Annex 7: Evidence tables

Hepatitis B virus

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

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Hou et al., 1993)		83 patients with AVHB	June 1985 to January 1992 Serum samples collected from 24 sex partners of 49 patients with AVHB who had sexual contact in the preceding 6 months and 16 sex partners of the controls were tested for HBV markers to determine whether the sex partner acted as a source of HBV infection	HBV sexual transmission	18/24 sex partners of patients were positive for HBsAg and HBV DNA All 16 sex partners of controls were negative		
(Huo et al., 1998)		5 index patients, married or engaged females Negative for HCV, HDV, HIV Spouses were all known to have been seropositive for HBsAg and developed antibodies	1989-1996	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. In contrast, there was significantly more variation (mean heterogeneity 6.1%, range 1-13.9%) in the region amplified between the five couples and between each couple and the controls (Fig. 1; $P < 0.001$).		

(Inaba et al., 1979)	Cohort observational-prospective JAPAN	68 HBsAg+ve pregnant females Their husbands Multicentre (35) Japan	Screening for HBV sAg and sAb. Comparison to: 28 HBV sAg negative pregnant women and their husbands Follow Up: 12-24 months	Screening for HBV sAg and sAb. Sputum before and after delivery Cervical mucus (18 women) Vaginal discharge (5 women) Husband testing - blood Dilution ½-1/256. Titres over ¼ were positive. IAHA; R-PHA for Ag PHA for Ab	30 husbands were positive (44.2%) 11.8% Ag +ve 32.4 Ab +ve Controls: None Ag +ve ONE Ab+ve Sputum and mucus did not contain HBV Ag Lochia contained HBsAg up to day 6 after delivery.	Sexual transmission of HBsAg seems to occur, particularly if sexual contact takes place during or immediately after menstruation.	The risk of transmission from extramarital sources was only verbally verified. Only 68 out of 195 HBV Ag+ve women were included in the study. Potential risk for bias as only a subgroup analysed
(Rosenblum et al., 1992)	Cross-sectional study USA	Women 18y an older Selling sex for money or drugs since 1978. Multiple affluent states Including detention centers, brothels, STD clinics. Drug treatments centers and on the street. 1368 females cer	Questionnaire. IDU ever injected. Number of sexual partners. STD's, sexual practice.	Serum tested for: HBV (anti HBc and antiHBs), HDV, HIV, syphilis.	Prevalence of HBV infection 56%. Increased with age, IDU use. Among IDU Increased risk of HBV included increasing age, black and Hispanic, duration of IDU use, number of partners, seropositivity for HIV or syphilis. Decreased risk of HBV in women using a diaphragm, spermcides, sponge or opal contraceptives, using condoms. Non-IDU Increased risk of HBV in penile-anal and penile oral intercourse, number of partners, HIV, syphilis seropositivity and decreased risk in penile -vaginal and use of vaginal sponge	Having anal intercourse and failure to use barrier contraceptives may facilitate transmission of HBV infection to women. 83% never used barrier contraceptives and 37% engaged in anal intercourse. Heterosexual transmission was the only risk factor for disease acquisition in 27% of females with a positive HBV test.	Study identifies a high risk population. No studied comparison group. The authors compare their findings with prevalences measured in other studies.

(Tufon et al., 2019)		The high prevalence (8.0%) of HBV infection in the Southwest region of Cameroon requires that we consider any HHC and/or SP of an HBV infected patient at risk of contracting the infection. 203 HBV infected participants 138 sexual partners (SP)	Crossectional study <u>Current infection</u>: positive for HBsAg and anti-HBc <u>Past infection</u>: positive for anti-HBc only <u>People with past and current infection</u>: people positive for HBsAg and anti-HBc + people positive for anti-HBc only	Questionnaire to obtain demographic data as well as information on vaccine status, condom use, marital status, nature of relationship, present living condition, and the number of years spent with HBV infected individual test for HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-hepatitis B core (anti-HBc) total	Of the 138 SPs 28 (20.3%) had taken the HBV vaccine 20/138 (14.5%) SP tested HBsAg positive 36/138 (26.1%) had evidence of past and current HBV infection Female SPs were significantly more associated with the infection compared to male SPs, and this proved to be statistically significant only with the crude OR (OR = 2.31, CI: 1.01–5.29) SPs who were cohabiting with their corresponding HBV infected SPs were significantly more associated with infection (OR = 3.95, CI: 1.73–9.04) compared to SPs who were not cohabiting		
(Katoonizadeh et al., 2018)		2590 HBsAg positive individuals and their 1454 spouses (1003 females, 451 males)		Measurement for HBsAg was performed on baseline stored serum samples of all GCS participants	HBsAg was positive in 2.3% (n = 33) of the spouses (4.2% in husbands and 1.4% in wives, P = 0.02) The rate of HBV-exposure (HBcAb positivity) was 48% (n = 480) in female spouses, 62.9% (n = 281) in male spouses Despite high virus exposure rate among spouses, the rate of HBsAg positivity among them was very low (2.3%).		

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

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Cao et al., 2016)	CS	Inclusion criteria: 1.HBV fathers (AgHbs+, HBV DNA + or -) 2.pregnant women, AgHBS-, HBVDNA -, AcHbs+ or - <u>Observational group:</u> 202 couples with AcHbs+ women <u>Control group:</u> 196 couples with AcHbs- women <u>Fathers' stratification on HBVDNA levels (IU/ml):</u> • 10e9-10e6 (53/52) • 10e6-10e4 (51/50) • 10e4-10e2 (48/52) • <10e2 (50/42)	Retrospective study March 2006 to May 2013	Measurement of HBV-M (chemiluminescence) and HBVDNA (fluorogenic qPCR) in infants (cord blood) In observational and control groups according to fathers' HBV DNA levels	**Positive HBV DNA in cord blood:** <u>Observational group:</u> • 9/53 in 10e9-10e6 HBVDNA group • 1/51 in 10e6-10e4 group • 0/48 and 0/50 in the two other groups Statically difference in positive HBV DNA in cord blood between groups 1 and 2 (p=0.009) <u>Control group:</u> • 11/52 in 10e9-10e6 HBVDNA group • 3/50 in 10e6-10e4 group • 1/52 and 0/42 in the two other groups Statically difference in positive HBV DNA in cord blood between groups 1 and 2 Binary logistic regression: elevated paternal HBVDNA: risk factor for HBV infection of the neonates.	Decreased HBV vertical transmission from father to infant with lower HBVDNA in paternal serum in both groups	Threshold of 10e4 IU/ml with HBsAb+ women and 10e2 with HBSAb-women Which HBsAb level to block paternal vertical transmission? Viral load testing before conception

WHICH TECHNIQUE (IUI/IVF/ICSI) FOR MEDICALLY ASSISTED REPRODUCTION SHOULD BE USED IN COUPLES WITH HEPATITIS B VIRUS?

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Nie et al., 2019)	CS	Women who had an HBsAg-positive husband, who received antiviral therapy during pregnancy, or who conceived after oocyte donation were excluded group 1: 125 women who underwent IVF-ICSI group 2: 126 women with natural conception	Between December 2014 and December 2017, consecutive pregnant women who received ART or natural conception and had a live birth and who were diagnosed as having chronic HBV infection (i.e., HBsAg was detectable in serum for more than 6 months before natural pregnancy or ART, with or without HBV DNA seropositivity	seropositive rate of HBsAg in children at birth. rate of HBV infection, (HBsAg, in children at 9–15 months of age)	no significant difference in the rate of HBsAg-positive children at birth between the two groups (6.3% [11/176] vs. 9.3% [12/129]; P=0.319). 145 and 31 children were born as a result of treatment with IVF and ICSI, respectively. 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 (P¼.451). 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]; P=0.202). All 23 HBsAg-positive children seroconverted to negative at 9-15 mo of age after HBIG therapy	assisted conception does not increase the risk for mother-to-child transmission of HBV compared with natural conception.	

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

DNA integration in semen/oocytes/embryo

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Jin et al., 2016)	CS	Long term follow-up. China May 2011-May 2014 Maternal information included the occurrence of pregnancy, the immunization and hepatitis B immune globulin (HBIG) treatment before and after delivery, the term of delivery, the test results of HBV, and the neonatal outcome.	31 couples where man was HBsAg positive and woman was HBsAg negative and 41 couples with a HBsAg positive woman and a HBsAg negative man.	Unfertilised oocytes and nonviable embryos in previous ART cycle were tested for HBV DNA, RNA. Vaccination was performed at birth, at 4 weeks and 6 month of age. Babies received HBIG within 8 hours after birth. Children were tested AFTER full vaccination at 9 and 24 months. Testing included: HBsAg, antibody to hepatitis B surface antigen (anti-HBs), antibody to hepatitis B core antigen (anti-HBc), hepatitis B e antigen (HBeAg) and antibody to hepatitis B e antigen (anti-HBe). If the HBV markers of the children suggested a possible HBV infection, the HBV DNA loads in the plasma were analyzed quantitatively using real-time polymerase chain reaction with an HBV fluorescence quantitative polymerase chain reaction kit.	A total of 72 HBsAg-positive couples were screened. One couple was lost to follow-up. A total of 24 babies were born from 23 deliveries, 12/24 newborns were born to couples with HBV-positive oocytes and/or embryos 2 couples with HBV DNA- positive oocytes and/or embryos; 7 couples with HBV RNA-positive oocytes and/or embryos; 3 couples with HBsAg-positive oocytes and embryos. Based on logistic regression analysis, the pregnancy outcomes were shown not to be associated with female HBeAg status, male HBeAg status, the type of reproductive technology, or the presence of HBV in oocytes and embryos. 20/24 children were anti-HBs-positive. 3/24 children were negative for HBsAg, anti-HBs, anti-HBc, HBeAg and anti-HBe. 1/24 was seropositive for anti-HBs, anti-HBc, and anti-HBe at 6 months of age. At 9 months of age, this child had seroconverted (anti-HBs- and anti-HBc-positive) and had no detectable HBV DNA load in the plasma. The mother of this child was positive for HBsAg, anti-HBc, anti-HBe, and HBV DNA at the time of delivery, and her un-fertilized oocytes and nonviable embryos were HBV RNA-positive.		The presence of HBsAg in oocytes and embryos may not result in the vertical transmission of HBV in the offspring of HBV carriers. In this long-term follow-up study, none of the children born to couples with HBV-positive oocytes and embryos remained chronically infected or became chronic carriers of HBV. This study provides direct evidence that the presence of HBsAg in oocytes and embryos may not result in vertical transmission of HBV to offspring of HBV carriers.

(Hu et al., 2011)		China 250 oocytes and 578 embryos analysed Oocytes: 139 +ve women 436 embryos from couples with woman +ve 75 embryos from 27 couples man +ve 67 embryos from 18 couples both +ve	To detect the presence and the expression of HBV in human oocytes and early embryos from patients with HBV infection and to evaluate the influence of the woman's serum HBV DNA levels, infection duration and mother's serum HBsAg status on the presence of HBV. **Oocytes**: germinal vesicle (GV) stage, metaphase I (MI) stage, metaphase II (MII) stage after failure to fertilize (HbsAg +ve women) **Embryos** were poor quality or polyspermy embryos (unsuitable for transfer or cryopreservation) Couples with one + partner. Oocytes and embryo from healthy couples=controls		Embryos had a 14.4% +ve rate Oocytes had a 9.6% +ve rate HBV positive embryos are either maternally or paternally dependent A significant increase in viral positivity in oocytes and embryos was found in those with a high serum HBV DNA level.	HBV DNA could enter the nuclei of human oocytes and embryos. The presence of HBV DNA was related to the serum HBV DNA level, to the serum HBsAg status of the woman's mother, and possibly to the duration of infection.	Downside is that only abnormal oocytes and embryo were analysed, This is not proof the virus integrates in healthy oocytes and embryos.
(Huang et al., 2003)		14 subjects 5 healthy controls 9 HBV infected	To evaluate the level of sperm chromosome aberrations in male patients with hepatitis B, and to detect whether HBV DNA integrates in sperm chromosomes of hepatitis patients.		233 analyzable sperm metaphase spreads in the hepatitis group, 33 (14.8%) complements contained chromosome aberrations, significantly higher than 5 (4.3%) chromosome aberrations in the control group($P<0.005$).	Sperm chromosomal aberrations are higher in HBV infected males. Only one patient had DNA integration in the sperm genetic material	Small study, one positive only.

(Kong et al., 2016)		China Ovarian tissues from 50 patients with gynaecological disease and HBV positivity Ovarian cysts 18 Ectopic pregnancy 2 Ovarian teratoma 6 Uterine cancer 24 Controls 6 (No HBV)	To clarify if HBV can replicate in the ovum, correlate serum levels and ovum infection.		Brown positive signals of HBcAg were detected in 6 ovarian tissues (12%, 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). Theree smaples 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	Serum HBeAg status and HBV DNA levels could influence HBV expression and replication in the ovum.	Exclude-cannot find any tables or figures
(Quint et al., 1994)	Report Holland	128 women had their embryos exposed to HBV infected serum used for culture media for 6 weeks. All women developed HB disease. 18 women were successful and became HBV positive during pregnancy. .	22 children born from the 18 infected women and 16 children born from 12 non-infected women. Blood samples for the detection of HBV DNA and other serologic parameters of hepatitis B infection (HBsAg, antibody to hepatitis B core antigen [anti-HBc], and antibody to HBsAg [anti-HBs]) were obtained from mothers and children at birth and in the first year of life. Lymphocytes for HBV DNA detection in infants were obtained at 4 and 12 months after birth.		Infants born from women infected because of IVF showed perinatal anti-HBc in their serum. Over a period of 6 to12 months, this anti-HBc became undetectable in all infants. HbsA g were not detected after birth. Passive transfer of anti-HBs was observed in 16 of the 22children. The six anti-HBs-negative children were born from three anti-HBs-negative mothers; two women were HBsAg positive at delivery, and one had just resolved HBsAg. All cord blood samples(n=22) and maternal serum samples(n=18) were HBVDNA negative, with the exception of 2 maternal serum samples from HBsAg- and HBeAg-positive mothers at delivery. In the control group (group II), all serum samples were HBc and HBV DNA negative. At delivery, all mothers and children were anti-HBc negative but anti-HBs positive due to active passive immunization. Lymphocytes from 17 of the 22children (group I) born from infected women could be tested for HBV DNA during follow-up. All results remained negative, as did the HBV DNA results for lymphocytes from 14 available samples from the control group (group II).	In this study, HBV DNA could not be demonstrated by PCR in any of the children of mothers exposed to HBV during IVF.	

Placenta

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Chen et al., 2013b)	China	171 pregnant women, HBV+ve.	Oct 2009-Oct 2011 Maternal venous blood and cord blood. 157 placental tissue samples HBV markers in serum and placenta, HBV DNA in serum and placenta	Maternal venous blood and cord blood. 157 placental tissue samples HBV markers in serum and placenta, HBV DNA in serum and placenta	HBV infection in decidual cells had the smallest risk for intrauterine infection of neonates, while HBV infection in villous capillary endothelial cells had the greatest risk for intrauterine HBV infection (OR=4.26, P<0.01) neonates of mothers with high HBV DNA levels ($\geq 10^6$copies/ml) were more likely to get intrauterine infection (P<0.01) compared with those with low levels($\leq 10^4$copies/ml) **IHC:** Rate of HBsAg+ in placental tissues was 36.9% (58/157), and HBcAg+ rate was 31.8%(50/157). There was no HBsAg+ or HBcAg+ staining in the negative controls. **RT-PCR:** 67 cases (42.7%) of placental tissues expressed HBV DNA, none detected in healthy placentas. **ISH:** The infection rate of HBV in decidual cells was 55.4% (87/157), 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 (trend test,P<0.01).		Intrauterine infection diagnosis based on HBsAg and HBV DNA in cord. Could it be maternal blood???

(Wei et al., 2015)	Cohort study China	155 placentae and blood specimens from HBsAg positive women and their newborns.	January 2005 to February 2009	ELISA for HBV markers (e antigen, anti-HBe and anti-HBc from mothers. Serum tested for HBsAg and anti-HBc from infants.	63 (40.65%) of mothers were HBsAg positive only, 54 (34.84%) were HBsAg+/eAg+/anti-HBc+, 27 (17.42%) were HBsAg+/anti-HBe+/anti-HBc+ 11 (7.09%) were HBsAg+/anti-HBc+ Mothers with HBsAg were divided into two groups (HBeAg positive and HBeAg negative) Placentae HBsAg+ve (58/155=37.4%) overall The rate of having a placenta positive for HBsAg is higher in HBeAg-positive mothers. OR (95% CI) value was 2.00 (1.02– 3.95) In addition, testing anti-HBc positive is evidence that one has been infected with HBV and that the infection may be resolved (HBsAg-negative) or ongoing (HBsAg-positive)	The results of this study not only show the relationship between HBV DNA levels and placental HBV infection, but that the risk of an HBsAg-positive placenta is higher with increasing maternal blood HBV DNA levels (the relative risk estimate OR was 3.24–3.85). In other words, if a mother's serum HBV DNA level does not exceed 104 copies/mL, placental HBV infection may be reduced.	Do newborns have antibodies against HBV???
(Xu et al., 2002)	Case control and pathology study China	402 HBsAg+ve pregnant women and their newborns Cases: 15 + newborns Controls: 387 neg newborns	1993-1997 Blood from women before delivery, within 24 h from newborns Pathology study: 101 HbsAg women, and 14 negative. Placental tissue		Antigen e +ve and PTL were associated with HBV infection (OR 14.46 and 6,66. 15/420 had serum HbsAg within 24h of birth (3.7%). If the woman was positive for both e and s antigen intrauterine infection rate was 9.8%. Maternal serum HBsAg titer was associated with intrauterine HBV infection. Similarly maternal serum HBV DNA concentration was significantly associated with intrauterine HBV infection. Overall placental infection rate was 44.6%. The HBV infection rates decreased gradually from the maternal side to the fetus side. HBsAg in 33.7% (34/101) HBxAg 37.6% (38/101 HBcAg 20.8% (21/101) HBV DNA 44.6% (45/101)		Same as Wei above

DOES HEPATITIS B VIRUS/TREATMENT OF HEPATITIS B VIRUS BEFORE MEDICALLY ASSISTED REPRODUCTION IMPACT THE OUTCOME OF MEDICALLY ASSISTED REPRODUCTION?

Female HBV infected

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Chen et al., 2014)	Retrospective case-control study. China	123 cycles in chronic HBV patients 246 cycles in non-infected couples. Matched for age, D3 serum FSH (follicle stimulation hormone) levels, body mass index and assisted reproductive technology approach used (IVF or ICSI), in a ratio of 1:2. All patients HBsAg tested. Chronic HBV infection was diagnosed when positive for 6 months. Exclusion: Acute Hepatitis or current antiviral therapy, abnormal liver function tests, other viral infections. Also, cycles were no embryo transfer was effected. Long protocol.	Study Group HBs Ag group=123 HBsAg positive women Control Group Seronegative women and husbands, matched for female age, day 3 serum FSH level, body mass index, and ART approach used (IVF or ICSI), in a ratio of 1:2. Semen preparation by density gradient+ swim up. ET on d2 or d3, younger than 35=2 embryos, over 35 y old= 3 embryos.	number of mature oocytes retrieved, fertilization rate, cleavage rate, proportion of high-quality embryos, and number of embryos transferred. Pregnancy was diagnosed by serum hCG estimation 14 days after embryo transfer. Clinical pregnancy was confirmed by transvaginal ultrasound examination 4 weeks after the positive pregnancy test. The primary outcome measures of the study were clinical pregnancy and implantation rate defined as number of gestational sacs per embryo transferred.	**HBsAg vs controls** Implantation rate (%): 30.52% (76/249) vs 28.34% (142/501) Clinical pregnancy (%) rate: 44.72% (55/123) vs 43.09% (106/246) Live birth rate (%): 42.28% (52/123) vs 40.65% (100/246)	Among women undergoing IVF/ ICSI HBsAg seropositivity is not associated with IVF/ICSI outcomes.	Retrospective nature. Selection bias? No indication if ALL HBC+ve women were included or how the selection was carried out if only some were selected. Also, the authors do not detail the study years.

(Lee et al., 2010)	Retrospective cohort Hong Kong, China	1676 couples undergoing their first ART cycle were included. First IVF cycle between January 2004 and December 2008. 131 (7.8%; 95% CI 6.6–9.2%) women were HBsAg positive and 161 (9.6%; 95%CI 8.2–11.1%) husbands were HBsAg positive. 13 (0.8%; 95% CI 0.4–1.3%) couples were both HBsAg positive.	The study was conducted to evaluate the prevalence of positive HBsAg in the attending population and to compare the outcomes of IVF treatment between couples with and without positive reactions for HBsAg.		No difference in treatment outcomes The ongoing pregnancy rates per cycle and per transfer were not significantly different among seropositive and seronegative women (26.7% versus 30.2% per started cycle; 31.5% versus 34.0% per transfer; respectively) or among seropositive and seronegative husbands (30.4% versus 29.9%per started cycle; 34.0% versus 33.8% per transfer, respectively). The ongoing pregnancy rate of couples with both partners being HBsAg positive was not significantly different from couples with discordant HBV serostatus and those couples both partners being HBsAg negative (23% versus 29%versus 30%) although the number was small. The live-birth rate was also not significantly different among the three groups (23% versus 27% versus 27%), while it was still not significantly different between the both partners seropositive and both partners seronegative (23.1% versus 26.9%). The live-birth rate was not significantly different when the analysis was confined to the comparison of couples with both partners seropositive with those with seropositive wife or seropositive husband (23.1% versus 23.9%versus 29.2%, respectively) in serodiscordant couples..	There were no significant differences in ongoing pregnancy rate and live-birth rate among HbsAg-positive and negative couples. This piece of information is of importance in the counselling of seropositive couples undergoing IVF treatment	Small numbers.

(Shi et al., 2014)	Retrospective CS	672 couples 77 with female HBsAg+ 136 male HBsAg+ 11 both HBsAg+ First IVF cycles For each HBV-seropositive cycle, two HBV-seronegative control cycles were matched. Criteria: age, cause of infertility, and date of ova retrieval (1 day). All women were tested for HBV, HCV, HIV, gonorrhea and syphilis within 6 months of the treatment cycles. Excluded if: seropositive for HCV, HIV and/or syphilis, acute hepatitis or received any antiviral treatment before IVF treatment. Cycles, which were cancelled in the case of no available embryo or if OHSS developed, were also excluded from this study. Study: 224 couples Control: 448 both HBsAg-seronegative couples.	Dec 2008-June 2012 Impact of HBV on sperm parameters, ovarian stimulation, and outcomes of the first IVF and embryo transfer treatment cycles between HBV-seropositive and HBV-seronegative couples.	Parameters analysed included: age of patients; type and duration of infertility; infertility aetiology; endometrial thickness; ovarian reserve evaluation (cycle day 3 serum level of FSH); total dose of gonadotropin treatment; serum estradiol levelon day of hCG injection; semen parameters on the day of oocyte retrieval; the numbers of oocytes retrieved, fertilized oocytes, two-pronuclear zygotes, cleaved embryos, top-quality embryos (grade I+II),and embryos transferred.	female HBsAg+ vs controls: implantation rate, 36.0% (54/150) vs. 38.5% (117/304) clinical pregnancy rate 48.1% (37/77) vs. 50.6% (78/154) HBV infection contributed significantly to fertilization rate (odds ratios (OR): 0.410, 95% confidence interval (CI): 0.186–0.906,P=0.028), but was not associated with successful pregnancy (OR: 1.173, 95% CI: 0.814–1.692,P=0.392).	Less top-quality embryo rate in couples with female partners being HBVseropositive and lower fertilization and 2PN rates in couples with one partner being HBV-seropositive during IVF treatment. Normal sperm morphology was significantly lower in HBV-infected male partners.	Analysis is on 213 Study and 426 control. NO explanation as to why!

(Wang et al., 2019)	Retrospective CS	10,208 patients undergoing their first IVF treatments. 8550 were studied, After exclusions: 180 HBsAg+ HBeAg+ patients, 714 HBsAg+ HBeAg- patients, and7,565 HBsAg negative controls were studied. patients who lacked HBV serostatus (n=157) or who also had another virus infection—including hepatitis C virus (n=22), human immunodeficiency virus (n=1), or syphilis (n=96)—were excluded from this study. Patients older than 38 years (n=579) and those treated with antagonist protocols (n=235) also were excluded. The cycles missing embryo information (n=234),with ovarian hyperstimulation syndrome (n=75), without clinical pregnancy data (n=1), with a chromosomal abnormality (n=89), or with intrauterine death (n=21), a medical abortion (n=3), stillbirth (n=17), or ectopic pregnancy (n=128) were also excluded from this study.	Jan 2010- April 2018 All patients received a routine luteal phase down-regulation protocol with gonadotropin-releasing hormone agonist. Briefly, hu-man chorionic gonadotropin (hCG) was administered to induce final oocyte maturation when two-thirds of follicles had reached a mean diameter of 18 mm. The serum E2concen-tration and endometrial thickness were measured on the day of hCG administration. The pregnancy outcomes included the implantation, clinical pregnancy, miscarriage, and live-birth rates. The maternal and neonatal outcomes included maternal pregnancy complications, delivery type (cesarean or eutocia), pre-term delivery (<37 gestational weeks), and number of live babies delivered.		The prevalence of HBsAg seropositive infection was10.5%, and 2.1% for HBsAgþ+ HBeAg+ infection in the study population. HBsAg+HBeAg+ vs HBsAg+HBeAg- vs controls The implantation rate in the HBsAg+HBeAg-group was lower than in the HBsAg seronegative control group (35.7% (607/1701) vs. 38.7% (6950/17939)) There was no statistically significant difference between the HBsAg+HBeAg+ group and controls (39.6% (158/399) vs 38.7% (6950/17939)) 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) live-birth rate 53.1% (93/175) vs 51.1% (360/704) vs 52.3% (3911/7480)	Although the implantation rate in the HBsAg+HBeAg- group was lower than in the HBsAg- controls, there was no association between HBV carriers and clinical pregnancy, miscarriage, or live birth outcomes. We found that HBV seropositivity was positively associ-ated with a high frequency of infertile patients with an ovula-tory disorder, one common cause of infertility in women.	

Male infected

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Cito et al., 2019b)	Retrospective CS	134 infertile couples undergoing IVF/ ICSI. 66 HBV+ men, HBV- woman 68 controls (both HBV-) Male age: 18-45 Female age: 18-40 Exclusion: 1.abnormal liver function or chronic hepatitis; 2) azoospermia or severe criptozoospermia; 3) cycles with donor semen or chromosomal aberrations; 4) couples who were seropositive for hepatitis immuno-deficiency virus (HIV) and/or hepatitis C virus (HCV), 5) positive history of parotitis; 6) antiviral therapy during thestudy period. Controls included both men and women who were negative for serum HBsAg, hepatitis B surface antibody (HBsAb), hepatitis Be antigen, hepatitis B e antibody, and hepatitis Bc antibody (HBcAb). Control couples were matched for age, ART approach used (IVF or ICSI) and cause of infertility. Semen analysis was evaluated according to the 2010 WHO criteria.	Jan 2011 to August 2018 Evaluate the influence of HBV men infection on IVF/ICSI outcomes, in a cohort of consecutive serodiscordant couples Baseline characteristics did not significantly vary between the HBV-positive and HBV-negative groups. Overall, the cause of couple infertility was: tubal factor (32.8%), endometriosis (20.9%), male factor (33.6%), unexplained (6.7%), mixed (6.0%).	Reproductive outcomes after IVF/ICSI	HBV vs controls 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) Live birth rate per cycle 21.2% (14/66) vs 19.1% (13/68) Clinical PR was not statistically different between groups after adjusting for confounding variables (odds ratio=1.28, 95% confidence interval=0.57–2.95, p=0.56	HBV infection proved to be able to affect fertilization and CRs in couples with HBsAg-positive men and negative women. However, clinical pregnancy out-comes, including implantation, pregnancy, miscarriage and live births rate were not influenced. In this setting, HBV infected men have the same chance to become father, compared to seronegative patients.	

(Lee, et al., 2010)	Retrospective CS	Evaluate the prevalence of positive HBsAg in centre's population and to compare the outcomes of IVF treatment between couples with and without positive reactions for HBsAg. 1676 couples 131- woman HBsAg+ 161 male HBsAg + 13 both positive The age of women, the age of their husbands and the duration of subfertility were comparable among seropositive and seronegative women. No exclusion criteria detailed	No specified		no significant difference between seropositive and seronegative men in ongoing pregnancy rates per started cycle (30.4% versus 29.9%)		
(Oger et al., 2011)	Matched case control study	Males HBsAg+ = 32 Control = 64 Controls and cases were matched by age (women within 2 years; men younger or older than 40 years old) and number of motile spermatozoa on the day of oocyte retrieval (less or more than 24.25 million which corresponds to the median value of this variable's distribution in the cases group). Only the first two IVF attempts were considered. Cases and controls with no oocyte on the day of retrieval, no spermatozoa available on that day or men with genetic abnormalities were excluded.	Jan 2005-March 2008 Measures: Patients: age; type, duration and infertility aetiology; ovarian reserve (d3 FSH and anti-Mullerian hormone concentrations; antral follicle count); and sperm parameters.		The number of cycles without embryo transfer was similar between cases and controls (15.6% versus10.9%). The reasons were complete fertilization failure (n= 6), poor embryo quality (n= 4), ovarian hyperstimulation syndrome (n= 1) and contamination of a culture well (n= 1). Implantation rates (13.5% versus 20.0%) and clinical pregnancy rates per cycle (18.8% (6/32) vs 31.3% (20/64)) and per transfer (23.1% versus 35.1%) were comparable between the two groups. Live birth rate per cycle 15.6% (5/32) vs 23.4% (15/64)	This study showed that HBV-infected men have spermatozoa with decreased motility before preparation of the semen. Couples in which male partners have a chronic infection with HBV, have a significantly higher risk of a LFR after IVF, which led to a slight decrease in the total number of embryos.	

(Shi, et al., 2014)	Retrospective CS	672 couples 77 with female HBsAg+ 136 male HBsAg+ 11 both HBsAg+ First IVF cycles HBV-seropositive = HBV group, HBV-negative – Control group For each HBV-seropositive cycle, two HBV-seronegative control cycles were matched. Criteria: age, cause of infertility, and date of ova retrieval (1 day). All women were tested for HBV, HCV, HIV, gonorrhea and syphilis within 6 months of the treatment cycles. Excluded if: seropositive for HCV, HIV and/or syphilis, acute hepatitis or received any antiviral treatment before IVF treatment. Cycles, which were cancelled in the case of no available embryo or if ovarian hyperstimulation syndrome developed, were also excluded from this study. Study: 224 couples Control: 448 both HBsAg-seronegative couples.	Dec 2008-June 2012 Impact of HBV on sperm parameters, ovarian stimulation, and outcomes of the first IVF and embryo transfer treatment cycles between HBV-seropositive and HBV-seronegative couples.	Parameters analysed included: age of patients; type and duration of infertility; infertility aetiology; endometrial thickness; ovarian reserve evaluation (cycle day 3 serum level of FSH); total dose of gonadotropin treatment; serum estradiol levelon day of hCG injection; semen parameters on the day of oocyte retrieval; the numbers of oocytes retrieved, fertilized oocytes, two-pronuclear zygotes, cleaved embryos, top-quality embryos (grade I+II),and embryos transferred.	male HBsAg+ vs controls: implantation rate, 38.5% (104/270) vs. 37.7% (206/547) clinical pregnancy rate 58.1% (79/136) vs. 53.7% (146/272) HBV infection contributed significantly to fertilization rate (odds ratios (OR): 0.410, 95% confidence interval (CI): 0.186–0.906,P=0.028), but was not associated with successful pregnancy (OR: 1.173, 95% CI: 0.814–1.692,P=0.392).	Less top-quality embryo rate in couples with female partners being HBVseropositive and lower fertilization and 2PN rates in couples with one partner being HBV-seropositive during IVF treatment. Normal sperm morphology was significantly lower in HBV-infected male partners.	Analysis is on 213 Study and 426 control. NO explanation as to why!

(Zheng et al., 2016) .	Retrospective CS	The HBV serostatus assessed within the year preceding IVF!!!!!! Group A : 121 seronegative controls Group B (active infection) – HBV DNA seropositive, n=92. Group C (convalescent infection) – HBsAg, HBeAb and HBcAb positive and HBV DNA negative, n= 125 Sperm: ejaculated or PESA/ TESA. NO mention if frozen!	2007-2012 All ICSI first cycle. Excluded: chromosomal abnormalities or acute or chronic infectious diseases aside from HB; couples in which the female partner tested positive for serum HBsAg, HBeAg, HBeAb or HBcAb, determined with diagnostic ELISA kits (Roche, USA), or HBV-DNA, determined by FQ-PCR with a cut-off of 103 copies/ml.		**Group A vs B vs C** <u>Ejaculated sperm</u> Implantation rate 28.3% (45/159) vs. 32.8% (39/122) vs. 23.0% (38/165) clinical pregnant 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) live birth rate 36.4% (28/77) vs. 49.2% (29/59) vs. 35.9% (28/78) no significant differences. <u>TESA/PESA</u> Implantation rate 31.1% (28/90) vs. 26.1% (18/69) vs. 25.3% (24/95) clinical pregnant rate 50% (22/44) vs 39.4% (13/33) vs 42.6% (20/47) early miscarriage rate 0% vs. 23.1% (3/13)vs. 5.0% (1/20) P<0.05 live birth rate 50% (22/44) vs. 27.3% (9/33) vs. 36.2% (17/47)	HBV actively infected men using PESA/TESA for ICSI had a lower 2PN fertilization rate, live birth rate and high early miscarriage rate compared to the convalescent infection and control group. There was no difference in ICSI outcome between the convalescent and control group. V	I find it challenging to look at ART outcomes over such a large time span as clinical and laboratory practice HAS CHANGED A LOT BETWEEN THOSE YEARS. The conclusions do not reflect the statistical evidence. The only significant finding was the increased miscarriage rate in testicular sperm Brings TESTICULAR SPERM to the table. Recall bias Testing was done Within the year (is an acute infection still present after 12 months???)

(Zhou et al., 2011)	CS	916 male patients 457 HBsAg positive 459 HBsAg negative Controls included men who were negative for serum HBsAg, hepatitisB surface antibody (HBsAb), hepatitis B e antigen (HBeAg), hepatitis B e antibody (HBeAb) and hepatitis B c antibody (HBcAb); control patients were also matched for days of sexual abstinence as well as time seeking fertility assistance Excluded: Patients with chromosomal abnormalities, concomitant varicocele, a history of surgery or congenital defects (urological or related to reproductive organs), long-term drug use and/or toxic or radiation exposure, those with a history of parotitis or genital tract infections as well as those receiving any antiviral therapy for fertility during the study period were excluded. 1824 cycles of ART-retrospective	Jan 2008-Dec 2009-positive Jan 2004-Dec 2009 ART Implantation rate was calculated by the number of intra- or extrauterine gestational sacs per embryo transferred. Clinical pregnancy rate was defined as the number of women with intrauterine gestational sacs (upon ultrasound scan) per cycle with successful embryo transfer.	**Reproductive outcomes in IVF and ICSI**	HBV vs controls <u>IVF</u> Implantation rate: 24.9% (284/1140) vs 26.7% (296/1108) Clinical pregnancy rate : 40.5% (217/535) vs 40.3% (210/521) Cancellation rate 8.9% (52) vs 11.2% (66). <u>ICSI</u> Significantly lower implantation and clinical pregnancy rates: Implantation rate: 18.3% (126/688) vs 24.2% (159/657) Clinical pregnancy rate: 31.2% (96/308) vs 39.3% (118/300) No difference in cycle cancellations 5.2% (17) vs 7.6% (25) HBV infection significantly contributed to lower implantation rate (OR: 0.57, 95% CI: 0.48–0.99, P=0.044) and clinical pregnancy rate IVF and embryo transfer outcomes in HBV-positive men were comparable with those in their healthy counterparts(P>0.05), though there was a trend of lower implantation rates among HBV-positive men. ICSI: Ovarian stimulation and response as well as the number of embryos transferred per cycle were similar in both study groups. After ICSI and embryo transfer, we observed lower rates of 2PN fertilisation (P=0.005), high-grade embryo acquisition (P=0.046), implantation (P=0.008) and clinical pregnancy (P=0.035) among HBV-positive men when compared to matched controls.	We also observed suboptimal ICSI and embryo transfer outcomes in the HBV-positive group (decreases in the rates of 2PN fertilisation, high-grade embryos acquisition, implantation and clinical pregnancy per cycle of embryo transfer). We conclude that HBV infection in men is associated with impaired ICSI and embryo transfer outcomes as well as impaired sperm quality.	Study suggest that cycle of ICSI where the male is HBsAg positive have poorer outcomes.

WHICH TECHNIQUES CAN BE USED TO PREVENT/REDUCE HEPATITIS B TRANSMISSION DURING ASSISTED REPRODUCTION?

Semen processing

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Condijts et al., 2020)		4 cHBV infected men 18-50 years old; 3 under nucleoside analogues treatment and one none, 2 with serum positive HBV load Semen and serum samples on the same day 96 sperm fractions after treatment	na	To compare HBV load in different fraction of spz after sperm selection through discontinuous gradient (90-45) +/- SU at different times (30mn, 1 and 2 h) in fresh and frozen sperm		No HBV DNA detection in all 96 samples except one (HBV3, NP fraction) sperm fraction, no viral DNA detection in motile fraction	Ghent University Hospital: cHBV infected men excluded from ICSI Suggestion that they may change their mind Need to increase samples number

DOES THE PLASMATIC VIRAL LOAD CORRELATE WITH HEPATITIS B VIRUS IN SEMEN?

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Ayoola et al., 1981)		100 Nigerians (50 M and 50 W) attending at the family planning clinic	None Not precised	Semen collection Menstrual blood collection Blood samples HBsAg detection, HBcAb, HBcAg, HBeAg and HBeAb if HBsAg+	HBsAg detection: • 9 serum samples (5 M and 4W) • 4 blood samples • 3 semen samples HBcAb detection: • - in 7/9 HBsAg+ • 2 semen samples • 2 blood samples No HBeAg or HBeAb detection		Old study

(Fei et al., 2015)		Observational cohort: 151 HBsAg+ and HBcAb+ chronic (>6 months) men from infertile couples: • HBeAg+ for 94 • HBeAg- for 57 Exclusion criteria: HBC and HIV positivity	March 2010 to October 2012	Serum collection: HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, HBV DNA (qRT-PCR) Semen collection: HBV DNA (qRT-PCR) Semen and blood samples: collection at the day of oocyte pick-up Sperm parameters (WHO) Separation of SL form spz though discontinuous gradient	Sperm parameters: No statistical differences for sperm volume, count and motility between HBeAg+ and HBeAg- patients. Lower normal forms in HBeAg+ / HBeAg- patients Lower volume et normal forms in seminal HBV DNA+ / HBV DNA- patients Distribution of serum and seminal HBD DNA levels *HBsAg+:* 51/151 serum HBV DNA>8 log10IU/ml 86/151 seminal HBV DNA >500IU/ml 143 serum HBV DNA+ 65 seminal HBV DNA+ *HBeAg+:* 51 serum HBV DNA>8log10IU/ml 34 seminal HBV DNA 3-4log10 IU/ml *HBeAg-:* 19 serum HBV DNA 3-4log10IU/ml 56 seminal HBV DNA<500 IU/ml Significant difference between serum and seminal HBV DNA levels, in HBsAg+ (6.5 and 0 log10 IU/ml), HBeAg+ and HBeAg- patients. Higher HBV DNA levels in serum and seminal levels in HBeAg+/HBeAg- patients. Predictive value of serum HBV DNA, HBsAg and HBeAg for seminal HBV DNA: • Higher serum HBV DNA and HBeAg in seminal HBV DNA+/HBV DNA- patients • Serum markers to predict the presence of seminal HBV DNA: serum HBV DNA (>6.9 log10IU/ml) HBsAg (<1791.5 S/CO), HBeAg (>14.8S/CO) • Combination of serum HBV DNA and HBeAg: high diagnosis (1000 sensitivity and 95.4% specificity) • AUC ROC: HBV DNA, HBeAG or both: 0.97, 0.94 and 0.97, respectively (higher than serum HBsAg (0.82)).	**Hepatitis B status may correlate with HBV in semen, with the combination of serum HBV DNA and HBeAg best predictor to identify those men with positive semen HBV DNA** HBeAg+ patients: greater risk of seminal HBV DNA positivity Serum HBeAg level is associated with HBV in semen In HbeAg- patients: seminal HBV DNA levels almost negative	Source of HBV DNA in sperm need to be determined (leucocytes?) No blood contamination Female partner's vaccination Sperm washing + ICSI???

