PRE-CONGRESS COURSE 1

Organised by the Paramedical Group

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PRE-CONGRESS COURSE 1 - PROGRAMME

Paramedical Group

Course Co-ordinator: H. Birch (UK)

Course description: The aim of this pre-congress course will be to familiarize participants with hepatitis B, hepatitis C and HIV screening, the implication of these tests and the impact on fertility treatment. The afternoon visit to an IVF unit in Barcelona will offer delegates to visit a unit offering treatment to couples affected by these diseases.

Target audience: Nurses, counselors and affiliated paramedics

Chairmen: D. Molero (E) & H. Van Ranst (B)

Programme

09.00 – 09.45	Introduction Aetiology of hepatitis B, hepatitis C and HIV Implications of testing for Hep B, Hep C and HIV (reliability and frequency of testing) – ***A.M. Fabregat and P. Girona Tomas (E)***
09.45 – 10.30	Screening for viral diseases in medically assisted reproduction: how to interprete tests, how to inform patients, how to protect the child – ***P. Lacor (B)***
10.30 – 11.00	***Coffee break***
11.00 – 11.30	The most appropriate treatment for positive patients - ***V. Vernaeve (E)***
11.30 – 12.00	Hepatitis B, C and HIV. Pregnancy, delivery and mental care - ***K. Roelens (B)***
12.00 – 12.30	Safety of staff and risks of cross contamination – ***J. Lemmen (DK)***
12.30 – 13.30	***Lunch***
13.30 – 17.00	Visit to an IVF Unit

AETIOLOGY OF HEP B, HEP C AND HIV.
IMPLICATIONS OF TESTING HEP B, HEPC AND HIV

Dra. A.M.Fabregat
Andrology Department
Instituto Bernabeu Alicante, Spain

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4-. HUMAN IMMUNODEFICIENCY VIRUS

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Introduction I

➢As part of the study for the application of any AR techniques, we need to do a serological analysis which involves:

- Abs (IgG) anti-HCV
- HBsAg (surface antigen)
- Abs anti-HIV
- RPR or VDRL

We can find several cases:

➢Female (+) / male (-):

- We need a specialist (hepatologist) report on the woman´s state and progress of the infection (chronic illness), viral load (HCV and HIV) or serological tests (HBV).

Introduction II

➢Female (-) / male (+):

- Sperm washing procedures (HCV or HIV) and viral detection using PCR (to check virus removal). Controversy exists if the infection is HBV, so we recommend the woman vaccination.

After AR treatments, we have to check both serological states (woman and child) by means of PCR.

➢Female (+) / male (+):

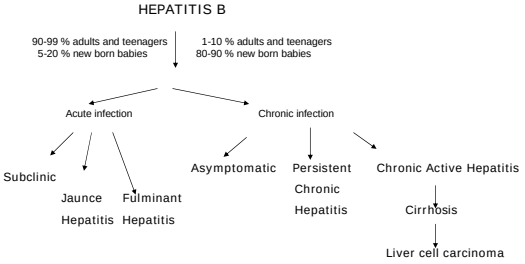
- Combination of the two previous measures.

Introduction I

- There are over 350 million chronic infectious carriers in the world.
- Hep B is one of the most common infectious diseases.
- In adults, chronification or persistence of a Hep B infection occurs in about 5 % of the cases.

- About 500.000 and 1.000.000 of infected people will die as a consequence of acute hepatitis, hepatic cirrhosis or liver cell carcinoma.
- Illnesses associated to Hep B infection are the tenth cause of death in the world and the liver cell carcinoma is the fith more frequent carcinoma in the world population.

Introduction II



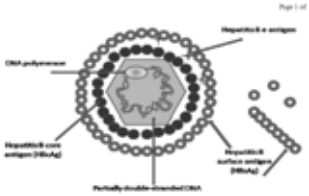
- The rate of progress from acute to chronic infection depends on the age when infection occurs:90% at neonatal stage, 20-50% between 1 and 5 years old, and 5% among adults.

Introduction III

- A safe and effective Hep B vaccine exists, even in the long term.
- Its rough effectiveness is 90 % in adults and teenagers and almost 100 % in newborn babies.
- We consider that a title anti-HBsAg is protective when it´s equal or over 10 mUI/ml. Aproximately, 5% of the vaccinated people don´t develop antibodies.
- Nowadays, we don´t recommend memory doses in general population.

- Prophylaxis after Hep B exposure with inmunoglobulins and with vaccine administration is highly effective and it has a special interest between health workers.

HepB : General Description



- Hepadnaviridae family: this family of virus infects preferably hepatocites (kidney, pancreas and mononuclear cells too).
- DNA viruses: circular and incomplete doble helix-strand DNA.

