZIKA VIRUS TO THE NEWBORN?

ELECTIVE CAESAREAN SECTION

No relevant studies could be found in the literature.

BREASTFEEDING

Evidence

A meta-analysis was identified including 9 case reports (10 mother-infant pairs). In 5 cases, the onset of maternal symptoms occurred before birth. In 2 out of 5 cases, the newborn tested positive for ZIKV by RT-PCR or nested PCR and one newborn's test result was inconclusive. In 4 out of 5 cases, the presence of ZIKV in breast milk was confirmed. In one case, the maternal symptoms started 2 days after delivery. The newborn tested negative for ZIKV at birth. The breast milk sample tested positive for ZIKV on the first day of breastfeeding; the infant tested positive for ZIKV the day after initiating breastfeeding. However, there was no replicative ZIKV detected in cell culture. In 2 cases, ZIKV-infection occurred after birth. In the first case the mother's breast milk tested negative for ZIKV, the infant was not tested. In the second case, the mother's breast milk tested positive and the infant was asymptomatic. In one case, infective ZIKV particles were found in the breast milk and the mother refrained from breastfeeding for the duration of her symptoms. The infant tested negative for ZIKV-infection (Sampieri and Montero, 2019).

Several case reports have been published and not included in the meta-analysis. A case report including four breastfeeding mother-infant pairs reported no ZIKV in the infants (Cavalcanti et al., 2017).

One case report showed secondary microcephaly, in an exclusively breastfed infant, in the presence of ZIKV-positive breast milk, but ZIKV-negative blood samples in both mother and infant (Siqueira Mello et al., 2019).

Conclusion

ZIKV has been found in breast milk of women with confirmed ZIKV-infection.

The possibility of transmission of ZIKV through breastfeeding has only been assessed in 12 mother-child pairs. This provides insufficient evidence to establish a recommendation.

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Summary

ZIKV	Male testing positive	Female testing positive	Couple testing positive
If detected before MAR	Postpone MAR treatment		
	For 3 months	For 2 months	For 3 months
If detected during MAR	Cancel cycle		
If detected after MAR	Caesarean section not recommended		
	Breastfeeding not contra-indicated		

Figure 6: Summary of management of medically assisted reproduction in patients testing positive for ZIKV.

PART G: SARS-CoV-2

In the circumstances of the current situation on the SARS-CoV-2 global pandemic, the guideline group considered introducing a chapter on this virus. However, the evidence is emerging and changing constantly. The best source of information with the latest evidence about the impact of this virus on MAR practice can been found here:

https://www.eshre.eu/Home/COVID19WG

PART H: Laboratory safety

With respect to the risk of cross-contamination by infectious agents in laboratory, the primary means of prevention is strict adherence to good laboratory practice, including careful management of cryobanks using appropriate methods of cryostorage.

PICO QUESTION: CAN SEPARATE CRYO TANK STORAGE PREVENT CROSS CONTAMINATION OF STORED MATERIAL?

Evidence

Bacteria, fungi and viruses have been shown to survive in liquid nitrogen (LN2) cryobanks (Mirabet et al., 2012). Viruses have been detected after storage at -70°C for HIV (9.1 years), HBV (4 years) and HCV (9 years) (Baleriola et al., 2011). HCV has also been shown to be stable for 6 months at -80°C (Halfon et al., 1996).

One study (Hawkins et al., 1996) 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 (Hawkins et al, 1996). Whilst this study did not involve reproductive cells or tissue, and also involved ethyl vinyl bags which are not used in MAR cryopreservation and have a 10% failure rate in liquid nitrogen (Khuu et al., 2002), viral transmission through LN2 was demonstrated.

A more recent study showed that no viruses could be detected in the LN2 used to vitrify oocytes (n=14) and embryos (n=10) from patients infected with HIV, HCV, and HBV using open devices (Cobo et al., 2012), nor detection of HCV in the LN2 from the long-term storage tank for a patient infected with HCV (Cobo, et al., 2012). However, the sample size was small.

In one animal study, screened bovine embryos were vitrified using open devices or in plastic cryovials, and then stored in LN2 experimentally contaminated with 3 bovine viruses (BVDV, BHV, BIV) for 3-5 weeks. Warmed embryos showed infection with BVDV & BHV-1 (83 batches, 13/61, 21.3%; but no infection with BIV (22 batches, 0/22, 0%) (Bielanski et al., 2000).