(Hadchouel et al., 1985)		Experimental group: 17 patients 9 with acute hepatitis B 8 chronic HBsAg+ (3 chronic active and 5 chronic persistent) Control group: 4 patients		- Detection of HBV sequences by southern bolt in SL and spz - Sperm parameters analysis - Sperm discontinuous gradient (SL and spz)	HBV DNA detection: *In acute hepatitis patients (9):* Positive in SL in 3 Negative in serum in 9 Positive in spz in 2/3 (positive SL) *In chronic HBsAg patients (8):* Negative in semen in 8 Positive in serum in 8 Restriction enzyme pattern consistent with integration in the spz genome	Presence of HBV DNA in the semen, at least during acute phase of HBV infection No blood contamination No viral multiplication in spz (as 3.2kb band not observed)	Small size
(Qian et al., 2005)		Observational study Experimental group: 4 patients 2 patients: HBsAg+, HBeAg+, HBcAb+ 2 patients: HBcAg+ Control group: 1 patient Negative for all HBV markers (s, e and c Ab, s and e Ag) All HCV- and HIV-	2003 to 2004	Serum collection: HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, HBV DNA (qRT-PCR) Liver function Sperm parameters analysis (WHO) Detection of viral DNA in serum, spz and SL by PCR (480pb) Quantification of HBV DNA in serum and semen by RT-PCR	Detection of HBV DNA in sera and semen by PCR: Positive in the 2 HBsAg+ patients Negative in the 2 HBcAb+ and control Quantification of HBV DNA in sera and semen by RT-PCR: Lower titer in semen/ serum in the 2 HBsAg+ patients	Reliable RT-PCR to quantify HBV DNA in sera and semen	

WHICH INTERVENTIONS CAN BE USED TO REDUCE/AVOID VERTICAL TRANSMISSION OF HEPAPTITIS B VIRUS TO THE NEW-BORN?

ECS

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Chen et al., 2019)	SR	18 studies and 11446 pairs. 7 for birth transmission and 13 (2 common) for 6 motnhs follow up.			**At birth:** 7 studies, 3904 mother-infant pairs 2147 CS vs 1918 vaginal delivery Serological HBV positive: 2.0-18.2% in CS (average 7.2%; 151/1940) vs. 0.0-60.0% (average 16.6%; 301/1813) in vaginal delivery OR 0.269 (95% CI 0.139–0.520) **At 6 months** 13 studies, 7542 mother-child pairs 4022 CS vs. 3540 vaginal delivery MTCT 1.6-21.4% (average 3.3%; 132/4022) in CS vs. 1.3-19.2% (average 4.1%, 145/3520) in vaginal delivery OR 0.790 (95% CI 0.614 to 1.016)		Latest study, good detail in terms of methodology, large numbers. Conclusions align with global guidelines on the topic.
(Lee et al., 1988)	CS	447 infants born to mothers positive for HBeAg and HBsAg received HBV immunization	Vaginal birth (n=385) vs CS (n=62)	Rate of transmission	Infants who received HBV vaccine alone had a similar rate of HBV infection whether delivered by caesarean section 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 caesarean section (3/53, <6%) than in those delivered vaginally (57/286, 19-9%, p < 003). At birth, HBV-DNA was detected in none of the sera from infants delivered by caesarean section, but was found in 13 of 67 infants delivered vaginally.		

(Peng et al., 2018)	CS	criteria: (i) positivity for HBsAg,(ii) no evidence of HCV infection (anti-HCV negative), (iii) the absence of HIV infection (anti-HIV negative) and TP infection (anti-TP negative), (iv) the exclusion of a husband with HBV infection, and (v) the absence of preexisting chronic diseases such as diabetes mellitus, hypertension, or heart diseases. 1384 pregnant women	Prospective cohort study June 2012-march 2017 Wuhan China 867 CS deliveries 517 vaginal deliveries		no significant difference in the proportion of positive HBV DNA neonates between the CS group vs the VD group (0.7% vs 1.7%, P = 0.066) a higher proportion of infants who were positive for HBsAg at birth was seen in the VD group compared with that in the CS group (12.5% vs 4.8%, P < 0.001). After follow-up, 0.6% infants (5 of 888) in the CS group and 1.7% infants (9 of 519) in the VD group were identified as having chronic hepatitis B infection. (p<0.001)		

Breastfeeding

Reference	**Study type**	**Patients**	**Interventions**	**Outcome measures**	**Effect size**	**Authors conclusions**	**Comments**
(Zheng et al., 2011)	SR	32 studies, 5650 infants	Breastfeeding versus bottle feeding	CHB infection in infants	Breastfeeding vs bottle feeding RD -0.8%, (95% confidence interval [CI]: -1.6%, 0.1%) BF is not associated with additional risk of infantile CHB infection concurred with that of all the individual studies, except one [11], which suggests that BF is associated with a lower risk than FF.		

(Azzari et al., 1990)		40 infants born to HBsAg positive mothers	20 infants breastfed 20 infants formula fed All infants received active and passive immunization		Breastmilk was tested and found HBsAg+, anti-HBcAg+ and anti-HBeAg+ But HBeAg and HBV DNA negative No data on seroconversion in the infants		
(Chen et al., 2013a)		296 HBsAg+ and anti-HBc positive women and their infants 248 HBsAg positive mothers and their 250 infants No HIV or HCV coinfections No antiviral treatment during or before pregnancy	Retrospective cohort study Cohort 1: 2002–2004 Cohort 2: January 2006 to December 2010 All infants received HBV vaccination and 53.3% received HBIG		397 (72.7%) were breastfed and 149 (27.3%) were formula-fed chronic HBV infection occurred in 1.5% (6/397) of breastfed children and 4.7% (7/149) of formula-fed children respectively, NS Of the 13 children, 5 were administrated both HBIG and hepatitis B vaccine after birth, but the 8 others were only vaccinated against HBV		

(Zhang et al., 2014a)		1186 HBsAg mothers and their infants	January 2008 to June 2012 Passive and active immunization was given to neonates born to HBsAg-carrier mothers.		HBeAg positive mothers 132 (30.3%) were breastfed 303 (69.7%) formula fed; *Formula feeding*: no significant differences in rates of HBV transmission between vaccine-only group (3/40, 7.5%) and HBIG plus vaccine (25/263, 9.5%), X2=0.167, NS *breast-feeding infants*: significant differences in rates of HBV transmission between vaccine-only group (7/26, 26.9%) and HBIG plus vaccine (4/106, 3.8%), X2=11.774, P=0.001. HBeAg negative mothers, 508(67.6%)were breastfed 243(32.4%) formula-fed. None of the infants was found to be HBV infected either in breast-feeding group or formula-feeding group no matter what immunizations they received.	The positive rates of both HBsAg and HBsAb have no significant differences between infants of breast feeding or formula feeding.	

Vaccination

Reference	**Study type**	**Patients**	**Interventions**	**Outcome measures**	**Effect size**	**Authors conclusions**	**Comments**
(Lee et al., 2006)	SR		HBV vaccination versus placebo	Infant HBV infection	Compared with placebo or no intervention, hepatitis B vaccination significantly decreased the risk of hepatitis B occurrence (relative risk 0.28, 95% confidence interval 0.20 to 0.40; four trials). Subgroup analyses between high quality and low quality trials, the mother's hepatitis B e antigen status, or time of vaccination were not significantly different		

(Chen, et al., 2019)	SR	Infants of HBsAG mothers	(1) infants were injected with a 3- or 4-dose vaccine series starting within 24 hours after delivery (HBVac); Vs placebo or no treatment		Three studies compared the efficacy of hepatitis B vaccine with placebo or no treatment among infants of HBV carrier mothers. At age 6 months or older, the infant HBsAg-positive rates were 20.2% (26/129) in the HBVac group and 59.3% (70/118) in the placebo/none group (RR, 0.33; 95% CI, 0.23–0.48; I2, 22.7%).		EXCLUDED: All included studies are also included in SR Lee et al., 2006 which is more complete
(Schillie and Murphy, 2013)	SR	Infants of HBsAg mothers		HBV vaccination efficacy	For infants of HBsAg-positive mothers (including those who did and did not receive HBIG), vaccine efficacy ranged from 79 to 98% in seven studies [21–27]. The median seroprotection proportion across all studies including HBsAg-positive and HBsAg-negative mothers was 98% (range 52%, 100%). 11 examined the immune responses of infants born to HBsAg-positive mothers. Ten trials included infants born to HBeAg-positive mothers. After the first dose, the median proportion with anti-HBs ≥10 mIU/mL and GMT (three and five trials, respectively) were 23% (range 11%, 100%) and 60 mIU/mL (range 3 mIU/mL, 161 mIU/mL). After the second dose, the median proportion with anti-HBs ≥10 mIU/mL and median GMT (six and eight trials, respectively) were 67% (range 30%, 100%) and 24 mIU/mL (range 8 mIU/mL, 228 mIU/mL).	The combined results demonstrate high effectiveness of hepatitis B vaccination initiated at birth to elicit titers of anti-HBs which correlate with protection against perinatal and early life acquisition of HBV infection	

(Schalm et al., 1989)	RCT	238 pregnant women detected, 193 children were born in the study period Newborns in group A were similar to those in group B as far as selected characteristics of the mothers are concerned (	1982-1984 Group A: early vaccination Within 2d of birth, at 1 and 2 months Group B: delayed vaccination 3, 4 and 5 months of age And a second injection of HBIG at 3 months All newborns of HBsAg-carrier mothers were given HBIg hepatitis B surface antibody within2h after birth		From 3 months onward, anti-HBs concentrations were higher in group B than in group A; statistically significant difference ($P < .05$) was observed at 11 and 24 months. None of the newborns had HBsAg-positive results at 3 months of age. Subsequently, a subclinical hepatitis B infection developed in two infants who were found to be HBsAg carriers. Their mothers had tested HBeAg positive, and immunoprophylaxis had been given according to schedule A. None of the infants in group B developed antigenemia		Early vs late vaccination
(Gonzalez et al., 1993)	CS	81 children, asymptomatic HBsAg carriers	May 1986-September 1986 Seventy-nine of these newborns were administered either heptatitis B (HB) vaccine only (the first 8 cases) or combined prophylaxis of vaccine and immunoglobulin (the following 71 cases).	identification of HBsAg, anti-HBc, HBeAg and anti-HBe and determination of anti-HBs titres	The seroconversion levels determined at these time points showed a decrease from 98.6% at 1 year to 87.9% and 87.5% at 5 and 7 years, respectively no response to vaccination: 1/71 at 1y, 8/66 at 5y, 7/56 at 7y		

(Wang et al., 2016)	CS	863 HBsAg positive mothers and their corresponding 871 infants No coviral infections On the basis of the status of HBeAg of mothers, different vaccination doses were given to the infants	Non-randomized, double blind Prospective observational cohort study July 2012 to April 2015 Infant follow-up at 7 and 12 months Group A: infants born to HBsAg+/HBeAg- mothers 100IU HBIG + 10ug vaccine within 2h after birth, 1 and 6 months Group B: infants born to HBsAg/ HBeAg+ mothers: 100IU HBIG+20ul vaccine within 2h after birth, 1 and 6 months		Group A: 565 infants Group B: 306 infants Total infant HBV infection rate was 1.84% (16/871). No immunoprophylaxis failure in group B Immunoprophylaxis failure in group A: 5.2% (16/306) All of the infants with immunoprophylaxis failure were born to HBeAg-positive mothers with HBV DNA >$4x10^7$ IU/mL.		

(Young et al., 2003)		HBsAg positive mothers	(I) vaccine at 0, 1, 6 month and HBIG 0.5 ml at 0 and 2 months; (II) vaccine at 2, 3, 8 months and HBIG 0.5 ml at 0, 2, 4 months; (III) vaccine at 0, 1, 2 months and HBIG 0.5 ml at 0, 2 months; (IV) vaccine at 0, 1, 6 months and HBIG 0.5 ml at 0, 2 months; (V) vaccine at 0, 1, 2 months and HBIG 1 mL at birth; (VI) vaccine at 0, 1, 2 months and HBIG 0.5 mL at birth. These groups fall into three vaccine schedules given at 0, 1, 6 months (schedule A), 2, 3, 8 months (schedule B) or 0, 1, 2 months (schedule C), with a variable HBIG regimen	HB vaccination efficacy	There was no statistical difference in the six study groups (I–VI) in their respective proportion of defaulters. The use of different doses of HBIG and the administration of boosters outside the study had no longlasting effects on the anti-HBs responses. Over the years, there was a gradual decline in the proportion of high anti-HBs level in all groups (Fig. 2). There was a significant difference between the three schedules at the sixteenth year, being higher (45.6%) for schedule B (2, 3, 8 months), than schedule A (0, 1, 6 months) (30.9%) or schedule C (0, 1, 2 months) (30.7%) A vs B and B vs C $p<0.05$ The use of schedule B offered an advantage over other schedules in protecting from anti-HBc seroconversion after 2 years of age ($X2 = 3.706$, OR = 0.373, $P = 0.054$) but not younger ($X2 = 0.002$, OR = 0.962, $P = 0.98$).		

HBIG

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Jin et al., 2014)	SR				Newborn injected with vaccine+HBIG vs vaccine in newborn and HBIG in mother during pregnancy At birth: 7 studies, 1061 infants, RR 0.66 (0.52, 0.84); 7-12 months of age 12 RCTs, 1453 infants, RR 0.54 (0.42, 0.69) > 12 months of age 7 RCT, RR 0.54 (0.42, 0.69). Meta-analysis showed that newborns in the experimental group had a higher amount of protective antibodies at birth and at 7–12 months of age, but not at more than 12 months of age RR 2.12 (1.66, 2.70), 291 infants, 4 RCT; RR 1.12 (1.03, 1.22), 8 RCT, 566 infants; RR 1.06 (0.96, 1.16), 5 RCT,		
(Machaira et al., 2015)	SR	9 studies 4RCTs	Vaccine only vs vaccine+HBIG in infants from HBsAg+/HBeAg- mothers		No difference in seroprotection rate (4 studies, 1323 patients, OR 1.24, 95% CI 0.97–1.58)		

(Beasley et al., 1983)	CS	infants of e-antigen-positive HBsAg carrier mothers.	Follow-up visits every 3mo until age 2 Group A infants received HBIG at birth and a second dose at 3 months of age, at which time vaccination was initiated Group B infants received HBIG at birth only and vaccination was initiated when they were 4-7 days old Group C infants received HBIG at birth only and vaccination was initiated when they were approximately 1 month old. Controls: 84 infants, no prophylaxis In all three groups the initial vaccination was followed by boosters a month and 6 months later.		Controls: 74/80 infected infants (92.5%) became HBsAg carriers and in 6/10 others high levels of anti-HBs developed, while 4 remained uninfected. 9/159 (5.7%) infants treated with any of the HBIG vaccine schedules became chronic HBsAg carriers, 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. 4/159 infants who had been given HBIG and vaccine and became HBsAg carriers (2 . 5%) were HBsAg positive before vaccination was started, and might be considered inevitable carriers who had probably been infected in utero.	All but 6% of infants of e-positive HBsAg carrier mothers were protected, which is a substantial improvement over the protection offered by either HBIG or vaccine alone.	
(Beasley et al., 1981)	RCT	202 infants who completed the 3-dose series	Group A: given 1 0 ml saline at birth, three, and six months; Group B: given 1.0 0 ml HBIG at birth, and saline at three and six months; Group C: given 0’ 5 ml HBIG diluted in 0’ 5 ml of immune serum globulin (ISG) (for a total volume of 1 - 0 ml) at birth, three, and six months.		Placebo: 33/35 infants were infected (HBsAg positive at 3 months) Group A: 2/35 (5.7%) were not infected Group B: 21% not infected 50% were permanently infected with HBsAg Group C: 25% not infected 23% developed permanent HBsAg The differences between each HBIG group and the placebo group and between the two HBIG groups are highly significant statistically (P<0. 01).		

(Guo et al., 2012)	CS	324 HBsAg-positive pregnant women	Prospective study All infants were injected with a 10 mg dose of HB vaccine at birth, at 1 month, and at 6 months (I) HBIG on the mother group pregnant women HBIG during pregnancy, but not their children [61 pairs (18.82%)]; (II) HBIG on the infant group children who were injected with HBIG and HBV vaccines within 24 h after birth (active and passive immunization) [114 pairs (35.19%)]; (III) HBIG on the mother and infant group both pregnant women and their children injected with HBIG (united maternal and child immunization) [135 pairs (41.67%)]; and (IV) no-HBIG group No HBIG for either pregnant women and their children [14 pairs (4.32%)].		Of the 324 infants, 18 (5.56%) were identified as HBsAg positive at the age of 6 months. Compared with the no-HBIG group, HBIG on the mother and infant group had the lowest HBsAg-positive rate [odds ratio=0.14, 95% confidence interval (CI)=0.02–0.90, P=0.039], whereas HBIG on the infant group had the lowest HBsAb-positive rate (odds ratio=0.07, 95% CI=0.02–0.23) The HBsAg-positive rate of the no-HBIG group was 14.29%.		

(Hu et al., 2012)	CS		August 2002 to July 2004 Retrospective cohort 419 children born to HBsAg positive mothers Control: 453 children born to HBsAg negative mothers		Of the 419 invited mothers with positive HBsAg, 298 (71.1%) mothers and their children participated in the study, while 328 (72.4%) of the 453 invited mothers who were negative for HBsAg and their children attended the investigation Of the children born to HBsAg positive mothers, 11 (3.7%) were HBsAg positive, and 16 (5.4%) were HBsAg negative but anti-HBc positive, indicating past resolved infection only 37.6% of the HBV-exposed infants received HBIG after birth 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 (Table 3). 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		Difficult to draw any conclusions due to the retrospective nature of the study

(Wei et al., 2018)	CS	All pregnant women were negative for hepatitis A virus (HAV), HCV, hepatitis D virus (HDV), hepatitis E virus (HEV) and HIV. The rates of loss to follow-up were comparable between two group No significant differences between the mothers at baseline	August 2009 to June 2011 The neonates born to mothers coded with odd numbers were treated with 100 IU HBIG (n=545) neonates born to mothers coded with even numbers were treated with 200 IU HBIG (n=632). All the infants received three doses of 10 μg HepB intramuscularly in the upper arm, following a schedule of 0 (within 12 h of birth), 1 and 6 months, combined with a dose of HBIG at birth in the contralateral arm.		<u>7 months of age:</u> perinatal infection between 100 IU and 200 IU groups, which were 1.5% (8/545) and 1.9% (12/632), respectively (p = .568). All the infected infants were born to HBeAg-positive Mothers In highly viremic mothers, who were HBeAg-positive and had viral loads of ≥ 7 log IU/mL, 5.5% (8/145) and 6.6% (12/181) transmitted the virus to their infants in 100 IU and 200 IU group, respectively (p = .677). <u>12 months of age</u> Protective levels of anti-HBs remained in 98.2% (431/439) and 97.1% (496/511) of the infants in 100 IU and 200 IU groups, respectively (p = .266).		
(Zhang et al., 2014b)	CS	1150 HBsAg+ mothers with their infants	January 2008 to June 2012 Prospective cohort 965 neonates received vaccination with HBIG 185 neonates received only HB vaccina at 24h-1 and 6 months 100IU HBIB and vaccination		209/1150 HBsAg-carrier pregnant women demanded an injection of 200 IU HBIG immunoprophylaxis failure rate was significantly higher in the vaccination only group than vaccination+HBIG group RR=0.371, 95 % CI [0.167, 0.825], p=0.015		

(Gong and Liu, 2018)			30 subjects in group A received the hepatitis B vaccine at 0, 1 and 6 months after birth at a dose of 10 μg each time. 30 subjects in group B received an intramuscular injection of 100 IU HBIG 2 h after birth before getting the same treatment as group A. Mothers of 30 subjects in group C received a total of three gluteus maxinus injections of 200 IU HBIG each time at 28 weeks of gestation, 4 weeks and 8 weeks later. And the same treatment as group B		The numbers of infants who were HbsAb-positive were 24, 27 and 29, respectively, in groups A-C, corresponding to blocking success rates of 80, 90 and 97%, respectively. A vs B $p<0.05$ A vs C $p<0.05$ when mothers were positive for both HBsAg and HBeAg. The successful blocking rate in group A was lower than those in both group B and C, and the differences were statistically significant ($p<0.05$). In addition, the successful blocking rate in group B was lower than that in group C ($p<0.05$).		
(Qiao et al., 2019)		4112 mother-infant pairs	cross-sectional study October 2017 and January 2018.		During 2016–2017, the programme achieved timely HBIG and HBvacc-BD coverage of 99% (4070/4112) and 98% (4045/4112) respectively MTCT rate of 0.9% (0.6–1.1%), with 35 children tested HBsAg positive, and a sero-protection rate of 96.8% (96.3-97.4%), with 3981 children tested anti-HBs positive. Out of 35 HBsAg-positive children, 94% were born to HBsAg- and HBeAg-positive mothers during this pregnancy. For children administered the HBvacc-BD between 12 and 24 h in birth, the adjusted odds of MTCT was 1.9 times higher than that of children immunized within 12h of birth (2.4% vs 0.6%, adjusted odds ratio [aOR] = 2.9, 95% confidence interval [CI]:1.4–6.3, P = 0.01). no significant association between MTCT and HBIG administration		

(Wheeley et al., 1991)		1010 infants	January 1986 to December 1987 Group A (Four lOmcg. doses of vaccine (“HBVax”) Group B (250IU HBIG at birth, combined with the same vaccine schedule as in treatment Group A).		the antibody titre was significantly higher in treatment Group B (infants who had received HBIG at birth) (P = <<< 0.001 at 1 month: P = <<< 0.001 at 2 months). However, at 6,9, and 18 months old there was no statistically significant difference in antibody titre between the two groups (P = 0.22 at 6 months: P = 0.24 at 9 months: P = 0.33 at 18 months infants born to HBeAg+ mothers: Group A: 1/8 HBsAg+ Group B: 1/8 HBsAg		

Hepatitis C virus

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

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Ackerman et al., 1998)	SR	38 studies 9 controlled studies among sexual contacts of non-haemodialysis, non-renal transplantation 29 uncontrolled studies 4250 stable sexual contacts (males and females)			573/4250 tested anti-HCV positive Calculated pooled prevalence among the spouses was 13.48% (95%CI, 12.48-14.55) (table 2) 9 Controlled studies: Spouses of anti-HCV-positive patients in areas non-endemic for HCV have a higher prevalence of anti-HCV than spouses of negative controls (15.2% vs0.9%, OR 20.57, 95%CI 6.05-84.08, P < 0.0001) (table 4) 2 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 sign higher than in controls (OR 2.14, 95% CI 1.12-4.08)		

(Kao et al., 2000)	CS	**prospective analysis** 112 HCV pos patients and their anti-HCV neg spouses Patient: anti-HCV and HCV RNA pos Heterosexual couples HBV neg Dec 1990 – Aug 1997 AntiHCV test 1x/y Follow up > 1y Location: Taiwan 75 men HCV+ 37 women HCV+	Questionnaires • Sexual behaviour (vaginal) • Shared toothbrush • IV drug use • Transfusion • Occurrence hepatitis • Duration relationship	(1) HCV transmission risk= no. Converted spouses/sum of follow up period per couple (person years, py) (2) genotyping	112 couples followed up for 12-92 mth (mean 45,9±22,3 mth) 1 seroconversion (female, after 20 mth) Transmission risk 2,33/1000py Genotype 1b in both male and female	Transmission risk of HCV is low in monogamous heterosexual couples More studies needed.	Relatively short period of follow up. There is positive correlation between duration of relationship and risk of transmission.
(Tahan et al., 2005)	CS	600 patients with chron HCV with their partners 320 male (M) 280 female (F) Multicentre Jan '99-Nov '02 Location Turkey **Retrosp. Cohort analysis** 600 spouses included **Prosp. Cohort analysis** 216 antiHCV neg spouses 101 F 115 M Excluded: HIV pos Patients with antiviral treatment	**Retrospective cohort** Questionnaire: Total acts of intercourse estimate Duration marriage Assessing risk factors **Prospective cohort** Annual antiHCV partners Questionnaire (same as above)	HCV transmission risk AntiHCV and HCVpcr Genotyping	**Retrospective cohort** 12/600 (2%) anti-HCV + • 4/280 (1,4%) F • 8 /320 (2,5%) M 11/600 (1,8%) spouses HCV RNA pos Genotyping all couples concordant type 1b Rate intercourse 1,73/wk HCV+ vs HCV- spouses: Duration marriage comparable 1521±506,7 wk vs 1532,4±670,2wk Follow up period patient 209,6±137,5wk vs 147±165,5wk (p=0,035) Risk factors spouses -4 several needle injuries -17 blood transfusion -14 multi sex partners -30 shared personal items -45 surgical/dental procedures **Prospective cohort** Mean follow up 35,7±6,3 mth Total no. sex acts 257,9±72,2 Rate intercourse 1,9/wk 0 HCV infected spouses	Risk of HCV infection by sexual transmission is very low. No increased risk for spouses to acquire HCV even in long lasting sexual relationship	Retrospective cohort data subjected to memory of participants No data on type of intercourse

(Vandelli et al., 2004)	CS	**Prospective cohort study** 895 heterosexual antiHCV and HCVpcr pos patients and their antiHCV neg partners elligible Location Italy Sep 1991- 2001 All subjects HIV neg Monogamous Antiviral therapy patients excluded 776 completed follow up (=7760 person-years, py) 119 completed 300 py Total 8060 py	Annual antiHCV test Questionnaire -Sexual behaviour incl anal interc -Risk assessment -Advised not to share personal items Physical examination (genital ulceration)	Transmission risk HCV per person-years (py) Genotyping AntiHCV and HCVpcr	Mean VL patients 6,7±1,7 MEq/L Mean freq intercourse 1,8/w (anal intercourse 0, condom use 0) 3 spouses infected Transmission rate 0,37/1000py Genotyping 2 concordant for 1b and 2a 1 discordant (dental implant prior to test) Transmission rate for concordant couples 0,25/1000py Phylogenetic analysis revealed different viral dna in concordant couples 1 HCV-concordant spouse seroconverted for HIV after occupational needle injury with HIV pos(HCV neg) patient	Strong evidence that sexual transmission of HCV in long term monogamous relationships is rare in HCV discordantly infected couples 2 spouses had major risk exposure in addition to living with infected partner Transmission risk may even be 0 based on phylogenetic comparison	No comment
(Caporaso et al., 1998)	CS	**Cross-sectional study** 585 anti-HCV and HCV RNA pos patient and their 1379/1509 household contacts participated (91,4%) Location Italy March- Dec 1995 Multicenter HBV neg patients 327 male patients 258 female patients Household contacts 455 spouses (33%) 738 offspring (53%) 63 parents (5%) 123 other (9%)	Questionnaire Assessing risk factors Duration relationship	Prevalence of HCV Anti-HCV and HCV RNA	Overall prevalence in household contact 101/1379 (7,3%) Spouses 71/455 (15,6%) Rest 30/924 (3,2%) (p<0,05; OR 6,5 [95% CI 3,8-8,6]) Relationship > 20y: 19,8% Relationship < 20y: 8% (p<0,05; OR 2,8 [95% CI 1,5-5,3]) aOR spous vs rest: 1,4 [95% CI 0,7-3,0]	In agreement with other authors we found an increased risk in spouses and correlation with the length of marriage Adjusting for confounders the findings suggest that sexual transmission does not play a role	No data on risk factors of spouses No data on sexual activity

(Hajiani et al., 2006)	CS	**Case control study** 60 patients HCVpcr pos with their 300 household contacts Control group: 360 first time blood donors Aug 1998-Sep 2003 Location Iran Patients HBV neg	Questionnaire Risk factor assessment Relationship duration	Prevalence HCV in household AntiHCV and HCVpcr	4/300 (1,33%) antiHCV pos household members 2 spouses (3,39%) antiHCV pos 1 HCVpcr pos Relationship duration of 2 anti-HCV pos spouses was > 15 y Prevalence of anti-HCV positive household contacts (1.33%) vs controls (1%) (p>0,06).	Spouses of HCV patients are more likely to be infected compared to other family members	No data on sexual behaviour
(Koda et al., 1996)	CS	**Cross sectional study** 121 patients with chron liver disease (antiHCV pos) and their spouses 116 patients HCVpcr pos Location Japan Jan 1992-Mar 1995 Patient characteristics 19 HCC 18 liver cirrhosis 67 chron active hepatitis Mean age 58,4y (28-85y)	NA	Prevalence of HCV Establish sexual transmission AntiHCV and HCVpcr genotyping	21/121 (17,4%) spouses antiHCV pos (19 HCVpcr pos) 12/19 (63,2%) concordant for genotype	Patients selected were suffering from advanced liver disease. Evidence supports Intra spousal transmission of HCV	Patients selected with severe liver pathology No data on sexual behaviour

(Terrault et al., 2013)	CS	**Cross sectional study** 2077 couples screened 672 (32%) eligible 500 enrolled and completed all requirements **Inclusion criteria** - Heterosexual -Monogamy both partn - ≥3 sex contacts in preceding 6 mth -HIV and HBV neg -Relationship ≥ 36 mth Exclusion -IV drug us -antiviral drug therapy Location USA Jan '00-May'03 **Characteristics** Median duration relationsh 15y (2-52y) HCV+ male 306 HCV+ fem 194	AntiHCV and HCVpcr Interview (M/F separately) -Sexual history in time intervals (type, freq, barrier protection) -Sharing grooming items -all other known risk factors Genotyping Sequencing/phylogenetic analysis	Prevalence of antiHCV Incidence per number of sexual acts Incidence density number of potential transmission events per total person-years (py)	**Intercourse** table 2 -Vaginal 499 (99,8%) -Vag during menses 326 (65,2%) -Anal 152 (30,4%) -Oral male receptive 456 (91,2%) -Oral fem receptive 462 (92,4) **Prevalence** 20/500 (4,0%) partners antiHCV pos 13/20 (65,0%) HCVpcr pos **Genotyping** 9/20 (45,0%) concordant 8/20 (40,0%) discordant 3/20 (15,0%) indeterminant **Sequencing/phylogenetics** 6/9 (66,6%) concordant couples analysed 3/9 (33,3%) same viral strain 3/9 (33,3%) different viral strain 3/9 (33,3%) unknown **Prevalence potentially attributable to sex contact** 3/500 (0,6%; 95% CI 0,0%-1,3%) Cumulative py 8377 **Estimated incidence** Min 3,6/10000 py [95% CI 0,0-7,7] based on 3 confirmed couples Max 7,2/10000 py [95% CI 1,3-13,0] based on 3 confirmed+3 unknown couples **Estimated risk/sex act** Min 1/380000 [95% CI 1/600000-1/280000] Max 1/190000 [95% CI 1/1,3 million-1/100000] **HCV conc vs HCV discord coupl** Vag interc during menses: 100% vs 65,6% [p=0,55] Anal intercourse: 66,7% vs 30,2% [p=0,22]	Sexual transmission of HCV in monogamous heterosexual relationships is extremely infrequent. Maximum prevalence 1,2% and maximum incidence 0,07%/y or 1/190000 sexact (includes couples with unknow viral strain relatedness) Limitation: cross sectional nature, relatively small sample size and small numbers of positive partners. Phylogenetically related viral strains is no genetic proof of of transmission, but strong evidence of sexual transmission.	No comments

(Tong et al., 1995)	CS	**Cross sectional study** 68 antiHCV and HCVpcr pos patients with chron hepatitis and their partners Patients All HIV and HBV neg Median age 50y (27-76) 11 chron persistent hep 27 chron active hepat 17 liver cirrhosis 13 no biopsy Spouses Mean age 50y (29-84y) Location USA Year not registered	Questionnaire patients and partners Assessing risk factors (Age, Gender, race, duration relationship, IV drug, needle exposure) Sexual behaviour AntiHCV and HCVpcr Genotyping	Prevalence of HCV among spouses	4/68 (5,9%) spouses antiHCV pos 3/68 (4,4%; 95% CI 1,5%-12,2%) no risk factors 1/68 had blood transfusion 2/4 spouses HCVpcr pos Genotyping 2/2 (100%) concordant type 1 Duration relationship HCVpos vs HCVneg spouses 25y (15-30) vs 10y (2-43y) P=0,02	Transmission of HCV between spouses is low. These findings suggest that sexual transmission occurred in these 2 couples. Sexual transmission of HCV is low but a risk factor in spouses in a long-term sexual relationship with a chronic hepatitis patient	No data on sexual behaviour
(Fadil-Romao et al., 2006)	CS	**Cross sectional study** 53 hemodialysis patients and their sexual partners 16 Patients were antiHCV and HCV PCR pos Location Brazil Year: Not reported HBV and HIV neg	Interview spouses before blood test Questionnaire: Risk factor assessment	HCV transmission rate AntiHCV and HCV PCR	0/16 (0%) spouses antiHCV pos	Very low risk of heterosexual HCV transmission. Larger studies needed	Very small sample size

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

Horizontal transmission

No studies could be identified reporting a threshold in serum HCV RNA load to below which horizontal transmission does not occur.

Vertical transmission

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

SHOULD IUI, IVF OR ICSI BE PREFERENTIALLY USED FOR MEDICALLY ASSISTED REPRODUCTION IN HEPATITIS C INFECTED COUPLES?

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Garrido et al., 2004)	CS	Total n= 91 couples (seropositive men, negative couple). Only HIV n=18; HIV+HCV n=33; Only HCV n=40. 134 ejaculates with sperm washing.	2 years prospective study. HCV RNA, sperm washing, ICSI.	1. Semen parameters: Concentration, Motility, Recovery rate. 2. Cycle results. 3. Women seroconversion.	Sperm washing should be offer to al HIV or HCV male patients in couples needing ART. Fertilization/MII oocyte: 59.3±5.3 Pregnancy rate/cycle: 40.1 Newborns: 4 None of the female partners seroconverted		This establish the effectiveness of the technique. The criteria to select a procedure IUI or FIV or ICSI is seminal quality or women infertility diagnosis, or both, not the virus.
(Nesrine and Saleh, 2012)	CS	Total n=60 women undergoing ICSI cycles. HCVAb+, RNA- n=30; HCVAb+, RNA+ n=30.	Cross sectional observational study		HCV transmission in ICSI cycles seems to be of low incidence in HCV RNA positive patientes, and absent in HCV Ab positive RNA negative.		

(Savasi et al., 2013)	CS	Total n=135 ART couples. IUI=14; ICSI=21. Males HCV RNA positive, Women HCV Ab negative.	Prospective study. IUI or ICSI. Sperm washing.	1. In women: HCV Ab before ART, 6 months after ART, and at delivery. 2. In children: HCV Ab after birth and at 18 months. 3. Pregnancy rate.	1. No horizontal or vertical transmission after sperm washing. in IUI or ICSI couples. 2. In HCV serodiscordant couples who required ART, sperm washing should be used to treat HCV positives semn before ART. 3. In HCV serodiscordante fertile couples is not necesary to treat if the don't need reproductive assistance.		

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

RNA in semen/oocytes/embryos

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Papaxanthos-Roche et al., 2004)	CS	24 unfertilized oocytes HCV RNA positive women, 10 negative controls and 20 positive controls (artificially contaminated).	To investigate the susceptibility of human oocytes from hepatitis C virus (HCV) RNA-positive women to HCV contamination during assisted reproductive technology (ART).	PCR for HCV RNA associated with zona-intact unfertilized human oocytes and in plasma and follicular fluid.	- 20 oocytes articifially exposed to HCV RNA positive plasma were positive 10 negative control unfertilized oocytes were negative 24 unfertilized - HCV RNA was associated with 17/24 (70.8%) oocytes (6/7 after ICSI and 11/17 after conventional IVF). - Oocyte contamination probably occurred during ovarian puncture by blood and contamination of follicular fluid. - HCV RNA was found in 19/20 (95%) follicular fluid samples. - A weak correlation was found between plasma and follicular fluid HCV RNA loads (r= 0.73, P< 0.001).	More studies are needed to evaluate the risk of HCV contamination to which oocytes/embryos are exposed and to establish good safety guidelines for oocyte/embryo manipulation and cryopreservation.	First report of PCR-detected HCV RNA associated with zona-intact unfertilized human oocytes.

Placenta

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Giugliano et al., 2015)					- 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.**		

DOES HEPATITIS C VIRUS/TREATMENT OF HEPATITIS C VIRUS BEFORE MEDICALLY ASSISTED REPRODUCTION IMPACT THE OUTCOME OF MEDICALLY ASSISTED REPRODUCTION?

Male infected

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Cito et al., 2019a)	CCS	153 couples Group 1: HIV-seropositive men, n=24 Group 2: HCV-seropositive men, n=60 Group 3: controls n=69	February 2011 to August 2018 Retrospective analysis Sperm-washing procedure was performed using a three-step system with fresh ICSI cycles <u>Sperm preparation</u> density gradient centrifugation method (95% - 50% gradient) 20 min at 300g Pellet resuspended and centrifuged 10 min at 250g Swim up 1h at 37°C	Seminal parameters, fertilization rate (FR), cleavage rate (CR), pregnancy rate per cycle (PR/C), miscarriage rate, implantation rate (IR) live birth rate	HCV vs controls 1. Fertilization rate: 61% vs. 75%, (p<0.01). 2. Implantation rate 11.1% (24/216) vs 14.1% (16/113) 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), and live birth per cycle 15.7% (16/102) vs. 15.9% (11/69) were not significantly different in the three groups. Therefore: The sperm-washing technique with ICSI may generate a promising way to improve pregnancy outcomes and to reduce the risk of viral transmission in these couples. No horizontal or vertical seroconversion		
(Pirwany et al., 2004)	CS	25 IVF-ET cycles in HBV and HCV serodiscordant couples. -13 HBV serodiscordant patients (10 males and 3 females). -12 HCV serodiscordant patients (9 males and 3 females). -Control group: 27 age matched patients.	Retrospective two years cohort study To examine the reproductive performance in serodiscordant HBV and HCV serodiscordant couples	COH response, fertilization rate, cleavage rate, implantation rate, pregnancy rate.	HCV vs controls Fertilization rate: 63.9% vs. 75.9% Clinical pregnancy rate: 0% (0/12) vs. 41% (11/27)		Nice design but maybe low n. No sperm washing in methods

(Prisant et al., 2010)	CS	232 cycles of IVF/ICSI for 130 serodiscordant HIV or HCV couples were compared with 232 cycles for 211 matched seronegative couples.	Five years prospective case control study. comparing outcomes of cycles of serodiscordant HIV or HCV couples performing sperm wash and IVF/ICS vs seronegative couples. <u>Sperm preparation</u> Density gradient centrifugation (45%-90%) Straw was discarded if HCV RNA was detected in the selected sperm final fraction If the woman was infected, cumulus–oocyte complexes were first rinsed three times in culture medium	Mature oocytes, fertilization rate, cleavage rate, transferred embryo per ET, implantation rate, clinical pregnancy per oocyte retrieval and per ET children born.	HCV vs controls 1. female HCV infected Fertilization rate: 71.1% vs. 70.2% implantation rate: 5.1% vs. 9.6% clinical pregnancy rate/ET: 10.8% vs 12.8% children born: 2/22 vs. 4/42 No significant difference 2. In 28 serodiscordant couples, males with HCV: -fertilization rates were significant different from those of controls: 54.7% vs. 68.2% - implantation rate: 12.8% vs. 4.2% clinical pregnancy rate: 17.5% vs. 7.0% children born: 8/28 vs. 2/46 No difference	Authors give more impact to no significant difference in clinical pregnancy rate in HCV serodiscordant couples compared to seronegative couples.	
(Yang et al., 2015)	CS	1424 couples undergoing IVF cycles: A: 90 couples where the female was HCV positive, B: 78 couples where the male was HCV positive, C: 1256 control seronegative couples.	A 5 year retrospective study comparing the IVF outcomes in both groups	Fertilization rate Clinical pregnancy rate Miscarriage rate	A vs B vs C Fertilitzation rate: 76.93±19.18 vs. 80.99±19.95 vs. 78.14±19.73 Clinical pregnancy rate: 45.6% (41/90) vs. 48.6% (36/74) vs. 55.0% (691/1256) Miscarriage rate: 23.3% (21/90) vs. 18.9% (14/74) vs. 19.0% (239/1256)	HCV infection has no affection on IVF treatment outcomes.	