- Hep B is made up by: external envelope (surface antigen) and central particle or core (nucleocapsid proteins, viral genome and a polymerase complex).
- Virals genotypes: A-G. D genotype in mediterranean area.

Epidemiology

- Central Europe, which has a prevalence of carriers of around 1-2 %, is considered a low prevalence area (but Spain and Italy are considered a medium prevalence country).

Transmission

- The most frequent transmission way depends on the illness prevalence in each area.

PREVALENCE	HIGH (10-20 %)	MEDIUM (3-5 %)	LOW (0.1-2 %)
GEOGRAPHIC DISTRIBUTION	-Sub-Saharan Africa -Southeastern Asia -China -Pacific Islands	-Mediterranean area -South America -East Europe -Central Asia -Middle East -Japan	-Western Europe -USA -Canada -Australia -New Zeland
TYPICAL AGE OF INFECTION	Perinatal and first childhood	First childhood	Adults
MORE FREQUENT INFECTION WAY	Maternal-fetal and percutanean	Percutanean and sexual	Sexual and percutanean

- Maternal-fetal transmission: during labour or after birth.
- Percutanean transmission: basically injected drug users who share syringes or needles.

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Introduction I

- The WHO estimates that around 170 million of people, which represents 3 % of the world population, can be infected by Hep C virus.
- Around 78 % of the infected people are chronic carriers of this virus.
- With a latency of 20-30 years, 20 % of the chronic carriers will suffer cirrhosis and 6 % of this population will suffer liver cell carcinoma.

Introduction II

- Because Hep C vaccine does not exist, prevention is important in identifying the infected peple.
- Behaviour to adopt in relation to males with positive antibodies anti-HCV isn´t clear, because a big controversy exists about sexual Hep C transmission role.
- In Hep C serodiscordant monogamous couples, the rate of seroconversion after 10 years is 2.5 % (Neumayr *et al.*, 1999; Zylberberg *et al.*, 1999).

HepC: General Description

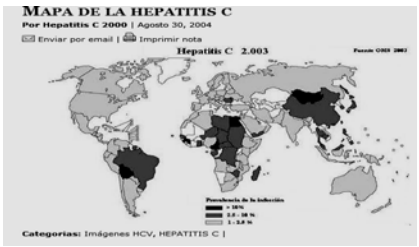
- Flaviviridae family.
- Spherical virus with a envelope glycoproteins which contains lipids.
- Genome: 1 copie of single-stranded RNA.

- Characteristics: high degree of genomic heterogeneity that originates differents genotypes.

Epidemiology I

- The genotype 1b is especially prevalent in South and East of Europe. Genotype 3a is the most prevalent in occidental countries between injected drug users.

Epidemiology II



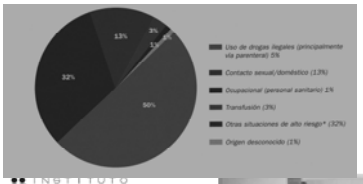
- European countries are between low prevalence countries (1.0– 2,5 %).

Transmission I

➢Percutanean transmission: blood receptors and blood derivatives (less than 1%), hemodyalisis, tatoos, injected drug users.

As for risk of healthy workers, risk of Hep C infection after accidental prick is low (around 2 %).

➢Transmission across the mucus: sexual act, perinatal.



-Injected drug users (5%).
-Sexual/domestic contact.
-Health workers (1%).
-Blood trasfusion (3%).
-Other high risk situations.
-Unknown origin (1%)

4-. HUMAN IMMUNODEFICIENCY VIRUS

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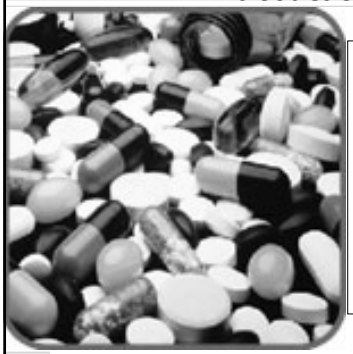
Introduction I



Great activity antiretroviral treatments have delayed and even avoided the AIDS progression:

- ✓Increasing survival.
- ✓Improving life quality
- ✓Turning AIDS into chronic illness.
- ✓So many seropositive people consider the possibility to have offspring.

Introduction II

Options:
Male HIV+ /
Female HIV- to have a baby:

- Baby adoption.
- RA treatments with donor´s sperm.
- Biological children:
  Unprotected sexual intercourse: risk of horizontal transmission between 0.08% and 0.2%.

  Unprotected sexual intercourse only at the ovulation moment: with a lower risk.