Recommendation

Since viruses can survive and be transmitted via liquid nitrogen (LN2), separate storage of reproductive cells according to viral positive and viral negative status is recommended.	GPP

Emptied and dried cryo tanks and transport shippers should be disinfected according to local standard operating procedures to reduce the potential of cross-contamination.	GPP

Individual clinics must risk assess to decide the number of cryo tanks needed.	GPP

Separate cryopreservation dewars should be used to quarantine gametes and embryos from patients with unknown infectious status.	GPP

Justification

There is a lot of variation in practice. Some clinics store all viral positive samples together, and some clinics have separate storage per type of virus.

Before attempting disinfection of cryo-tanks, contact the manufacturers regarding the warranty conditions. We are unaware of any disinfection procedure for the absorbent material in dry transport shippers.

PICO QUESTION: CAN THE TYPE OF CRYOSTORAGE ENVIRONMENT (LIQUID VERSUS VAPOUR/OPEN VERSUS CLOSED SYSTEMS) PREVENT CROSS CONTAMINATION OF STORED MATERIAL?

Evidence

Liquid versus vapour

Unlike LN2 vapour phase (LNVP) storage vessels, LN2 storage vessels will accumulate particulate matter from the atmosphere. This includes pathogenic organisms which may remain viable by immersion in LN2. Pathogens can accumulate on the surface of cryodevices placed into LN2 storage, creating a contamination risk, particularly when removed from storage and warmed (Grout and Morris, 2009). Contamination of samples in LNVP also carries potential risk.

Mirabet et al. aimed to identify microbiological agent in the liquid nitrogen containers, while comparing different types of tanks (liquid nitrogen vs. gas phase vs. half gas half liquid). The vapour phase tank yielded less contamination than the liquid phase (Mirabet, et al., 2012).

Contamination found in the lower part of the liquid nitrogen vessels after thawing are generally environmental germs and exclusively contamination of water without cross-contamination of the samples themselves (Molina et al., 2016).

In an animal study investigating bovine embryos and viruses (BVDV and BHV-1), contaminated and non-contaminated samples of embryos and semen were stored in proximity in LNVP in open containers (dry

shippers) for 7 days. No cross-contamination took place, indicating that LNVP is a safe means for short-term storage of embryos and semen in dry shipper dewars (Bielanski, 2005).

Open versus closed systems

No original research studies could be found.

Recommendation

Vapour phase cryopreservation could be considered over liquid nitrogen in terms of safety to reduce the risk of cross-contamination.	Conditional ⊕○○○

Provided the cryomaterial is not compromised, cryodevices, such as sealed semen straws/vials, should be cleaned with a disinfectant wipe after removal from LN2 storage to mitigate risk of transmission of pathogens from the cryodevice surface.	GPP

Justification

No storage environment can guarantee 100% prevention of cross-contamination. Current evidence shows that the risk of cross-contamination is smaller with the use of vapour phase as compared to liquid nitrogen.

Many embryologists are understandably cautious about storing vitrified oocytes/embryos in vapour phase. The risks of undesired warming in vapour phase need to be weighed against the risk of cross-contamination in the liquid phase. The risk of warming is less for cryopreserved sperm samples compared to vitrified oocytes/embryos, as the rate of warming of frozen sperm is slower, hence the vapour may be considered more acceptable for sperm samples.

Open versus closed systems

Joaquim et al 2017 states:

- devices must be chosen carefully to minimize the risk of disease transmission
- there is no scientific consensus on the safety of claimed high-security closed methods in comparison with current open vitrification systems. More investment should be made to make closed systems more efficient (Joaquim et al., 2017)

Dubaut et al. state that for ZIKV, “the specimen should be labelled and stored separately reflecting potential increased risk, and if liquid nitrogen is required, open carriers should not be used”.

It is recommended that dry shippers should be used in accordance with manufacturer’s instructions, as “dry” shippers rather than filled with liquid nitrogen.

PICO QUESTION: CAN THE TYPE OF VIALS PREVENT CROSS-CONTAMINATION OF STORED MATERIAL?

Evidence

LN2 infiltrates inner thread vials and may cause a high rate of microbial contamination (and risk of explosion on thawing). Vials with a heat-sealable/ hermetically-sealable membrane are resistant to LN2 penetration and contamination (Chen et al., 2006).