Female infected

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Shaw-Jackson et al., 2017)	CCS and SR	25 cycles (subgroup of 13 patients with active replication) of HCV sero-positive women compared to those of 107 cycles of an uninfected control group. HBV/HIV coinfected women were excluded as well as couples with HCV/HBV/HIV infected partners	17 years retrospective study 1998-2015 first IVF cycles	IVF outcomes were evaluated for patients with active viral replication (HCV RNA positive).	For the CCS, results on seropositive patients: HCV vs controls - fertilization rates (67% vs. 86%)and implantation rates (6% (3/47) vs. 23% (11/47), Significantly reduced - clinical pregnancy 12% (3/25) vs. 36% (9/25) Miscarriage: 4% 1/25 vs. 12% (3/25) Children born: 2/25 vs. 7/25 Not statistically different Patients with active replication - implantation rate: 0% (0/22) vs. 26% (6/23) significantly reduced - No live birth on the HCV RNA patients with active replication.	More and larger studies with well-defined groups are needed to clarify the eventual impact of the HCV on IVF outcomes.	
(Englert et al., 2007)	CS	42 IVF/ICSI cycles in HCV-seropositive women and 84 matched control cycles. All of the couples tested negative for HBV and HIV No cycles included were both partners tested positive for HCV	January 1990 and May 2005	COH response and IVF/ICSI outcomes in both groups. To analyze the impact of seropositivity with hepatitis C virus on invitro fertilization outcomes	- Statistically significantly more cycles were cancelled among HVC-seropositive women. - A statistically significantly higher amount of FSH was administrated to HCV-seropositive women. - -fertilization rates: 56.4% vs 59% Implantation rates: 19% vs. 19.2% and Pregnancy rates/transfer: 28.5% (8/28) vs. 29.3% (22/75) were not statistically different.		

(Hanafi et al., 2011)	CS	A: 40 women HCV PCR positive, and two HCV PCR negative control groups: B: HCV PCR- seropositive C: HCV PCR- seronegative.	4 years retrospective study of patients underwent ICSI meeting the criteria of one of the three groups.	A comparison of the three groups regarding the ovarian response to stimulation, embryo quality and pregnancy rates.	1. Lack of ovarian response to stimulation was higher in HCV RT –PCR positive and sero-positive females than sero-negative controls. (52% vs 30% vs 5%) 3. The fertilization rate (28% vs. 32% vs. 67%) implantation rate (33.3% vs. 45% vs. 52%) pregnancy rate 5% (2/40) vs. 32.5% (13/40) vs. 47.5% (19/40) was significantly reduced in the HCV–PCR-positive group com5pared with the PCR negative/HCV sero-positive and HCV sero-negative control groups. 4. There was a negative correlation between number of oocytes and viral load. HCV infection in females undergoing ICSI has a negative impact on the outcome, and the impact is higher in PCR positive cases.		
(Pirwany, et al., 2004)	CS	25 IVF-ET cycles in HBV and HCV serodiscordant couples. -13 HBV serodiscordant patients (10 males and 3 females). -12 HCV serodiscordant patients (9 males and 3 females). -Control group: 27 age matched patients.	Retrospective 2 years cohort study	COH response, fertilization rate, cleavage rate, implantation rate, pregnancy rate.	HCV vs controls Fertilization rate: 63.9% vs. 75.9% Clinical pregnancy rate: 0% (0/12) vs. 41% (11/27)		

(Prisant, et al., 2010)	CS	232 cycles of IVF/ICSI for 130 serodiscordant HIV or HCV couples were compared with 232 cycles for 211 matched seronegative couples.	Five years prospective case control study. comparing outcomes of cycles of serodiscordant HIV or HCV couples performing sperm wash and IVF/ICS vs seronegative couples. <u>Sperm preparation</u> Density gradient centrifugation (45%-90%) Straw was discarded if HCV RNA was detected in the selected sperm final fraction If the woman was infected, cumulus–oocyte complexes were first rinsed three times in culture medium	Mature oocytes, fertilization rate, cleavage rate, transferred embryo per ET, implantation rate, clinical pregnancy per oocyte retrieval and per ET children born.	HCV vs controls 1. female HCV infected Fertilization rate: 71.1% vs. 70.2% implantation rate: 5.1% vs. 9.6% clinical pregnancy rate/ET: 10.8% vs 12.8% children born: 2/22 vs. 4/42 No significant difference 2. In 28 serodiscordant couples, males with HCV: -fertilization rates were significant different from those of controls: 54.7% vs. 68.2% - implantation rate: 12.8% vs. 4.2% clinical pregnancy rate: 17.5% vs. 7.0% children born: 8/28 vs. 2/46 No difference	Authors give more impact to no significant difference in clinical pregnancy rate in HCV serodiscordant couples compared to seronegative couples.	
(Yang, et al., 2015)	CS	1424 couples undergoing IVF cycles: 90 couples where the female was HCV positive, 78 couples where the male was HCV positive, and 1256 control seronegative couples.	A 5 years retrospective study comparing the IVF clinical outcomes of both groups.		All experimental and control groups (HCV-positive men, HCV-positive women, and controls) had similar sperm parameters, ovarian stimulation, fertilization and pregnancy results. Conclusion: HCV infection has no affection on IVF treatment outcomes.		

WHICH TECHNIQUES CAN BE USED TO PREVENT/REDUCE HEPATITIS C VIRUS TRANSMISSION DURING MEDICALLY ASSISTED REPRODUCTION?

Semen processing

The evidence on semen processing will be discussed in detail in the next section

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

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Bourlet et al., 2009)	CS	86 couples with male HCV+ and women HCV- 76 men HCV+/HIV- 10 men HCV+/HIV+ Mean age man 39,4 (29-59 y) Mean age women 35,1 (22-43 y) Prospective cohort study Jul '01-Dec '05 Multicentre (3 centres) 148 blood sample 181 seminal plasma samples (all ejaculated) 153 sperm cell fractions	1 ml semen centrifuged at 800g and seminal plasma frozen 1 ml semen centrifuged over gradient (50,70,90%) Pure sperm and spermatozoa recovered from 90% fraction divided in 2 aliquots. 1 aliquot frozen for virological analysis and 1 aliquot for swim up and then frozen for ART HCV analysis: rtPCR in blood and semen (91% performed with Roche/9% with Abbott) HCV load in semen was estimated	(i) Prevalence of HCV in seminal plasma and sperm fraction used for ART (ii) influence of seminal HCV on semen parameters and ART outcome (iii) HCV serological status of babies conceived through ART tested 3 mth after birth	37/181 (20,4%) of seminal plasma HCV RNA pos 20,2% of men HCV+/HIV- 22,2% of men HCV+/HIV+ All 153 sperm fractions were HCV neg Semen parameters (volume, motility, concentration, morphology) showed no difference between seminal plasma HCV+ vs HCV- ART outcomes (no. Oocytes, fert. Rate, % top-quality embryos, preg. Rate, life birth rate) showed no difference between seminal plasma HCV+ vs HCV- 135 ART cycles: 10 IVF/78 ICSI/12 FET/35 IUI- cycles 36 pregnancies 28 life birth 0 babies HCV+	The absence of detection of HCV RNA in seminal plasma in a single sample does not exclude an intermittent shedding of viral genome in this compartment, so a negative test one day can does not mean it can be positive another day. Due to the sensitivity of the test, a semen sample can never be categorized as absolutely negative. PCR test on sperm fraction is not standardized. Small sample size, but no difference is semen parameters between seminal plasma HCV+ vs HCV-. 0 transmission of HCV to newborn. More ICSI-cycles due to decreased sperm motility after freezing.	Women not tested for HCV after ART so horizontal transmission of HCV through MAR unknown Couples with HCV+/HIV+ male were not infertile and received MAR for transmission risk reduction of HIV. Couples with HCV alone were infertile and sperm processing was not performed to reduce horizontal transmission. Not documented how many HCV+/HIV- couples had IUI or IVF or ICSI. Not plausible that IUI can lead to HCV transmission to newborn. 58 couples analyzed where ART led to pregnancy 11 couples undergoing ART not analyzed due to ongoing ART

(Bourlet et al., 2002)	CS	32 HCV + men requiring ART All HCV RNA pos All patients: HIV and HBV negative No antiviral medication	Semen processing Fraction 1 (seminal plasma): 1mL ejaculate centrifuged 800xg and cryopres. Supernatant Fraction 2 (sp. zoa): 1mL ejaculate centrifuged on 3-layer gradient (50,70,90%). Cryopreserv. Sp zoa Fraction 3 (swim up)	(1) VL HCV RNA blood serum (log cop/mL) (1) Qualitative HCV RNA seminal plasma (pos/neg) Sens: 40 cop/mL (=1,6 log) (1) RT PCR HCV RNA seminal plasma and motile sperm fractions (log cop/mL) Sens: 100 cop/mL (=2 log) (2) genotype HCV (3) ART outcome and HCV transmission to child	Blood serum (n=32) Mean VL 5,97 ±0,51 [4,97-7,34] Seminal plasma qual. Pos n=4 (12,5%) Neg n=28 Fraction 2 qual.: All neg (n=4) 1 patient: neg plasma, but pos fraction 2 Fraction 3 qual. All neg (4 sampl, 1 ptn) Blood serum VL minus Seminal plasma VL= 2,83-5,34 log cop. /mL 1 patient: 7 semen samples all pos HCV but not all detectable semen VL **Plasma semen pos vs neg**: Mean blood VL 6,52±0,55 vs 5,88±0,46 [p=0,002] Genotype blood is identical as genotype semen ART 11 attempts 5 women pregnant (1 tripl, 2 twins, 2 singlt) 9 babies 0 babies HCV RNA pos at birth and at 6 mth	12,5% of HCV infected are pos HCV in semen. Lower than other publications but they include HIV coinfected patients. Positive correlation between viral load blood and seminal plasma All HCV pos seminal plasma had blood VL > 500 cop/mL No compartmentalization of HCV RNC between blood en semen	VL blood is not comparable with semen. There is a positive correlation between blood en semen VL Small study group (n=4) Old paper Comparison blood with fraction 2 is after sperm washing No data on HCV transmission to women undergoing ART

(Canto et al., 2006)		Semen samples from 20 HIV+/HCV+ men HIV VL undetectable All men HCV PCR pos in blood Men not requiring ART	Ejaculated sperm divided into 2 aliquots 1 aliquot frozen, total sperm 1 aliquot processed (i) two layer (45,90%) gradient centrifugation (ii) divided into 3 fractions (motile sperm, seminal plasma and dead sperm/cellular elements) 100uL for pcr and 900uL--> (iii) 3 fractions washed 3 times by RPMI and resuspension of pellet (iv) swim up	Real time PCR on total sperm and 3 processed fractions (pre wash and post wash)	PRE WASH Total sperm • 100% HIV pos • 5% HCV pos Seminal plasma • 50% HIV pos • 0% HCV pos Non motile cells • 30% HIV pos • 0% HCV pos Motile sperm • 5% HIV pos • 0% HCV pos POST WASH All 3 fractions • 0% HIV pos • 0% HCV pos	HCV is detected intermittently in total semen. High degree of sensitivity of multiplex PCR and effectiveness of HIV/HCV extraction methods. Semen washing, together with the relevant reproductive technology and HAART, reduce the risk of viral transmission. Semen purification is highly beneficial for HIV/HCV coinfected individuals	Study group did not require ART. No data documented on ART or transmission. Small group.

(Cassuto et al., 2002)	CS	35 couples with HCV+ male requiring ART All men HCV RNA pos in blood No antiviraltreatment 7 mth pior to ART No HBV No HIV All couples infertile -5 tubal pathology -5 anovulation/ovarium disease -19 male factor -1 unexplained infert. -5 mixed 50 semen samples -26 for IVF -24 for ICSI	Sperm processing: Density gradient (45% and 90%) centrifugation HCV RNA test: -200 µl semen dilution with 400 µl PBS -centrifugation -supernatant discarded and pellet lysed with 600 µl lyses buffer -standard HCV RT-PCR IVF/ICSI with sperm fraction after 90% density gradient centrifugation	HCV RNA detection (i) total sperm (ii) 45% fraction (iii) 90% fraction (iv) embryo culture media Pregnancy outcome ART HCV transmission to women and children after IVF/ICSI	7/50 (14%) samples total semen HCV RNA+ Viral load semen all < 600 IU/ml) 1/50 (2%) 45% fraction samples HCV RNA+ 0/50 (0%) 90% fraction samples HCV RNA+ 0/50 (0%) embryo culture media HCV RNA+ 4 women pregnant after IVF (2 miscarriage, 2 ongoing) only 1 child born at time of end follow up 10 women pregnant after ICSI (2 miscarriage, 8 ongoing) 0 born at time of end follow up 0 women HVC+ 0 children	Modified RT-PCR technique efficiently decrease inhibiting factors in seminal fluid. High sensitivity of modified RT-PCR is efficient overcomes false negative results and the necessity to test 2 or more diluted semen samples as reported in other studies. Contamination of HCV in women or children cannot be excluded. Standard IVF or ICSI does not increase the risk of HCV transmission to women.	No data on HCV status of all children conceived (only 1 child born at the end of follow up period) No data on time of HCV test women or type of HCV test.
(Garrido et al., 2006)	CS	7 male patients with TMC < 2 mill spz • 6 HIV+/HCV+ • 1 HIV+/HCV- Goal ART to reduce viral transmission risk 5 men on antiretroviral therapy 1 man had detectable viral load 8 sperm samples included	(i) 1:1 (vol/vol) dilution with human tubal fluid medium and centrifuged at 400g 3x (i) One half of pellet frozen for PCR and one half for ART	Nested PCR Presence of HIV RNA, HIV DNA and HCV RNA	0 sperm samples positive for HIV (DNA/RNA) or HCV 1 sample no HCV result available	Men with severe male factor should be accepted into assisted reproductive programs when, after modified sperm washes, molecular viral absence is confirmed and motile sperm are detected, regardless of their quality.	No data documented on ICSI and transmission to women or child

(Leruez-Ville et al., 2013)	CS	4 HCV-infected 'azoospermic' men • 1 anejculation, tetraplegic • 2 obstructive azoospermia • 1 cryptospermia All men detectable blood viral load Age 34, 39, 40, 41	2 men TESE (3and4) 2 men MESA and TESE(1and2) 3 men bilateral TESE(2,3,4) 2 MESA samples: Centrifuged on a 2-layer gradient Puresperm(45% and 90%) and washed twice afterwards pellets resuspended and frozen 4 TESE-samples: Centrifuged on one gradient Puresperm(45%) and washed twice afterwards pellets resuspended and frozen	HCV Real Time RNA (RT-RNA) test on all upper layers of gradient, remaining TESE tissue and volume of one straw Treshold 240cop/mL Outcome ICSI (life birth) HCV transmission female partner (HCV tested 2-12 months after ICSI) Health child	Upper layer gradient (2 MESA, 7 TESE): all HCV detected Final processed sperm samples (2 MESA, 4 TESE): no HCV detected 11 ICSI cycles, 1 LB 0 women HCV detected 1 healthy child	Washing process is effective for risk reduction of HCV after TESE or MESA ICSI is safe for women No comparison to ejaculated sperm so indication that retrieved sperm is saver than ejaculated sperm. Only limited to azoospermic men. Confirmation other centers needed	HCV test in women unclear (pcr or antibodies) Unclear if child was tested for HCV (only mention that child is healthy) Very small sample size No genotyping of HCV mentioned
(Meseguer et al., 2002)	CS	34 HIV+ male 21 co-infected with HCV 41 semen washing 97% received antiretroviral therapy Prospective controlled trial	(i)Sperm wash Triple gradient (90,70,45%) centrifugation and swim up (ii)Nested PCR vs one round PCR on motile sperm fraction	% HIV DNA and RNA And HCV RNA	Nested PCR 5/41 (12,2%) HIV+ 5/21 (23,8%) HCV+ One round PCR 0% HIV+ 0% HCV+	Semen samples that are considered as negative by use of commercial methods for HIV/HCV detection are not absolutely free of virus, since nested PCR results were positive	No couples undergoing ART. No data one transmission

(Molina et al., 2014)	CS	93 couples requiring ICSI with male positive for HIV, HCV or HBV 33 HIV+ men (23 HCV co-infected, 1 HBV co-infected, 1 HBV/HCV co-infected, 2 female partners HIV pos, 1 female partner HCV pos) 23 HCV+ men (1 HBV co-infected, 1 female partner HCV+) 37 HBV+ men (34 female partners adequately vaccinated) 62 washed semen samples for ICSI from 59 couples (33hiv+23hcv+3hbv) 173 ICSI cycles in 93 couples (incl cycles with non-washed semen from HBV+ men) 48 cycles with HCV+ men	Retrospective cohort study Semen processing (i)Density gradient (80-40%) centrifugation (ii) pellet washed (1:2 vol/vol) at 300g for 8 min Half of pellet frozen for ART and one half for virological analysis	1.Viral load HIV, HCV and HBV semen after sperm washing with real time PCR 2.outcome ICSI 3.seroconversion rate after ART: PCR on blood of women 3 weeks, 3 mth, 6 mth after ART and children at birth and age 3 mth. 4. obstetric and neonatal outcomes	1.none semen samples were HIV, HCV or HBV pos after washing 2.no significant differences in number (mature) oocytes, fertilization rate, number embryos transferred, number embryo's cryopreserved per retrieval, pregnancy rate between HIV+, HCV+ and HBV+ men (table 3) 3.No seroconversion detected in 62 women and 34 newborns (8 newborns from HCV+ men) 4. no significant differences in obstetric or neonatal results (table 4)	Sperm washing and ICSI is a safe and effective option for reducing risk of transmission or super infection in serodiscordant or concordant couples who wish to have a child.	Included HCV concordant couple. Data on outcome ICSI with washed semen was pooled with data outcome ICSI with non-washed semen. Outcome ICSI with washed semen not documented separately Number of semen samples from HCV pos men not documented (neither for HIV or HBV pos men) No p-value documented in tables. Conclusion is unfounded because reduction of transmission was not aim of study (no comparison of ICSI with washed semen vs ICSI with not washed semen).
(Savasi, et al., 2013)	CS	35 HCV discordant coupl M+/F- requiring ART All detectable VL No antiviral medication Jan 08-dec 10 14 couples IUI-MOH 21 couples ICSI-COH	Spermwashing/swim up No pcr on semen	(1) outcome IUI and ICSI (2) seroconversion rate women and children	IUI: n=14 No cycles 47 No LB: 6 Seroconv women: 0 Infected children 0 ICSI: n=21 No cycles 38 No LB: NM Seroconv women: 0 Infected children: 0	0 seroconverted mothers and 0 infected children. Although risk of sexual transmission of HCV is low ART with sperm washing should be offered to infertile discordant HCV-infected couples	No measurement of HCV RNA Small group Small chance of HCV transmission through sexual intercourse No comparison to spermprocessing without swim up No LB after ICSInot mentioned

(Savasi et al., 2010)	CS	16 blood and semen samples from 16 HCV/HIV1 pos men requiring ART None treated for HCV 15 men on HAART 8 men HIV VL undetect	Ejaculated semen Semen processing: (i)Density gradient (40,80%) centrifugation 3 fractions after centrifugation (1) seminal plasma (2) non sperm cells (NSC's) (3) motile sperm (1) was filtered and frozen (2) was washed 2x in PBS and frozen (3) was resuspended and washed (3a) swim up and frozen (3b) remaining spermatozoa washed 2x in PBS and frozen	HCV RNA with nested PCR in blood en semen fractions	Blood: 81,3% HCV RNA pos (3 men HCV undetect.) (1) 12,5% HCV RNA pos (2) 19 HCV RNA pos (3a) 0% HCV RNA pos (3b) 0% HCV RNA pos 1 patient with HCV pos seminal plasma was HCV RNA undetect in blood.	HCV RNA can be found in seminal plasma and NSC's (31,5%). All washed motile sperm samples before swim up and after swim up HCV RNA negative. Correlation between blood serum HCV and seminal plasma HCV level is unpredictable. Sperm washing should be performed for each semen sample of HCV patients before ART	Small sample size. No data on outcome ART documented. No data on transmission.

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

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Bourlet, et al., 2009)	CS	86 couples with male HCV+ and women HCV- 76 men HCV+/HIV- 10 men HCV+/HIV+ Prospective cohort study Jul '01-Dec '05 Multicentre (3 centres) 148 blood sample 181 seminal plasma samples (all ejaculated) 153 sperm cell fractions 58 couples analyzed where ART led to pregnancy 11 couples undergoing ART not analyzed due to ongoing ART	1 ml semen centrifuged at 800g and seminal plasma frozen 1 ml semen centrifuged over gradient (50,70,90%) Pure sperm and spermatozoa recovered from 90% fraction divided in 2 aliquots. 1 aliquot frozen for virological analysis and 1 aliquot for swim up and then frozen for ART HCV analysis: rtPCR in blood and semen (91% performed with Roche/9% with Abbott) HCV load in semen was estimated	(i) Prevalence of HCV in seminal plasma and sperm fraction used for ART (ii) influence of seminal HCV on semen parameters and ART outcome (iii) HCV serological status of babies conceived through ART tested 3 mth after birth	37/181 (20,4%) of seminal plasma HCV RNA pos 20,2% of men HCV+/HIV- 22,2% of men HCV+/HIV+ All 153 sperm fractions were HCV neg	The absence of detection of HCV RNA in seminal plasma in a single sample does not exclude an intermittent shedding of viral genome in this compartment, so a negative test one day can does not mean it can be positive another day. Due to the sensitivity of the test, a semen sample can never be categorized as absolutely negative. PCR test on sperm fraction is not standardized. 0 transmission of HCV to newborn. More ICSI-cycles due to decreased sperm motility after freezing.	Women not tested for HCV after ART so horizontal transmission of HCV through MAR unknown Couples with HCV+/HIV+ male were not infertile and received MAR for transmission risk reduction of HIV. Couples with HCV alone were infertile and sperm processing was not performed to reduce horizontal transmission. Not documented how many HCV+/HIV- couples had IUI or IVF or ICSI. Not plausible that IUI can lead to HCV transmission to newborn.

(Canto, et al., 2006)		Semen samples from 20 HIV+/HCV+ men HIV VL undetectable All men HCV PCR pos in blood Men not requiring ART	Ejaculated sperm divided into 2 aliquots 1 aliquot frozen, total sperm 1 aliquot processed (i) two layer (45,90%) gradient centrifugation (ii) divided into 3 fractions (motile sperm, seminal plasma and dead sperm/cellular elements) 100uL for pcr and 900uL--> (iii) 3 fractions washed 3 times by RPMI and resuspension of pellet (iv) swim up	Real time PCR on total sperm and 3 processed fractions (pre wash and post wash)	PRE WASH Total sperm • 100% HIV pos • 5% HCV pos Seminal plasma • 50% HIV pos • 0% HCV pos Non motile cells • 30% HIV pos • 0% HCV pos Motile sperm • 5% HIV pos • 0% HCV pos POST WASH All 3 fractions • 0% HIV pos • 0% HCV pos	HCV is detected intermittently in total semen. High degree of sensitivity of multiplex PCR and effectiveness of HIV/HCV extraction methods. Semen washing, together with the relevant reproductive technology and HAART, reduce the risk of viral transmission. Semen purification is highly beneficial for HIV/HCV coinfected individuals	Study group did not require ART. No data documented on ART or transmission. Small group.
(Cassuto, et al., 2002)	CS	35 couples with HCV+ male requiring ART All men HCV RNA pos in blood No antiviraltreatment 7 mth pior to ART No HBV No HIV All couples infertile 50 semen samples -26 for IVF -24 for ICSI	Sperm processing: Density gradient (45% and 90%) centrifugation HCV RNA test: -200 µl semen dilution with 400 µl PBS -centrifugation -supernatant discarded and pellet lysed with 600 µl lyses buffer -standard HCV RT-PCR IVF/ICSI with sperm fraction after 90% density gradient centrifugation	HCV RNA detection (i) total sperm (ii) 45% fraction (iii) 90% fraction (iv) embryo culture media Pregnancy outcome ART HCV transmission to women and children after IVF/ICSI	7/50 (14%) samples total semen HCV RNA+ Viral load semen all < 600 IU/ml) 1/50 (2%) 45% fraction samples HCV RNA+ 0/50 (0%) 90% fraction samples HCV RNA+ 0 women HVC+ 0 children	Standard IVF or ICSI does not increase the risk of HCV transmission to women.	No data on HCV status of all children conceived (only 1 child born at the end of follow up period) No data on time of HCV test women or type of HCV test.

(Garrido, et al., 2006)	CS	7 male patients with TMC < 2 mill spz • 6 HIV+/HCV+ • 1 HIV+/HCV- Goal ART to reduce viral transmission risk 5 men on antiretroviral therapy 1 man had detectable viral load 8 sperm samples included	(i) 1:1 (vol/vol) dilution with human tubal fluid medium and centrifuged at 400g 3x (i) One half of pellet frozen for PCR and one half for ART	Nested PCR Presence of HIV RNA, HIV DNA and HCV RNA	0 sperm samples positive for HIV (DNA/RNA) or HCV 1 sample no HCV result available	Men with severe male factor should be accepted into assisted reproductive programs when, after modified sperm washes, molecular viral absence is confirmed and motile sperm are detected, regardless of their quality.	No data documented on ICSI and transmission to women or child
(Leruez-Ville, et al., 2013)	CS	4 HCV-infected 'azoospermic' men • 1 anejculation, tetraplegic • 2 obstructive azoospermia • 1 cryptospermia All men detectable blood viral load Age 34, 39, 40, 41	2 men TESE (3and4) 2 men MESA and TESE(1and2) 3 men bilateral TESE(2,3,4) 2 MESA samples: Centrifuged on a 2-layer gradient Puresperm(45% and 90%) and washed twice afterwards pellets resuspended and frozen 4 TESE-samples: Centrifuged on one gradient Puresperm(45%) and washed twice afterwards pellets resuspended and frozen	HCV Real Time RNA (RT-RNA) test on all upper layers of gradient, remaining TESE tissue and volume of one straw Treshold 240cop/mL	Upper layer gradient (2 MESA, 7 TESE): all HCV detected Final processed sperm samples (2 MESA, 4 TESE): no HCV detected 0 women HCV detected 1 healthy child	Washing process is effective for risk reduction of HCV after TESE or MESA No comparison to ejaculated sperm so indication that retrieved sperm is saver than ejaculated sperm. Only limited to azoospermic men. Confirmation other centers needed	HCV test in women unclear (pcr or antibodies) Unclear if child was tested for HCV (only mention that child is healthy) Very small sample size No genotyping of HCV mentioned

(Molina, et al., 2014)	CS	93 couples requiring ICSI with male positive for HIV, HCV or HBV 33 HIV+ men (23 HCV co-infected, 1 HBV co-infected, 1 HBV/HCV co-infected, 2 female partners HIV pos, 1 female partner HCV pos) 23 HCV+ men (1 HBV co-infected, 1 female partner HCV+) 37 HBV+ men (34 female partners adequately vaccinated) 62 washed semen samples for ICSI from 59 couples (33hiv+23hcv+3hbv)	Retrospective cohort study Semen processing (i)Density gradient (80-40%) centrifugation (ii) pellet washed (1:2 vol/vol) at 300g for 8 min Half of pellet frozen for ART and one half for virological analysis	1.Viral load HIV, HCV and HBV semen after sperm washing with real time PCR 2.outcome ICSI 3.seroconversion rate after ART: PCR on blood of women 3 weeks, 3 mth, 6 mth after ART and children at birth and age 3 mth. 4. obstetric and neonatal outcomes	1.none semen samples were HIV, HCV or HBV pos after washing 2.no significant differences in number (mature) oocytes, fertilization rate, number embryos transferred, number embryo's cryopreserved per retrieval, pregnancy rate between HIV+, HCV+ and HBV+ men (table 3) 3.No seroconversion detected in 62 women and 34 newborns (8 newborns from HCV+ men) 4. no significant differences in obstetric or neonatal results (table 4)	Sperm washing and ICSI is a safe and effective option for reducing risk of transmission or super infection in serodiscordant or concordant couples who wish to have a child.	Included HCV concordant couple. Data on outcome ICSI with washed semen was pooled with data outcome ICSI with non-washed semen. Outcome ICSI with washed semen not documented separately Number of semen samples from HCV pos men not documented (neither for HIV or HBV pos men) No p-value documented in tables. Conclusion is unfounded because reduction of transmission was not aim of study (no comparison of ICSI with washed semen vs ICSI with not washed semen).

(Savasi, et al., 2010)	CS	16 blood and semen samples from 16 HCV/HIV1 pos men requiring ART None treated for HCV 15 men on HAART 8 men HIV VL undetect	Ejaculated semen Semen processing: (i)Density gradient (40,80%) centrifugation 3 fractions after centrifugation (1) seminal plasma (2) non sperm cells (NSC's) (3) motile sperm (1) was filtered and frozen (2) was washed 2x in PBS and frozen (3) was resuspended and washed (3a) swim up and frozen (3b) remaining spermatozoa washed 2x in PBS and frozen	HCV RNA with nested PCR in blood en semen fractions	Blood: 81,3% HCV RNA pos (3 men HCV undetect.) (1) 12,5% HCV RNA pos (2) 19 HCV RNA pos (3a) 0% HCV RNA pos (3b) 0% HCV RNA pos 1 patient with HCV pos seminal plasma was HCV RNA undetect in blood.	HCV RNA can be found in seminal plasma and NSC's (31,5%). All washed motile sperm samples before swim up and after swim up HCV RNA negative. Correlation between blood serum HCV and seminal plasma HCV level is unpredictable. Sperm washing should be performed for each semen sample of HCV patients before ART	Small sample size. No data on outcome ART documented. No data on transmission.

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

No studies could be found investigating this PICO question.

DOES THE PLASMATIC VIRAL LOAD CORRELATE WITH HEPATITIS C VIRUS IN SEMEN?

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Bourlet, et al., 2002)	CS	32 HCV + men requiring ART All HCV RNA pos All patients: HIV and HBV negative No antiviral medication	Semen processing Fraction 1 (seminal plasma): 1mL ejaculate centrifuged 800xg and cryopres. Supernatant Fraction 2 (sp. zoa): 1mL ejaculate centrifuged on 3-layer gradient (50-70-90%). Cryopreserv. Sp zoa Fraction 3 (swim up)	(1) VL HCV RNA blood serum (log cop/mL) (1) Qualitative HCV RNA seminal plasma (pos/neg) Sens: 40 cop/mL (=1,6 log) (1) RT PCR HCV RNA seminal plasma and motile sperm fractions (log cop/mL) Sens: 100 cop/mL (=2 log) (2) genotype HCV (3) ART outcome and HCV transmission to child	Blood serum (n=32) Mean VL 5,97 ±0,51 [4,97-7,34] Seminal plasma qual. Pos n=4 (12,5%) Neg n=28 Fraction 2 qual.: All neg (n=4) 1 patient: neg plasma, but pos fraction 2 Fraction 3 qual. All neg (4 sampl) Blood serum VL minus Seminal plasma VL= 2,83-5,34 log cop. /mL 1 patient: 7 semen samples all pos HCV but not all detectable semen VL **Plasma semen pos vs neg**: Mean blood VL 6,52±0,55 vs 5,88±0,46 [p=0,002] Genotype blood is identical as genotype semen ART 11 attempts 5 women pregnant (1 tripl, 2 twins, 2 singlt) 9 babies 0 babies HCV RNA pos at birth and at 6 mth	12,5% of HCV infected are pos HCV in semen. Lower than other publications but they include HIV coinfected patients. Positive correlation between viral load blood and seminal plasma All HCV pos seminal plasma had blood VL > 500 cop/mL No compartmentalization of HCV RNC between blood en semen	VL blood is not comparable with semen. There is a positive correlation between blood en semen VL Small study group (n=4) Old paper Comparison blood with fraction 2 is after spermwashing No data on HCV transmission to women undergoing ART

(Bradshaw et al., 2015)	CS	Prospect cohort analysis 70 men with chron (> 12 mth) or acute HCV (< 6 mth) [CHCV or AHCV] with and without HIV A: 18 AHCV/HIV+ B: 22 CHCV/HIV+ C: 26 CHCH/HIV- D: 4 AHCV/HIV- Baseline comparable Exclusion: HCV RNA neg at enrollment Group D due to small size	Paired blood and semen samples 12-24 wks after enrollment 35 men repeat samples (incl other STI) Processing semen -centrifuged 2x (800x, 1350xg) -cryopres. Supernatant -pellet resuspended -Centrifuged 2x (700x, 12000xg) -Cryopres pellet HCV RNA blood and semen (after validation for semen: lower detection level 1,8e10 IU/mL)	(1) Correlation between plasma and semen HCV viral load and HIV VL blood (log IU/mL) VL seminal plasma (log IU/mL) HCV RNA detection in semen pellet (pos/neg)	VL blood median A: 5,8 [4,4-6,2] B: 6,4 [5,5-6,7] C: 6,1 [5,6-6,4] VL sem. plasma median A: 2,2 [1,9-3,3] B: 2,3 [1,8-3,4] C: 2,0 [1,8-2,4] P=0,431 VL blood vs VL seminal plasma (r2=0,142; p=0,02) Group C with semen HCV + vs semen HCV- Med blood VL: 6,2 [5,7-6,7] vs 6,0 [5,3-6,2] P=0,105 Total 29 men (43,9%) semen HCV + Median VL blood: 6,2 [5,8-6,7] vs Total 37 men (56,1%) semen HCV - Median VL blood: 5,8 [4,8-6,3] P=0,02 Semen pellet 4 men pos in pellet and plasma 5 men neg in pellet and plasma 2 men neg pellet and pos in plasma	HIV coinfection is not associated with increased levels of seminal HCV RNA. But too few individuals in group D Weak correlation between HCV VL blood and seminal plasma HCV in seminal plasma undetectable if VL blood > 5,0 log IU/mL	Patients with HIV coinfection. Subgroup HIV neg Subgroup analysis shows there is a difference between blood VL en seminal VL for HCV A higher blood VL correlates presence of HCV RNA in semen (but not with VL in semen) Blood VL is compared to VL semen after washing

WHICH INTERVENTIONS CAN BE USED TO REDUCE/AVOID VERTICAL TRANSMISSION OF HEPAPTITIS C VIRUS TO THE NEW-BORN?