  Sperm washing and AR treatments and IUI or IVF.

HIV: General Description

Spheric form with 120 mm in diameter.

✓ External envelope: lipidic bilayer which belong to host-cell originally but provided with virus proteins GP 41 and GP 120.

In the viral core:
- Conical capsid: It´s formed from p24.

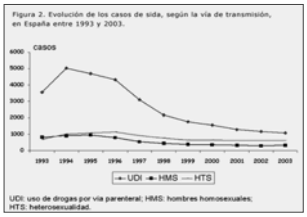
Genome: 2 copies of single-stranded RNA.
- Enzimes: Protease, Integrase, Reverse Transcriptase, nucleoproteins.

Epidemiology

- Prevalence in Central Europe countries is between 0.1-0.5%, however Spain and Italy have a prevalence higher than Central Europe countries (between 0.5-1 %).

Transmission

➢ Blood way (injected drug users), sexual way (spem is a HIV reservory) or vertical way (from mother to fetus: 15-20 %).



"Evolution of the HIV cases, depending on the transmission way, in Spain between 1993 and 2003".

"Red colour": injected drug users.

"Blue colour": homosexual males.

"Green colour": heterosexual males.

➢Spain is the Western Europe country with the highest number of AIDS pediatric cases.

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THANK YOU FOR YOUR ATTENTION

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SPERM WASHING AND HBV, HCV, HIV DETECTION

Pilar Girona Tomás
Andrology Department
Instituto Bernabeu Alicante ,Spain

- **Detection Methods: HCV, HIV , HepB.**
  - **Antibodies detection**
  - **Antigen detection**
  - **Viral load (PCR)**
- **Sperm washing**

Hepatitis B detection

TABLE 5

Interpretation of serologic testing in patients with HBV infection.

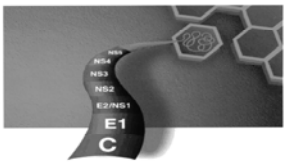
HBsAg	HBsAb	HBcAb	HBeAg	HBeAb	Possible interpretation
+	−	IgM	+	−	Acute HBV infection, highly infectious
+	−	IgG	+	−	Chronic HBV infection, highly infectious
+	−	IgG	−	+	Late acute or chronic HBV infection, low infectivity
+	+	IgG/IgM	+/−	+/−	1. HBsAg of one subtype and heterotypic anti-HBs (common) or, 2. Process of seroconversion from HBsAg to anti-HBs (rare)
−	−	IgM	+/−	+/−	1. Acute HBV infection 2. Anti-HBc window
−	−	IgG	−	+/−	1. Low-level HBsAg carrier 2. Remote past infection 3. False positive
−	+	IgG	−	+/−	Recovery from HBV infection
−	+	−	−	−	1. Immunization 2. Possible remote infection 3. False positive

See: Glossary of Hepatitis B Terms.

Practice Committee. Hepatitis and reproduction. Fertil Steril 2004.

Hepatitis C detection

- Specific antibodies detection: IgG against HCV virus (don´t differentiate between acute or chronic infection). EIA

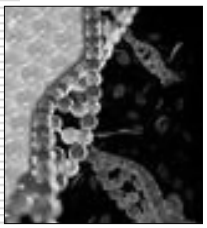


- Confirmatory analysis or inmunoblott (HCV antigens detection: C, E1, E2/NS1, NS2, NS3, etc...).

- RNA HCV detection PCR: viral load.

HIV detection

- Specific antibodies detection against HIV (EIA, Confirmatory analysis by Western Blot).



- Virus detection:
  - Virus antigen detection: gp120, p24, etc...).
  - Proviral DNA detection: PCR
  - Viral RNA quantitative detection by PCR: viral load.

  - 1st generation: > 10.000 copies/ml.
  - 2nd generation: 200-400 copies/ml.
  - 3rd generation: 20-50 copies/ml.

ART and sperm washing (HCV/ HIV)

- Sperm washing
- HCV/ HIV detection in sperm washed
- Efficacy and safety of sperm washing
- In which cases is it recommended?
- Natural conception in serodiscordant couples.

Sperm Washing

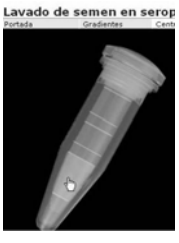
Precursor: **Augusto E. Semprini** in 1992.

➢Add 1-1.5 ml of 90% isotonic solution.