Recommendation

Hermetical sealing of cryovials with additional covers could reduce the risk of cross-contamination of stored material.	Conditional	⊕○○○

Justification

Liquid nitrogen infiltrates inner thread vials, therefore increasing the risk of cross-contamination or explosion of the vial upon warming. Sealing the vials, using a membrane resistant to liquid nitrogen can overcome this issue. Application of such a secondary enclosure has been termed "double bagging" (Bielanski, 2014).

PICO QUESTION: CAN HIGH SECURITY STRAWS PREVENT CROSS CONTAMINATION OF STORED MATERIAL?

Evidence

When three types of straws (24 each of PVC, PETG and IR) were filled with HIV-1, then sealed either ultrasonically or thermally, prior to storage in LN2, only thermal sealed ionomeric resin (IR) straws showed no contamination on the outside of the straw upon thawing (Letur-Konirsch et al., 2003). Similarly, IR straws filled with HCV+ seminal plasma, and thermally sealed prior to LN2 storage, showed no contamination on the straw exterior upon thawing (Maertens et al., 2004).

Recommendation

The use of high security straws in combination with thermal sealing is the preferred approach as it minimises the risk of cross-contamination.	Strong	⊕○○○

At the time of thawing, decontamination of the exterior of the straw and the single use of sterile scissors will reduce the risk of contaminating the stored contents with potential pathogens.	GPP

Justification

Cryostorage devices should provide closure integrity and sample stability, without risk of infection from the cryo-tank.

Caution should be used to specifically consider avoidance of cross-contamination upon sample removal from the cryo-tank.

PICO QUESTION: CAN THE USE OF SEPARATE LABS PREVENT CROSS CONTAMINATION?

Evidence

One study showed that for baseline swabs (n=79) taken in a total laboratory automation (TLA) system during routine clinical use after running a small number of high-titre HCV samples, low level HBV (n=10) and HCV (n=8) contamination was detected on equipment and exposed surfaces, even when good lab practice was adhered to (Bryan et al., 2016).

When specimen containers were exteriorly coated with a fluorescent powder to enable the visualization of gross contamination using UV light, experienced lab technologists using standard personal protective equipment (PPE) showed contamination of PPE (gloves and laboratory coat cuffs), bare hands, biosafety cabinets (8/36; 22% tests) and testing accessory items (29/32; 91% tests) (Yarbrough et al., 2018).

For dentistry, after sterilization of invasive medical instruments with 2% glutaraldehyde, HBV was still detectable on the sterilized instruments (Zhou et al., 2006).

Contamination of samples by LN2-borne bacteria (S. minor Sclerotia) during cooling in controlled rate freezers, in vitrification procedures or in vapour phase vessels has been demonstrated (Grout and Morris, 2009).

A study by Cobo et al. included 24 patients testing positive for HBV having oocyte vitrification or IVF. No virus particles were detected in follicular fluid, oocyte or embryo culture media after IVF (Cobo, et al., 2012).

Recommendation

Given that personal protective equipment (PPE), laboratory equipment and exposed surfaces can be contaminated even after good laboratory practice, disinfection and changing PPE between cases can reduce the risk of cross-contamination.	GPP

The recommended procurement, processing, release and storage procedures should be used for all samples, not only virally positive samples.	GPP

Justification

We found no studies investigating if the use of separate labs will enhance the prevention of cross-contamination of samples.

Good practice according to the ESHRE guideline should be applied in all MAR laboratories, acknowledging that each center and country may have different legislation.

A large multi-center study is needed to investigate the need of separate labs for viral positive samples.

Use of single use devices (e.g. ICSI pipettes) mitigates the risk of cross-contamination.

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Annexes

Annex 1: Guideline development group

Annex 2: Abbreviations

Annex 3: Recommendations for research

Annex 4: Methodology

Annex 5: Stakeholder consultation

Annex 6: Literature study: flowcharts, list of excluded studies

Annex 7: Evidence tables

Annex 1: Guideline development group

This guideline was composed of (previous) members of the SIG Safety and Quality in ART, Ethics and Law and the former task force on Viral Infections, with addition of experts in the field that replied on a call for experts to the ESHRE audience.