ECS

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Ghamar Chehreh et al., 2011)	SR	Selection criteria ECS or EmergencyCS (EmCS) vs vaginal delivery Follow up > 18 mth Excluded HIV+ women Incomplete data Lost to follow up 8 prospect observational studies included (total 641 mother-child)		Perinatal transmission defined by 2 pos pcr on separate occasions or 2 pos anti-HCV > 18 mth infants age	A: 510 (79,5%) vag delivery B: 131 CS (20,5%) Seroconv infant A: 36/510 (7%) B: 8/131 (6,1%) B vs A: OR 1,1 [95% CI 0,45-2,67] which means that C/S does not decrease perinatal HCV transmission from HCV-RNA+/HIV- mothers to infants. Most infected infected seroconverted at age 3-4 mth Heterogeneity not signif I^2=0,1% Likelihood of publication bias not signif (P_{Begg}=0,2; P_{Egger} =0,5)	CS does not decrease HCV transmission from mother to infant Unable to account for potential confounders e.g. VLmother, ECS or EmCS, instrument-aided vag dlivery	

Breastfeeding

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Cottrell et al., 2013)	SR	14 cohort studies (2 good, 2 fair, 10 poor quality) Follow up > 1 y Total 2971 mother-infant Exclusion: HIV co-infected women unless < 10% of study group HIV+ or data on HIV-negative women reported separately			None reported association between breast feeding of HCV infected women and the risk of transmission to infants.		5/14 did not repot RR 1/14 did not repot transmission rate Included a few studies with HIV-co-infected women

Human immunodeficiency virus

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

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Baggaley et al., 2010)	SR	15 studies		HIV transmission to unaffected partner	Infectiousness of anal intercourse per act Summary estimate 1.8% (95% CI 0.3–3.2)	HIV type (1 or 2) not specified in manuscript	
(LeMessurier et al., 2018)	SR	14 studies		HIV transmission to unaffected partner	<u>the index case is taking ART (with varying levels of viral load)</u> 23 linked transmissions were identified over 9922 person-years (pooled incidence 0.23 transmissions/100 person-years, 95% CI 0.15–0.35, 10 studies) <u>couples where the index case has suppressed viral load</u> 0 transmissions over 1327 person-years were identified (pooled incidence 0.00 transmissions/100 person-years, 95% CI 0.00–0.28, 2 studies). <u>serodiscordant couples who reported "always" using condoms</u> 1.14 HIV transmissions per 100 person-years (95% CI 0.56–2.04)	HIV type (1 or 2) not specified in manuscript	

(Colombe et al., 2019)	CS	289 couples	The follow-up period started either from the start of the relationship or from the first positive HIV result for the baseline individual. The follow-up period ended either at the spouse's seroconversion date, or at the end of the relationship, or at the last sero-survey for which a spouse had an available HIV-1 test result and remained HIV seronegative.	Risk of HIV seroconversion	105/289 serodiscordant couples 63.8% (67/105) of couples had a male baseline individual and a female serodiscordant spouse. 14/105 people HIV-seroconverted, 13 of which women Female spouses thus had a rate of seroconversion 8.77 [1.15–67.04] times higher than male spouses (p = 0.036).		HIV-1
(de Vincenzi, 1994)	CS	256 couples	22 months of follow-up	Risk of HIV seroconversion	Seroconversion occurred in 12/256 partners (8 women and 4 men), seroconversion rate of 2.3/100 person-years (95% CI 1.2-4.0) 0/256 seroconversions in couples with consistent condom use 12/256 seroconversions in the group with inconsistent condom use Seroconversion rate: 4.8/100 person-years (95% CI 2.5-8.4)		HIV type (1 or 2) not specified in manuscript

(Deschamps et al., 1996)	CS	475 HIV infected patients and their 475 HIV-negative regular sex partners. Sexual activity was discontinued by 298 of the 475 discordant couples (63%) within 6 months of study entry. The other 177 couples (37%) were sexually active during all or part of the prospective study period. The sexually active (n = 111) and sexually inactive (n = 298) HIV-infected patients were similar in age, sex, and level of education	Prospective cohort study The Group Haitien d'Etude du Sarcome de Kaposi et des Infections Opportunistes (GHESKIO) Couples were evaluated every 3 months if they were sexually active and every 6 months if they were not	Risk of HIV seroconversion	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. The seroconversion rate was similar in couples who never used condoms (14.4% [13 of 90 persons]) and couples who used condoms irregularly (13.3% [6 of 45 persons]) (P > 0.2; relative risk, 1.08 [CI, 0.44 to 2.66]) The rate of female-to-male transmission of HIV was 7.6 per 100 person-years (5 of 34 persons); the rate of male-to-female transmission was 4.8 per 100 person-years (15 of 143 persons) (P > 0.2)		HIV type (1 or 2) not specified in manuscript

(Ma et al., 2019)	CS	Inclusion criteria were as follows: (1) discordant HIV infection couples, if one spouse was HIV-positive and the other was HIV-negative; or concordant HIV infection couples, if both spouses were HIV-positive, while one spouse was infected with HIV via sexual transmission by the other; (2) the fixed partner has no history pertaining to the risk of HIV infection, such as intravenous drug abuse, multiple sexual partners, blood transfusion, etc.; (3) the couples had a stable marriage and lived together for more than 6 months; (4) the age of couples was in	patients with HIV/AIDS who had a fixed partner and lived in Lu'an during January 1999 to August 2016	The dependent variable was seroconversion occurrence among HIV-negative partners during the follow-up period.	45/231 couples had already had HIV transmission between spouses at the time of first detection in the first spouse The transmission rates between male-to-female and female-to-male spouses were 21.56% (36/167) and 14.07 (9/64), NS <u>186 HIV serodiscordant couples</u> A total of two couples (1.08%) were seroconverted to concordant HIV-positive, with a seroconversion rate of 0.39 per 100 person-years (2/507.7). The HIV transmission rates between male-to-female and female-to-male spouses were 0 (0/131) and 3.62 (2/55), respectively. These seroconversions occurred in couples where the index case did not immediately received antiviral treatment		HIV type (1 or 2) not specified in manuscript

(Operskalski et al., 1997)	CS	18 (10 male, 8 female) HIV index cases and their 19 partners (11 female, 8 male)			2 partners were anti-HIV positive when first observed (1 male, 1 female) 4 partners seroconverted during 23 person-years of observation (1 male, 3 female) Below an estimated level of 3.75, none of the six recipients transmitted to their partners, compared with five of 12 above that level.		HIV-1
(Quinn et al., 2000)	CS	415 couples 228/415 men were infected with HIV-1 187/415 female infected with HIV-1	Retrospective study 30 months follow-up		90 seronegative partners seroconverted during the study (50 female and 40 male) The rate of transmission from male partners to female partners was not significantly different from the rate of transmission from female partners to male partners (12.0 per 100 person-years vs. 11.6 per 100 person-years). Among couples in which the initially HIV-1–negative partner seroconverted, the mean serum HIV-1 RNA level of the HIV-1–positive partner was significantly higher than that of the HIV-1–positive partner in couples in which the HIV-1–negative partner remained seronegative (mean, 90,254 copies per milliliter vs. 38,029 copies per milliliter; P=0.01). The rate of 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 per milliliter.		HIV-1

(Ragni et al., 1998)	CS	39 partnered HIV infected hemophilic men In a relationship for 6mo or more No ART or safe sex guidance			5 (13%) of men were transmitters The proportion of transmitters with HIV RNA>100.000 copies/ml was sign higher than the proportion of non transmitters with that level of viral load 3/5 vs 3/34 The median HIV RNA in transmitters was 121.800 copies/ml was 10-fold higher than the median HIV RNA in non transmitters		HIV type (1 or 2) not specified in manuscript
(Rodger et al., 2016)	CS		The PARTNER study was an prospective observational multicenter study of serodifferent couples, heterosexual and men who have sex with men (MSM), in which the HIV-positive partner is taking ART. 888 couples (548 heterosexual and 340 MSM) contributed 1238 eligible couple-years of follow-up; 1251 when including periods of follow-up time in which the HIV-RNA load was suppressed at the beginning of the period but during which the load became elevated.		A total of 11 of the originally HIV-negative partners were observed to acquire HIV during eligible follow-up, but there were no phylogenetically linked transmissions Given that there were no linked transmissions (even when considering periods during which the HIV-RNA load became elevated [representing a total of 13 couple-years of follow-up]), the estimated rate for transmission through any condomless Sex with the HIV-positive partner taking ART with HIV load less than 200 copies/mL was zero, with an upper 95% confidence limit of 0.30 per 100 couple-years of follow-up (0.29 when including periods of follow-up time in which the HIV-RNA load was suppressed at the beginning of the period but during which the load became elevated).		HIV-1

(Tang et al., 2016)	CS	4481 HIV serodiscordant couples Sero-different couples with the HIV-negative spouse seroconverting at least 3 months after the previous negative diagnosis during cohort observation period were labeled as ªcase couplesº. The ªcontrol couplesº were selected randomly from the same cohort that did not have the HIV-negative spouse seroconversion during the same period.	Retrospective cohort study October 1, 2010, and September 30, 2012, and followed-up to December 31, 2012,		53 seroconversions within 5218 person-years of follow-up The incidence rate was 1.02 (95%CI: 0.76±1.33) per 100 person-years.		HIV type (1 or 2) not specified in manuscript
(Wawer et al., 2005)	CS	ART was not available at the time of the study 414 HIVdiscordant couples who subsequently received at least 1 followup visit that permitted the retrospective assessment of HIV transmission.	10 month survey visits Retrospective cohort study		239/414 couples reported to be monogamous 72/239 the unaffected partner seroconverted The overall rate of HIV transmission per coital act was 0.0012 (95% CI, 0.0009–0.0015). Transmission per act was highest in the interval immediately after the acquisition of HIV by the index partner (0.0082/coital act [95% CI, 0.0039–0.0150]), under the assumption of ~2.5 months of exposure for the HIV negative partner During the subsequent 10-month interval (~6–15 months after seroconversion by the index partner), the rate of transmission decreased to 0.0015/ coital act (95% CI, 0.0002–0.0055), which was not significantly different from that observed among partners of prevalent index partners (0.0007/coital act [95% CI, 0.0006–0.0011]).		HIV-1

(Zheng et al., 2018)	CS	Inclusion criteria: 15 years old and above; had a spouse/partner newly registered as HIV positive; not have a positive test for HIV at baseline themselves; and been married, divorced, currently single but married before, or a separated spouse to the newly registered HIV positive spouse/partner	Observational cohort study 12 months of follow-up		Compared with males in this cohort, the risk of HIV acquisition was higher among female partners, the OR value was 2.09 (95% confidence interval [CI]:1.67, 2.63) times higher for females at the 6-month follow-up (P<0.0001). Higher risk for HIV acquisition was observed in the 55- to 64-year age group population with an OR value 2.23 (95% CI: 1.87, 2.66), married HIV individual with 2.45 (95% CI: 1.30, 4.63), and illiterate HIV/AIDS with 6.66 (95% CI: 2.31, 19.24). Compared with those who were taking ART, the OR value in ART-naïve group was 1.14 (95% CI: 0.91, 1.43), there was no significant difference between ART and ART naïve group.		HIV type (1 or 2) not specified in manuscript

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

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Attia et al., 2009)		11 eligible cohorts reporting on 5021 couples and 461 HIV transmission events in 16 publications or abstracts from eight countries			**untreated HIV-infected individuals** The estimated probability of HIV transmission per coital act, after controlling for age, ranged from 0.0001 when viral load was below 1700 copies/ml (sexual intercourse 10.4 times per month) to 0.0023 when viral load was greater than 38 500 copies/ml (sexual intercourse 7.9 times per month) **HIV-infected individuals on ART** The overall HIV transmission risk patients to heterosexual partners, irrespective of viral load and other sexually transmitted infections, was 0.46 (95% CI 0.19–1.09) per 100 person-years, based on five episodes of HIV seroconversion **HIV transmission from people not on antiretroviral therapy** Amongst people with viral load below 400 copies/ml, irrespective of sexually transmitted infections, the transmission rate was 0.16 (95% CI 0.02–1.13) per 100 person years, based on one episode of HIV transmission in six studies [9,11–14,17]. The transmission rate increased with increasing viral load to 9.03 (95% CI 3.87–21.09) per 100 person years amongst individuals with viral load at least 50 000 copies/ml (Fig. 2).	There were insufficient data to allow estimation of summary rates of transmission through sexual intercourse without condoms, or to separate female–male and male–female transmission. The available studies found no episodes of HIV transmission in discordant heterosexual couples if the HIV-infected partner was treated with ART and had a viral load below 400 copies/ml, but the data were also compatible with one transmission per 79 person-years	Type of HIV (1 or 2) not specified in the manuscript

(Rodger, et al., 2016)	CS	A total of 11 of the originally HIV-negative partners were observed to acquire HIV during eligible follow-up	The PARTNER study was an prospective observational multicenter study of serodifferent couples, heterosexual and men who have sex with men (MSM), in which the HIV-positive partner is taking ART. 888 couples (548 heterosexual and 340 MSM) contributed 1238 eligible couple-years of follow-up; 1251 when including periods of follow-up time in which the HIV-RNA load was suppressed at the beginning of the period but during which the load became elevated.	HIV transmission	there were no phylogenetically linked transmissions Given that there were no linked transmissions (even when considering periods during which the HIV-RNA load became elevated [representing a total of 13 couple-years of follow-up]), the estimated rate for transmission through any condomless Sex with the HIV-positive partner taking ART with HIV load less than 200 copies/mL was zero, with an upper 95% confidence limit of 0.30 per 100 couple-years of follow-up (0.29 when including periods of follow-up time in which the HIV-RNA load was suppressed at the beginning of the period but during which the load became elevated).		HIV-1

(Pedraza et al., 1999)	CS	38 highly exposed heterosexual couples Inclusion criteria: HIV infection of the index case, a longterm relationship with the infected partner (at least 1y), the only risk factor for HIV transmission was unprotected sexual intercourse with the index case	Index cases and partners were seen every 6mo		In 10/38 HIV transmission occurred Higher viral loads in transmitters vs non-transmitters 21.139 vs 5.484 RNA copies/ml resp. P=0.03 Viral isolation was obtained in 9/10 transmitters vs 8/18 non-transmitters		HIV-1

SHOULD IUI, IVF OR ICSI BE PREFERENTIALLY USED FOR MEDICALLY ASSISTED REPRODUCTION IN HUMAN IMMUNODEFICIENCY VIRUS INFECTED COUPLES?

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Barnes et al., 2014)	SR	24 studies **HIV-1** IUI Range age of feamel partners in serodiscordant couple with HIV+ men: 28.8y - 34.6y ART: Range age of HIV+ women: 35.5y-36.3y Range age of female partners of HIV+ men: 32.8y- 38y for ART HIV+ men: ART: CD4 (377 – 608 cells/mm3) 55%-90% undetectable viral loads. IUI: CD4: 409-612 cells/mm3) 36-100% undetectable load HIV+ women: ART: cD4 200-712 cells/mm3 48-95% undetectable viral loads	Meta analysis published paper through April 2013, Sample size ranged from 19 to 854. 14 studies on IUI 12 studies serodiscordant couple with HIV+ male, 4 HIV+ women 15 studies on ART: 12 studies on couples with HIV+ male and 7 HIV+ female.	Clinical pregnancy rate Multiple pregnancy rate Miscarriage rate	% Clinical Pregnancy per cycle: IUI HIV+ males (n=2393 patients --> probably cycles, as the conclusion is on the same numbers and it is stated cycles): 17% (95CI: 15%-20%) HIV+ women (n=28: 14% (95CI: 25%-35%) ART HIV+ males (n=780): 30% (95CI: 25%-35%) HIV+ women (n=253: 16% (95CI: 13%- 20%) Multiple pregnancy rate: IUI: HIV+ males (n=2359): 10% (95CI: 6%-14%) HIV+ (n=25): 14% (95CI: 1%-36%) ART: HIV+ males (n=415): 33% (95CI: 25%-41%) HIV+ (n=68)29% (95CI: 7%-59%) Miscarriage rate: IUI: HIV+ males (n=2393): 19% (95CI: 14%-25%) HIV+ females (n=25): 13% (95CI: 1%-34%) 8212 HIV+ males in IUI: ZERO transmission to seronegative partner 1254 HIV+ males ART: ZERO transmission	Serodiscordant couples with HIV who do not meet the criteria for AIDS have a reasonable chance of pregnancy through MAR. Male and female candidates for IUI seems to have a pregn. Rate comparable to the general ferti population. MK rate seems to be similar to those for HIV-subfertile couples for IUI and higher for ART. ZERO seroconversion	Including studies where patients have co-infections with HCV and HBV.

(Vitorino et al., 2011)	SR	**HIV-1 or HIV1/2 not specified in text** Inclusion: Male HIV+ partners: Stable viral load in the previous 4 to 6 months. CD4+T count >200cells/mm3 Consistent use of condoms Median age: male IUI: 33.2y (range 30-35.5y) IVF/ICSI: 33.2y (33.4 - 36.6y) Female: IUI: 33.3y (range 31.9-38y) IVF/ICSI: 37.2y (33.7-39y)	Meta-analysis, papers published before dec. 2007. Databases: PubMed, LILACS, SciELO, Scirus, Cochrane, Scopus and university thesis. Included: 11 studies. 3900 IUI cycles (1184 couples) 738 IVF/ICSI cycles (579 couples) Semen preparation: Density gradient + swim-up. 4/11 studies: post wash PRCR included in the study. HIV+ after wash: range: 2.5% - 7.7%.	Clinical pregnancy rate Cumulative pregnancy rate Miscarriage rate	% pregnancy per cycle: (median) IUI: 18% (14.5- 23%) IVF/ICSI: 38.% (24.8-46.2%) Cumulative pregnancy rate: (median) IUI: 50% (40-63.1) IVF/ICSI: 52.9 (41-67.5%) Abortion rate (median): IUI: 15.6% (9.5-24.7%) IVF/ICSI: 20.6% (9.3-29.5%)) ART safety: HIV seroconversion (PCR) and vertical transmission: NO seroconversion in female partners, no vertical transmission to children born (at birth or at 3-6 months after delivery).	Cumulative evidence shows that ART (IUI and IVF/ICSI) is safe and effective in serodiscordant couples were men are HIV+ and there is no horizontal or vertical transmission of HIV.	Also co-infected patients with HCV in 3 studies. IUI is effective in HIV serodiscordant couples as it is in the general population. The median pregnancy rate is actually higher than in the general population, probably due to the fact that there is no infertility in the couples.

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

DNA integration in semen/oocytes/embryo

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Baccetti et al., 1994)	Original basic research study – theory testing study SPERM	15 seronegative and 15 seropositive sperm donors <u>Exp1</u> 2 ml HIV-1 stock ($10^{4.7}$ TCID50) added to 2 ml sperm Incubation at 37°C, 5%CO2 for 5h Negative control= uninfected HIV-1 C8166 T cells Positive control= infected HIV-1 C8166 T cells <u>Exp 2</u> Coculture of spermatozoa with 72h infected C8166 T cells for 5-12h.	<u>Techniques used</u>: Electron microscopy immunohcytochemistry In situ hybridisation at electron microscopy level IVF Fluorescence immunocytochemistry	Detection of HIV particles	**<u>EM:</u>** Sperm-associated retrovirus like particles are visible in sperm of HIV infected persons or of seronegative donor sperm that was incubated with **HIV-1.** 3 types of particles found inside sperm: Between plasma membrane and outer acromosomal membrane in the sperm head or in the neck or mitochondrial districts, in the region between the nucleus and the acrosomal membrane. -> some particles have the diameter of a virus particle, but they never show the nucleoid-like core. Spermatozoa of seronegative donors are always free of virus particles. Co-incubation with HIV in time course of binding and penetration: virus like particles were found only on the outer surface of spermatozoa when incubated for 2h. At 6h: virus-like particles inside the sperm cytoplasm. **<u>Immunocytochemistry:</u>** Anti-HIV sera binding in 8/15 infected patients and 15/15 seronegative incubated semen appearing exclusively in acrosomal region and mitochondrial region. **<u>In situ hybridisation:</u>** HIV-1 presence in the perinuclear region close to the acrosome in HIV infected men and in 8/15 HIV co-incubated sperm of seronegative men. **<u>Transfer during fertilisation:</u>** IVF of oocytes with sperm of HIV+ men -> fixed for EM: embryos contain virus particles identical to those present in the sperm of the HIV men. Alternative HIV receptor detection: Antigalactosylceramide antibodies to bind the midpiece and the equatorial segment of all living or fixed human spermatozoa.	Virus particles containing HIV-1 antigen can penetrate in human spermatozoa in vitro and in vivo. We believe that these particles are HIV virions. We agree that the virus particles found in the sperm cytoplasm represent infecting and not replicating virions.	Certain details are sometimes lacking concerning numbers e.g. IVF experiment: how many oocytes were used for this exp? No data on co-infection in the patient population

(Baccetti et al., 1999)	Original study OOCYTE	**15 seronegative women**, 100 oocytes HIV stock from chronically infected H9 cell line (H9 IIIb). In some experiments, cumulus cells were stripped and then co-incubated with HIV.	<u>Techniques used:</u> PCR TEM immunocytochemistry	Detection of HIV particles	**Lack of virus infection in HIV-1** exposed oocytes. Absence of HIV-1 associated antigen in immunoEM. No virus-like particles as found in previous study in sperm. No detection of HIV receptors: GalAAG (putative receptor for HIV), CD4, CXCR5 and CCR5 (= main receptor complex for entering HIV).	No viruslike particles were found in HIV co-incubated oocytes of seronegative women and there is an absence of HIV receptors on oocytes (lacking on granulosa cells and in the whole zona pellucida)-> this study shows the failure to HIV-1 infect oocytes directly.	
(Bertrand et al., 2004)	Researc hletter 4 cases OOCYTE	4 HIV infected women with seronegative partners Age (30-38-35-31) ¾ HIV undetectable viral load, 1 patient: 3600 cop/ml in blood.	24 follicular fluids 15 follicular flushes 1 cumulus cells sample In total: 39 samples for HIV-RNA, 1 HIV-DNA From 4 patients out of 7 IVF or ICSI cycles. HIV tested on Cobas Amplicor HIV-1. Detection limit: >50 cop/ml.	Detection of HIV particles	Patient with **detectable viral load**: **HIV-1 RNA detected** in follicular fluid (9111 cop/ml and in 1 flush sample (601 cop/ml) and 2 other flushes were negative. All other patients with **undetectable viral load, no** viral RNA was found in follicular fluid or flushes and the 1 cumulus sample that was tested was HIV- 1 DNA negative. 2/4 patients had a live birth from IVF – all seronegative. 1 patient undetectable viral load 1 patient detectable viral load	No detection of HIV-1 RNA and DNA in follicular fluid or cumulus cells of patients with undetectable viral load.	No information on co-infection status

(Deleage et al., 2011)	Original research paper SPERM	Seminal vesicles were obtained from **seronegative persons** undergoing prostatectomy and had not received hormone treatment. HIV-1 infection in seminal vesicle explants Tissue sections from HIV infected men (9 persons) all on HAART and 7/9 patients had undetectable viral load and were deceased. 7/9 donors were also co-infected with HCV, HBv or both.	<u>Techniques used:</u> PCR Immunohistochemistry Statistics SAS	Detection of HIV particles	<u>Detection of potential **HIV-1** target cells in human seminal vesicles.:</u> HIV co-receptors: CCR5 and CXCR4 were detected in primarily CD163+ macrophages and to a lesser extend CD3+ T lymfo and CD4+ cells whereas stromal CD8+ cells were scarce. <u>Analysis of seminal vesicles of HIV infected men</u>: HIV p24+ cells were detected in seminal vesicles of 7/9 patients. HIV Gag RNA+ cells co-localized with CD163 staining. No correlation between the number of HIV p24+ cells in the seminal vesicles and the number of CD3 T lymfo, HLA-DR and macrophages ($p>0.05$). Infected cells were observed either in the stroma, close to the epithelium or in the lumen of the seminal vesicles. -> which suggests they can contribute to the semen contamination.	Human semen vesicles support HIV infection in vitro and in vivo and can contribute virus to the sperm.	Rationale to look at seminal vesicles -> vasectomy has little effect on the seminal shedding of HIV-1 RNA -> testis and epididymis are probably not the primary source of HIV in semen.
(Dussaix et al., 1993)	Original research paper SPERM	**17 healthy volunteers** Co-infection experiment	<u>Techniques used</u>: EM CD4 expression exp. Reverse transcriptase (RT) activity	Detection of HIV particles	RT activity in co-infected spermatozoa, not in negative controls. Binding of **HIV-1** to spermatozoa confirmed by EM: binding to the plasma membrane of the cell, but also inside the nucleus of the sperm and the space between the nuclear membrane and the karyoplasm. In the nucleus, HIV particles were found in the vacuoles in the apical region. CD4 was not detected on the spermatozoa surface in this study.		Particles observed in sperm were composed of amorphous material surrounded by a membrane, unlike mature HIV 1 particles that contain a well-condensed core. These particles were not found in seronegative samples.

(Miller et al., 2019)	OBs	**10 HIV infected persons** Age 27 to 52y. Underwent elective bilateral orchiectomy for gender affirmation. At time of surgery: all were on antiviral therapy for at least 6 months. Immunostaining techniques on frozen tissue slices.	Investigation of within-host proviral burden, genetic diversity and compartmentalization in 10 HIV+ men undergoing orchiectomy. **Blood vs testicular tissue.** HIV penetrates into the testes early during infection and subsequently persist there.	Detection of HIV particles Question is: are proviruses who persist in the testes different than those from blood?	The testis is a site of HIV persistence. The principle mechanism by which blood and testis reservoirs differ is not via seeding but rather via differential clonal expansion of latently infected cells. There is a difference in quantity and distribution of identical HIV sequences between hosts and between anatomical sites within each individual shows that it will be very difficult to eradicate HIV completely in the body.		(these phylogenetic studies on viruses are very complex)
(Quayle et al., 1997)	Original research paper SPERM	22 **HIV-1**+ men CD4 + cell count>200µl 10/22 were on reverse transcriptase inhibitors 12/22 had never taken or had stopped 6 months previously 13HV+ men, randomly recruited Unknown CD4 count and unknown medication status 12 seronegative men	<u>Techniques used:</u> Immunohistology P24 assay Isolation of cell population by immunomagnetic bead assay PCR quantification of HIV DNA Statistical analysis: ANOVA Semen and blood collected at the same time Semen was prepared density and swimup.	Detection of HIV particles	Median number of CD45+ leucocytes and CD68+ macrophages are not different in semen of different patient populations in this study. The presence of HIV provirus in blood T cells and macrophages was shown. In 8 matched semen samples, 6 T cell and 3 macrophage samples were HIV provirus +.	T cells are most commonly infected with HIV (75% of the samples), followed by macrophages (38% of the samples). Viral DNA was never detected in motile spermatozoa or immature germ cell populations. Germ cells are not the vectors of HIV transmission.	No information on possible co-infections

(Steenvoorden et al., 2012)	Original research paper OOCYTE	Human oocytes: 41 used in experiment of **Seronegative donor** 28oocytes were injected via ICSI with HIV, 13 oocytes = negative controls. Cat oocytes: 543 oocytes injected with concentrations of HIV+ 376 oocytes = negative controls	Febr 2007 – Febr 2009 <u>Techniques used</u> Injection via ICSI of HIV+ media: Human oocytes injected with $4x10^4$ **HIV-1** Cat oocytes injected with $4x10^4$ $4x10^2$ 40 copies QPCR to determine copy number integration	Detection of HIV particles	Human oocytes: 11% (3/11) oocytes HIV+ Cat oocytes: $4x10^4$: 6%-49% HIV+ $4x10^2$:2%-7% HIV+ 40 copies: 0.6% - 1.8% HIV+ -> significant correlation ($p<0.001$). Viral integration of injecting 40 copies of HIV is <2%. The detection limit of PCR for washed semen is nowadays 10-40 cop/ml.	The probability of virus integration is extremely low when small amounts of virus particles are injected through ICSI. The theoretically calculated chance of integration in human oocytes through ICSI is 0.00002%. The chance of the female partners and/or future child gets infected with HIV-1 after ICSI with washed sperm is extremely low	COOL paper! VERY NICE of the group of Repping
(Young et al., 2019)	Original research paper SPERM	**Healthy donors** Sperm co-incubated with HIV. Sperm was washed free from seminal plasma and lipid extraction	<u>Techniques used:</u> P24 antigen ELISA Computational modelling		Does HIV bind to sperm via lipid binding, since washing can remove it? HIV binds in a dose dependent manner to motile sperm and at higher level to immotile sperm in co-incubation experiment with HIV, probably due to intact receptors on the surface. When HIV-bound sperm was prepared 1) simple washing: HIV withstood the washing at low speed 2) gradient centrifugation (45/90): A reduced binding ability in the interfaced immotile sperm. HIV binding was only around the background signal in the 90% layer.	The binding of **HIV-1** to sperm does not happen with high affinity and likely superficially on the sperm surface since HIV-1 can be removed upon centrifugation through 45%-90% percoll gradient.	

Placenta

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Dictor et al., 2001)	CS	39 pregnancies in 37 HIV-1 infected women	Between 1989 and 1993 Prospective cohort study Placental tissue was collected, generally from each side (87 tissue blocks)		34 fullterm deliveries (one stillbirth) 5 elective abortions 4/39 children were HIV infected 12/37 placentas Synctiotrophoblast and villous mesenchymal cells stained positive for HIV IHC Only 36 samples (18 placentas) were amplifiable for PCR 3/18 placentas tested positive Only for 1 placenta, the tests were concordant		Old data, old detection techniques used
(Peuchmaur et al., 1991)	CS	75 pregnant HIV-1 infected women After abortion or delivery the placentas were collected and tissue samples (2 blocks of the central area and 2 blocks of the free membranes were immediately snap frozen	Januari 1987-May 1988		No cells positive for HIV proteins were found in the frozen sections of the 75 placentas via IHC ISH showed no HIV proteins regardless of the clinical status of the mother and even in those with grade IV disease and those with disease progression		Old data, old detection techniques used

(Mattern et al., 1992)		Placentas were obtained within 6h of delivery 27 placentas 19 from HIV positive mothers 4 from HIV negative mothers 4 from untested mothers who were considered low-risk for HIV infection		Immunoperoxidase staining for HIV core antigen	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 1 1 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 One of the 8 culture-negative placenta specimens from seropositive pregnancies was immunoperoxidase positive, and the remaining 7 culture-negative specimens were negative in the p24/55 antigen immunoperoxidase assay.		Old data, old detection techniques used Type of HIV (1 or 2) not specified

DOES HUMAN IMMUNODEFICIENCY VIRUS/TREATMENT OF HUMAN IMMUNODEFICIENCY VIRUS BEFORE ASSISTED REPRODUCTION IMPACT THE OUTCOME OF ASSISTED REPRODUCTION?

Male infected

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Bujan et al., 2007)	CCS	84 HIV-1 serodiscordant couples (HIV+ men, HIV- women). HIV serodiscordant couples: 294 IUI cycles Control couples: 320 IUI-D cycles 96.4% received HAART (more than 2 different drugs) 3.6% no treatment. HIV blood mean viral load: 633±3696 cop/ml HIV semen mean viral load: 581±1377 cop/ml CD4 count mean:610±243mm^3. <u>Inclusion:</u> HIV-1+ men: good health, clinically asymptotic, CD4 >200mm^3 , stable viral blood load at least 4 months. Women: HIV-1 neg <u>Exclusion</u>: azospermic or severe oligospermic <u>Control group</u>: Matching on period of treatment (same period) IUI-D due to male sterility in heterosexual couples. 90 couples	June 2000 – Oct 2003 <u>Donor sperm processing:</u> Cfr. WHO: Sperm Freeze (Fertipro): ratio 1:2, 0.3ml CBS straws, liquid nitrogen storage. <u>HIV+ partner sperm processing:</u> density (50/70/90) PureSperm – 90% fraction: 2x wash BM1 medium – swim up (37°C, 60 min, angle 45°). Aliquot min. 2x10^6 spermatozoa for HIV-1 test, rest frozen liquid nitrogen. 2 semen samples were provided with 45-60 min interval. <u>HIV-1 test:</u> Plasma: HIV-1 RNA :Amplicor HIV-1 Monitor Test (Roche)- detection limit = 20 cop/ml Semen: HIV-1 RNA and DNA (also Roche). HIV-1 DNA detection limit= 5 cop/10^6 cells. HIV-1 RNA detection limit = 20 cop/10^6 cells. <u>IUI protocol:</u> Stimulation: FSH (Gonal F, Merck Serono) or Metrodin (Organon France). HCG (5000IU) trigger lead foll ≥18-20mm. <u>Thawing straw:</u> 5 min at RT – 5-10min 37°C - wash with culture medium – dilution in 2 volumes of medium + centrifuged 600xg, 10min – resuspend pellet in 0.3ml culture medium = sample in polyethylene catheter for IUI (30-60s) - patient remained recumbent 10-15 min. No supplement progesteron. Beta HCG test after 14-16d post insemination.	Pregnancy rate/ IUI cycle Pregnancy rate/couple Baby take home rate Miscarriage rate seroconversion	Pregnancy rate/ IUI cycle HIV+ 18.03% Contr 14.69% P>0.05 Pregnancy rate/couple HIV+63.10% Contr 52.22% P>0.05 Baby take home rate: HIV+ 52.4% Contr 41.1% P>0.05 Miscarriage rate: HIV+ 16.98% Contr 21.28% P>0.05 ->No seroconversions in serodiscordant HIV- women: P24 antigen and HIV-1antibody and HIV-1 RNA negative, 6 months after HCG- IUI and after delivery. 44 children born	Chance of pregnancy is patients with viral infections is not different than in patients without viral infection for IUI. There is no risk for seroconversions	1) semen processing: 2 semen samples for the HIV+ men: are they combined in 1 semen prep or not? not clear in paper. 2) paper also describes a second control group undergoing ICSI -> this was excluded in this table, because of differences in treatment (IUI vs ICSI). 3) no exact p values given, only <0.05 4) no congenital malformations are given 5) HIV detection in the children is not given -> no co-infected patients in the data set

(Cito, et al., 2019a)	CCS	24 serodisc HIV couples 69 control couples <u>Inclusion criteria:</u> Female age range: 18-40y Male age range: 18-45y Serodiscordant couple: Man HIV+ seronegative CD count >200/mm Stable viral load <4 months before ART All patients were on HAART <u>Exlusion:</u> Men with azospermia and severe cryptozospermia Controle group: Seronegative males, female partners normo-ovulatory females with tubal factor infertility No other gynaeco pathologies.	Between February 2011 to August 2018 All ICSI <u>Sperm preparation:</u> Ejaculate through masturbation, 2-7 days abstinence. Liquefication incubator 37°C- 30 min Density gradient centrifugation 50%/95% (PureSperm Nidacon) (1ml/1ml). 1 ml semen onto the gradient. 300g, 20 min Supernatants removed / pellet transferred into new tube + suspent in 2.5ml Sperm medium (Origio) 250g, 10 min. Swim up: 45°, 1h at 37°C. 1ml recoverd and divided in 2 parts. 1 part for HIV post-prep testing, other for ICSI treatment. <u>Stimulation:</u> Recombinant FSH 225-375 IU (Gonal F). ≥14mm follicles: short protocol with GnRH antagonist. When at least 2 foll 17-18mm: 250mcg Rec HCG subcutaneously -> oocyte pickup 36h later. Luteal support: 50mg intramuscularly progesterone (Protogest). ET on day 3 or day 5	IPR = #gestational sac / #embryo transferred CPR= HCG>50mU/L + ultrasound visualisation intrauterine sac with heartbeat (5-6 weeks) PregnLoss= before 20 weeks and all biochemical pregn LBR= #cyles leading to a live birth	<u>HIV serodis vs controle</u> IPR 12.1% vs 14.1 (P=0.3) CPR 21.7% vs 20.3% (p=0.3) PrgLoss 20% vs 28.5% (p=0.38) LBR: 17.4% vs 15.9% (p=0.6)	No statistical diff between serodisc and controls	Paper also included HCV patients, but they are analyzed separately, so in this table only HIV data. How the matching occurred is not described! ‘**we extracted the cycles from our database’.....** Reason for exclusion? Patients screened for HIV-1/2, but no further specification in the manuscript text

(Prisant, et al., 2010)	CCS	Inclusion 28 serodiscordant couples (HIV-1+ men, HIV-women). Mean age females: 35.3±4.3y Matched control: 4 criteria: age, etiology of infertility, rank of oocyte retrieval, IVF or ICSI) CD4 count > 200mm^3 Stable viral load assessed less than 4 months before ART Control group: Same period 41 seronegative couples Mean age females: 35.5±4.4y Matching: Age Etiology of infertility Rank of oocyte retrieval Type of MAR	Between dec 2002 and June 2007 IVF or ICSI – Day 2 ET (dET or tET) 44 cycles in each arm Sperm preparation: Semen, density gradient (45/90) Puresperm + suspension of pellet in IVF medium. HIV testing: Seminal plasma: HIV-1 RNA. Semen: proviral RNA and DNA (Cobas, Roche M2000sp), sensitivity: 50cop/mL. Luteal phase: Micronized progesterone vaginally during luteal phase. BetaHCG between 9-11 days after ET Two-sample Wilcoxon test for quantitative variabels, comparion of rates: chi-squared test. All tests were 2-sided. P<0.05 SAS	Implantation rate: BetaHCG between 9-11 days post ET Clinical pregn= betHCG+ and gestational scan with heartbeat ultrasonically 4-5 weeks post ET.	IR Serodiscordant 10.7% vs 7% control CPR/Oocyte retrieval Serodiscordant 18.2% vs 9.1% control CPR/ET Serodiscordant 22.2% vs 10.8% control Children born Serodiscordant 6 vs 4 control	No differences in IR and CPR	1) single center? Probably, not specified. 2) no separate data on IVF and ICSI but taken into the matched control **3) more data in the paper, also on HCV and female HIV+. The data here is the subdata on the HIV+ males -> results are on HIV infected only** 4) no details on stimulation protocol 5) no data on HAART 4) statistical analysis details are on embryo development, not on PR.

(Sauer and Chang, 2002)	CCS	<u>Inclusion</u> 43 serodiscordant couples HIV-1+ males Mean male age: 37.2±0.8y Years since HIV diagnosis: 8.7±0.9y Viral load: 3726±2970 cop/ml CD4 count: 512±51x10^6/L Female partners were HIV-1 neg Female age: 34±0.6y 25 (58.1%) were on HAART -> 34 couples included because 9 were canceled. <u>Control group:</u> Matched via age of women. Randomly selected through SPSS 78 couples age: 23-45 years	From Aug 1997 - Dec 2000 55 ICSI cycles in serodiscordant couples All cycles had ET, up to 4 embryo's for ET 55 IVF/ICSI cycles of 50 controle patients were used <u>Semen preparation:</u> WHO criteria and Kruger's strict morphological criteria Masturbation, abstinence 2 days. Liquification 15-20 min or longer if needed. 1-2ml semen on density gradient (47/90), spin 10-20 min 300g + pellet transferred to clean tube + 5ml HTF (+5% 'HSA), spin 10 min 300g + pellet resuspended in 3ml HFT-'HSA at max for 5 min. Pellet resuspended in small vol of HTF-'HSA. <u>Stimulation:</u> Long protocol, gonadotropin-releasing hormone analogs and recombinatnt FSH or human menopausal gonadotropins). Ovarian suppression: leuprolide acetate for 10-14 days starting on day 2 of the menstrual cycle. HCG trigger (10 000IU) when at least 3 foll: 18-20 mm and E2 was rising. OPU 34-36h after trigger <u>HIV testing:</u> HIV-1 DNA (PCR): sensitivity: 10cop/ml SPSS statistics, t test, analysis of variance or chi-square. Wilcoxon and Kruskall-Wallis for non-parametric data. P<0.05	Clinical pregnancy rate Ongoing pregnancy rate Babies born	3 months after delivery, mothers and babies were tested. Patients who did not become pregn or who had miscarriages: HIV enzyme assay 3 and 6 months later. CPR/ ET Serodisc 45% vs <40% (~38%) Ongoing PR/ET Serodisc 31% 17 babies born from serodiscordant couples	No significant diff in pregnancy outcomes of miscarriages. No complications were reported. No seroconversions. All mothers and babies were HIV-1 neg. After 3 months.	1) power calculations for this study show that 376 pairs needed to enroll. This report is an interim analysis 2)outcome data on control is shown in a table, but no exact numbers are described. 3) in the paper is stated that women aged 40y could receive up to 7 embryos per transfer No co-infections of HCV or HBV stated in the paper.

Female infected

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Marques et al., 2015)	SR	342 HIV+ women Mean age: 35.4y CD4 count: >200 to 712cells/mm^3. Range:48% - 100% had undetectable viral load Range: 44% - 95% was on antiviral treatment. Matching: 6/10 age-matched cohort studies. 2/10: clinical prospective studies 2/10 case-matched studies (age, etiology of and duration of infertility, history of pelvic surgery, type of pituitary inhibition) & (age, etiology of infertility, rank of oocyte retrieval, type of ART)	Pubmed search selection: until July 2014 516 IVF/ICSI cycles 10 papers Stimulation: Need for higher doses of gonadotropins to achieve satisfactory results	Clinical pregnancy rate Vertical transmission	Cancellation of cycles: 1 study showed significance (15.2% HIV+ vs 4.9% controls). CPR/stimulation cycle: Range:6.7% to 24.1% HIV+ CPR/ET: Range: 9.1% to 63% HIV+ 6 matched control studies 2 studies: lower PR% in HIV+ 3 studies: PR not significant vs controls (but lower values) 1 study: PR not significant vs controls (but higher values) Vertical transmission = zero	PR in IVF/ICSI in couples with HIV+ women is 'conflicting' it is not clear if they have worse outcome. No specified which type of HIV (1 or 2)	No information on co-infections in this SR

WHICH TECHNIQUES CAN BE USED TO PREVENT/REDUCE HUMAN IMMUNODEFICIENCY VIRUS TRANSMISSION DURING MEDICALLY ASSISTED REPRODUCTION?