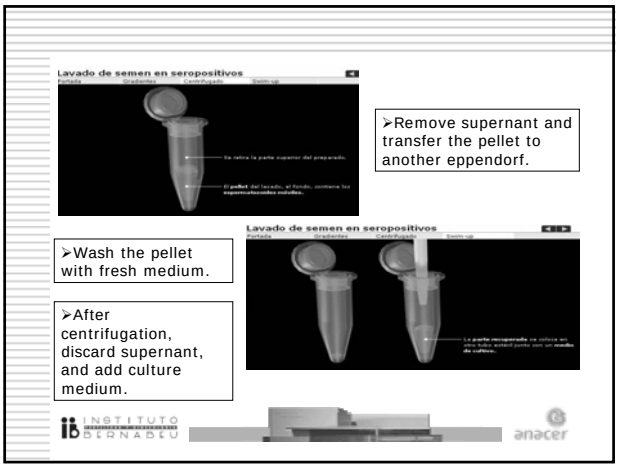
➢Add 1-1.5 ml of 70% isotonic solution.

➢Add 1-1.5 ml of 50% isotonic solution.

➢Finally, add 1-1.5 ml of semen sample.



➢Centrifugation: 20 minutes at 1300 rpm.



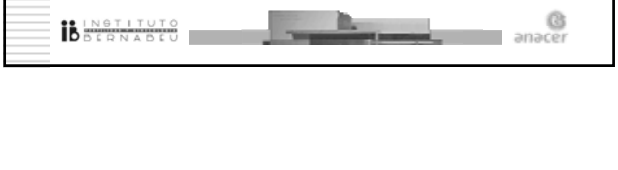
➢Remove supernant and transfer the pellet to another eppendorf.

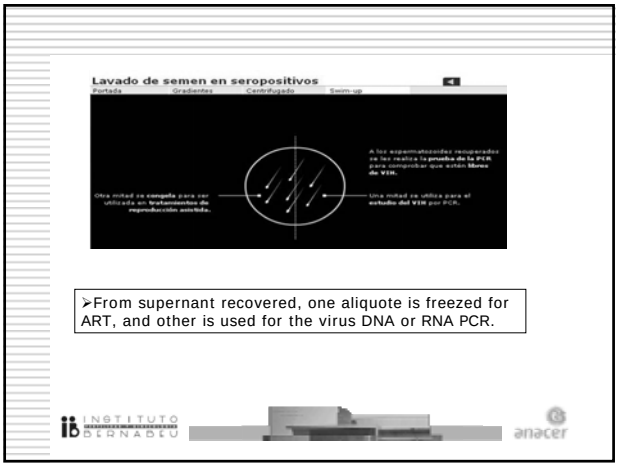
➢Wash the pellet with fresh medium.

➢After centrifugation, discard supernant, and add culture medium.

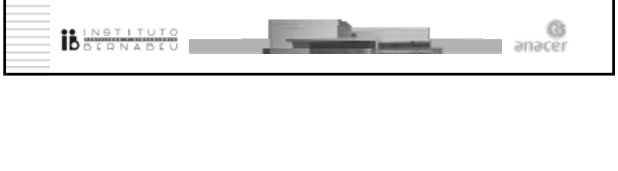
➢Incubate the eppendorf as showed in the picture for 45-60 minutes at 37ºC (swim-up).

➢Supernant contains motile spermatozoas.



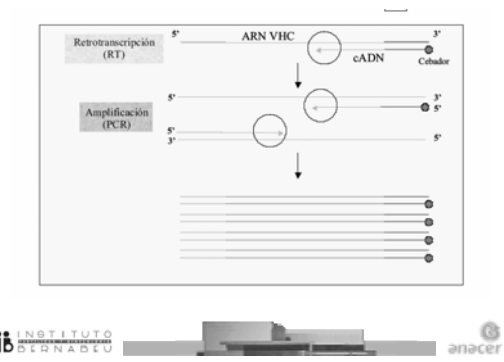


➢From supernant recovered, one aliquote is freezed for ART, and other is used for the virus DNA or RNA PCR.



PCR RNA HCV

- **Viral load quantification: Amplicor HCV Monitor test (Roche Diagnostics)**
  - Biotinylated primers ⇨ Biotinylated products ⇨ easy detection by colorimetric reaction (streptavidin peroxidase).
  - Internal quantitative standard: control RNA added to the sample before extraction (amplificated with RNA problem).



PCR DNA HIV

- **Proviral DNA Detection : AMPLICOR HIV-1 kit (Roche)**
  - Sperm chromatin decondensation by DTT treatment.
  - Primers: SK462 Y SK431. Locus *gag* amplification of virus genome.