Chair of the GDG

Edgar Mocanu	Rotunda Hospital, Department of Reproductive Medicine, Royal College of Surgeons in Ireland, Dublin (Ireland)

GDG members

Andrew Drakeley	Liverpool Women's Hospital, Department of Reproductive Medicine, Liverpool (UK)
Markus S. Kupka	Gynaekologicum Hamburg, Department Gynaecology and Obstetrics, Hamburg (Germany)
Evelin E. Lara-Molina	IVI RMA Global Barcelona, Department of Egg Donation, Barcelona (Spain)
Willem Ombelet	Genk Institute for Fertility technology, ZOL Hospitals, Genk Faculty of Medicine and Life Sciences, Hasselt University, Hasselt (Belgium)
Catherine Patrat	APHP.centre – University of Paris, Cochin, Service de Biologie de la Reproduction - CECOS, Paris (France)
Guido Pennings	Bioethics Institute Ghent (BIG) Ghent University, Department of Philosophy and Moral Science, Gent (Belgium)
Augusto Enrico Semprini	University of Milan, Department of Biomedical and Clinical Sciences, Milan (Italy)
Kelly Tilleman	Ghent University Hospital, Department for Reproductive Medicine, Gent (Belgium)
Mauro Tognon	University of Ferrara School of Medicine, Department of Medical Sciences, Ferrara (Italy)
Nino Tonch	Amsterdam University Medical Centre, location AMC, Department of Reproductive Medicine, Amsterdam (Netherlands)
Bryan Woodward	X&Y Fertility, Leicester (UK)

Invited expert

Mark Atkins *(clinical microbiologist/virologist)*	Micropathology Ltd, Coventry (UK)

Methodological support

Nathalie Le Clef	European Society of Human Reproduction and Embryology (Belgium)

DECLARATIONS OF INTEREST

All members of the guideline development group were asked to declare possible conflicts of interest by means of the disclosure forms (see *ESHRE Manual for Guideline Development*).

	Conflicts of interest
Edgar Mocanu	None declared.
Andrew Drakeley	Research fees from Ferring and Merck Consulting fees from ferring Sponsering for scientific meetings from Ferring, Gedeon-Richter and Pharmasure
Markus S. Kupka	None declared.
Evelin E. Lara-Molina	None declared.
Willem Ombelet	None declared.
Catherine Patrat	Speakers fees from Merck and MSD
Guido Pennings	None declared.
Augusto Enrico Semprini	None declared.
Tilleman Kelly	Speakers fees from Cooper Surgical and Ferring. Consultancy for educational purposes as member of the advisory board BioTeam of Ferring.
Mauro Tognon	None declared.
Nino Tonch	None declared.
Bryan Woodward	None declared.
Nathalie Le Clef	None declared.

Annex 2: Abbreviations

Ab	Antibody
Ag	Antigen
CI	Confidence interval
CNP	Combined neonatal prophylaxis
CS	Caesarean section
DNA	Desoxyribonucleic acid
EIA	Enzyme immune assay
ELISA	Enzyme-Linked Immuno Sorbent Assay
ET	Embryo transfer
FISH	Fluorescence in situ hybridization
GDG	Guideline development group
GPP	Good practice point
HBcAg	Hepatitis B core antigen
HBeAb	Hepatitis B e-antibody
HBeAg	Hepatitis B e-antigen
HBIG	Hepatitis B Immune globulin
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HBxAg	Hepatitis B x-antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HR	Hazard ratio
HTLV	Human T-cell lymphotrophic virus
ICSI	Intracytoplasmic sperm injection
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHC	Immunohistochemistry
IQR	Interquartile range
ISH	In situ hybridization
IU	International unit/infectious units
IUI	Intra-uterine insemination
IVF	In vitro fertilization
LN2	Liquid nitrogen
LNVP	Liquid nitrogen vapour phase
MAR	Medically assisted reproduction
MD	Mean difference
MESA	Microsurgical epididymal sperm aspiration
MTCT	Mother-to-child-transmission
NAT	Nucleic acid testing
NVP	Nevirapine
OR	Odds ratio
RD	Risk difference
PCR	Polymerase Chain Reaction
FTCT	Father-to-child-transmission
RCT	Randomized controlled trial

RDT	Rapid diagnostic test
RNA	Ribonucleic acid
ROC-AUC	Receiver operating characteristic – area under the curve
RR	Relative risk/risk ratio
SET	Single embryo transfer
SMD	Standardized mean difference
TESE	Testicular sperm extraction
WMD	Weighted mean difference
ZIKV	Zika virus
ZDV	Zidovudine

Annex 3: Recommendations for research on MAR in couples with a viral infection/disease

From the literature and discussion of the available evidence, several topics were identified for which evidence is inconsistent, insufficient or non-existing. For the benefit of patients with a viral infection/disease, the GDG recommends that future research, where possible in well-designed RCTs, should focus on these research gaps.