Semen processing

The evidence on semen processing will be discussed in detail in the next section

PrEP

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

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

Ejaculated sperm

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Zafer et al., 2016)	SR	40 studies included (37 papers, 3 abstracts) (18 prospective studies, 21 retrospective studies, 1 both prospective and retrospective data). 4257 HIV discordant couples 11915 ART cycles Men: age range: 29-58y Women: age range: 29-40y 27.6% (641) men: no antivirals at the moment of semen prod. 52.1% (985) men: not virally suppressed at the time of semen wash. CD4 levels range: 200-608cells/µl Not mentioned in SR methods if : HIV-1 and/or HIV-2. It is known that certain papers contained co-infected patients. It is not specified in the SR, but from analysing individual papers part of this SR; it is known.	Period: through Dec 2014 <u>Semen preparation:</u> method by Semprini et al. 1989 (29 studies) (reference to 2013 paper: gradient + wash + swim up). Table with techniques: (38 studies reported) Density gradient + swim up: 29 studies (4 studies HIV+ after semen prep (1.3%-7.7% (RNA and or DNA)? Density gradient only: 8 studies (1 study HIV+ (after semen prep (2.9% (DNA)) Double swim up: 1 <u>Analysis</u>: GRADE methodology Data from studies were pooled. 95%CI were calculated of HIV transmission risk per cycle and per couple. StataCorp v12.0		HIV RNA + post semen wash: 5 studies (1.3%7.7% of samples). No seroconversions in women (n=3994) after ART with washed HIV- semen in 11915 cycles. No vertical transmissions reported in 1026 newborns. Semen washing is safe and effective 93.8% of women had HIV test available before and after exposure to washed semen.	0% (0/3994) seroconversion in women (11585 ART cycles) HIV transmission risk is significantly lower ($p<0.001$) per ART cycle than the historical risk assessment of 0.1% per act in unprotected intercourse 0% vertical transmission (0/1026 newborn)	No difference between gradient and simple semen wash comparison. All studies performed at least gradient density centrifugation and most of then an extra swim-up. Density gradient + swimup: 4375 couples Semen post prep: HIVRNA+ [1.3 - 7.7] HIVDNA+ [2.6 - 5.6] Density gradient: 56 couples Semen post prep: HIV- Double swimup: 25 Semen post prep: HIV-

(Fourie et al., 2015)		HIV+ men (n=95) No info on co-infections in the paper Recent blood counts (<3 months prior to semen donation). Exclusion: CD4 <300 cells/µl 2 semen samples per week for 1-2 consecutive weeks. 1st sample = diagnostic sample 2nd sample= processed for therapeutic use	2008-2012 <u>Semen preparation:</u> Gradient (40/80) with the use of the proInsert tube without swimup. <u>HIV-1 testing:</u> RNA semen: Cobas ampliprep, Roche (detection limit: 20 cop/ml). HIV DNA semen: MangaPure 32 (Roche).		186 semen samples tested. 53.8% of neat semen samples were HIVDNA+(13.4%), HIVRNA+ (11.3%) or both+ (29%). Patients with undetectable viral load: 32.7% had semen sample HIV+RNA. Disagreement of viral load in blood and semen was random. After semen wash: 98.1% of semen samples were HIV-	ART in combination with semen preparation by gradient centrifugation and use of ProInsert is safe. Processed semen should be used of ART only when confirmed virus free by PCR.	Validation of the pro_insert tube Density gradient (pro Insert) 186 samples Semen post prep: HIVRNA+ 1.9%

(Inoue et al., 2017)		129 serodiscordant couples 183 ejaculates Average age male HIV-1+ : 37.2±4.3y CD4 count: 444±220x10^6 cells/ml viral load: 62±48.1 cop/ml 84% on HAART Average age female: 35.6±6.1y Patients could have co-infections with HBV, HCV or syphilis. <u>Exclusion</u> Female age ≥42y, Females were confirmed HIV- before treatment Azoospermia cases	Jan 2002 and April 2012 Single center (Kei university hospital) <u>Semen preparation:</u> Abstinence: 3-5 days Liquification: 15-60 min at RT Semen sample divided in 2 aliquots. Gradient centrifugation (continuous gradient (0%-80%). + swim-up (sample was introduced through an insert tube. <u>Ovarian stimulation:</u> GnRH agonist logn protocol or GnRH antagonist protocol – recombinant FSH as HMG. GnRH started midluteal of the previous cycle. GnRH antagonist started when 1 or more foll 14mm. GnRH administered until day of trigger: HCG (10000IU) when 3 or more foll ≥18mm (34h before OPU). ICSI was performed. Culture medium was tested for HIVRNA and HIVproviral DNA. HIV testing: QIAmp Ultrasens Virus Kit (Qiagen), nested PCR with proven possibility to detect a single virion in the presence up to 8x10^6 spermatozoa.		Implantation: HCG >25IU/L or gestational sac Clinical pregn: detection of gestational sac Births >22 weeks = abortions Births: 37-42 weeks = full term HIV testing in females: Not pregn: HIV AB tet 3 months after ET Pregn: HIV test at 36 weeks of gestation, at delivery and 6 months after birth 2.2% of semen samples after wash HIV+ 1 embryoculture HIV+ consistent with the sequence of the HIV+ partner.	No obvious malformations of babies (1 case of hydrocephalus and 1 case of glucose-6-Phosphate dehydrogenase deficiency). 91 live births, no horizontal infections of female partners, no vertical transmission in babies.	Semen prep with ET tube (proInsert like setting) Density gradient + swimup (insert tube) 129 couples Semen post prep: HIVRNA+ 2.2%

(Leruez-Ville et al., 2002)	CS	125 HIV-1+men No information on co-infections given. 34 men, not treated 25 men with nucleoside analogues (2 or 3 reverse transcriptase inhibitors) 66 received HAART	Oct. 1995 – Febr. 2000 <u>Semen preparation:</u> Sperm diluted 1:1 + (45/90) gradient) + pellet spermatozoa: 2x wash with medium (600xg and 200xg). HIV RNA blood: Monitor 1.5 (Roche), detection limit: 200cop/ml. Samples <200cop/ml, were tested with ultrasensitive protocol: limit: 20 cop/ml HIV RNA semen: threshold 20-340 cop/ml depending RNA extraction protocol cfr. PCR inhibitors (4x10^6 spermatozoa) used forRNA extraction. HIV DNA semen: treshold		In total: 40.7% (46/113) seminal fraction samples HIV RNA+ = 6.4% (5/12) men were HIV+ RNA 1.6% (2/125) men were HIV+DNA in semen <u>Untreated men (34)</u>: Seminal plasma: 78.8% HIV+RNA Spermatozoa: 17.6% HIV+RNA, 2.9% HIV+DNA Blood:88.2% HIV+RNA <u>Nucleoside analogues (25)</u> Seminal plasma: 62.5% HIV+RNA Spermatozoa: 4% HIV+RNA, 4% HIV+DNA Blood:76% HIV+RNA	In our study, semen processing with density gradient and pellet washing did not always completely eliminate HIV from fraction of spermatozoa. Probably because no swim up was performed. The use of unprocessed semen without prior viral validation could be discussed as a possibility for men with well-controlled blood and seminal plasma viral loads, it should be	In their conclusion, authors state: 'in our study, semen processing with density gradient and pellet washing did not always completely eliminate HIV from fraction of spermatozoa -> in methods section: only 2x wash is described? Probably there is a typing error: 'serum samples as 2ml undiluted portions or 1:1 diluted were put on two-layer (45-90%) gradient) -> probably this is 'semen' instead of 'serum'
(Pasquier et al., 2000)		32 HIV+ men 62.5% (n=20) anti-HIV antibodies in serum, 16 patients, HCV RNA in blood. 51 semen samples	Single center Toulouse, France <u>Semen preparation:</u> Semen pelleted 11000xg + gradient (50/70/90). Sperm pellet was washed 2x + swim up: pellet overlaid with 1.1ml medium 37°C, 5%CO2 60 min 45°. <u>HIV testing blood:</u> HIV-1 RNA plasma: amplicor Roche (detection limit: 20cop/ml) <u>HIV seminal plasma</u>: Amplicor HIV-1 monitor V1.5 assay (detection limit: 100cop/ml. <u>HIV testing in spermatozoa</u>: 2x 10^6 cells pelleted. Cell lysis by thermal shock (3x 15sec liq. Nitrogen and 30sec 60°C + incubation 1h 60°C. Proteinase K inhibition: 10 min 95°C. RNA precipitated with ETOH.		16/51 seminal plasma samples: HIV-1 RNA+ In spermatozoa sample: 0% HIV+ (RNA or DNA). Swim ups were HIV- After gradient: 50% fraction showed HIV+	The absence of HIV-1 in blood is not system systematically correlated with absence in seminal plasma. Absence in seminal plasma is not correlated with no virus in seminal cells. Motile 90% fraction after swim up was always HIV- The use of density gradient plus swim up reduces HIV-1 in the spermatozoa of doubly infected men	Co-infection with HCV. No data on HIV only.

(Persico et al., 2006)		55 HIV+ men Median age: 36y (28-43y) 74% (n=41) were on HAART. 14 patients did not take any drugs for treatment. 28 patients had viral load of <250cop/ml. HIV-1 RNA copies in blood: Average: 134 cop/ml (range 49-370 000). Mean CD4 cell count: 406±32 x 10^5/ml. No info on co-infections.	semen fraction after liquification was kept for HIV testing. <u>Semen preparation</u>: Density gradient (47-90) (30 min, 1600g) + seminal plasma was filtered using 0.2µm filter and stored at -80°C. Intermediate layer: 2x wash and stored. Semen pellet: wash (10min 1600xg + swim up 37°C, 5%CO2 60min. Upper layer was stored. HIV RNA in blood: Amplicor Roche detection limit: 50cop/ml For different semen cells suspension, different extractions were used. Statistical analysis: Bravias-pearson linear correlation. Spearman's rank correlation. Fisher's exact.		HIV-1 RNA + in 76% of blood samples. HIV-1 RNA + in 4% neat semen samples. HIV+RNA in 13% seminal plasma HIV+RNA in 3% non-semen cells HIV+RNA in 2% of samples after gradient and before swim up. HIV-1 RNA negative in all 46 samples after gradient and swim up. HIV-1 RNA detection limit: 50 cop/ml. HIV-1 DNA + in 100% of blood samples. HIV-1 DNA negative in all neat semen samples.	Why so low HIV RNA+ % in neat semen? probably die to PCR inhibitors. In processed semen, they are eliminated. On the other hand, the amount of HIV-1 RNA in the whole ejaculate could have been diluted below the detection limit. This confirms HIV-1 DNA in seminal compartments where 15% non-spermic cells were HIV+ despite 0% DNA+ in whole semen. Typical sperm wash techniques must include a final swim-up and HIV-1 RNA/DNA must be conducted on purified seminal compartments.	Density gradient + swimup: 55couples Semen post prep: All HIV- after swim up

(Zamora et al., 2016)	CS	Serodiscordant couples. No tresholds for viral load for accessing treatment. M&M: HBV and HCV were tested in the patients but no information is given in the patient characterstics.	Jan. 2006-Sept. 2013 269 sperm wash procedures 183 couples 234 completed ICSI cycles (105 cycles own oocytes, 129 donor oocytes) Monocentric study: Spain <u>Semen preparation:</u> Semen diluted 1/1 with sperm medium Centrifugation (400xg, 20min), supernatants discarded. Pellet resuspended (=1ml sperm medium) + gradient (45/70/90) (1/1/2 ml) (300xg, 20 min). Pellet resuspended in 5ml sperm medium centrifuged 250xg, 10min), second wash in 2.5ml. Pellet resuspend in 1-12ml en swim up (45°, RT, 1h HIV-1 RNA (amplicor roche) (sensitity: 400cop/ml) and HIV DNA		5/263 semen samples= 1.86% were HIV+ after triple gradient	In the light of the difference in viral load between blood and semen, we see no reason to offer extended semen wash to all serodiscordant couples, regardless of serological viral load	No information on transmission rate Density gradient + swimup: 263 samples Semen post prep: HIVRNA+: 1.86%

Surgically retrieved sperm

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Garrido et al., 2009)	Case report	1 couple, HIV+ male 40y old, no detectable viral load in blood. CD4+: 242 cells/ml No co-infections. Female partner = seronegative. No info on HIV-1 or HIV-2	<u>Semen preparation</u> The TESE fragments were minced mechanically with sterile slides. The suspension was transferred to a falcon tube and centrifuged at 600g for 5 minutes. The pellet was suspended in bicarbonate buffered medium and incubated at 37°C, 5% CO2 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 0.5-1ml.		3 OPU, 22 oocytes obtained in total. 10 MII, 6 Zygotes, 3 embryos transferred in 2 embryo transfer procedures. No pregnancies.	TESE-ICSI treatments in azospermic seropositive males is a viable approach, together with sperm washing and PCR confirmation of viral absence should be performed.	
(Leruez-Ville, et al., 2013)	Case report	2 HIV-1 + males Non-obstructive azospermia Undetectable blood viral load, under HAART CD4: 929/mm^3 and 169/mm^3	Semen collection= TESE for HIV-1 + males: <u>Preparation:</u> The testicular tissue pieces were washed to eliminate blood contamination. Sterile needles were used for seminiferous tubules dilacerations. The suspension was centirfuges at 300g for 20 min. on 1 ml 45% Puresperm. The sperm pellet was collected and resuspended in 5 ml medium and centrifuges for		Resulting pellet after semen prep was sent for HIV-1 RNA detection -> negative.	ICSI was done in 1 couples only, as no sperm was found in the TESE prep. TESE prep of both patients was sent for HIV-1 RNA and were both negative. No pregnancies. HIV testing in the female partners was negative after the ICSI	No info on co-infections are given -> however, this paper also describes 4 HCV males. It could be assumed that it are mono-infectious persons

			10 min. at 600g. After 2 washes, the pellet was resuspended and used for ICSI HIV-1 RNA detection: COBAS Ampliprep Total nucleic acid isolation kit. Treshold 100IU/ml for semen. For blood: 12 cop/ml.			cycle.	
(Nicopoullos et al., 2004) Nicopoullos, J. D., Frodsham, L. C., Ramsay, J. W., Almeida, P. A., Rozis, G. and Gilling-Smith, C. Synchronous sperm retrieval and sperm washing in an intracytoplasmic sperm injection cycle in an azoospermic man who was positive for human immunodeficiency virus. Fertil Steril. 2004; 81 (3): 670-4.	Case report	1 HIV+ male Obstructive azospermia due to vasal aplasia. Viral load: 500.000 cop/ml CD4: 830x10^6/L Female partner = HIV negative	MESA collection + sperm washing: No details on semen preparation only reference to previous work: Nicopoullos, J. D., Almeida, P., Vourliotis, M., Goulding, R. and Gilling-Smith, C. A decade of sperm washing: clinical correlates of successful insemination outcome. Hum Reprod. 2010; 25 (8): 1869-76. <u>Testing for HIV-1 RNA</u> Nucleic acid testing for HIV-1 RNA, detection limit: >25cop/10^6sperm.		Following sperm washing: HIV-1 RNA testing was undetectable. ICSI was performed on 6 MII oocytes, 3 ET was performed. HCG test was negative.	Sperm washing can be applied in cases of sperm retrieval where sperm volume and density if low allowing treatment of azospermic HIV pos men.	

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

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Zafer, et al., 2016)	SR	40 studies included (37 papers, 3 abstracts) (18 prospective studies, 21 retrospective studies, 1 both prospective and retrospective data). 4257 HIV discordant couples 11915 ART cycles Men: age range: 29-58y Women: age range: 29-40y 27.6% (641) men: no antivirals at the moment of semen prod. 52.1% (985) men: not virally suppressed at the time of semen wash. CD4 levels range: 200-608cells/µl Not mentioned in SR methods if : HIV-1 and/or HIV-2. It is known that certain papers contained co-infected patients. It is not specified in the SR, but from analysing individual papers part of this SR; it is known.	Period: through Dec 2014 <u>Semen preparation:</u> method by Semprini et al. 1989 (29 studies) (reference to 2013 paper: gradient + wash + swim up). Table with techniques: (38 studies reported) Density gradient + swim up: 29 studies (4 studies HIV+ after semen prep (1.3%-7.7% (RNA and or DNA)? Density gradient only: 8 studies (1 study HIV+ (after semen prep (2.9% (DNA)) Double swim up: 1 <u>Analysis</u>: GRADE methodology Data from studies were pooled. 95%CI were calculated of HIV transmission risk per cycle and per couple. StataCorp v12.0		HIV RNA + post semen wash: 5 studies (1.3%7.7% of samples). No seroconversions in women (n=3994) after ART with washed HIV- semen in 11915 cycles. No vertical transmissions reported in 1026 newborns. Semen washing is safe and effective 93.8% of women had HIV test available before and after exposure to washed semen.	0% (0/3994) seroconversion in women (11585 ART cycles) HIV transmission risk is significantly lower ($p<0.001$) per ART cycle than the historical risk assessment of 0.1% per act in unprotected intercourse 0% vertical transmission (0/1026 newborn)	No difference between gradient and simple semen wash comparison. All studies performed at least gradient density centrifugation and most of then an extra swim-up.

(Fiore et al., 2005)		8 semen samples (normospermia WHO) of 8 HIV- men used for spiking experiment. PBMC from HIV+ patient (no info on co-infection is given) Viral loads: $1x10^3$ // $5x10^3$ // $1x10^4$ //$5x10^{4//}$ $1x10^5$/// $5x10^5$ // 1 x 10^6 // $3x10^6$ cop/ml Incubation semen + viral load: 10 min, 37°C Samples were tested for HIV-1 RNA (detection limit:80cop/ml).	<u>HIV+ semen preparation:</u> Sample divided in 3 aliquots: 500-700µl and filled up to 1ml of different dilutions of HIV+ PBMC) -> exp 1-2-3. <u>Semen preparation</u>: Spiked semen add 4ml sperm buffer centrifugation 1200rpm, 10min + supernatant add 2 ml sperm buffer + density (40/80) (2300rpm, 10 min) *+ pellet wash 4 ml sperm medium (1200rpm, 10min) + swim up (30 min 37°C (pellet add 1 ml sperm medium). HIVRNA test before swim up* (4x250µl of semen pellet) and after swim up (4x250 µl final suspension).		<u>HIV-1 RNA testing results:</u> $1x10^3$ // $5x10^3$ // $1x10^4$ // $5x10^4$ // -> HIV negative after gradient and after swimup $1x10^5$// $5x10^5$ // -> HIV positive (¼ samples) after gradient and negative after swimup 1 x 10^6 // $3x10^{6\ ->}$ HIV positive after gradient and postive after swimup (2/4 samples)	Efficiency of sperm washing in removing HIV-1 varies according to the amount of virus present in the sample. Viral evaluation of processed semen in HIV serodiscordant couples is mandatory before ART.	*Spiking experiment*

(Inoue, et al., 2017)		129 serodiscordant couples 183 ejaculates Average age male HIV-1+ : 37.2±4.3y CD4 count: 444±220x10^6 cells/ml viral load: 62±48.1 cop/ml 84% on HAART Average age female: 35.6±6.1y Patients could have co-infections with HBV, HCV or syphilis. <u>Exclusion</u> Female age ≥42y, Females were confirmed HIV- before treatment Azoospermia cases	Jan 2002 and April 2012 Single center (Kei university hospital) <u>Semen preparation:</u> Abstinence: 3-5 days Liquification: 15-60 min at RT Semen sample divided in 2 aliquots. Gradient centrifugation (continuous gradient (0%-80%). + swim-up (sample was introduced through an insert tube. <u>Ovarian stimulation:</u> GnRH agonist logn protocol or GnRH antagonist protocol – recombinant FSH as HMG. GnRH started midluteal of the previous cycle. GnRH antagonist started when 1 or more foll 14mm. GnRH administered until day of trigger: HCG (10000IU) when 3 or more foll ≥18mm (34h before OPU). ICSI was performed. Culture medium was tested for HIVRNA and HIVproviral DNA. HIV testing: QIAmp Ultrasens Virus Kit (Qiagen), nested PCR with proven possibility to detect a single virion in the presence up to 8x10^6 spermatozoa.		Implantation: HCG >25IU/L or gestational sac Clinical pregn: detection of gestational sac Births >22 weeks = abortions Births: 37-42 weeks = full term HIV testing in females: Not pregn: HIV AB tet 3 months after ET Pregn: HIV test at 36 weeks of gestation, at delivery and 6 months after birth 2.2% of semen samples after wash HIV+ 1 embryoculture HIV+ consistent with the sequence of the HIV+ partner.	No obvious malformations of babies (1 case of hydrocephalus and 1 case of glucose-6-Phosphate dehydrogenase deficiency). 91 live births, no horizontal infections of female partners, no vertical transmission in babies.	Semen prep with ET tube (proInsert like setting) Density gradient + swimup (insert tube) 129 couples Semen post prep: HIVRNA+ 2.2%

(Kuji et al., 2008)		Spiking experiment without use of semen. Only artificial mixtures were used. HIV-1 LAI strain produced from chronically infected MOLT-4 cells = viral source Viral load in the sample was approx. 10cop/20µl. HIV-1 LAI strain (0.2ml) was mixed with 2.5ml 65% Percoll or 2.5ml 50% Pureception. Final concentration: $1.15x10^6$/ml.	<u>Preparation:</u> Pureception and Percoll was used. 1) Isopyknic 65% Percoll: 16400g 20min 50% Pureception: 11400xg 20min Aliquots 0.25ml were fractionated beginning at the bottom of the tube. 2) preparation: Continuous gradients: 80% Percoll or 90%Pureception. Centrifugation: 1600g for 5, 10,20 ane 40 minutes. <u>PCR testing:</u> RT-PCR p24 antigen using MiniVidas (Biomérieux)		<u>HIV-1 RNA testing results:</u> RNA loads highest at 1.042g/cm^3 When using continuous gradients: most HIV-1 particles were found at gravity less than 1.04 even after 40 minutes of centrifugation in both gradients. In Pureception small viral accumulations were observed at the bottom of the tube, in Percoll this finding was absent.	The calculated bouyant density of HIV-1 was approximately 1.042 in isopyknic centrifugation. Most HIV-1 particles were found at gravity less than 1.04. Small viral accumulations were found at the bottom of the tube.	*Spiking experiment*

(Leruez-Ville, et al., 2002)	CS	125 HIV-1+men No information on co-infections given. 34 men, not treated 25 men with nucleoside analogues (2 or 3 reverse transcriptase inhibitors) 66 received HAART	Oct. 1995 – Febr. 2000 <u>Semen preparation:</u> Sperm diluted 1:1 + (45/90) gradient) + pellet spermatozoa: 2x wash with medium (600xg and 200xg). HIV RNA blood: Monitor 1.5 (Roche), detection limit: 200cop/ml. Samples <200cop/ml, were tested with ultrasensitive protocol: limit: 20 cop/ml HIV RNA semen: threshold 20-340 cop/ml depending RNA extraction protocol cfr. PCR inhibitors ($4x10^6$ spermatozoa) used forRNA extraction. HIV DNA semen: treshold $2x10^6$ spermatozoa used) 5 cop/10^6 spermatozoa		In total: 40.7% (46/113) seminal fraction samples HIV RNA+ = 6.4% (5/12) men were HIV+ RNA 1.6% (2/125) men were HIV+DNA in semen <u>Untreated men (34)</u>: Seminal plasma: 78.8% HIV+RNA Spermatozoa: 17.6% HIV+RNA, 2.9% HIV+DNA Blood:88.2% HIV+RNA <u>Nucleoside analogues (25)</u> Seminal plasma: 62.5% HIV+RNA Spermatozoa: 4% HIV+RNA, 4% HIV+DNA Blood:76% HIV+RNA <u>HAART (66)</u>: Seminal plasma: 8.9% HIV+RNA Spermatozoa: 1.5% HIV+RNA, 0% HIV+DNA Blood:1.5% HIV+RNA	In our study, semen processing with density gradient and pellet washing did not always completely eliminate HIV from fraction of spermatozoa. Probably because no swim up was performed. The use of unprocessed semen without prior viral validation could be discussed as a possibility for men with well-controlled blood and seminal plasma viral loads, it should be prohibited for men with partially or poorly controlled HIV infection	In their conclusion, authors state: 'in our study, semen processing with density gradient and pellet washing did not always completely eliminate HIV from fraction of spermatozoa -> in methods section: only 2x wash is described? Probably there is a typing error: 'serum samples as 2ml undiluted portions or 1:1 diluted were put on two-layer (45-90%) gradient) -> probably this is 'semen' instead of 'serum' No information on transmission rate Density gradient + 2 wash: 113 samples Semen post prep: HIVRNA+: 6.4%

(Pasquier, et al., 2000)		32 HIV+ men 62.5% (n=20) anti-HIV antibodies in serum, 16 patients, HCV RNA in blood. 51 semen samples	Single center Toulouse, France <u>Semen preparation:</u> Semen pelleted 11000xg + gradient (50/70/90). Sperm pellet was washed 2x + swim up: pellet overlaid with 1.1ml medium 37°C, 5%CO2 60 min 45°. <u>HIV testing blood:</u> HIV-1 RNA plasma: amplicor Roche (detection limit: 20cop/ml) <u>HIV seminal plasma</u>: Amplicor HIV-1 monitor V1.5 assay (detection limit: 100cop/ml. <u>HIV testing in spermatozoa</u>: 2x 10^6 cells pelleted. Cell lysis by thermal shock (3x 15sec liq. Nitrogen and 30sec 60°C + incubation 1h 60°C. Proteinase K inhibition: 10 min 95°C. RNA precipitated with ETOH.		16/51 seminal plasma samples: HIV-1 RNA+ In spermatozoa sample: 0% HIV+ (RNA or DNA). Swim ups were HIV- After gradient: 50% fraction showed HIV+	The absence of HIV-1 in blood is not system systematically correlated with absence in seminal plasma. Absence in seminal plasma is not correlated with no virus in seminal cells. Motile 90% fraction after swim up was always HIV- The use of density gradient plus swim up reduces HIV-1 in the spermatozoa of doubly infected men	Co-infection with HCV. No data on HIV only.

(Persico, et al., 2006)		55 HIV+ men Median age: 36y (28-43y) 74% (n=41) were on HAART. 14 patients did not take any drugs for treatment. 28 patients had viral load of <250cop/ml. HIV-1 RNA copies in blood: Average: 134 cop/ml (range 49-370 000). Mean CD4 cell count: 406±32 x 10^5/ml. No info on co-infections.	semen fraction after liquification was kept for HIV testing. <u>Semen preparation</u>: Density gradient (47-90) (30 min, 1600g) + seminal plasma was filtered using 0.2µm filter and stored at -80°C. Intermediate layer: 2x wash and stored. Semen pellet: wash (10min 1600xg + swim up 37°C, 5%CO2 60min. Upper layer was stored. HIV RNA in blood: Amplicor Roche detection limit: 50cop/ml For different semen cells suspension, different extractions were used. Statistical analysis: Bravias-pearson linear correlation. Spearman's rank correlation. Fisher's exact.		HIV-1 RNA + in 76% of blood samples. HIV-1 RNA + in 4% neat semen samples. HIV+RNA in 13% seminal plasma HIV+RNA in 3% non-semen cells HIV+RNA in 2% of samples after gradient and before swim up. HIV-1 RNA negative in all 46 samples after gradient and swim up. HIV-1 RNA detection limit: 50 cop/ml. HIV-1 DNA + in 100% of blood samples. HIV-1 DNA negative in all neat semen samples.	Why so low HIV RNA+ % in neat semen? probably die to PCR inhibitors. In processed semen, they are eliminated. On the other hand, the amount of HIV-1 RNA in the whole ejaculate could have been diluted below the detection limit. This confirms HIV-1 DNA in seminal compartments where 15% non-spermic cells were HIV+ despite 0% DNA+ in whole semen. Typical sperm wash techniques must include a final swim-up and HIV-1 RNA/DNA must be conducted on purified seminal compartments.	Density gradient + swimup: 55couples Semen post prep: All HIV- after swim up

(Zamora, et al., 2016)	CS	Serodiscordant couples. No tresholds for viral load for accessing treatment. M&M: HBV and HCV were tested in the patients but no information is given in the patient characterstics.	Jan. 2006-Sept. 2013 269 sperm wash procedures 183 couples 234 completed ICSI cycles (105 cycles own oocytes, 129 donor oocytes) Monocentric study: Spain <u>Semen preparation:</u> Semen diluted 1/1 with sperm medium Centrifugation (400xg, 20min), supernatants discarded. Pellet resuspended (=1ml sperm medium) + gradient (45/70/90) (1/1/2 ml) (300xg, 20 min). Pellet resuspended in 5ml sperm medium centrifuged 250xg, 10min), second wash in 2.5ml. Pellet resuspend in 1-12ml en swim up (45°, RT, 1h HIV-1 RNA (amplicor roche) (sensitity: 400cop/ml) and HIV DNA		5/263 semen samples= 1.86% were HIV+ after triple gradient	In the light of the difference in viral load between blood and semen, we see no reason to offer extended semen wash to all serodiscordant couples, regardless of serological viral load	No information on transmission rate Density gradient + swimup: 263 samples Semen post prep: HIVRNA+: 1.86%

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

No studies could be found investigating this PICO question.

DOES THE PLASMATIC_VIRAL LOAD CORRELATE WITH HUMAN IMMUNODEFICIENCY VIRUS IN SEMEN?

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Ball et al., 1999)	CS	34 HIV-1+ persons Various CD4 cell counts No anti-viral therapy in the preceding 3 months No info on co-infections	Cross-sectional comparative study 5ml blood EDTA processed within 6h after collection 250µl semen diluted 1/1 RPMI +2% formaldehyde Blood and semen: pelleted 1500g, 5 min. Pellets suspended in 10ml RPMI. Semen: centrifugation 1500g, 20 min PBMC prep by ficoll. Final pellets resuspended in 100µl RPMI, divided in 2 aliquots and stored at -70°C. HIV RNA determined using NASBA using 50µl as input. Cut-off: 2000 viral particles/ml? DNA extraction using DNA extraction kit from Strategene (10µg/ml proteinase K for 18h at 37°C. Nested PCR was performed. Statistics: spearman's rank correlations between paired titres and Wilcoxon's signed rank correlation.		Proviral DNA Viral RNA 32 Paired samples: blood and semen Proviral DNA Blood: 100% (31/31) Median 496 cop/ml [9-5678] Semen: 47% (15/32) Median: <6 cop/ml [<6-2171] Viral RNA Blood: 76% (26/34) Median: 18600 cop/ml [<2000-977600] Semen: 63% (19/30) Median: 5600 cop/ml [<2000 - 667800] -> proviral DNA in semen was associated with concomitant viral RNA in semen (p<0.05 Fisher's exact). -> proviral DNA and viral RNA were higher in blood compared to corresponding semen sample (p<0.0001). (in 2/19 patients this trend was reversed). -> a strong (= actuallly moderate) correlation existed between the blood and semen viral RNA titres (r= 0.5156, p<0.005).	Positive correlations between: 1) semen proviral DNA and semen viral RNA titres 2) semen and blood RNA titres 3) semen proviral titres and blood proviral titres. Blood proviral titres were inversely correlated to blood CD4 cell counts (r= -0.3683, p>0.05)	

(Bujan et al., 2004)		94 HIV+ infected HIV patients, no info on co-infection status. 281 paired blood and semen samples. 1st visit: median age: 37y, range [25-50y]. Median duration of HIV infection: 144.5 months [range 10.3 - 238.7]. All persons were clinically asymptotic. 92.5% (78 persons) were on antiretroviral therapy = 17% (16) on 2 nucleoside inhibitors and 75% (71) receiving more than 3 or more drugs.	Between April 1998 and Jan 2001 HIV RNA Blood: HIV-1 RNA quantified on amplicor HIV-1 monitor v1.5 using the ultrasensitive protocol. Detection limit >20 cop/ml. Semen: nuclisense protocol and same test as blood. Detection limit for semen >100 cop/ml. HIV DNA Spermatozoa: amplicor HIV-1 monitor v1.5. detection limit for HIV DNA: >5 cop/10^6 cells. Statistics: Mann-whitney to compare semen qualitative data. Fisher's exact to compare qualitative data.		HIV RNA blood 72.2% (68/94) patients had detectable viral load in blood. 53.7% (151/281) blood samples were HIV+ for RNA: 123 cop/ml [range 3-130 000). HIV RNA seminal plasma HIV+ RNA was detected in 38 semen samples: 201 cop/ml [range 5-277 500 cop/ml]. (233 samples were HIV- and 10 samples could not be quantified due to PCR inhibitors). HIV-1 RNA concentrations in blood and seminal plasma were not correlated (r=-0.2, p>0.05). When blood HIV RNA was detected, 19.4% of semen samples were HIV+ for RNA. When blood HIV RNA was undetectable, 7.9% of seminal plasma was HIV+ for RNA. HIV-1 DNA was detected in 8.7% of native semen samples. The median blood CD4 count tended to be lower when HIV-1 RNA was detectable in seminal plasma than when it was undetectable.	6-10% of the sperm samples were DNA HIV+ where their seminal plasma samples were negative for HIV-1 RNA. Undetectable seminal plasma RNA levels do not mean absence of HIV-1 genomes in sperm cells. Although HAART may reduce blood RNA to undetectable levels, this does not mean that there are no viral genomes in semen. Negative HIV RNA in semen does not mean negative HIV DNA in semen. Negative results for DNA and RNA in semen on one day, does not predict the result on any of the following days.	

(Cheret et al., 2017)	CT	19 patients Inclusion: Primary HIV-1 infection (PHI) CD4 <500 cells/µl blood PHI = HIV-RNA in plasma 19 patients were studied: 12 intensive CART group 7 standard triple drug group CART = combined anti-retroviral therapy No info on co-infection status	HIV reservoir substudy Blood and semen during PHI (day 0) and at 24 months. Semen and blood: HIVRNA: Cobas Ampliprep Taqmann assay v2 (Roche). Det limit below 100cp/ml Total HIV-DNA extracted using QIAamp DNA microkit (Qiagen) quantified using realtime PCR. Det limit 5 cop/PCR. 1mg was used per PCR (equivalent of 150.000 cells). Total DNA was quantified by 260nm and stored at -20°C. Statistics using SAS and R. Results on HIV-1 subtypes		During PHI: Blood HIV-RNA > semen HIV-RNA 5.66 vs 4.22 log10 copies/ml p<0.0001). HIV-DNA detected in 10/19 patients in semen. After 2 years of cART: all patients had undetectable HIV-RNA in blood and semen. Semen HIV-RNA load correlated well with blood HIV-RNA in patients with acute infection (r=0.81, p=0.015), but not in those with recent infection.		
(Du et al., 2016)		19 HIV+ men undergoing antiretroviral therapy for 6 months. No info on co-infection status. Median age: 33y Median CD4: 418cells/µl Paired semen and blood samples were taken	HIV -1 RNA quantification in semen and blood: BioMérieux BV assay, detection limit >50 cop/ml. HIV V3 loop B and C are present in HIV-1		Blood HIV RNA was undetectable in 17/19 persons. Seminal HIV RNA was detectable in 16/19 persons.	HIV RNA was undetectable in plasma of most patients, whereas HIV RNA could be detected in most semen samples.	No r or p value on correlation statistics

(Ferraretto et al., 2014)		88 HIV-1+ men 306 semen samples All patients were on antiretroviral therapy and had undetectable viral load (<50 cop/ml) in blood for more than 6 months. No info on co-infection status.	Jan 2006 – Dec 2011. HIV RNA in semen quantified on Roche COBAS Ampliprep detection limit: >200 cop/ml		7.5% (23/306) were HIV+ RNA (>200cop/ml) in seminal plasma in patients with an undetectable viral load in blood.	We show intermittent shedding for HIV-1 RNA in semen of patients given efficacious antiretroviral therapy.	No r or p value on correlation statistics
(Gupta et al., 2000)	CS	18 HIV-1+ persons, asymptomatic, no info on co-infection status No patient was taking potent anti-viral therapy. Median CD4 count 343 range [117-935] Paired blood and semen samples were collected weekly.	Blood Heparinized blood pelleted at 122g RT. Semen Seminal cells were pelleted from whole semen by centrifugation 800-1000g for 10min. Supernatants frozen at -70°C. Pellets resuspended in 5ML HBSS -> ficoll gradient centrifugation. Seminal mononuclear cells were collected and frozen in DMSO at -130°C. HIV RNA nuclisense kit on NASBA. Detection limit 200-400 cop/ml.		3 patterns of prevalence of HIV-1 RNA in semen. Intermittent shedder: Plasma HIV viral load quite stable, semen fluctuates. Persistent shedder: Plasma HIV viral load and viral load in semen shows some relation. Non shedder: Ver low to median viral load in blood, no viral detection in semen. There was no relationship between the pattern of virus load in blood and in semen.	The source of HIV-1 in semen is complex and is related to the pattern of shedding in semen. The data shows that subjects with intermittent shedding of HIV-1 in semen, that the virus population in semen was distinct from that in blood and there was no correlation between the level of virus in blood and semen.	The emphasis of this study is on the shedding of HIV in semen in 18 HIV + persons -> but this gives an idea on why certain studies do find a correlation (probably because the small patient cohorts can constitute of persistent shedders – correct?) No r or p value on correlation statistics

(Kalichman et al., 2008)	SR	19 empirical studies Total number of HIV+ men included across the studies: 1226 No info on co-infection status in the SR	Studies reported on correlation between blood viral load and semen viral load. 15/19 studies detecting HIV RNA on NASBA (nucleic acid sequence based amplification) (nuclisense assay) = an assay that is relatively unaffected by factors in semen that inhibit HIV RNA detection. Nuclisense assay is on HIV-1		Correlation ranged between 0.07 to 0.64 for HIV RNA viral load detection between blood and semen. The mean correlation was 0.45 (SD= 0.20, median: 0.45). There is little evidence that the association between blood and semen viral load is influenced by disease stage.	Semen viral load was generally lower than blood viral load, but this was variable across studies.	
(Kariuki et al., 2020)		43 HIV+ men No antiretroviral therapy Mean age: 29y Median CD4: 519cells/µl Median blood viral load 4.10log10 cop/ml HIV-1 detected in semen when blood viral load >10.000 cop/ml	Between June 2015 and January 2017 Semen prep: 1/1 diluted semen with PBS Underlaid 19% Nycodenz in PBS (1000g, 20 min) to separate semen cells from other cells		Log10 viral loads in semen correlated moderately with log 10 viral loads in blood R^2= 0.1556 p=0.026 There is an independent HIV-1 replication in the male genital tract resulting in shedding into the seminal plasma. Even when undetectable viral loads exist in blood, it is not always sufficient to suppress shedding.		

(Lambert-Niclot et al., 2012)		304 HIV patients No info on co-infection status. 628 paired blood and semen samples. Each patient provided 1 to 8 samples. All patients were on HAART with blood viral load <40 cop/ml for more than 6 months.	Between Jan 2002 and June 2011 HIV-1 RNA detection on Cobas Taqman HIV-1 assay (Roche): Detection limit blood: 20-40 cop/ml Detection limit semen: 100-200 cop/ml.		6.6% (20/304) patients had at least one HIV RNA+ seminal sample although the plasma viral load was undetectable. The HIV RNA viral load ranged from 135 to 2365 cop/ml.	HIV-1 secretion is intermittent. There was an association between HIV-1 RNA in plasma and HIV-1 DNA in blood, but both were not associated with seminal HIV-1 RNA.	No r or p value on correlation statistics

(Leruez-Ville, et al., 2002)	CS	125 HIV-1+ men, no info on co-infection status 34 no anti-viral therapy 25 therapy: 1 or 2 reverse transcriptase inhibitors 66 HAART	Between Oct 1995 and Febr 2000 Cross sectional study to assess the burden in semen of untreated HIV+ men Longitudinal study (18 months) to evaluate the dynamic evolution of HIV shedding in response to prolonged HAART. Paired blood and semen. Blood: HIV RNA (HIV-1 monitor Roche) detection limit: 200cop/ml. When <200cop/ml than ultrasensitive protocol: detection limit: <20 cop/ml Semen: nuclisense extraction kit protocol, detection limit: 20-340 cop/ml		Blood HIV+ RNA in 50/125 (40%) men. Semen HIV+ RNA in 46/113 seminal plasma fractions (40.7%) Untreated men: Blood Median RNA load: 18000 cop/ml [<200 – 570000] Seminal plasma: 5500 cop/ml [<20-1 000 000]; Treated transcriptase inhibitors Blood Median RNA load: 6000 cop/ml [<200 – 450000] Seminal plasma: 700 cop/ml [<20-12700]. HAART Blood All (except 1) < 200 cop/ml. Ultrasensitive protocol: 17.5% residual blood viral load between 20-200 cop/ml median: 56 cop/ml [22-160] Seminal plasma: 1419 cop/ml [84-3224]. -> HIV RNA loads in blood and seminal plasma correlated significantly (Spearman Rank r=0.75, p<0.0001). 6.4% of the men showed HIV RNA is spermatozoa fraction. 1.6% of the men showed proviral DNA in spermatozoa fraction.	HIV detection in spermatozoa was more likely to be positive for men with high HIV replication level in blood or seminal plasma.	

(Liuzzi et al., 1996)	Cross sectional study	Blood and semen paired samples of 23 HIV-1+ patients. Age between 26 – 38y. All patients are NOT on therapy. CD4+ cell count range [79 – 728x10^6/L].	RNA extraction using guanidinium thiocyanate methods. HIV RNA detection through RT-PCR. Statistics: spearman's rank Check HIV-1 HIV1/2		Blood HIV RNA median: 14 817cop/ml Range [167-254 880] Semen HIV RNA median: 162 cop/ml Range [0-72 080] Viral load significantly lower in semen that in plasma ($p<0.0001$). RNA levels between plasma and semen were not correlated ($r=0.199$, $p>0.05$).	Semen and plasma HIV viral load are not correlated and viral load in semen is independent of the clinical stage of HIV-1 infection and of CD4+ cell count. HIV infected individuals are potentially infectious at all stages of immunodeficiency.	
(Pasquier et al., 2017)		1396 paired semen and blood samples collected from 362 HIV+ men. Mean age at first consultation: 39y±6y (median 39y). Mean duration of HIV infection was 11±6 years (median 11y). 92% (299/362) were treated in the study period and 83% (299/362) with a triple or more Co-infections with HBV and HCV are given in the study (up to 40% of patients)	Between Jan 1998 and December 2013 HIV-1 RNA testing: Cobas taqman HIV-1 assay (Roche) detection limit >20 cop/ml, for semen, detection limit: >200cop/ml. Statistics: Chi square, Fisher's exact or Mann Whitney U. (SAS)		Seminal shedding occurred in 13% of the patients (46/362). The blood viral load of 52% (187/362) was always undetectable and always detectable in 95 men (26%). HIV seminal shedding was 4x less frequent: 5.3% and at least 5x less abundant (mean 213 cop/ml (range [<200-4388].	Residual HIV shedding occurred in 6.1% of patients on antiretroviral therapy.	This study also looks at the differences between shedders and none shedders, on shedding patterns and on shedding in relation to treatment. This is not in the PICO but could be good information. Although statistical analysis is described in M&M, no information on statistics in report

(Politch et al., 2016)	CS	60 HIV+ men on HAART for at least 3 months., no info on co-infection status 8/60 men had detectable viral loads in blood and were excluded. -> 52 patients had undetectable viral load in blood and were included. Median age: 42.5y [24-59] Median CD4+ count: 518.5 cells/mm^3 [108 – 1492].	Blood, pre-ejaculatory (PE) sample and semen collected. PE samples were centrifuged at high speed (15 600g) for 40 sec. Semen was spun at 600g, 10 min. Supernatant was collected and semen pellet resuspended in PBS. HIV-1 RNA quantified using nuclisense protocol. Detection limit >40 cop/ml for blood, >80 cop/ml for PE and semen.		Undetectable viral load in blood: 19% (10/52) HIV men had HIV RNA in semen (range [59-800 cop/ml] (none had RNA in PE samples).	High levels of RNA in pre-ejaculate fluid, however, none of the men on stable HAART with undetectable viral load in blood had HIV in pre-ejaculate samples, but they had HIV RNA in semen.	Although the emphasis of the study is on pre-ejaculatory secretions, it is of interest that 52 men had undetectable viral loads in blood and 19.2% of these patients had HIV RNA in semen. No r or p value on correlation statistics
(Politch et al., 2012)		101 HIV+ men 101 paired semen and blood samples Median age: 43y. Median CD4 count: 513 cells/mm3. 80% on HAART for > 1year and 72% had been on their current HAART for > 6 months.	HIV-1 RNA quantification RT-PCR: Detection limit > 80 cop/ml. HIV-1 DNA: PCR: detection limit: >100cop/ml. Statistics: mann whitney U, spearman rank, Fisher exact		HIV RNA+ Blood: 18% men Median 560 cop/ml (range [80-640 000]. Semen: 30% (HIV RNA and/or DNA) Men with HIV RNA in blood had a higher prevalence of HIV in semen than men without HIV RNA in blood (p=0.049).	HAART does not completely eliminate HIV from semen.	No r or p value on correlation between semen and blood

(Sheth et al., 2009)	CS	25 HIV+ men having no therapy Patients were also tested for syf, chlamydia, herpes, simplex (HSV) and CMV. 36% HSV+ and 100% CMV+ No info on HCV or HBV status. Blood and semen collected at 0,2, 4, 8, 12, 16, 20 and 24 weeks. In total 116 paired samples tested.	HIV-1 RNA analysed using Versand HIV-1 RA 3.0 assay (Bayer); Detection limit: 50 cop/ml for blood and 300 cop/ml for semen.		At week 16 all persons had undetectable viral loads and 23/15 also had undetectable viral loads in semen. Semen shedding was present during 19/116 study visits (16.4%) with undetectable blood HIV RNA. No association was found between isolated semen shedding and specific antiretroviral agents of classes.	Isolated HIV RNA semen shedding was detected in many participants despite the viral suppression in blood. Antiretroviral therapy is likely to substantially reduce HIV transmission at a population level, but substantial interindividual heterogeneity in semen viral load despite undetectable blood viral load suggests that some individuals remain sexually infectious.	No r or p value on correlation statistics
(Xu et al., 1997)		74 HIV+ men NOT on antiviral therapy. 53 blood samples and 74 semen samples No info on co-infection status	Between Febr. 1989 – April 1993 HIV-1 DNA detection trough PCR analysis and gel electrophoresis.		HIV-1 DNA detected in 100% of blood Range [20-2500 cop/ml] HIV-1 DNA detected in 65% of semen Range [<10 – 5000 cop/ml] Correlation between HIV DNA in blood and semen: $r=0.35$, $p<0.05$. The concentration of HIV-1 DNA was sign. Higher in blood than in semen ($p<0.0001$). There was a sign. Inverse correlation between CD4 cell count and HIV-1 DNA in semen.	HIV-1 DNA in semen correlated significantly with HIV-1 DNA in blood	

WHICH INTERVENTIONS CAN BE USED TO REDUCE/AVOID VERTICAL TRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS TO THE NEW-BORN?

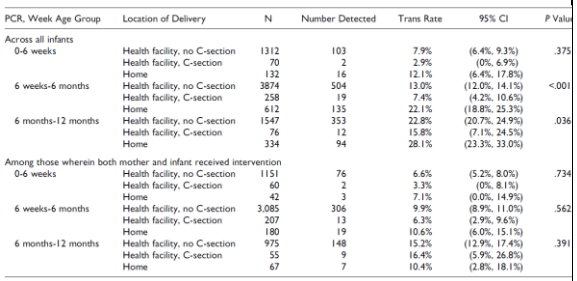
ECS

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Kennedy et al., 2017)	SR		Studies investigating risk of vertical transmission of HIV with ECS RCTs CS	Infant HIV infection	**Morbidity:** 6 studies, ECS was associated with increased odds of all morbidities compared with vaginal delivery (OR 3.12, 95% CI 2.21–4.41) but the OR was lower when compared with all other modes of delivery (OR 1.52, 95% CI 1.06–2.20) **Infant HIV infection** The RCT found significantly fewer HIV infections among infants delivered by ECS (1.7%) versus vaginal delivery (10.6%) (OR 0.2, 95% CI 0.0–0.5, 1 RCT, 385 infants). The OR was closer to one and nonsignificant for women who received zidovudine in pregnancy (OR 0.4, 95% CI 0–1.4) compared with the OR for women who received no zidovudine in pregnancy (OR 0.2, 95% CI 0–0.8). In meta-analysis of all observational studies, ECS was also associated with a decreased odds of infant HIV infection (Table 3). The OR for infant HIV infection comparing ECS to vaginal delivery was 0.43 (95% CI 0.30–0.63, 13 studies, 16204 infants, moderate heterogeneity) Stratifying to patients receiving cART, the relationship between ECS and lower infant HIV infection was no longer statistically significant (OR 0.82, 95% CI 0.47–1.43, 4 studies, 8823 infants) versus vaginal delivery		Info on HIV type not available in some studies, probably HIV-1 Information on coinfections not available in some studies

(Aho et al., 2018)			1993–2013 Registry study vaginal, elective CS emergency CS		212 women with altogether 290 children including four pairs of twins No perinatal HIV transmissions occurred. The overall rate of vaginal delivery was 74.5% and that of both elective and emergency CS 12.8% 2000–2013. During this time period, 80.0% of the women achieved undetectable viral load before the delivery and 78.8% of them delivered vaginally		HIV type (1 or 2) not specified
(Edathodu et al., 2010)	CS	Elective CS in 28 pregnancies Vaginal delivery in 11 pregnancies			The median CD4+ T-lymphocyte count at about the time of delivery was 536 cells/mm3 (mean 574, range 183-1142 cells/mm3) All were on antiretroviral therapy during pregnancy and delivery. All the newborns were tested at the end of 18 months and tested negative for the HIV-1 screening and PCR assay	Elective cesarean delivery was recommended until 2003 after which, vaginal delivery became the standard in mothers with viral load below 1000 copies/mL, unless the patient opted for cesarean delivery	HIV-1

(Livingston et al., 2016)	CS	2297 women 1055 vaginal delivery 798 ECS 444 NECS	Prospective cohort study "elective cesarean"(ECS), "non-elective cesarean" (NECS) or "vaginal". For study purposes, ECS was defined as a scheduled cesarean prior to the onset of labor and prior to ruptured membranes or rupture of membranes ≤ 5 minutes prior to delivery. NECS was defined as a cesarean performed after the onset of labor or ruptured membranes ≥ 5 minutes prior to delivery.	Associations between mode of delivery and the maternal morbidity outcomes	No maternal deaths after adjusting for the last CD4 count, viral load and CDC classification during pregnancy and clinical diagnoses during pregnancy, ECS and NECS remained significantly associated with higher odds of any maternal morbidity, surgical wound/vaginal delivery laceration/wound complications, and infections, as compared to vaginal delivery. Last viral load of pregnancy (copies/ml) ≤ 400: 93% vaginal, 73% ECS, 79% NECS Infants infected with maternal viral load ≤ 400: 4 (0.4%) vaginal, 1 (0.1%) ECS, 0 NECS; NS Infants infected with maternal viral load > 400: 2 (0.2%) vaginal, 3 (0.4%) ECS, 2 (0.5%) NECS; NS		HIV-1
(Mayaux et al., 1995)	CS	848 mother-infant pairs Inclusion: All infants born to women known to be HIV-1 seropositive before or at the time of delivery Mothers were advised not to breastfeed HIV-2 excluded	Prospective cohort study Mode of delivery determined by the local obstetrician No data on ART use in the mothers	Mother-to-infant rate is based on only data for all infants born >18 months	CS: 121 children Vaginal: 723 children 171/848 children were infected → 20.2±2.7% Risk of transmission did not differ according to the type of delivery RR 1.0 (0.7-1.4), NS		HIV-1

(Orbaek et al., 2017)	CS	Women living with HIV (WLWH) Excluded: Women testing positive after deliver Mode of delivery unknown Cut-off for vaginal delivery was VL<1000 HIV-1 RNA copies/ml Definitive exclusion of an HIV diagnosis of the child was based on two negative virological test results prior to or at 18 months of age. Undetectable VL was defined as HIV RNA < 40 copies/mL.	Retrospective case-comparison 1 January 2002-31 December 2014 CS was classified as elective when planned ahead of birth and taking place before labour or rupture of the membranes. All other unplanned CS performed acutely during pregnancy or labour were classified as emergency CS.	Mode of delivery: Vaginal, ECS or EmCS	389 HIV pregnancies 130 vaginal (33.4%) 158 ECS (40.6%) 101 EmCS (26%) All women were on ART at delivery (median VL < 40 copies/mL; IQR <40–230 copies/mL) and there were no cases of MTCT in the study group.		HIV-1
(Simpson et al., 1997)		children whose mothers were identified as being infected with HIV before delivery were enrolled	Prospective cohort study		259 children Period 1 (without maternal zidovudine): The risk of transmission of HIV was 20.6% (28/136; 95% CI: 14.1–28.4%) 27/132 delivered by CS Period 2 (with maternal zidovudine): The risk of transmission of HIV was 9.8% (12/123; 95% CI: 5.1–16.4%) 24/125 delivered by CS Of the 250 children whose mode of delivery and infection status could be determined, 14.8% (30/203) of those born vaginally and 14.9% (7/47) of those born by cesarean section were infected with HIV (p = 0.98).		HIV-1

(Tibaldi et al., 2019)		Eligible women were offered vaginal delivery and provided a signed	Retrospective cohort study 2012-2017 "elective cesarean section" (ECS), "non-elective cesarean section" (NECS) or "vaginal delivery". ECS was defined as a scheduled cesarean prior to the onset of labor and prior to ruptured membranes or rupture of membranes ≤ 5 min prior to delivery. NECS was defined as a cesarean performed after the onset of labor or ruptured membranes > 5 min prior to delivery.		142 (24.5%) had a vaginal delivery 323 (55.7%) had an elective cesarean section 115 (19.8%) had a non-elective cesarean section. <u>Vertical transmission</u> Vaginal delivery: 0% (0/139) ECS: 0.3% (1/316) NECS: 0.9% (1/113)		HIV type (1 or 2) not specified
(Torpey et al., 2012)		All infants, aged 0 to 12 months, born to HIV-positive mothers, and who underwent a DNA PCR test, were eligible to be included.			the differences were not statistically significant when both the mother and infant received PMTCT interventions (Table 3). In the multivariable logistic regression model, only breast-feeding status was consistently associated with vertical transmission across all ages 		HIV type (1 or 2) not specified

PCR, Week Age Group	Location of Delivery	N	Number Detected	Trans Rate	95% CI	P Valu
Across all infants						
0-6 weeks	Health facility, no C-section	1312	103	7.9%	(6.4%, 9.3%)	.375
	Health facility, C-section	70	2	2.9%	(0%, 6.9%)	
	Home	132	16	12.1%	(6.4%, 17.8%)	
6 weeks-6 months	Health facility, no C-section	3874	504	13.0%	(12.0%, 14.1%)	<.001
	Health facility, C-section	258	19	7.4%	(4.2%, 10.6%)	
	Home	612	135	22.1%	(18.8%, 25.3%)	
6 months-12 months	Health facility, no C-section	1547	353	22.8%	(20.7%, 24.9%)	.036
	Health facility, C-section	76	12	15.8%	(7.1%, 24.5%)	
	Home	334	94	28.1%	(23.3%, 33.0%)	
Among those wherein both mother and infant received intervention						
0-6 weeks	Health facility, no C-section	1151	76	6.6%	(5.2%, 8.0%)	.734
	Health facility, C-section	60	2	3.3%	(0%, 8.1%)	
	Home	42	3	7.1%	(0.0%, 14.9%)	
6 weeks-6 months	Health facility, no C-section	3,085	306	9.9%	(8.9%, 11.0%)	.562
	Health facility, C-section	207	13	6.3%	(2.9%, 9.6%)	
	Home	180	19	10.6%	(6.0%, 15.1%)	
6 months-12 months	Health facility, no C-section	975	148	15.2%	(12.9%, 17.4%)	.391
	Health facility, C-section	55	9	16.4%	(5.9%, 26.8%)	
	Home	67	7	10.4%	(2.8%, 18.1%)	

Breastfeeding

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Assefa et al., 2017)		566 breast-fed 291 formula-fed infant simple random sampling technique was used and the study subjects were selected from the clinic computerized registry	Retrospective cohort study Breast feeding: Exclusive breastfeeding for the first 6 months and introduce complementary feeding at 6 months and continue breastfeeding until 12–18 months. Formula feeding: Exclusive replacement of feeding for the first 6 months and adequate complementary feeding and formula thereafter for those who fulfill the AFASS criteria. HIV-free survival-child is alive without acquiring HIV infection.	HIV free survival	Three quarter of infants 421 (77.2%) in breast-fed and 207 (75.0%) formula-fed infants groups were on SD NVP + AZT for 7 days ARV prophylaxis The cumulative probability of HIV free survival for breastfed infants and young children in the first 180 and 360 days were 95% and 93%, respectively, but it was 97% in the two above mentioned durations for formula-fed infants and young children.		Type of HIV (1 or 2) not specified
(Coutsoudis, 2000)		549 pregnant, HIV infected women 156 bottle feeding 288 mixed feeding 103 exclusive breastfeeding	Prospective cohort study follow-up clinic when their infants were 1 week, 6 weeks, and 3 months of age and thereafter every 3 months until 15 months		The transmission rate at 3 months in 156 children who were never breastfed was 18.8% compared to 24.1% in the 288 infants who had received breast milk together with other feeds. However, among the 103 infants who were exclusively breastfed, 14.6% were infected, which is significantly different from the rate in those receiving mixed breastfeeding (p = 0.03) and not very different from those who had never been breastfed		HIV-1

(De Martino et al., 1992)	CS	961 at risk children born to HIV positive women 168 breastfeeding 793 bottle feeding	Part of infants were enrolled prospectively and part retrospectively		Median duration of breastfeeding was 2 months The estimated adjusted infection ratio for one day of breastfeeding versus bottle feeding was 1.19 (95% CI 1.10-1.28)		HIV-1
(Imade et al., 2010)		A total of 318 HIV-positive pregnant women on their third trimester and on antiretroviral (ARV) drugs were recruited for this study. The exclusive breast-feeding was for a period of 6 months after which infants born to these women were all screened for HIV using polymerase chain reaction (PCR).	Prospective cohort study Breastfeeding - On ARV - Not on ARV No breastfeeding		HIV infection in children Breastfeeding 32/77 positive (41.56%) No breastfeeding 22/241 (9.17%) The prevalence of post-natal HIV infection was significantly higher (P<0.0001) in breast-fed infants compared with their non-breast-fed counterpart and breastfeeding was a risk factor for acquiring HIV infection among infants (OR=7.079 95%CI 3.268-13.300) The use of ARV during breastfeeding was not associated with post-natal HIV infection among infants (OR=0.018 95%CI=0.004, 0.091). None use of ARV during breastfeeding period was significantly (P<0.0001) associated with post-natal HIV infection (OR=54.944 95%CI=10.938, 276.00		HIV-1

(Kagaayi et al., 2008)	CS	182 infants born to HIV positive women	Prospective cohort study Compare mortality and HIV-free survival among formula-fed and breast fed infants born to HIV-infected mothers Exclusive breastfeeding Mixed feeding Bottle feeding		182 infants 75 (41%) of mothers chose to formula-feed 107 (59%) mothers chose to breast-feed. The proportion of HIV infected infants at one month were 13.0% (12/92) among the breast-fed compared to 4.4% (3/69) among the formula-fed infants (P-value = 0.06)		Type of HIV (1 or 2) not specified
(Magoni et al., 2005)	CS	306 infants born to HIV positive mothers	Prospective cohort study 30 September 2000 to 30 October 2002 EBF group comprised children who received only breastmilk with no other concomitant fluid or feed. EFF group included infants who were formula-fed only and who were never breastfed. MF group included children	HIV transmission rates	week 6, transmission rates EFF: 4/117 children (3.4%) vs. EBF: 17/152 (11.2%) vs. MF: 6/35 children At month 6, transmission rates EFF: 3.7% (4/108) vs. EBF: 16.0% (19/119) vs. MF: 20.4% (10/49) no statistically significant risk difference between the EBF group and the MF group (hazard ratio for the MF group, 1.4; 95% CI, 0.6–3.3;		Type of HIV (1 or 2) not specified

(Mbori-Ngacha et al., 2002)	RCT	425 women enrolled in the study 213 women formula feeding arm 212 women to the breastfeeding arm	1992 and 1998,		204 infants in the formula feeding arm 197 in the breastfeeding arm. Ninety-two infants acquired HIV-1 infection during the study, 31/204 (21%) in the formula feeding arm 61/197 (37%) in the breastfeeding arm no significant difference in 2-year mortality rates between infants randomly assigned to be formula fed or to be breastfed		HIV-1
(Njom Nlend et al., 2018)	CS	Children born to HIV-1 positive women exclusive breastfeeding (EBF) or exclusive replacement feeding (ERF), with emphasis to avoid mixed feeding (MF) practice.	Retrospective cohort study 24 months of follow-up April 2008-December 2013		1086 eligible infants maternal ARV experience 566 (52.12%) received triple ART, 411 (37.85%) received AZT 109 (10.04%) had no ARV. infant feeding options the first 3 months of life, 663 (61.05%) were on ERF, 408 (37.57%) on EBF 15 (1.38%) on MF Vertical transmission: EBF (2.72%); ERF (3.80%); MF (21.43%) according to exposure to ARVs, HIV vertical transmission rates were 1.7% (10/566) from ART group, 1.9% (8/411) from AZT-group, and 19.2% (21/109) from ARV-naïve group, $p < 0.0001$.		HIV-1

(Olayinka et al., 2000)	CS	236 Infants born to HIV-positive mothers (1) Breastfeeding only (2) Mixed feeding (3) Formula feeding only	Prospective cohort study 1992-1995 24 months of follow-up		95/236 infants acquired HIV-1 More than 50% (120/203; 59.1%) of all infants by the age of 3 months were exclusively breastfed, while 81 (39.9%) and two (1.0%) of the 203 infants were mixed and formula-only fed, respectively. HIV-1 incidence at 3 months 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		HIV-1
(Peltier et al., 2009)	CS	All enrolled women received HAART from 28 weeks of gestation irrespective of the study group. all newborn infants exposed to HIV received NVP 2 mg/kg at birth and AZT 4 mg/kg twice-daily for seven days	non-randomized, interventional cohort study May 2005 and January 2007,		240(42.7%) preferred BF under HAART and 322(57.3%) women chose FF Overall, seven children were infected with HIV-1 of which six in utero (three in each infant feeding group). Only one child in the BF group became infected between month 3 and month 7 and no child acquired HIV infection between birth and nine months in the FF group. In the BF group, the cumulative probability of HIV-1 transmission at six weeks and nine months was 1.3 (95 CI 0.4 4.1) and 1.8 (95%CI 0.7 4.8), respectively. In the FF group, these cumulative probabilities were similar at six weeks and nine months estimated to be 1 (95 CI: 0.3 3.0). Over the first nine months, the probability of HIV-1 transmission was not statistically different between both groups (log-rank test, P=0.43).		HIV-1

(Tess et al., 1998)	CS	HIV infected women, naïve for zidovudine 432 children 168 (32%) were breastfed 264 were never breastfed	Jan 1988-April 1993 Retrospective cohort study	Risk of HIV transmission	Children who were breasfed had a significantly higher risk of being infected than those who were never breastfed (21% vs 13%, p=0.01) No clear pattern in risk of transmission by duration of breastfeeding was observed		HIV-1

CNP

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Chiappini et al., 2013)	SR	A total of 5285 mother–infant pairs were defined as at high risk for MTCT and included in the study		HIV transmission rate	Neonatal prophylaxis was administered to 4623/5285 (87.5%) infants, of whom 3518 (66.6%) received one drug and 1105 (23.9%) received CNP; most infants on CNP received three drugs (n ¼ 677; 61.3%), with the remaining 428 (38.7%) receiving two MTCT rates were 3.4% (95% CI 2.7–4.0), 6.3% (95% CI 4.8–7.6) and 17.7% (95% CI 13.9–21.5) for one-drug neonatal prophylaxis, CNP and no neonatal prophylaxis, respectively Crude MTCT rates were 1.8% (39/2140) and 4.2% (29/681; aOR 1.97; 95% CI 1.14–3.39; P ¼ 0.014) in one drug and CNP groups, respectively, among infants whose mothers received antenatal ART; 7.0% (18/257) and 5.9% (8/134; aOR 0.86; 95% CI 0.28–2.64; P ¼ 0.804) in those whose mothers received no antenatal or intrapartum antiretroviral prophylaxis; 8.0% (42/523) and 13.7% (27/198) (aOR 1.57; 95% CI 0.81–3.08; P = 0.178) among those whose mothers received only intrapartum prophylaxis.		
(Chigwedere et al., 2008)	SR	10 studies		HIV transmission rate	The combined transmission rate for arms that used ARVs (both in mothers to reduce viral load as in neonates as prophylaxis) is 10.6% (95% CI: 8.6–13.1), while the combined transmission rate for arms that used placebo is 21.0% (95% CI: 15.5–27.7). Using the combined transmission rates above, the efficacy of using ARVs to re duce MTCT is approximately 50% (1–10.6/21.0).		

(Beste et al., 2018)	SR	4 studies included	Efficacy of multidrug regimens in high-risk infants versus a 4-6 week regimen of AZT (birthdose was NVP in 1 study)	HIV transmission rate	Transmission rates for infants receiving single-drug prophylaxis: ranging from 2% (95% CI 0.3-5.2% to 4.8% (95% CI3.2-7.1%) In the multidrug arm: 2.2% (95% CI 1.2-3.9%) in the 2-drug arm, and ranging from 0.4% (95% CI 0.1-1.4%) to 2.4% (95% CI 1.4-4.3) In the EPPICC study, however, transmission rates were higher in the multidrug group [6.3% vs. 3.4%, odds ratio: 1.41, 95% CI: 0.97–2.05, P = 0.07]. The higher transmission rate in this study—and in contrast with other studies—is likely the result of severe confounding by indication:	there is currently no evidence that 3-drug regimens are superior to 2-drug regimens in preventing intrapartum HIV transmission in high-risk infants.	

(Aizire et al., 2012)	RCT	350 randomized infants to receive SMON or placebo 57 infants enrolled to receive SWEN	February–August 2007. secondary data analysis of the HIV Prevention Trials Network (HPTN) 046 protocol 6-months NVP (SMON) or placebo in a 1 : 1 ratio stratified by maternal antiretroviral drug use during pregnancy (PMTCT, maternal treatment or neither) after the release of the 6-weeks NVP (SWEN) trial results suggesting a 50% reduction in mother-to-child transmission risk of HIV-1 [5], the following protocol design changes were implemented starting 10 August 2007: Enrolled infants were not randomized but started on open-label SWEN regimen Infant follow-up: 2, 4, 6, and 8 weeks and 3, 4, 5, 6, 9, 12, and 18 months.	efficacy and safety of NVP prophylaxis against breast milk transmission of HIV-1.	Infant HIV infection during follow-up was determined in four of 146 (2.7%) versus seven of 97 (7.2%) in the SMON and placebo arms, respectively, P = 0.12, and three of 57 (5.3%) in SWEN group.		HIV-1

(Chasela et al., 2010)	RCT	2369 mother–infant pairs to undergo randomization Baseline demographic and laboratory characteristics of the 2369 mother–infant pairs who underwent randomization were well balanced among the three groups Loss to follow-up before 28 weeks occurred among 12% of mother–infant pairs in each study group.	<u>maternal-regimen group</u> Combivir 2x daily and nevirapine at 200 mg 1x daily for 2 weeks and 2x daily thereafter until 28 weeks. (later replaced by 2x daily nelfinavir 1250 mg; nelfinavir was replaced with 2x daily 400 mg lopinavir +100 mg of ritonavir) <u>infant-regimen group</u> a dose of nevirapine that increased according to age, ranging from 10 mg daily in the first 2 weeks to 30 mg daily for weeks 19 through 28. <u>control group</u> no extended postnatal ART All mothers in labor and their newborn infants received a single dose of oral nevirapine In addition, all mothers received zidovudine and lamivudine as a single tablet (300 mg of zidovudine and 150 mg of lamivudine) every 12 hours from the onset of labor to 7 days after birth. All infants also received twice-daily zidovudine (2 mg per kilogram of body weight) and lamivudine (4 mg per kilogram) for 7 days. Mother–infant pairs were followed at 1, 2, 4, 6, 8, 12, 18, 21, 24, 28, 32, 36, 42, and 48 weeks after birth.	rate of detection of HIV-1 infection at 28 weeks among infants	By 2 weeks, infants in each of the three study groups had a similar estimated risk of infection: 5.4% (95% confidence interval [CI], 3.9 to 7.4) in the control group (reference group), 5.5% (95% CI, 4.1 to 7.2; P = 0.97 with the use of a z statistic) in the maternal-regimen group, and 4.4% (95% CI, 3.2 to 6.0; P = 0.35) in the infant-regimen group. Among infants who were HIV-1–negative at 2 weeks, the estimated risk of HIV-1 infection by 28 weeks was 5.7% in the control group (reference group), 2.9% in the maternal-regimen group, and 1.7% in the infant-regimen group In an analysis that included all infants who underwent randomization regardless of infection status at 2 weeks, the estimated risk of HIV-1 infection by 28 weeks was 10.9% (95% CI, 8.7 to 13.6) in the control group, 8.2% (95% CI, 6.5 to 10.3) in the maternal regimen group, and 6.0% (95% CI, 4.5 to 7.8) in the infant-regimen group		HIV-1

(Fowler et al., 2014)	RCT	1,522 infants were randomized at age 6 weeks 759 NVP 763 placebo Maternal and infant demographics and maternal clinical status were similar across the two study arms	phase 3, randomized, double-blind, placebo-controlled trial that assessed the efficacy and safety of extension of once-daily NVP to 6 months of age or until cessation of breastfeeding infants had received NVP prophylaxis until 6 weeks of age and then randomized to receive - Extended daily NVP - placebo		220/752 (29%) of mothers was on ART in NVP arm 219/753 (29%) of mothers in the placebo arm HIV infections at <u>6 months</u>: 1.1% (95% CI 0.3-1.8%) in the NVP arm versus 2.4% (95% CI 1.3-3.6%) in the placebo arm, p=0.049 following discontinuation of study product at 6 months, HIV infection rates were no longer significantly different from 9 through 18 months. <u>18 months</u>, 16 infections in the NVP arm versus 23 infections in the placebo arm, with a cumulative postnatal infection rate of 2.2% (95% CI 1.1-3.3%) versus 3.1% (95% CI 1.9 – 4.4%, p=0.28) ; translating into HIV-free rates of 97.8% in the NVP arm versus 96.9% in the placebo arm Among the 149 mothers (85 NVP arm and 64 placebo arm) with CD4 cell counts < 350 cells/mm3 at randomization and not on ART, the cumulative infection rates were high, but not statistically different by study arm For the 924 women with CD4 cell counts ≥ 350 cells/mm3 at randomization and not considered eligible for ART, (451 NVP arm and 473 placebo arm), cumulative postnatal infection risk was consistently lower in the infant NVP arm than the placebo arm but NS		HIV-1

(Jamieson et al., 2012)	RCT	1829 mother-infant pairs Inclusion: women had to have been pregnant ≤ 30 weeks be aged ≥ 14 years, have a CD4 count of 250 cells per µL or more and have used no ART drugs Baseline characteristics were well balanced across the control and antiretroviral intervention groups in all mother–infant pairs	April 21, 2004, and Jan 28, 2010. <u>maternal antiretroviral,</u> according to ruling regulations <u>infant-nevirapine,</u> 10 mg daily in the first 2 weeks, 20 mg daily between weeks 3 and 18, and 30 mg daily from week 19 to week 28. All antiretroviral interventions were stopped after mothers reported cessation of breastfeeding or after 28 weeks <u>control groups</u> no intervention after the initial 7d Irrespective of ART intervention group, all mothers in labour and their newborn babies were offered one dose of oral nevirapine (mother 200 mg; infant 2 mg/kg) and zidovudine and lamivudine to be taken twice a day for 7 days (mothers 300 mg zidovudine and 150 mg lamivudine in one tablet		The cumulative risk of HIV-1 transmission by 48 weeks was significantly higher in the control group (7%, 95% CI 5–9) than in the maternal-antiretroviral (4%, 3–6) or the infantnevirapine (4%, 2–5) groups The reduction in risk of HIV transmission from 2 weeks to 48 weeks was 48% (95% CI 23–74) in the infant-nevirapine group and 38% (9–67) in the maternal-antiretroviral group. Of all infants randomly assigned, irrespective of infection status at 2 weeks, the risk of HIV infection by 48 weeks was 12% (9–15) in the control group compared with 10% (7–12) in the maternal-antiretroviral (p=0·1436) and 8% (6–10) in the infant-nevirapine groups (p=0·0063		HIV type (1 or 2) not specified

(Omer, 2011)	RCT	1890 infants	SWEN trials. Enrollment started in February 2001 in Ethiopia; in August 2002 in India; and in July 2004 in Uganda-2007 Single dose arm: one dose of 200 mg NVP for mothers self-administered at the onset of labor and a 2 mg/kg oral dose of NVP for their newborns. 6-week extended-dose arm: arm consisted of the single-dose regimen and 5mg oral NVP to infants daily from 8 to 42 days of age		the study population for the modified intention-to-treat analysis consisted of 1890 infants with 987 in the single-dose group and 903 infants in the extended-dose group 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 (risk ratio 0.87, 95% CI: 0.65–1.15; P=0.33). the impact of extended-dose NVP was highest in the infants of mothers with CD4 cell count more than 350 cells/ml compared to infants of mothers with CD4 cell count 200 cells/ml or less and infants of mothers with CD4 cell counts between 201 and 350 cells/ml		2 regimens HIV-1
(Shapiro et al., 2009)	RCT	1200 HIV infected mothers	Mothers received all ZVD antenatal and intrapartum Group 1: ZDV prophylaxis for 1 month + 1 dose placebo for mother and infant at birth (Formula fed arm) Group 2: ZDV prophylaxis for 1 month + 1 dose NVP for mother and infant Group 3: ZDV prophylaxis for 6 months (breastfeeding arm) After 17 months of enrollment, the study was modified and all infants received single-dose NVP Infant DNA PCR testing was performed at birth, at 1, 4, 7,		There was a nonsignificant trend for early protection from maternal receipt of NVP in the FF arm Breastfeeding arm:late MTCT occurred in 24 (4.4%); it occurred in 15 infants before 4 months of age, in 6 during the period from month 4 to 6, and in 3 during the period from month 7 to 24 In the FF arm, 2 infants became infected after the 1 month visit, and 2 became infected at an undetermined time point. Four (16.7%) of the 24 late transmissions occurred among infants whose prophylactic ZDV had been stopped prior to their first positive HIV test result. Maternal receipt of single-dose NVP did not predict late MTCT.		

(Taha et al., 2011)	RCT	3126 infants born to HIV infected women no baseline differences by study arm in maternal age, CD4 count or presentation time/delivery mode or in infant gender, birth weight, adherence to regimen or breastfeeding before discharge. single-dose NVP prophylaxis during labor for mothers unless late presenters	April 2004 and completed follow-up in September 2009 PEPI trial <u>N=1004 Control</u>: single-dose oral NVP plus 1 week of daily oral ZDV <u>N=1071 control + extended oral daily NVP</u> (ExtNVP) from day 8 to 14 weeks of age; <u>N=1051 control plus extended oral daily NVP plus ZDV</u> (ExtNVP/ZDV) from day 8 to 14 weeks of age. Postnatal follow-up visits were at 1, 3, 6, 9, and 14 weeks, and 6, 9, 12, 15, 18, and 24 months of infant age	Infant infection at 9 months of those uninfected at birth (Taha 2003)	HIV infection in infants: 136/1004 in the control arm, 95/1071 in the ExtNVP arm, 98/151 in the ExtNVP/ZDV arm <u>9 months</u>, HIV infection had occurred in 11.1% (95% CI: 9.3 to 13.3) of control arm 5.0% [95% CI: 3.8 to 6.6] of the ExtNVP and 6.0% (95% CI: 4.7 to 7.7) of the ExtNVP/ZDVarm (P< 0.001) <u>24 months:</u> HIV infection had occurred in 15.6% in control 10.8% in ExtNVP (p=0.003 vs control) 11.2% in ExtNVP/ZDV arms P =0.008 vs control).		

Human Papilloma virus

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

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Burchell et al., 2010)		Women (age 18-24) attending university or college in Montreal, Canada, and their male partners Eligible women had a current male sexual partner for which the relationship duration was no more than 6 months; had an intact uterus and no history of cervical lesions/cancer; and were not pregnant or planning to become pregnant in the next 24 months. Eligible male partners were aged 18 and older.	(N = 263 couples) May 2005 - August 2008 each HPV type was considered as its own observation, such that participants could have as many as 36 HPV-type outcomes	**HPV prevalence** HPV presence in genital specimens (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) by Linear Array HPV genotyping assay (LA-HPV)	Prevalence among women and men with 10 or more lifetime partners was 15.4 (95% CI: 5.9-40.2) and 9.5 (95% CI: 4.4-19.8) times higher than among those with 1 partner 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: 38.0-80.1) and men (OR = 58.7, 95% CI: 39.8-86.3) if their partner harbored the type under consideration	Frequent condom use was protective in men, particularly if his partner was HPV-infected (OR = 0.64, 95% CI: 0.50-0.82). This effect was attenuated among women with an infected partner (OR = 0.88, 95% CI: 0.69-1.11).	HPV can be spread in sexual partners. The risk of HPV infection increases with the number of sexual partners. MORE PARTNERS MORE HPV-POSITIVE CASES Condom is protective

(Dillner et al., 1996)		mean age was 26 years (range, 16-48). 1002 women visiting family planning or youth clinics in Sweden, an age-matched subsample of 274 women stratified according to lifetime number of sex partners was analyzed.	intensive interview (146 items) by experienced midwives regarding sexual history, sexual practices, selfperception, and substance use. Cervical, vaginal, urethral, and serum specimens were tested for a panel of microbiologic agents, notably HSV-2, Neisseria gonorrhoeae, C. trachomatis (culture), and HPV (Southern blotting).	association of seropositivity to human papillomavirus (HPV) capsids of types 11, 16, 18, or 33 with sexual behavior	The proportion of HPV-16-seropositive subjects increased linearly at approximately 4% per partner (P < .001), from 4% among those with 1 lifetime partner to 35% among those with >5 lifetime partners. HPV-33 and HPV-18 seroprevalences were linearly dependent on the number of partners (P < .001, increase with 4% per partner, and P = .008, increase with approximately 3% per partner, respectively), providing serologic confirmation that the important mode of transmission of HPV-16, -18, or -33 infection in women is sexual.	HPV serology appears to be suitable as a marker of sexual behavior in populations.	Serological tests confirm that the important mode of transmission of HPV-16, -18, or -33 infection in women is sexual.
(Hernandez et al., 2008)		Heterosexual, non-pregnant, monogamous couples (25 men, 25 women)	February 2005-November 2006 Study visits at 2 months interval Average study follow-up: 7.5 mo For males: separate genital specimens from the penis glans/corona, penis shaft, scrotum, and inner foreskin (uncircumcised men), semen were collected for women: pap smear, swabs from ectocervix and endocervix, including the transformation zone both: anal, oral and hand swab, urine	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). Sexual transmission involved 13 different oncogenic and nononcogenic HPV types; 8% were vaccine-covered types transmitted between partners. Male-to-female transmission was observed in 7 couples. All infections transmitted from male to female partners originated in the penis with or without additional involvement of the scrotum. Female-to-male transmission was observed in 12 couples. Transmission from the cervix and/or urine to the male genitals	These results have implications for HPV prevention and control strategies, including the targeting of prophylactic vaccines	This study confirms that the HPV infection can be transmitted during vaginal and anal intercourses.