Considered are:

- HPV and the association with infertility
    - HPV as a cause of male infertility
    - HPV subtypes involved in infertility
- Semen processing in HBV and HCV: comparison between standard semen processing and advanced semen processing
- Semen processing for HTLV I/II
- Association between SARS-CoV-2 and infertility
- Laboratory safety procedures for SARS-CoV-2

Annex 4: Methodology

GUIDELINE DEVELOPMENT

The European Society of Human Reproduction and Embryology (ESHRE) guidelines are developed based on the Manual for ESHRE guideline development (N. Vermeulen, N. Le Clef, S. Mcheik, A. D'Angelo , K. Tilleman, Z. Veleva, W.L.D.M. Nelen, Manual for ESHRE guideline development, version 2019), which can be consulted on the ESHRE website (www.eshre.eu/guidelines). The principal aim of this manual is to provide stepwise advice on ESHRE guideline development for members of ESHRE guideline development groups. The manual describes a 12-step procedure for writing clinical management guidelines by the guideline development group, supported by the ESHRE methodological expert:

The current guideline was developed and funded by ESHRE, which covered expenses associated with the guideline meetings (travel, hotel and catering expenses) associated with the literature searches (library costs, costs associated with the retrieval of papers) and with the implementation of the guideline (printing, publication costs). Except for reimbursement of their travel expenses, GDG members did not receive any payment for their participation in the guideline development process.

The scope of the guideline and first version of the key questions were drafted by members of the ESHRE Special Interest Group Safety and Quality in ART, Ethics and Law and members of the former task force on Viral Diseases. A call was launched for experts in the field interested in joining the guideline development group. All applications were reviewed, and experts were selected based on expertise and geographical location. We strived towards a balance in gender and location within Europe. A meeting of the guideline development group was organized to discuss the key questions and redefine them through the PICO process (patients – interventions – comparison – outcome). This resulted in a final list of 9 key questions for 6 viruses each. Based on the defined key words, literature searches were performed by the methodological expert (Dr. N. Le Clef). Key words were sorted to importance and used for searches in PUBMED/MEDLINE, the Cochrane library, EMBASE and GIM. We searched the databases from inception up to 3 November 2020.

Literature searches were performed as an iterative process. In a first step, systematic reviews and meta-analyses were collected. If no results were found, the search was extended to randomized controlled trials, and further to cohort studies and case reports, following the hierarchy of the levels of evidence. References were selected or excluded by the methodological expert and expert GDG member based on title and abstract and knowledge of the existing literature. If necessary, additional searches were performed in order to get the final list of papers. The quality of the selected papers was assessed by means of the quality assessment checklist, defined in the ESHRE guideline manual. Furthermore, the

evidence was collected and summarized in an evidence table according to GIN format (http://www.g-i-n.net/activities/etwg). The quality assessment and evidence tables were constructed by the expert GDG members.

Normally, summary of findings (SoF) tables would be prepared following the GRADE approach for intervention studies with at least 2 studies per outcome. However, since the body of evidence mainly consisted of cohort and cross-sectional studies, case series and case reports, which were often difficult to compare in a direct manner, the added value of summarizing the evidence in SoF tables was limited and thus not performed for this guideline. The critical outcomes for this guideline were:

- Safety:
  - risk of horizontal transmission to partner/family/healthcare providers
  - risk of vertical transmission to the infant
- Efficacy: implantation rates, pregnancy rates, live birth rates, miscarriage rates

GDG meetings were organized to discuss the draft recommendations and the supporting evidence and to reach consensus on the final formulation of the recommendations. In a final step, all evidence and recommendations were combined in the ESHRE guideline: "Medically assisted reproduction in patients with a viral infection/disease".

FORMULATION OF RECOMMENDATIONS

We labelled the recommendations as either ''strong'' or ''conditional'' according to the GRADE approach. We used the words ''we recommend'' for strong recommendations and ''we probably recommend'' for conditional recommendations. Suggested interpretation of strong and conditional recommendations by patients, clinicians and health care policy makers is as follows:

Implications for	Strong recommendation	Conditional recommendation
Patients	Most individuals in this situation would want the recommended course of action, and only a small proportion would not	The majority of individuals in this situation would want the suggested course of action, but many would not
Clinicians	Most individuals should receive the intervention Adherence to this recommendation according to the guideline could be used as a quality criterion or performance indicator Formal decision aids are not likely to be needed to help individuals make decisions consistent with their values and preferences	Recognise that different choices will be appropriate for individual patients and that you must help each patient arrive at a management decision consistent with his or her values and preferences Decision aids may be useful in helping individuals to make decisions consistent with their values and preferences
Policy makers	The recommendation can be adopted as policy in most situations	Policy making will require substantial debate and involvement of various stakeholders

For each recommendation it is mentioned whether it is strong or conditional and what the quality of the supporting evidence was. In the justification section, more data are provided on the considerations taken into account when formulating the recommendations: balance between desirable and undesirable effects, certainty of the evidence of effects, certainty in how people value the outcome, acceptability and feasibility of the intervention. Impact on health equity and resource impact were only discussed where relevant.

STRATEGY FOR REVIEW OF THE GUIDELINE DRAFT

After finalization of the guideline draft, the review process was initiated. The draft guideline was published on the ESHRE website, accompanied by the reviewers' comments form and a short explanation of the review process. The guideline was open for review between 18 February and 1 April 2021.

To notify interested clinicians, we sent out an invitation to review the guideline by email to all members of the ESHRE SIG Safety and Quality in ART, Ethics and Law, SIG embryology and ESHRE members with an interest in viral disease.

Selected reviewers were invited personally by email. These reviewers included:

- *Coordinators and deputies of the ESHRE SIG Ethics and law and the ESHRE SIG Quality and Safety in ART.*
- *Contact persons of patient organizations across Europe.*
- *Contact persons of international and national societies focused on IVF/ICSI across Europe.*

All reviewers are listed in annex 6. The Reviewer comments processing report, including further information on the review and a list of all comments per reviewer with the response formulated by the GDG will be published on the ESHRE website.

GUIDELINE IMPLEMENTATION STRATEGY

The standard dissemination procedure for all ESHRE guidelines comprises publishing and announcement.

Each guideline is published on the ESHRE Website and in Human Reproduction Open. The announcement procedure includes a news item in "Focus on Reproduction", a newsflash on the ESHRE website homepage and a short presentation at the ESHRE Annual meeting. All participants in the annual ESHRE meeting will be informed about the development and release of new guidelines; all related national societies and patient organizations are informed about the guideline release. They are asked to encourage local implementation by, for instance, translations or condensed versions, but they are also offered a website link to the original document.

Patient versions of the guideline will be developed by a subgroup of the GDG together with patient representatives. The patient version is a translation of the recommendations in everyday language, with emphasis on questions important to patients. It aims to help patients understand the guideline's recommendations and facilitates clinical decision-making.

To further enhance implementation of the guideline, the members of the GDG, as experts in the field, will be asked to select recommendations for which they believe implementation will be difficult and make suggestions for tailor-made implementation interventions (e.g. option grids, flow-charts, additional recommendations, addition of graphic/visual material to the guideline).

SCHEDULE FOR UPDATING THE GUIDELINE

The current guideline will be considered for revision in 2025 (four years after publication). An intermediate search for new evidence will be performed two years after publication, which will inform the GDG of the necessity of an update.

Every care is taken to ensure that this publication is correct in every detail at the time of publication. However, in the event of errors or omissions, corrections will be published in the web version of this document, which is the definitive version at all times. This version can be found at www.eshre.eu/guidelines.

For more details on the methodology of ESHRE guidelines, visit www.eshre.eu/guidelines

Annex 5: Stakeholder consultation

As per routine development procedures, the guideline draft was open for review for 6 weeks, between 18 February and 1 April 2021. All reviewers, their comments and the reply of the guideline development group are summarized in the review report, which is published on the ESHRE website as supporting documentation to the guideline. The list of representatives of professional organization, and of individual experts that provided comments to the guideline are summarized below.

Representative	Organization
Harish M Bhandari	British Fertility Society, UK

Reviewer	Country
Thomas Mitchell, James Duffy, Anastasia Mania, Niki Konsta, Ippokratis Sarris	UK
Pierre Boyer	France
Carlos Calhaz-Jorge	Portugal
Stefan Matik	North Macedonia
Qianhong Ma, Fang Ma	China
Charalampos Siristatidis	Greece
Liana Bosco	Italy
Kimball O. Pomeroy	USA
Janek von Byern	Austria