(Kjaer et al., 2001)	CS	a cohort of 11,088 women (20–29 years) was included from a randomly selected general population sample of women from Copenhagen in a random sample of 1000 women, 15% was HPV DNA positive 100 virgins and 105 monogamous women	May 1991 to January 1993 Group A: virgins for 2 years n=30 Group B: virgins who initiated sexual contact N=70 Group C: monogamous women N=78 Group D: monogamous women having new sexual partners N=27	Women were examined twice with 2-year interval - interview, - cervical swabs, - Pap smear, - blood samples determining HPV DNA and HPV-16 Ab	all of the virgins who stayed virginal throughout the study continued to be HPV DNA negative at follow-up. Results show that sexual intercourse is important in the transmission of HPV, and that HPV 16 VLP seroconversion and the development of cervical lesions only occur after HPV transmission. Remarkably, no cervical lesions were found in HPV 16 DNA positive women who had seroconverted.	Although based on small numbers, this may suggest that the development of antibodies had a protective effect	HPV transmission during the intercourse has been proved by the investigators No cervical lesions were found in HPV 16 DNA positive women who had seroconverted. Small sample size
(Widdice et al., 2013)		25 couples Women were eligible if they had an incident HPV infection (ie, a new HPV type not detected at the previous visit), were 18 years or older, and had a partner willing to participate, whom they were in a heterosexual relationship with for at least 3 mo, no genital warts and no medication use in the genital area.	The parent study was initiated in 1990 and again in 2000 5 visits	HPV transmission between partners each partner completed a self-administered questionnaire on sexual habits. Female samples for HPV DNA were obtained from the intra-anal canal, vulva and vagina, cervical samples Male samples: glans (including corona sulcus), shaft, inner foreskin if applicable, scrotum, and perianal area Both: hand, mouth, tongue	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 V1 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		

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

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Hahn et al., 2013)	CS	469 pregnant women and neonates Type of HPV not specified	36 weeks of gestation Two months after birth	HPV viral loads of HPV-positive mothers were measured to investigate the relationship between vertical transmission and viral load.	HPV was detected in 72 of 469 pregnant women (15.4%) and in 15 neonates (3.2%) of the 72 infected women NS association of higher maternal load with infected neonates (p=0.089) no differences in maternal HPV copy number infected per cervical cell between HPV-positive and HPV-negative neonates (p = 0.880).	Vertical transmission of HPV is associated with vaginal delivery and multiple HPV types in the mother; however, neonatal HPV infection through vertical transmission is thought to be a transient.	the risk factors associated with vertical transmission of HPV infection from mothers to neonates were confirmed
(Kaye et al., 1994)		15 pregnant women with HPV-16 infections were studied	Eight of these women had infants who were positive for HPV-16 DNA at genital and/or buccal sites	Viral load Viral copy number	Transmitters vs non-transmitters: - viral load (mean 2 standard deviation) 4.35 ± 2.84 U/PCR sample vs. 1.83 2 1.12 U, P < 0.05 - viral copy number 35 to 5 x 10^6 copies/ PCR sample (629,886 ± 1,765,883) vs. between 17-195 copies (70.8 ±65.25 copies: P < 0.05).	No clear cut-off, All women with a viral load <4.0U (325 copies) transmitted, viral load of >1.6 U (22 copies) results in no transmission The viral load is an important, but not the sole, determinant for the transmission of HPV-16 from mother to infant	High HPV DNA load is an important parameter to be considered during the mother to child transmission of this viral agent

WHICH TECHNIQUE FOR MEDICALLY ASSISTED REPRODUCTION SHOULD BE USED IN COUPLES WITH HUMAN PAPILLOMA VIRUS?

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

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

DNA integration in semen/oocytes/embryo

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Foresta et al., 2011a)		Sperm of a male who in a previous study tested positive for HPV-16		Visualization of HPV-16 by FISH	HPV can infect human sperm, it localizes at the equatorial region of sperm head through interaction between the HPV capsid protein L1 and syndecan-1. Sperm transfected with HPV E6/E7 genes and sperm exposed to HPV L1 capsid protein are capable to penetrate the oocyte and transfer the virus into oocytes, in which viral genes are then activated and transcribed.	Sperm might function as vectors for HPV transfer into the oocytes, and open new perspectives on the role of HPV infection in males and are particularly intriguing in relation to assisted reproduction techniques.	
(Kaspersen et al., 2011)	CS	The presence of 35 types of HPV was examined on DNA from semen samples of 188 Danish sperm donors using a sensitive HPV array.	To examine whether HPV was associated with the sperm, in situ hybridization were performed with HPV-6, HPV-16 and -18, and HPV-31-specific probes.	Association between sperm and HPV	Sperm samples positive for HPV-6, HPV-16, HPV-18, or HPV-31 were hybridized with a specific probe against the respective type, and the HPV-probe-sandwiches were visualized (Figures 3A–3I and 4). This revealed characteristic protrusions at or near the equatorial segment of the sperm head (Figures 3A–C). Likewise, when sperm from HPV-6, -18, or -31 positive donors were hybridized with HPV-6, -18, or 31-specific probes, similar protrusions were identified (Figures 3D–I and 4). However, when sperm from an HPV negative donor were hybridized with an HPV specific probe, there was no specific binding (Figures 3J–L and 4)	These data indicate that HPV-6, -16, -18, and -31 bind to the sperm cell head particularly at or near the equatorial segment in vivo.	

(Lai et al., 1996)	CT	24 randomly selected patients who attended Fertility Clinics at the Chang Gung Memorial Hospital.	Possible presence and expression of human papillomavirus viruses (HPV) in human plasma and sperm cells.		HPV type 16 E6 and E7 DNA and RNA sequences were found in two and zero (no transcription) seminal plasma specimens, respectively, and in six and two (RNA transcription) sperm cells specimens, respectively. DNA and RNA sequences of HPV type 18 were found in eight and two seminal specimens and in 11 and 5 sperm cells specimens, respectively.	HPV cannot only infect human sperm cells, certain HPV genes are expressed actively in infected sperm cells. The virus-infected sperm cells conceivably can behave as vectors or carriers for the transmission of HPV, to sexual partner during sexual contact, to fetuses through fertilized eggs, or both.	Small number of cases. Interesting findings
(Schillaci et al., 2013)		Specimens of semen were collected from 308 male partners of couples undergoing IVF		The presence of HPV DNA was researched by the combined use of two HPV assays and a highly sensitive nested polymerase chain reaction assay followed by HPV genotyping. To examine whether HPV was associated with the sperm, in situ hybridization (ISH) analysis was performed.	Results of HPV investigation were compared with sperm parameters and ISH analysis. Twenty-four out of 308 semen samples (7.8%) were HPV DNA positive, but HPV infection did not seem to affect semen quality. ISH revealed a clear HPV localization at the equatorial region of sperm head in infected samples.	Oncogenic HPV genotypes were detected on spermatozoa from asymptomatic subjects, but a role of the infection in male infertility was not demonstrated.	

(Capra et al., 2019)		Semen samples of 22 patients aged between 22 and 44 years were obtained by masturbation after 3–5 days of sexual abstinence.	January 2016 to December 2016 Total semen and SU fraction from each patient were processed in order to carry out a differential lysis with consequent DNA extraction from the semen components separately		Forty-five per cent (10/22) of patients had the infection in the semen sample (Total semen). HPV test was positive in three samples also after swim up technique (SU fraction). the viral DNA can be detected in every fraction of semen: Total sperms, Cell fraction and seminal plasma. never found HPV DNA in the sperm heads recovered after the swim up and the differential lysis procedures		

Placenta

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Hahn, et al., 2013)	CS	Evaluates the rate of HPV infection in pregnant women and their neonates, and the risk factors associated with vertical transmission of HPV infection from mothers to neonates.	Cervical HPV testing was undertaken in pregnant women over 36 weeks of gestation, and mouth secretions and oral mucosa of neonates were tested for HPV immediately after delivery. HPV-positive neonates were rechecked 2 months postpartum to identify the persistence Of HPV infection.		HPV was detected in 72 of 469 pregnant women (15.4%) and in 15 neonates (3.2%). Maternal HPV positivity was associated with primiparity and abnormal cervical cytology. The rate of vertical transmission was 20.8%. No cases of HPV infection were found in the infants at 2 months postpartum no HPV was detected in placenta, cord blood or maternal blood by PCR or IHC.	Vertical transmission of HPV is associated with vaginal delivery and multiple HPV types in the mother; however, neonatal HPV infection through vertical transmission is thought to be a transient.	
(Koskimaa et al., 2012)	CS	HPV genotypes present in 329 pregnant women, their newborns,	cord blood, and placenta samples were determined by molecular techniques, including using pure DNA for nested polymerase chain reaction. HPV antibodies were tested using multiplex HPV serology.	HPV positivity of placental tissue and risk of vertical transmission	HPV DNA was detected in 17.9% of oral samples from newborns and in 16.4% of the cervical samples of the mothers. At delivery, mother-newborn pairs had similar HPV-genotype profiles, but this concordance disappeared in 2 months. HPV DNA was detected in 4.2% (13 of 306) of placental samples, in 3.5% (11 of 311) of cord blood samples, and in 4.1% (9 of 220) of breast milk samples. Oral HPV carriage in newborns was most significantly associated with the detection of HPV in the placenta (OR=14.0; 95% CI, 3.7-52.2; P=.0001).	HPV is prevalent in oral samples from newborns. The genotype profile of newborns was more restricted than that of the maternal cervical samples. The close maternal-newborn concordance could indicate that an infected mother transmits HPV to her newborn via the placenta or cord blood.	HPV transmission is possible via placenta or cord blood

(Rombaldi et al., 2008)	CS	The study included 49 HPV DNA-positive pregnant women at delivery.	This paper aimed at studying the transplacental transmission of HPV and looking at the epidemiological factors involved in maternal viral infection.	HPV positivity of placental tissue and risk of vertical transmission	12/49 placentas (24.5%) had a positive result for HPV DNA. 5/12 fetal side of placenta HPV+ 2/12 maternal side of placenta HPV+ 5/12 both sides of placenta HPV+ Eleven newborn were HPV DNA positive in samples from the nasopharyngeal or buccal and body or cord blood. In 5 cases (10.2%, n = 5/49) there was HPV type-specific agreement between genital/placenta/newborn samples. A positive and significant correlation was observed between transplacental transmission of HPV infection and the maternal variables of immunodepression history (HIV, p = 0.011).	the study suggests placental infection in 23.3% of the cases studied and transplacental transmission in 12.2%. It is suggested that in future HPV DNA be researched in the normal endometrium of women of reproductive age.	

DOES HUMAN PAPILLOMA VIRUS IMPACT THE OUTCOME OF MEDICALLY ASSISTED REPRODUCTION?

Male infected

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Depuydt et al., 2019)	CS	Seven hundred thirty-two infertile couples	None	Biochemical and clinical pregnancy rate in IUI cycles with HPV-positive or HPV-negative semen.	HPV prevalence in sperm was 12.5%/IUI cycle. When infectious HPV virions were detected in sperm, a significant decrease in clinical pregnancies was observed when compared with HPV-negative cycles (2.9% vs. 11.1 %/cycle). Above a ratio of 0.66 HPV virions/spermatozoon no pregnancies occurred (sensitivity 100%, specificity 32.5%)	Women inseminated with HPV-positive sperm had 4 times fewer clinical pregnancies compared with women who had HPV-negative partners. Detection of HPV virions in sperm is associated with a negative IUI outcome and should be part of routine examination and counseling of infertile couples.	Specific study with a lead on the specifics of the influence of the presence of HPV virions in sperm and decrease pregnancy rates, when the workout of the sperm used was standard and was employed for IUI.
(Depuydt et al., 2018)		514 donor sperm samples form 3 different sperm banks	Sperm samples were retrospectively examined for 18 different HPV types.	Presence of in sperm samples HPV	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%		

(Garolla et al., 2016)	CS	226 infertile couples	Male partners were evaluated by means of fluorescence in situ hybridization (FISH) for HPV on semen. After a diagnostic period, female partners underwent intrauterine insemination (IUI) or intracytoplasmic sperm injection (ICSI).	Seminal parameters and FISH analysis for HPV in sperm head. Spontaneous or assisted pregnancies, live births, and miscarriages were recorded.	Fifty-four male partners (23.9%) had HPV semen infection confined to sperm, confined to exfoliated cells, or in both cells. During the diagnostic period, noninfected couples showed spontaneous pregnancies. IUI and ICSI treatments were performed in, respectively, 60 and 98 noninfected and in 21 and 33 infected couples, with 38.4% and 14.2% cumulative pregnancy rates, respectively. The follow-up of pregnancies showed a higher miscarriage rate in infected couples (62.5% vs. 16.7%).	A reduction in natural and assisted cumulative pregnancy rate and an increase in miscarriage rate are related to the presence of HPV at sperm level.	This study compiles on a representative subset (226 couples). FISH is highly specific diagnostic tool for sperm. Plus, it clearly highlights the need to understand the mechanisms by which infected sperm can impair infertility outcomes from effective pregnancy to miscarriage. To be included
(Perino et al., 2011)	P	199 infertile couples	Patients were treated with standard procedure involving ovarian stimulation, sperm treatment and IVF and ICSI procedures	The association between pregnancy and miscarriage for demographic and clinical variables	Couples who underwent ART cycles experienced an increased risk of pregnancy loss when HPV DNA testing was positive in the male partner, compared with noninfected patients (66.7%–15%, P<.01). It is worth noting that all pregnancies in HPV-positive couples resulted in miscarriage, whereas there was a 15.9% overall miscarriage rate in HPV-negative couples (P<.001). statistically significant risk of miscarriage correlated with male age and to the presence of male HPV infection	HPV DNA testing in male partners of infertile couples could be useful in that it would allow clinicians to follow up individuals with infected sperm. In these cases, the possibility of delaying IVF procedures until the viral infection has been cleared could be taken into consideration.	This article puts the spot light on the occurrence of miscarriage with male HPV positivity and makes an accent on the requirement to test sperm before treatment, make a follow-up, strictly, of these patients until the viral infection of these patients is cleared. To be included

Female infected

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Xiong et al., 2018)	SR	Eighteen studies were included.	PubMed, Medline, Embase, and the Cochrane Library were searched until December 16, 2016.	Subgroup analysis of HPV genotype infection (high-risk HPV [HR-HPV] or indiscriminate genotype)	Eight studies revealed no significant association between HPV infection and spontaneous abortion (OR 1.40, 95% CI 0.56-3.50). However, subgroup analysis showed indiscriminate genotype HPV infection increased the ratio of spontaneous abortion with OR of 2.24 (95% CI 1.37-3.65), while HR-HPV infection had no significant effect (OR 0.65, 95% CI 0.21-1.98). The results indicated that HR-HPV infection was a risk for sPTB with a pooled OR of 2.84 (95% CI 1.95-4.14). HPV infection was found to be independent of the ART-based clinical pregnancy rate (RR 1.04, 95% CI 0.64-1.70) and spontaneous abortion of ART pregnancy (RR 1.47, 95% CI 0.86-2.50).	Indiscriminate HPV genotype infection can increase the risk of spontaneous abortion and HR-HPV infection was a risk factor for sPTB. However, there was not enough evidence to indicate the association between HPV infection and pregnancy rate of ART, and spontaneous abortion of ART pregnancy. Different genotypes of HPV infection may play a discrepant role in adverse pregnancy outcomes.	Specific to infected female and outome of pregnancy only. Subtype risk observed too. Yet, not enough evidence, again(!), to indicate influence of HPV over ART pregnancy outcomes.
(Wang et al., 2008)	CS	1044 Chinese women undergoing IVF for tubal infertility or, in their partners, abnormal semen.	Cervical scrapes, digital colposcopies, and cervical biopsies	clinical signs of cervical inflammation rate of HPV detection	There were no associations between IVF-ET outcome and infection rate, degree of cytopathologic abnormality, detection of HPV, or results of digital colposcopy and cervical biopsy. Cytologic results did not correlate with any of the clinical parameters of IVF-ET.	No association was found between IVF-ET outcome and cervical infection, cytopathologic result, HPV detection, or result from the colposcopy or biopsy. Extensive testing and treatment for cervical infection do not appear necessary in IVF-ET candidates.	Significant amount of patients. Disproves, extensive testing upstream IVF-ET, yet, must be noted that this outcome might depend on the subtype of HPV (e.g. clonal or not…).

WHICH TECHNIQUES CAN BE USED TO PREVENT/REDUCE HUMAN PAPILLOMA VIRUS TRANSMISSION DURING MEDICALLY ASSISTED REPRODUCTION?

Vaccination

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Garolla et al., 2018)	CS	retrospectively enrolled 151 infertile couples with detection of HPV in semen, between January 2013 and June 2015	Patients were counseled to receive adjuvant HPV vaccination. 79 accepted vaccination (vaccine group) 72 did not (control group).	Evaluate HPV viral clearance: semen analysis, INNO-LiPA and FISH for HPV in semen cells after 6 and 12 months from basal evaluation. Spontaneous pregnancies, miscarriages live births	Progressive sperm motility and anti-sperm antibodies were improved in the vaccine group at both time points ($p < 0,05$ vs control arm). 41 pregnancies, 11 in the control group 30 in the vaccine group, (respectively 15% and 38,9%, $p < 0,05$) Control group: 4 deliveries 7 miscarriages Vaccine group: 29 deliveries 1 miscarriage ($p < 0,05$ vs control). HPV detection on sperms was predictive of negative pregnancy outcome. and live births.	Adjuvant vaccination associated with enhanced HPV healing in semen cells and increased rate of natural pregnancies	

(Foresta et al., 2015)		179 out of 619 infertile patients, showing HPV-DNA detection in semen by FISH analysis, were enrolled. 91 vaccine-sensitive (VSPs) 88 nonvaccine-sensitive patients (NVSPs) by INNO-LiPA. 19 VSPs showed vaccine-type specific seroconversion at recruitment.	All patients underwent specific counselling. 42 seronegative VSPs were randomly assigned to receive quadrivalent vaccination in 6 months, 49 VSPs, 19 seroconverted and 30 seronegative, served as controls.	The prevalence of HPV-DNA semen infection and serology was studied in a follow-up of 24 months.	Compared to seronegative patients, VSP seroconverted at recruitment showed absence of multiple infections and 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 b 0.001 at 12 months vs seroconverted VSP), achieving clearance in 12 months.	Humoral immunity has a major role in healing from HPV infection. Elder ART patients with HPV semen infection may benefit by the union of both specific counselling and available prophylactic vaccination.	

Semen processing

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Brossfield et al., 1999)	Rest	More than HPV-negative 500 sperm samples	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. Sperm parameters were measured on a Hamilton Thorn HTM-C analyzer. Sperm DNA were extracted and polymerase chain reaction (PCR) was carried out targeting the L1 consensus gene of HPV and the designated sentinel gene, 17q21 spanning the D17S855 gene. Amplified products were analyzed in 2% agarose gel electrophoresis	The objective was to compare three types of sperm washing procedures for their capacity to remove exogenous human papillomavirus (HPV) DNA from infected (infected is not the appropriate term; exposed is correct) sperm.	PCR analyses detected the consensus L1 HPV gene in sperm after they were processed through either of the three procedures. Controls were negative for the L1 gene. Extracted DNA were verified by PCR amplification of 17q21 spanning the D17S855 gene. Transfected sperm had higher percentages of total motility and progression compared with the control. Centrifuged, washed, transfected sperm exhibited a greater curvilinear velocity and hyperactivation	The data showed that washing would not remove exogenous HPV DNA from sperm cells. The viral DNA was tenaciously bound to the sperm, suggesting an internalization into the sperm. The viral DNA also increased the motility of the sperm by affecting the velocity and progression of the sperm, which suggested either an increase in metabolism, an enhancement of the calcium-regulated motility mechanism, or an artifact of PCR reagents.	

(Fenizia et al., 2020)	OS	15 clinically HPV-positive male subjects	Freshly ejaculated semen was collected and readily processed by gradient separation followed by swim-up from the washed pellet. .	The resulting fractions were seminal plasma, cell pellet, round cells, non-motile spermatozoa and motile spermatozoa. All fractions were then tested for the presence of HPV DNA	67% were positive in at least one of the seminal fractions. If any postivity was detected, the plasma was always HPV positive. No consistent pattern was observed throughout different samples in the cell pellet, round cell and non-motile spermatozoa fractions. However, after the sperm-wash procedure, the fraction of motile spermatozoa was never found to be HPV-positive.	The sperm-washing technique, which was previously successfully used to remove HIV, can efficiently remove HPV from spermatozoa. However, the present study was conducted on a small population so a larger follow-up study is recommended. HPV screening should be performed in sperm samples and, upon HPV positivity, sperm-washing should be considered before MAR.	Representative of study of washing different fractions. Importantly, the fraction of motile spermatozoa was never found to be HPV-positive. Claim to be confirmed and later clinically assessed in further studies.
(Foresta et al., 2011b)	CS	32 infertile patients positive for semen HPV	To determine the effectiveness of three sperm washing protocols for removing human papillomavirus (HPV)-infected cells from semen samples of infertile patients	polymerase chain reaction (PCR) and in-situ hybridization in sperm and exfoliated cells	Evaluation of sperm parameters and presence of HPV, performed in semen samples before and after procedures of sperm selection. RESULT(S): All native samples showed the presence of infected sperm with a mean percentage of positivity (24.7% +/- 8.9%) higher than exfoliated cells (13.8% +/- 4.3%). Fifteen samples had HPV DNA on sperm and exfoliated cells. Sperm washing centrifugation showed no changes in the number of infected samples and in the percentage of infected cells. Ficoll and swim-up protocols induced a slight reduction in the number of infected samples (30 and 26, respectively).	This study demonstrated that conventional sperm selection rarely eliminates HPV sperm infection. More attention should be paid to the reproductive health of infected patients because, not only can HPV be transmitted, but it may also have a negative effect on development of the fetus.	HPV is rarely washed out upon sperm selection, this could related to the stage of the viral cycle (i.e. virions attached to the sperm head or only in seminal plasma) at which the samples are in.

(Garolla et al., 2012)	CS	22 infected male patients 13 control male subjects	direct swim-up and modified swim-up (with added Heparinase-III) in all samples to assess sperm clearance outcome	Evaluation of sperm parameters, terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling test to evaluate DNA fragmentation and fluorescence in situ hybridization or immunohistochemistry for HPV 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 ~24% (P< 0.01), while modified swim-up is able to remove completely HPV DNA both from naturally and artificially infected sperm. Enzymatic treatment with Heparinase-III tended to decrease sperm motility, viability and DNA integrity but the effects were not significant	This study shows that Heparinase-III treatment seems not to affect spermatozoa in vitro and suggests that this treatment should be investigated further as a means of preparing sperm from patients who are infected with HPV in order to reduce the risk of HPV infection when using MAR.	Only modified washing procedure on full fractions provided in the literature, yet showing that it doesn't have effect on HPV removal.
(Olatunbosun et al., 2001)	CS	semen samples from 85 volunteers 45 with historical or clinical evidence of genital HPV infection (study group) 40 were healthy, clinically HPV-negative semen donors	detect human papillomavirus (HPV) in semen and find if sperm washing removes HPV DNA	Amplification by nested polymerase chain reaction (PCR) was used to detect viral DNA sequences in semen samples	We detected HPV DNA in the sperm cells of 24 of 45 subjects (53%) with past or current HPV infections in contrast to three of 40 healthy subjects (8%) (P <.001). Overall, 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.	We suggest that HPV DNA testing should be done on the semen of prospective donors, and those with positive tests should be excluded from donation.	Simple sperm washing does not clear HPV in sperm. Confirmation of first papers on the subject using common washing.

DOES THE PLASMATIC VIRAL LOAD CORRELATE WITH HUMAN PAPILLOMA VIRUS LOAD IN SEMEN?

We identified no publications investigating the correlation between plasmatic and semen HPV load.

WHICH INTERVENTIONS CAN BE USED TO REDUCE/AVOID VERTICAL TRANSMISSION OF HUMAN PAPILLOMA VIRUS TO THE NEW-BORN?

ECS

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Chatzistamatiou et al., 2016)	SR	clinical studies reporting the prevalence of human papillomavirus (HPV) in the offspring of HPV-infected women in association to their mode of delivery	meta-analysis (8 studies)	The number of caesarean sections needed to prevent one case of perinatal infection (number needed to treat or NNT)	Our pooled results, showed that caesarean section is associated with significantly lower rates of HPV transmission than vaginal birth (14.9% vs. 28.2%, risk ratio or RR: 0.515, 95% confidence interval or CI: 0.34-0.78). The number of caesarean sections needed to prevent one case of perinatal infection (number needed to treat or NNT) would be 7.5.	As a conclusion it should be noted that caesarean section decreases the risk for perinatal HPV transmission by approximately 46%. Perinatal transmission still occurs in approximately 15% of the children born by caesarean section.	Expected outcome in straightforward manner, proper methodology for meta-analysis.
(Zouridis et al., 2018)	SR	This SR was made according to the PRISMA statement. They searched PubMed and Scopus.. Data from the selected articles were plotted, and the pooled percentage of antenatal vertical HPV transmission among HPV-positive mothers as well as the pooled relative risk of antenatal vertical HPV transmission between cesarean and vaginal delivery among HPV-positive women were calculated	SR		9 studies including 421 HPV-positive mothers and their offsprings were selected.The pooled percentage of antenatal vertical HPV transmission was 4.936% (95% CI 1.651–9.849), with moderate heterogeneity between the studies (I2=72.22%). The pooled relative risk of antenatal vertical HPV transmission between cesarean and vaginal delivery among HPV-positive women was 0.912, with no statistical signifcance (95% CI0.226–3.674) and homogeneity between the studies	**The low quality of the existing studies** creates the need of a new, carefully designed study to evaluate the precise rate of intrauterine HPV transmission.	No conclusion possible

(Summersgill et al., 2001)	Rest	268 healthy infants, children, and adolescents who were < or = 20 years old.	Sociodemographic information was obtained. Oral squamous cells were collected from swabs with young children and from oral saline solution rinses with older children and adolescents	Sociodemographic information Extracted DNA was evaluated for HPV by polymerase chain reaction, dot blot hybridization, and DNA sequencing	HPV was detected in 6.0% of the participants. HPV frequency among young children (<7 years old) was 8.7% (11/127), and among adolescents (13-20 years old) it was 5.2% (5/97). HPV was not detected in children aged 7 to 12 years old (0/44). Fifty-four percent (6/11) of HPV-positive children were 1 year of age or less; 3 of the HPV-positive children (<7 years old) were delivered by cesarean section. No statistically significant association was found between the detection of HPV in the oral cavity and method of delivery or gender; parent's race, education, HPV-related conditions, smoking history, or number of sex partners; or adolescent's smoking history or history of sexual activity.	This study suggests that HPV is present in the oral cavity primarily in children 2 years old and younger and in adolescents 13 years and older. Cesarean delivery was not protective against oral HPV infection; in fact, half of the HPV-positive infants were born by cesarean delivery.	Illustrates the prevalence in children and the mode of delivery. Again, c-section is not a protective mode to contamination. Plus, the age of children presenting the infection (2 -13 years old in oral cavity) is put forward in the prevalence estimates.
(Wang et al., 1998)	Rest	73 pregnant women on their third-trimester examinations.	Samples of fetal membranes, amniotic fluid and nasopharyngeal swab were obtained from the parturients and their neonates.	The presence of HPV types 16, 18 and 35 deoxyribonucleic acid was detected by polymerase chain reaction (PCR) and endonuclease method.	The maternal-fetal transmission rate of HPV was 50% (7/14) for spontaneous vaginal delivery, and 33.3% (4/12) for cesarean section.	HPV can be transmitted from mothers to their babies not only through the placenta during pregnancy, but also through the genital tract during delivery.	HPV can be transmitted through placenta and through cervical contact too.

Breastfeeding

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Louvanto et al., 2017)	CS	included 308 where the mother was breast feeding her offspring	Mothers collected the milk samples manually at day 3, and at months 2, 6 and 12. Cervical and/or oral samples were collected from all family members. HPV testing was performed using nested polymerase chain reaction and Luminex-based Multimetrix kit.	prevalence and persistence of HPV in breast milk in the Finnish Family HPV cohort study	Breast milk HPV DNA was found in 10.1% (31/308), 20.1% (39/194) and 28.8% (17/59) of samples at day 3, months 2 and 6, respectively. The following HPV genotypes were detected: 6, 16, 18, 33, 45, 53, 56, 59, 66 and 82. Breast milk HPV persisted among 5.5%	HPV in breast milk is prevalent among the lactating mothers and HPV can also persist in breast milk. The breast milk is a potential vehicle for HPV transmission to oral mucosa of the spouse but not of the offspring.	Specific, it stresses the actual question in detail. Good methodology
(Glenn et al., 2012)	Rest	40 normal lactating women	determine if viral sequences are present in human milk from normal lactating women	Standard (liquid) and in situ polymerase chain reaction (PCR) techniques were used to identify HPV and EBV in human milk samples from normal lactating Australian women	High risk human papillomavirus was identified in milk samples of 6 of 40 (15%) from normal lactating women - sequencing on four samples showed three were HPV 16 and one was HPV 18. Epstein Barr virus was identified in fourteen samples (33%).	The presence of high risk HPV and EBV in human milk suggests the possibility of milk transmission of these viruses. However, 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.	As above, this implies the presence of HPV in breast milk but not its transmission to infants as seen by the rarity of viral associated malignancies in youngsters.

(Yoshida et al., 2011)	rest	80 breast milk samples (n=80) were analysed for high-risk HPV DNA.		The domain including HPV E6 and E7 was amplified by polymerase chain reaction using consensus primers, and HPV serotype determined by electrophoresis after restriction enzyme digestion.	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.	It was concluded that the infection of HPV in maternal milk is rare (2/80); verticaltransmission through maternal milk was not detected in thisstudy (0/80).	No vertical transmission seen with breast feeding

HTLV I/II

WHAT ARE THE RISKS OF HTLV I/II TRANSMISSION THROUGH VAGINAL/ANAL INTERCOURSE?

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Roucoux et al., 2005)	CS	Prospect. cohort analysis Inclusion: 85 HTLV + blood donors and their HTLV neg partn. 30 HTLV I (7 M/23 F) 55 HTLV II (17 M/38 F) Relationship > 6 mth Exclusion: same sex coupl false pos lab result Incomplete interview	Follow up 1990-2003 2x/y HTLV-ab testing partn 2x/y interview blood donors and partn (condom use, monogamy, sex history.	(1) incidence rate (IR) sexual transmission in person years (x/100y) (2) risk factors associated with sex. transm T= transmitters NT= non transmitters	2 transmiss HTLV I (1 M-->F; 1 F-->M) 2 transmiss HTLV II (1 M-->F; 1 F-->M) IR HTLV I: 0,9/100y [CI 95%: 0,1-3,3] IR HTLV II: 0,5/100y [CI 95%: 0,06-1,8] IR M-->F: 1,2/100y [CI 95%: 0,1-4,3] IR F-->M: 0,4/100y [CI 95%: 0,05-1,6] Overall IR: 0,6/100y [CI 95%: 0,2-1,6] Median relation duration: 72 mth (NT) vs 57 mth (T) [p=NA] Proviral load: HTLV I: 4,46e10 (T) vs 2,91 (NT) [p=0,19] HTLV II: 3,20e10 (T) vs 1,59 [p=0,11]	IR M-->F > F-->M IR HTLV I > HTLV II Study group relatively small Too small for formal evaluation and detection of statistical significancy Lower IR than Stuver SO, Mueller NE (2,5/100y)	Small group Stuver SO, Mueller NE included patient with STD's, higher age and longer relationship duration

(Stuver et al., 1993)	CS	Prospect. cohort analysis 534 married couples in 2 villages in Japan Inclusion: 97 HTLV I discordant coupl 33 husband pos (H+) 64 wives pos (W+) 95 HTLV concordant 342 negative concordant	1984-1989 1x/y serological screening of HTLV I neg spouses No questions regarding sexual practices due to cultural norms Duration of marriage was calculated dependent on information given by H or W	(1) Analysis factors associated with transmission (2) Cumulative incidence rate [CI] (number seroconverted/number at risk)	Number infected couples rises with age (median 50-59) A man was 6.8 times more likely to be seropositive if the wife was positive. (59.7% vs 8.8%) A woman was 4.7 times more likely to be seropositive if the husband was positive (74.2% vs 15.8) H-->W seroconv: male age > 60 vs < 60 RR 12,2 {p=0,05} Age W at seroconv: post menopausal Titer > 1:1024 and anti-tax pos 6 seroconversions in HTLV disc coupl 4 H-->W and 2 W-->H 1 seroconv in HTLV neg concord coupl **A:** CI HTLV I disc: 7,5% (6/80) **B:** CI HTLV I neg conc: 0,18% (1/549) **A vs B**: RR 41,2 [CI 95%: 5,0-338]{p<0,001} **C:** CI H-->W 14,8% (4/27) **D:** CI W-->H 3,8% (2/53) **C vs D:** RR 3,9 [CI 95%: 0,77-20,1]{p=0,19}	(1) older age, longer marriage and high titer levels associate with higher risk of transmission (2) 7,5% in discordant couple 3,9 times higher for women vs men No information on extramarital sexual contacts Small number of seroconversion (W>>H)	No data on sexual practices could be obtained 2 seroconverted spouses (1 H and 1 W) reported to have had a blood transfusion 10 y prior to study Old study with possible bias in data collected

IS THERE A THRESHOLD BELOW WHICH TRANSMISSION OF HTLV I/II IS UNLIKELY?

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Kaplan et al., 1996)	CS	A total of 546 HTLV-seropositive donors were enrolled in the study. Of these, 382 (70.0%) reported having a current sexual relationship of >=6 months duration-113 men and 269 women Of these, 40 men (35.4%)and 85 women (31.6%) brought their partners in to enroll in the study	1990 at least 6 months	Risk factors for transmission	Female partners of male donors: 15/40 tested positive Male partners of female donors: 17/84 tested positive Among 31 couples in whom HTLV-infected men likely transmitted infection to their partners (11 HTLV-I and 20 HTLV-II) and 25 male-positive, female-negative couples (8 HTLV-I and 17HTLV-II), HTLV transmitter men had been in their relationships longer (mean 225 months vs. 122 months)and had higher viral loads (geometric mean 257,549 vs. 2,945 copies/300,000 cells for HTLV-I; 5,541 vs.118 copies/300,000 cells for HTLV-II) than non-transmitters (P = 0.018 and P = 0.001 for duration of relationship and viral load, respectively, logistic regression analysis	antibody titers were not as strongly associated with male-to-female transmission as viral load. These results suggest that antibody titers in the male partner are useful markers for male-to-female sexual transmission, put probably less so than viral load.	

(Paiva et al., 2017)	CS	178 HTLV-1-positive patients had spouses, 107 of which had tested partners, thus increasing the initial sample size (46 men and 61 women). 26 seroconcordant couples; Individuals co-infected with HTLV-2 or human immunodeficiency virus were not included in the analysis	Between January 2013 and May 2015		PVL was higher among couples in which both partners were infected (19 couples) than among discordant pairs (37 couples); the mean and median among seroconcordant couples were 363 (SD 433) and 179 (5-597) copies/104 PBMC, respectively, and 145 (SD 145) and 8 (0-143) copies/104 PBMC, respectively, among serodiscordant couples (P = 0.03). Among serodiscordant couples, there was no statistically significant difference in the distribution of PVL between 12 HTLV-positive men with seronegative wives, with a mean of 142 (DP 294) and median of 17 (0-173) copies/ 104 PBMC.	The apparent association between high circulating levels of provirus and seroconcordance rate among couples suggests that proviral loads contribute markedly to the risk of sexual transmission, regardless of gender index.	
(Stuver, et al., 1993)	CS	married couples enrolled in the Miyazaki Cohort Study and HTLV-I-seropositive (H^/ wife seropositive (W+), 33 HVW" , 64 H"^, and 342 H'/W-	between November 1984 and April 1989 were After 5 years of follow-up, sev	n seroconversions occurred and clustered significantly among serodiscordant pairs (relative risk [RR] = 41.2); the rate of transmission was 3.9 times higher if the carrier spouse was male (P = .19). Among HVW" couples, husband's age ^60 years strongly predicted seroconversion in the wives (RR = 11.5). All	4 carrier husbands whose wives seroconverted had HTLV-I titers ≥1:1024 (P = .04) and were anti-tax antibody positive (P = .06).	Overall, sexual transmission of HTLV-I was primarily from older infected husbands to their wives, with husbands' viral status being an important factor.	Threshold HTLV-I titers ≥1:1024

WHICH TECHNIQUE FOR MEDICALLY ASSISTED REPRODUCTION SHOULD BE USED IN COUPLES WITH HTLV I/II?

No studies have compared different techniques for MAR in couples where one partner is infected with HTLV I/II.

CAN HTLV I/II VIRUS DNA BE DETECTED IN OOCYTES/ SPERM/ PLACENTA?

DNA integration in semen/oocytes/embryo

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Nakano et al., 1984)		3 ATLA positive males	1983-1984	A monoclonal antibody GIN-14 to ATLV p19 and p28 was used for detec tionof ATLA.9) We examined 35 pairs (neonate and mother) for the appearance of ATLA in the cultured cells. ATLA was definitely demonstrated in the cells from 29 mothers. Percentages of ATLA-positive cells in each	Mononuclear cells were separated by the Ficoll-Conray method, cultured for 2-3 weeks and examined for ATLA by immunofluorescence Expression of ATLA in 1% pf the cells from the semen.	The transmission of ATLV via semen during sexual contact is also suggested to occur as one of several possible routes of horizontal transmission.	

Placenta

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Fujino et al., 1992)	Case report	Placental villi were obtained from 12 pregnant women at term: 9 from HTLV-1-seropositive women and 3 from HTLV-I seronegative.	No data	Immunochemistry and PCR of placental tissue	placental epithelial cells were positive with double staining 22% of placentas from HTLV-I seropositive mothers were infected by HTLV-I.,	the frequency of HTLV-I transmission from mother to cord-blood lymphocytes is 7%.' The difference between the frequency of HTLV-I infection of placenta and that of HTLV-I transmission to cord-blood lymphocytes suggests defence mechanisms against HTLV-1 infection at the maternofetal interface.	

DOES HTLV I/II /TREATMENT OF HTLV I/II BEFORE MEDICALLY ASSISTED REPRODUCTION IMPACT THE OUTCOME OF MEDICALLY ASSISTED REPRODUCTION?

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Mansouri Torshizi et al., 2014)	non-randomized cohort study (CS)	2007- 2011 One IVF center ICSI cycles HTLV I infected women	**Study group:** 32 ICSI cycles in HTLV I infected women **Control group** 62 ICSI cycles in age matched non-infected	fertilization rate (FR), embryo quality, implantation rate (IR), clinical pregnancy rate (PR), abortion rate (AR).	**Study vs control group** Fertilization 65% vs 73% (p=0.15), implantation 22.6% vs. 18.4% (p=0.33), pregnancy rate 46% (15/32) vs. 45% (28/62) (p=0.12) No of transferred embryos 2.9 ± 0.9 vs. 2.8 ± 0.7 (p=0.79) cryopreserved embryos: 4.4 ± 3.9 vs. 4.5 ± 4.3 (p=0.68) multiple pregnancies 6% vs. 10% (p=0.09) abortion rate 20% vs. 17% (p=0.21)	The results suggest that the embryo quality and ICSI outcome are not affected by HTLV-1 infection in serodiscordant couples. The major finding of this study is that the outcome of ICSI in HIV-I-infected patients and seronegative controls is similar.	Moderate quality, Risk of performance bias Women in the HTLV-1 group hab been 2 years younger

WHICH TECHNIQUES CAN BE USED TO PREVENT/REDUCE HTLV I/II TRANSMISSION DURING MEDICALLY ASSISTED REPRODUCTION?

We found no evidence comparing different semen processing techniques in HTLV I/II infected patients.

DOES THE PLASMATIC VIRAL LOAD CORRELATE WITH HTLV I/II IN SEMEN?

We found no studies investigating the correlation between viral load in semen and serum in HTLV I/II infected patients.

WHICH INTERVENTIONS CAN BE USED TO REDUCE/AVOID VERTICAL TRANSMISSION OF HTLV I/II TO THE NEW-BORN?

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Boostani et al., 2018)	SR	54 citations; of these, 96 potentially relevant articles were identified. After reviewing the 96 full-text articles in detail, 7 reports met the inclusion criteria for this review. Absolute numbers are not mentioned	1989-2004	Pooled odds ratio (OR) and risk difference (RD) of HTLV-I transmission in the breastfed group compared to the bottle-fed infants	Breast feeding versus bottle feeding OR = 3.48, 95% CI: 1.58-7.64 exclusive breast feeding up to 6 months compared to bottle feeding does not increase transmission rate of HTLV-I infection (pooled OR = 0.912, CI: 0.45-1.80; exclusive breastfeeding >6 months compared to bottle feeding: OR: 3.83, CI: 1.80-8.10	the current meta-analysis showed that short period (less than 6 months) of breastfeeding did not increase risk of HTLV-I infection transmission from mother to child among breastfeeders and more than 6 months of breastfeeding significantly increased the risk of HTLV-I infection. However, our meta-analysis shows that refraining from breastfeeding can decrease the risk of vertical HTLV-I transmission.	
(Ando et al., 1987)	non-randomized cohort study (CS)	35 mothers **HTLV-I** seropositive	24 breast feed 11 bottle feed	HTLV-I antigenpositive cells in peripheral blood samples At birth 1, 3, 6, 12 months after birth	HTLV-I antigenpositive cells were detected in peripheral blood samples obtained 12 months after birth. 11/24 breastfed infants 1/11 bottle-fed infants of HTLV-I seropositive mothers.	We conclude from this study that HTLV-I infection from mother to infant occurs mainly via breast milk, not via the placenta during pregnancy or delivery, and that bottle-feeding is an effective way of avoiding this route of transmission.	Thus bottle-feeding appears to be an effective method to avoid HTLV-I transmission from HTLV-I seropositivem others to infants. Study is 32 years old

(Hisada et al., 2002)	CS	150 mothers and their 154 children who had been followed up for at least 18 months.	January 1989 and August 1990, Peripheral blood samples from the mothers at delivery Blood samples from the children were obtained every 6 weeks for the first 6 mo, every 3 mo to 2 years, every 6 mo thereafter		The mean antibody titer among the 28 mothers who transmitted HTLV-1 was 18,870, compared with 11,316 among mothers who did not transmit. Compared to non-infected children, breastfeeding for ≤6 months OR 10.8 (95% CI 2.0-57.8) Compared with children who were breast-fed for ≤6 months, the risk of transmission among children who were breast-fed for 6.1–12 months was 4.4 fold higher and among those who were breast-fed for >12 months was 10.2-fold higher	In summary, the risk of infection for breast-fed children born to HTLV-1–positive mothers appears to be primarily determined by the provirus load to which they are exposed in the absence of passively transferred maternal antibody. The lower limit of detection for provirus load was 10 provirus copies/105 cells.	
(Paiva et al., 2018)	CS	192 mothers with HTLV-1 infection resulting in 499 exposed offspring, 288 (57.7%) of whom were tested for HTLV-1, making up the final sample for the study, along with their 134 respective mothers.	June 2006 and August 2016	Vertical transmission rate	253/288 children were breastfed, 41/288 tested positive for HLTV-I Risk factors: Mother's proviral load ≥100 copies/104 PBMC Breastfeeding over 12 months (OR 6.15, 95%CI 2.62-14.41)	Overall, the mother/child positivity rate was 14.2%, reaching 50% for infected Asian-descendant mothers.	

(Wiktor et al., 1993)	CS	34 index children Two to three years later, 36 seropositive mothers were recontacted	from 1983 to 1985.		seventeen of 74 (23%) [95% CI 15—34%] children were seropositive. Breastfeeding for >6 mo 4/19 (21%) index children Breastfeeding ≤6 mo 1/15 (7%) index children RR 3.2; CI 0.4-22.1	We conclude that mother-to-child transmission of HTLV-I in Jamaica is associated with longer duration of breast-feeding, older age, and higher HTLV-I antibody titer,	

Zika virus

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

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Counotte et al., 2018)	'living' systematic review up to April 2018	Mixture of study designs included. 36 cases presumed to have occurred sexually	Retrospective, prospective, case reports 24 studies 36 couples with a primary partner with ZIKV infection	sexual transmission.	Various reported. Absolute risk of sexual transmission not reported. 34/36 male to female Unclear how many male to female cases were vaginal or anal. 1 case male to male. 1 case female to male	Sexual transmission possible but low risk. Zika virus favours semen and the testis. Shorter period of infectiousness (3 months) based on viral cultures, than previously thought when testing for RT/PCR alone.	Part funded by WHO. Refs [6,78,79,82,84,85,87,88,90,91,96,100,102,105,113,115, 119-121,123,124,127,131,137]
(Coelho et al., 2016)	Retrospective cohort	29301	2015-2016 Zika and Dengue in Rio de Janeiro	incidence	90% more female infections than male for Zika and 30% for Dengue The regression results indicated a significantly higher Zika incidence for sexually active women (1.7767, 95% confidence interval (CI) 0.500 to 3.053, p = 0.006). Sex alone was not a significant predictor of Zika incidence (0.2120, 95% CI 1.207 to 0.783, p = 0.676).	Women of reproductive age more likely to be infected than men and also to seek medical input	

(García-Bujalance et al., 2017)	Case series	5 patients with ZIKV infection acquired in endemic areas after returning to Spain	Serial semen RT-PCR and viral culture	Duration of infectivity	The female partners of male patients 1, 4 and 5 were symptomatically infected for ZIKV. The female partner of patient 1 had positive serology for ZIKV, but we did not perform ZIKV RT-PCR in her serum. Detection of ZIKV RNA in serum and urine was analyzed from female partners of patient 4 and 5. Blood sample tested from female partner of patient 5 was negative.	Male reproductive system acts as a reservoir for Zika. Viral culture not easy to do. Unclear what the relationship between infectivity, RT-PCR & viral culture is?	
(Sánchez-Montalvá et al., 2018)	CS	11 Spanish travellers (6 men, 5 women) and 6 sexual contacts.	Prospective cohort study 12 months in 2016 in 2 centres.	Serial RT-PCR testing in different body fluids	Persistence in male and female genital fluids was up to 45 days. The sexual contacts of all index cases tested negative for Zika IgM and IgG	In this study sexual transmission did not occur (to non travelling partner), however the RT-PCR levels were low in the infected patients studied.	
(Sokal et al., 2016)	CS	17 patients attending a Paris travel clinic over 4 month period	Prospective cohort 4 month screening	Blood work	Leucopenia 6/17 and thrombocytopenia 2/17 observed. All recovered.	Travel from endemic countries in a symptomatic patient should raise the possibility of Zika.	

(Yarrington et al., 2019)	Case report	1	Woman had a frozen embryo transfer. Husband travelled to an endemic area. The couple had sexual relations in early pregnancy.	microcephaly	Placental tissue tested positive for ZIKV RNA	Couples having ART / MAR where the male travels to a Zika area should use barrier contraception on return	

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

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.

WHICH TECHNIQUE FOR MEDICALLY ASSISTED REPRODUCTION SHOULD BE USED IN COUPLES WITH ZIKA VIRUS INFECTION?

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.

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

DNA integration in semen/oocytes/embryo

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Joguet et al., 2017)	CS	15 males with ZikV tested sequentially.	4 months	Blood, urine and semen tests at 7, 11, 20, 30, 60 & 90 days	Eleven 90% fractions (25%) containing only spermatozoa were ZIKV RNA-positive. All were from semen with high ZIKV RNA load in seminal plasma (>5 log copies/ml). All these positive 90% fractions were submitted to a swim-up method to isolate motile spermatozoa. Seven (64%) swim-up fractions later tested positive for ZIKV RNA (maximum 7·20 log copies/2×106 cells). No sample was positive in cell fractions obtained after sperm preparation if native semen was negative. Seminal plasma was positive and semen cells of native semen were negative in 7 of 45 samples (15·5%), and seminal plasma was negative and native semen cells were positive in 2 of 45 samples (2%).	ZikV detected in blood the longest	Later studies have suggested that this does not equate to being infectious.
(Filho et al., 2019)	Case report	1 having MAR, female had ZikV (male neg)	RT-PCR of follicular fluid, cumulus & oocytes,	Embryos not created or replaced	Blood sample tested positive for ZIKV 2/7 oocytes tested positive for ZikV RNA follicular fluid & cumulus was negative	Embryo could carry ZikV, thus testing of couple important. The negative oocytes might have been previously positive as RNA degrades quickly	Interesting to MAR field, albeit for case report.

Placenta

(Bhatnagar et al., 2017)		Case series of 52 women suspected of being infected with Zika virus during pregnancy Of which 32 were effectively infected And different tissues from 8 infants with microcephaly who died	Placentas, fetal tissue, brains,	RT-PCR & in situ hybridisation	Brain and placental tissues from 32/52 (62%) case patients were positive by both Zika virus RT-PCR assays The time frame from maternal symptom onset to detection of Zika virus RNA by RT-PCR in placentas was 15–210 (mean 81) days. 12/17 case-patients with adverse pregnancy outcomes, Zika virus RNA was detected by RT-PCR in placentas/umbilical cord/fetal tissues; all had symptom onset during the first trimester. relative levels of Zika virus RNA in the first trimester placentas (13.10 [1.718–99.87] copies/cell) were 25-fold higher than those in the second or third trimester or full-term placentas.	Zika virus replicates and persists in fetal brains and placentas, providing direct evidence of its association with microcephaly. Tissue-based reverse transcription PCR extends the time frame of Zika virus detection in congenital and pregnancy-associated infections	
(de Noronha et al., 2018)		Case series of 24 women who contracted ZV in different stages of pregnancy: 1st trimester: 5 cases 2nd trimester: 8 cases 3rd trimester: 6 cases Unknown: 5 cases	All placental tissue was sampled at delivery independent of time of infection Except 1 from a spontaneous abortion at 12 weeks		Villous immaturity most common anomaly found In 15/24 cases, no pathological evidence of Zika infection was found in H&E sections, however, 3/15 cases presented with congenital disorders. Immunohistochemical (IHC) analysis of the placental tissue samples using anti-flavivirus MAb (4G2) and anti-ZIKV MAb showed immunostaining in the Hofbauer cells, regardless of the gestational age when ZIKV infection occurred enhancement of the number of syncytial sprouts was observed in the placentas of women infected during the third trimester, indicating the development of placental abnormalities after ZIKV infection.	Hofbauer cell placental hyperplasia could be considered for ZV when serum samples not available	

(de Noronha et al., 2016)		Case series 5	Case 1: miscarriage at week 12 Case 2: baby girl born at 38.4 weeks of gestation and died within 6h. Case 3: baby boy born at 9 mo of gestation and died within 20h Case 4: baby boy born at 35 weeks of gestation and died the day after birth Case 5: healthy baby	Histology from different fetal body parts	Case 1: placental immunopositivity in Hofbauer cells Placental tissue positive in Zika RT-PCR Case 2: positive RT-PCR test for ZIKV and histopathological changes in placental tissue Case 3: no placental tissue tested Case 4: no placental tissue tested Case 5: viral RNA by RT-PCR was detected in placenta. Umbilical cord blood and newborn serum samples were negative for ZIKV.	transplacental transmission of ZIKV through the detection of viral proteins and viral RNA in placental tissue samples from expectant mothers infected at different stages of gestation. We observed chronic placentitis (TORCH type) with viral protein detection by immunohistochemistry in Hofbauer cells and some histiocytes in the intervillous spaces. We also demonstrated the neurotropism of the virus via the detection of viral proteins in glial cells and in some endothelial cells and the observation of scattered foci of microcalcifications in the brain tissues. Lesions were mainly located in the white matter	ZIKV crosses placenta.
(Lum et al., 2019)		Case series 3 placentas, mothers infected in first, second and third trimesters	The fullterm placentas were investigated	Histology, immunology and transcriptomics by trimester	ZIKV infection did not induce any overt adverse placenta pathology. ZIKV protein co-localised to Hofbauer cells (Figure 1c), in line with previous reports. → positive infection of the placenta, regardless of the pregnancy trimester in which ZIKV infection occurred.	these data showed that ZIKV proteins were present in the placenta up to delivery, without causing any physical harm to the newborn infant.	

(Pomar et al., 2019)		Retrospective case control 291 fetal samples / placentas from ZIKV infected women Pregnant women were defined as ZIKV-positive either by a positive RT-PCR result in blood and/or urine, or by the presence of specific ZIKV IgM after anti-ZIKV antibody detection in the blood, confirmed by a micro-neutralizing assay in cases of suspected co-infections with other arboviruses	Placenta ZIKV infection status was classified into three categories as follow: 1) Control placentas stemming from pregnant women who tested negative for ZIKV up to delivery, 2) Exposed placentas stemming from proven ZIKV-infected pregnant women without reported congenital infection in the newborn (i.e. “expZIKV”), 3) Infected placentas stemming from proven ZIKV-infected pregnant women with proven congenital infection in the newborn (i.e. “congZIKV”) no differences in baseline maternal characteristics and birth parameters between groups	Monthly USS and placental analysis 76 transplacental infection 16 congenital ZIKV 11 preg loss	Placentomegaly (thickness>40 mm) was observed more frequently in infected placentas (39.5%) compared to exposed placentas (17.2%) or controls (7.2%) Among infected placentas (congZIKV), 27/43 (62.8%; 95%CI 48.3-77.2) demonstrated pathological Anomalies Among infected placentas (congZIKV), positive RT-PCR at birth were found in 51/58 (87.9; 95%CI 76.7-95.0) of placentas tested	early placentomegaly may represent the first sign of congenital ZIKV infection, which could be particularly useful in low income countries where the access to tertiary centers may be restricted.	

(Reyes et al., 2020)	Retrospective cohort	128. 89 had pregnancy outcome data. Most women (55%) were aged 20–29 years	ZIKV symptom onset was comparable across the three trimesters of pregnancy (23–24%) but unknown for 21% of women	Amniotic fluid testing Birth defects	Amniotic fluid - 39/68 samples were positive - 15/68 ZIKV infections were identified in AF only -29/68 AF samples negative but detected in other samples - 16 patients with both AF and serum samples taken on same day: - 9/12 AF positive, serum negative - 3/12 both AF and serum positive	Amniotic fluid another source of body fluid diagnosis of ZikV. It's presence makes birth defects more likely than if absent.	

(Santos et al., 2020)	CS	Case report	Full characterization of the placenta.	Placental pathological review	Evidence of maternal vertical transmission. Hyperplasia of placental Hofbauer cells in chorionic villi and numerous histiocyte-like cells in the decidua were observed. The decidua, fibroblasts, and chorion, as well as circulating cells in the intravascular compartment stained positive forZIKVenvelopprotein.Deciduitiswasprese ntonthematernal surface ofthe placenta, with aprevalence of lymphocytesassociated with vasculitis. A high level of uncommitted CD3+ T lymphocytes were present, in addition to CD4+ and CD8+ cells. Elevated expression of the apoptosis inhibitor, Bcl-2, was observed in syncytiotrophoblasts. For ZIKV envelop protein. Deciduitis was present on the maternal surface of the placenta, with a prevalence of lymphocytes associated with vasculitis. A high level of uncommitted CD3+ T lymphocytes were present, in addition to CD4+ and CD8+ cells. Elevated expression of the apoptosis inhibitor, Bcl-2, was observed in syncytiotrophoblasts.		

(Schaub et al., 2017)	CS	Prospective case series 8 cases	Ultrasound, maternal (including amniotic fluid) and fetal testing	Fetal assessment	At the time of diagnosis, Zika virus RNA was detected in all amniotic fluid samples. Retesting in 6 cases: 3/6 remained positive for Zika virus And 3/6 second test was negative for Zika virus infection (2–10 weeks after the positive sample).	abnormal fetal biological parameters highly suggestive of hepatic dysfunction and potentially anaemia. Amnio can be +ve then subsequently -ve. ? fetal immune response or false neg. Placental only +ve in 3/8 so limited use.	
(Sobhani et al., 2019)	CS	Retrospective cohort of 4 twin pregnancies			Zika virus PCR testing revealed discordance between dichorionic twins, between placentas in a dichorionic pair, between portions of a monochorionic placenta, and between a neonate and its associated placenta. Of the 8 infants, 3 (38%) had an abnormal neonatal outcome. Of 6 infants with long-term follow-up, 3 (50%) have demonstrated ZIKV-related abnormalities	Neonatal PCR testing, placental findings, and infant outcomes can be discordant between co-twins with antenatal ZIKV exposure. Each twin should be evaluated independently for vertical transmission.	

(Venceslau et al., 2020)	Case series	17 placentas from ZikV +ve mothers		Placental characterization	14/17 placentas positive for ZIKV genome (RT-PCR) The most common morphological and anatomical pathological findings were increased stromal cellularity, villitis, calcification, maternal vascular malperfusion, placental hypoplasia, and maternal– fetal hemorrhage (intervillous thrombi)	The detection of ZIKV in the placenta after several months of initial symptoms suggests that this tissue may be a site for viral persistence during pregnancy.	

DOES ZIKA VIRUS/TREATMENT OF ZIKA VIRUS BEFORE MEDICALLY ASSISTED REPRODUCTION IMPACT THE OUTCOME OF MEDICALLY ASSISTED REPRODUCTION?

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

WHICH TECHNIQUES CAN BE USED TO PREVENT/REDUCE ZIKA VIRUS TRANSMISSION DURING MEDICALLY ASSISTED REPRODUCTION?

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Joguet, et al., 2017)	CS	Prospective cohort study 15 men infected with zika virus	Observation of spontaneous clearance of zika virus in infected men. Effect on sperm count in acute zika virus infections	Sperm count repeated at intervals	Mean reduction from 117million per ml to 70 million per ml at day 60. Recovered by day 120.	Viral effect on testis and epididymis. Frequency of shedding and high viral load in semen, together with the presence of replicative virus in a motile spermatozoa fraction, can lead to Zika virus transmission during sexual contact and assisted reproduction procedures. 3/14 patients with motile sperm had zika virus RNA after sperm washing	Whole blood seems to be the best specimen for Zika virus RNA detection, diagnosis, and follow-up. However the presence in semen is more relevant to fertility advice and treatment.

(Cassuto et al., 2018)	Case report	1 man	Man presenting for ART / MAR 1 month after developing Zika symptoms	Serum and semen testing, before and after sperm prep sperm through a bilayer gradient centrifugation, usually performed in ART.	First sample of prepped sperm negative but repeat sample positive for zika. Usually, in ART, the semen preparation by bilayer density gradient centrifugation coupled with intra-cytoplasmic sperm injection (ICSI) is known to decrease the virus transmission risks. We know that hepatitis C virus is not present in the last fraction used for ART, while the ZIKV and other viruses such as HIV and hepatitis B	recommend to not consider the sperm fraction free of risk in sperm samples manipulation from ZIKV contaminated men.	

DOES THE PLASMATIC VIRAL LOAD CORRELATE WITH ZIKA VIRUS IN SEMEN?

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Barzon et al., 2018)	Prospective cohort	13 women, 17 men 2 pregnant	Serial testing of ZIKV RNA in plasma, whole blood, urine, saliva, and semen Mean follow up duration 38 days (max 480)	ZIKV RNA in plasma, whole blood, and semen ZIKV by RT-PCR	Mean plasma clearance 11.5 days. Lasted longer of whole blood was tested. Viral shedding maximises 7 days after symptom onset. Mean time to ZikaV clearance in semen was 25 days, though one case was 370 days. no significant association was observed between viremia and detection of ZIKV RNA in semen.	2 pregnant women were +ve for ZikV in plasma but negative by amniotic fluid culture. Their babies were unaffected at birth.	
(Joguet, et al., 2017)	Prospective observational cohort	15 men with acute zika	Serial blood, urine and semen samples	ZIKV RNA in serum/whole blood/seminal plasma by RT-PCR	3 different patterns of viral seminal shedding (Figure 2): A) non-shedding patients, with consistently negative ZIKV RNA detection in seminal plasma during follow-up (n=4/15); B) seminal shedders with concomitant sera and/or urine shedding (n=6/15) C) persistent seminal shedders after virus clearance in sera and urines, i.e. discordant shedding patients (n=5/15) Intermittence of seminal excretion was observed for 3/5 patients from this group C.	Has reproductive implications for men. Suggests ZikaV RNA persisted longer in blood than semen / urine, though differing patterns of excretion possible. Semen characteristics can be modified by Zika virus.	

(Mead et al., 2018)	Prospective observational cohort	225 enrolled, 185 men participated Men were excluded if they were incarcerated or did not speak English or Spanish. Baseline information was obtained, and a collection kit with return postage was mailed to the participant's home.	Urine and semen samples at various intervals until two consecutive samples tested negative.	ZIKV RNA assay by RT-PCR	Twice monthly testing for 6 months. ZikaV RNA found in 7% urine and 30% semen 60/184 men had at least 1 RNA-positive semen sample 61% of samples submitted within 30 days of disease onset tested positive for ZIKV 7% or less tested positive after 90 days or more after illness onset	Most ZikV RNA levels declined after 3 months but in 1 man persisted for 281 days.	
(Musso et al., 2017) (28711704)	Case series	14 asymptomatic blood donors testing positive for ZikaV RNA	Whole blood, semen, urine and saliva serially tested	ZIKV RNA in blood, semen by RT-PCR	5/7 (35%) tested semen positive. ZikaV RNA tested positive 7-54 days after blood donation, though viral cultures were negative. Plasma collected at the same time as the positive semen tested negative for ZIKV RNA for 6/8 ZIKV RNA-positive semen collections.	NAT test blood donors and confirm with semen testing	

(Paz-Bailey et al., 2018)	Prospective cohort	55 men, 50 women.	Serum, urine, saliva, semen, vaginal secretions weekly for 1 month then at 2, 4 and 6 months.	ZIKV RNA by RT-PCR in serum, semen, vaginal secretions	Median serum loss of ZikV RNA in blood was 14 days, max 80 days (2%). 1/15 (2%) tested positive via vaginal secretion. Median semen loss of ZikV was 34 days max 125 days (4%).	Suggests prolonged persistence in serum compared to other flavivirus'	

WHICH INTERVENTIONS CAN BE USED TO REDUCE/AVOID VERTICAL TRANSMISSION OF ZIKA VIRUS TO THE NEW-BORN?

ECS

No relevant studies could be found in literature.

Breastfeeding

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Sampieri and Montero, 2019)	SR	9 studies included with 10 cases and 3 with documented follow up	Zika transmission through breastfeeding Different stages of maternal infection from delivery: 5 just prior to delivery; 1 was 0-4 days after birth; 2 were 8 weeks to 6 months and 2 were afte 6 months from birth	Zika transmission to the newborn	No long term newborn sequelae	Re-affirm WHO recommendation that breastfeeding benefits outweigh risk	
(Cavalcanti et al., 2017)	Case series	4 cases of zika in breastfeeding mothers Brazil		Zika transmission to the newborn	Nil infected offspring	Zika virus may not be transmitted via breastmilk	Case report

(Siqueira Mello et al., 2019)	Case report	1 woman	Woman whose previous child had severe microcephaly delivered a normal child who was exclusively breast fed. One month later the child head circumference was unaltered, i.e. secondary microcephaly.	Zika transmission to the newborn		studies are needed to better define the dynamics of Zika virus transmission via breast milk and its potential harm to newborns	

Laboratory safety

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

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Baleriola et al., 2011)		No patients - compared 3 viruses (HCV, HIV, HBV) at difference storage conditions	Stability of HCV RNA. (i) in 7 samples stored for 1.2 years at -20°C and -70°C. (ii) in 35 samples stored for 1 year at -20°C (iii) in 22 samples stored for up to 9 years at -70°C Stability of HIV-1 RNA. (i) in 22 samples stored for 2.3 years at -20°C (ii) in 19 samples stored for up to 9.1 years at -70°C Stability of HBV DNA. (i) in 30 samples stored for 1.8 to 5 years at -20°C (ii) in 31 samples stored for up to 4 years at -70°C	Outcome measure is the potential harm if the virus remains infectious after storage.	there was a decline in the mean viral load for HCV RNA-positive samples stored at -20°C and -70°C. The mean of the difference in viral loads within identical samples stored at -20°C and -70°C was statistically significant, indicating a greater loss of HCV viral titer following storage at -70°C The mean viral load of 22 HIV-1 RNA Samples showed RNA loss There was a significant decline in the mean HBV DNA load of four samples measured with the CAP-CTM assay before and after storage at -20°C for 1.8 years	Despite decline in the viral load, the virus is still infectious	demonstrates viral stability at low temperatures

(Cobo et al., 2012)		24 BBV+ patients having oocyte vitrification or IVF/ET. Of all the patients, 6 HIV, 11 HCV, 6 HBV, 1 HCV & HBV	No viruses detected in FF, oocyte or embryo culture medium after IVF. Only one patient had a high viral load (HCV-2,295,000 copies/mL). With open vitrification, no virus was detected in the LN2, surrounding the oocyte is virtually impossible.	No cross-contamination of HIV, HBV, HCV.	Outcome measure was detectable virus in spent FF, media or LN2.	No detection of HIV, HBV, HCV after incubation/storage, even for the patient with a high HCV-viral load. Authors conclude the solid state of vitrified oocytes prevents transmission.	Concerns of cross-contamination disproved. However, low patient numbers and only one patient had a high viral load.
(Halfon et al., 1996)		7 patients with HCV- serum samplles	Serum samples stored: (1) immediate quantification, (2) at room temp 5 days (3) 4C, 5 days (4) -20C, 5 days (5) -80C, 5 days (6) 5 freeze-thaw cycles (7) blood unspun for 4h at room temp, then centrifuged and stored at -80C, 5 days (8) 4C for 6 months (9) -20C for 6 months (10) -80C for 6 months.	HCV RNA titers after each intervention	No HCV RNA titers after storage at RT, 5 days and then storage at 4C, 6 months. Decrease of HCV RNA titer (15.6%) in sera stored -20C, 5 days. 16% decrease after 5 freeze-thaw cycles resulted 29.5% loss after 4h RT, centrifugation 6month stability at -80C 23% loss at -20C.	Storage & handling affect HCV RNA in sera	For HCV+ serum, the virus is detectable up to 5 days at at RT. Long-term stability (6 months) was observed at -80C

(Hawkins et al., 1996)	CASE	6 patients. Contracted HBV from storage of bone marrow in a LN2 tank contaminated with HBV	6 patients had either bone marrow transplantation (BMT) or peripheral blood stem cell transplantation (PBSCT). They then developed HBV infections over a 28-month period as a result of contamination of a LN2 bone marrow storage tank.	HBV infection	Incomplete sealing of cryopreservation bags was proposed as the most likely to cause the cross-contamination. LN2 had leaked into the infectious bag and then into the LN2	Incomplete sealing of infectious cells/tissue can cause cross-contamination of HBV to other cells/tissue. .	Cross-contamination is prevented from separate storage and correct sealing of devices.
(Khuu et al., 2002)		Patients with hematologic malignancies, solid tumors, or genetic diseases, and HLA-matched first-degree related donors	Cryopreservation of PBPC and lymphocyte products in poly(ethylene co-vinyl acetate) (EVA) plastic bags before January 23 2002 and polyfluoroethylene polyfluoropropylene (FEP) bags thereafter. The bags were placed into aluminum presses and transferred to the pre-chilled chamber (4°C) of a controlled-rate freezing device. After sealing in an overwrap bag, they were placed into a prechilled aluminum cassette and vertically aligned racking system in the LN2 freezer,		1204 bags a total of 68 failures occurred in the 1204 cryopreserved product bags		

(Bielanski et al., 2000)	Animal study	PATIENTS No. Of patients Patient characteristics + group comparability No patients. Rather, bovine viruses were used as models for human viruses, to demonstrate if transmissible through LN2 to open devices	Bovine embryos were vitrified in closed or open devices & plunged into infected N2	Viral contamination of the embryos. 21.3$ batches exposed to the viruses tested positive in open devices (though not BIV). All closed devices were not infected.	Effect size- 83 batches of embryos	Cites: *Transmission of papovavirus via LN2. *Possible transfer of herpes simplex virus 1 & adenovirus type 2 on cotton wool via LN2.	Animal study

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

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Grout and Morris, 2009)		Agar plates infused with a suspension of cultured ascospores of the soil-borne agricultural plant pathogen Sclerotinia	Two simple assay systems to demonstrate potential of LN2 as a vector that can introduce contaminants into freezing equipment and storage containers used in cryopreservation. To see of vapour phase contaminates less than liquid nitrogen	Controlled rate freezer Cooling chamber was filled with normal and contaminated liquid nitrogen Different dewars were tested	Unlike LN2 vapour phase (LNVP) storage vessels, LN2 storage vessels will accumulate particulate matter from the atmosphere with time. 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	Greater consideration should be given to issue of external contaminants preserved in LN2 as a storage or transport medium and the use of LN2-free controlled rate freezers and long-term storage vessels might be appropriate in some circumstances to reduce this risk. Regular cleaning of storage vessels is recommended	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).

(Molina et al., 2016)		five cryopreservation containers. Each one can store about 600 embryos and/or oocytes from couples with negative serology for viral agents that cause serious diseases (HIV, HCV, and HBV).	The bank sterility conditions was evaluated for 4 consecutive months, samples of LN from each of the five containers were evaluated at two key moments: just before filling and right after filling	Assessment of the Contamination Risk Between Open and Closed Devices	A total of 40 LN samples were collected and evaluated from five containers. Contaminants of bacterial origin were found in all of the cryostorage containers both before and after their filling with LN. There was no relationship between the time that each container had been used and the presence of microbiologic contaminants in LN. Furthermore, the number of stored samples was not associated with a higher degree of contamination in the cryostorage containers **Open vs closed systems:** The storage periods were 12–18 months for open devices and 1–2 years for closed devices. The results of microbiologic study of the 32 samples from open and closed devices were negative for both bacteria and fungi. No microbiologic contaminant was found in the drop of clean unspent devitrification media used as negative control.		
(Mirabet et al., 2012)	Assessment of human tissue bank	Microorganisms cultured from different samples (frozen detritus and swabbing the surfaces inside the tank) in liquid or gas phase of nitrogen.	(a) to identify microbiological agents in the liquid nitrogen containers of our cell and tissue bank (b) to determine the effect of liquid nitrogen exposure on virus detectability.	a) Testing of frozen sediment and swabs of the outer surface of stored products (b) type of tank: liquid nitrogen vs gas phase vs half gas half liquid	we have mainly detected environmental and water-borne bacteria and fungi in our nitrogen tanks (Table 1). In addition, according to the experimental study by Bielanski [1], the vapour phase yielded less contamination than liquid phase (Table 1). .	Liquid nitrogen is a vehicle for infectious agent diffusion.	The vapour phase tank yielded less contamination than the liquid phase.

(Bielanski, 2005)	Experimental - animal	Transmission of selected bacterial and viral pathogens by the vapour phase of LN2 to embryos and semen in dewars designed for short-term storage and transportation of biological specimens. Bull semen and embryos	Transmission of *Pseudomonas aeruginosa*, E *coli*, *Staph.aureus*, BVDV, and BHV-1 was examined from: (1) contaminated dry shippers to germplasm; (2) between contaminated & non-contaminated cryopreserved germplasm; (3) between stock culture of pathogenic agents and germplasm. Contaminated	Contaminated and non-contaminated samples of embryos and semen were stored in proximity in the vapour phase LN in open containers for 7 days prior to testing for the presence of microbes.	Three experiments, e.g. Expt 1 embryos stored in OPS or 0.25mL straws (*n*D36), semen (*n*D18) or culture media (*n*D6) after 7 days of storage in the vapour phase in contaminated dry shippers	It is unlikely that LN vapours serves as a vehicle for microbe transmission between adulterated and non-adulterated germplasm during short-term storage or transportation in dry shipper dewars. Sealed containers are advised.	Animal study

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

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Chen et al., 2006)	Other (expt)	10 vials heat-sealed vs 10 vials not heat-sealed. Submerged in LN2. 3 x 15 vials with Vero cells with different heated-seals exposed to microbial infection.	Does heat-sealing plastic around the vial prevent LN2 and microbial entry.	LN2 and microbial contamination of a vial.	15 vials of each cell line were separated into 3 groups, according to different protocols. Partial membrane sealing process could completely protect the vials from LN2 penetration	Heat-sealing around a vial prevents LN2 and microbial entry.	INCLUDED: Heat-sealing of vials is effective for preventing LN2 entry and microbial infection.

CAN HIGH SECURITY STRAWS PREVENT CROSS CONTAMINATION OF STORED MATERIAL?

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Letur-Konirsch et al., 2003)	Expta ;	24 of each type of straw were used PVC, PETG, IR	Each straw was filled with 100ul HIV positive supernatant Straws were sealed ultrasonically at the free non-cotton plugged end IR straws were filled with a pump and sealed by thermosoldering of both ends Each straw was placed in an empty conical 15ml tube, capped and placed in a container of liquid nitrogen for 7 days	2 experiments 2nd: decontamination of outside surface after filling and sealing	PVC straws: 3 types of possible contamination PETG straws 1 type of contamination (defective sealing procedure) IR straws Safe for HIV-1	Under cryopreservation conditions, IR straws seem safe for HIV-1 storage in ART. For PVC and PETG straws, ultrasonic sealing could be the weak safety link.	
(Maertens et al., 2004)		Semen samples from 11 HCV negative men seminal fractions were spiked with a blood plasma containing 5 3 106 IU/ml of HCV RNA taken from a chronically HCV-infected patient. High-security IR straws were filled with 100 ul of seminal plasma using a pump and thermosealed	straws were submitted to different treatments: (i) disinfection of the extremity of the straw with no subsequent cryopreservation; (ii) no disinfection and no cryopreservation; (iii) disinfection before cryopreservation and before thawing; (iv) disinfection only before cryopreservation; (v) disinfection only before thawing; and (vi) cryopreservation without disinfection	No HCV RNA could be detected in any of the samples. Additional samples included the rinsing water from straws sealed by thermo-solder and from the heating wire used for HCV-positive semen.	the cryoprotectant did not inhibit the RT-PCR assay. absence of contamination of the exterior part of the straws via the sealing system and during cryopreservation in liquid nitrogen, even in the absence of disinfection steps.	IR straws are safe om LN2 tanks when used with samples containing high loads of HCV RNA. These straws are recommended for use by ART laboratories with semen from subjects with chronic viral diseases.	

CAN THE USE OF SEPARATE LABS PREVENT CROSS CONTAMINATION?

Reference	Study type	Patients	Interventions	Outcome measures	Effect size	Authors conclusions	Comments
(Bryan et al., 2016)	Exptal	No patients. 79 baseline swabs for nucleic acids performed on the TLA system, 10 were positive for HBV and 8 for HCV	Environmental swabs followed by PCR for HBV & HCV were taken from a chemistry TLA system during routine clinical use and after running a small number of high-titer HCV samples. Control experiments were performed to ensure the recovery of DNA and RNA viruses by swabs from a representative nonporous surface.	Viral nucleic acid was consistently detected from swabs taken from the distal inside surface of the decapper discharge chute, with areas adjacent to the decapper instrument and the centrifuge rotor also positive for HBV or HCV nucleic acid.	After running known HCV-positive samples, at least one additional site of contamination was detected on an exposed area of the line.	low level of viral contamination of automated clinical laboratory equipment occurs in clinical use. Given the risks associated with highly infectious agents, there is a need for risk-mitigation procedures when handling all samples.	For baseline swabs (n=79) taken in a total laboratory automation (TLA) system during routine clinical use after running a small number of high-titer 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 too.
(Cobo, et al., 2012)	Expt	24 BBV+ patients having oocyte vitrification or IVF/ET. Of all the patients, 6 HIV, 11 HCV, 6 HBV, 1 HCV & HBV	No viruses detected in FF, oocyte or embryo culture medium after IVF. Only one patient had a high viral load (HCV-2,295,000 copies/mL). With open vitrification, no virus was detected in the LN2, surrounding the oocyte is virtually impossible.	No cross-contamination of HIV, HBV, HCV.	Outcome measure was detectable virus in spent FF, media or LN2.	No detection of HIV, HBV, HCV after incubation/storage, even for the patient with a high HCV-viral load. Authors conclude the solid state of vitrified oocytes prevents transmission.	Concerns of cross-contamination disproved. However, low patient numbers and only one patient had a high viral load.

(Grout and Morris, 2009)		Agar plates infused with a suspension of cultured ascospores of the soil-borne agricultural plant pathogen Sclerotinia	Two simple assay systems to demonstrate potential of LN2 as a vector that can introduce contaminants into freezing equipment and storage containers used in cryopreservation. To see of vapour phase contaminates less than liquid nitrogen	Controlled rate freezer Cooling chamber was filled with normal and contaminated liquid nitrogen Different dewars were tested	Unlike LN2 vapour phase (LNVP) storage vessels, LN2 storage vessels will accumulate particulate matter from the atmosphere with time. 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	Greater consideration should be given to issue of external contaminants preserved in LN2 as a storage or transport medium and the use of LN2-free controlled rate freezers and long-term storage vessels might be appropriate in some circumstances to reduce this risk. Regular cleaning of storage vessels is recommended	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).
(Yarbrough et al., 2018)	Exptal	No patients Testing was performed by two experienced laboratory technologists using standard laboratory PPE and sample-to-answer instrumentation.	Detection of contamination during routine analysis of patient specimens	. Remnant specimens were spiked with the nonpathogenic bacteriophage MS2 at 1.0 _ 107 PFU/ml, and contamination was assessed using reverse transcriptase PCR (RT-PCR) for MS2.	Fluorescence was noted on the gloves, bare hands, & lab coat cuffs of the laboratory technologist in 36/36 (100%), 13/36 (36%), and 4/36 (11%) tests performed, respectively. Fluorescence was observed in biosafety cabine in 8/36 (22%) tests, on test cartridges/ devices in 14/32 (44%) tests, and on testing accessory items in 29/32 (91%) tests	Lab surfaces may become contaminated with blood-borne viruses during routine clinical laboratory testing. Adherence to the use of standard PPE and universal precautions protected the laboratory worker and instrumentation.	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 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)

(Zhou et al., 2006)	Audit	No patients. Investigation the viral contamination of invasive medical instruments in dentistry and to provide health administrative institutions with surveillance data	Sterilized samples were randomly collected from the department of dentistry to detect HBV-DNA, HCV-RNA, HIV-RNA and HBsAg.	Of the invasive medical instruments that were sterilized with 2% glutaraldehyde, one of the samples was positive for HBV-DNA, and another sample was positive for HBsA	Out of a total of 430 instruments in the group, there were two positive results, one positive PCR signal for a bur and one positive RIA for a turbine handpiece.	Though massive virus contamination of invasive medical instruments in dentistry has been reduced to a low level, the occurrence of contamination still remains	For dentistry, after sterilization of invasive medical instruments with 2% glutaraldehyde, HBV was still detectable on the sterilized instruments (Zhou et al, 2006).

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