PCR RNA HIV

- **Viral RNA Detection : HIV-1 MONITOR kit (Roche Diagnostics). RT-PCR**
  - RNA extraction
  - Reverse transcription: obtaining cDNA from RNA
    - ✓Primers: SK462 Y SK431 (gag locus)
  - cDNA PCR amplification
  - Hibridation of cDNA amplificated with HIV-1 specific probes
  - Detection by Enzyme Immunoassay (EIA): signal intensity depends on quantity cDNA.

Efficacy and safety of sperm washing

➢ **Marina F**, 2001 Spain (n= 610 sperm washing)
Virus presence in a 3.6% of sperm washing.
96,4% of samples after washing are useful for ART.

➢ **Veiga A et al**, 1999 Spain (n=209 sperm washing)
7,7% of PCR were positives after washing. These samples weren´t used.

➢ **Dulioust E et al**, 1998 France
Analysis of 31 men in different stages of infection:

94% HIV in blood sample and 84% HIV in seminal plasma.
In sperm washed:
17% showed HIV RNA positive
4% showed HIV DNA positive.

In which cases is recomended?

Assisted procreation in cases of hepatitis B, hepatitis C or human immunodeficiency virus infection of the male partner.
P. Honeck[1,2], M. Weigel[2], S.T. Kwon[1], P. Alken[1] and S. Bross[1]
Human Reproduction 2006 21(5):1117-1121; doi:10.1093/humrep/dei459

Table I. Virus proof in ejaculate and sperm processing for hepatitis B, hepatitis C and human immunodeficiency virus (HIV)

	HBV	HCV	HIV
Proof of virus			
Seminal plasma	Yes	Yes	Yes
Cell fraction	Yes	Yes?	Yes
Sperm's integrated genome?	Yes	No	No
Sperm processing	No (vaccination of partner)	Density gradient + swim-up	Density gradient + swim-up + testing

HBV? hepatitis B virus; HCV? hepatitis C virus

Couples in which the husband carries AgHBs don´t require sperm washing methods if the female partner has immunity for Hepatitis B (antiHBS+). There is about 5 % of vaccinated people that don´t develop immunity, in these cases it´s necessary an ART with sperm washing.

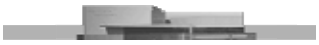
Natural conception in VIH serodiscordant

Is natural conception a valid option for HIV-serodiscordant couples?

Pablo Barreiro[1,3], **José Antonio Castilla[2], Pablo Labarga[1] and Vincent Soriano[1]**

Human Reproduction 2007, Jul, doi:10.1093/humrep/dem226

- "The very low risk of HIV transmission to the negative partner and to the baby if HIV-positive individuals have undetectable viremia under HAART is the basis for accepting natural pregnancy as an alternative option".
- "Restriction of unprotected sexual intercourse to woman's fertile days is of major importance to minimize the risk of HIV transmission and to maximize the chances of natural pregnancy. Attempts of natural pregnancy should not be done for >6–12 pinpointed ovulations".
- "Risk of HIV transmission can be minimized, but never eliminated".



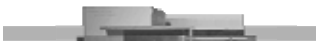
Natural conception in HIV serodiscordant

Safety of sperm washing and ART outcome in 741 HIV-1-serodiscordant couples.

V. Savasi[1], **E. Ferrazzi, C. Lanzani, M. Oneta, B. Parrilla and T. Persico**

Human Reproduction 2007 22(3):772-777; doi:10.1093/humrep/del422

- "Counselling unprotected intercourses on the day of ovulation to fertile couples reduces the risk of horizontal transmission of HIV, but this 'reduction' would condemn 5% of women to be infected by their partners (Mandelbrot *et al.*, 1997). Highly active antiretroviral therapy could further reduce this risk but does not guarantee an undetectable virospermia".
- "We still do not support the idea that these couples should be allowed to try to conceive naturally, just focusing on the best ovulation window (Barreiro *et al.*, 2004). This is an unacceptable option".



Screening for viral diseases in medically assisted reproduction:

how to interprete tests•how to inform patients•how to protect the child

About the speaker

Patrick Lacor, MD
Internal Medicine – Infectious diseases Department
Aids-reference Centre
Universitair Ziekenhuis Brussel, Belgium

Fields of interest: internal medicine, hiv-infection, medical ethics

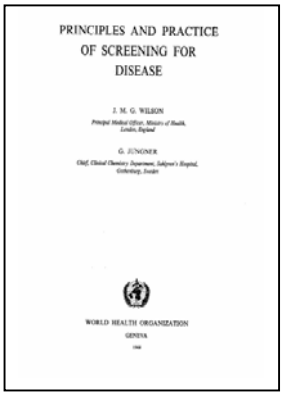
no conflicts of interest

Learning objectives

- to understand the principles of screening
- to interprete the results of screening tests
- to inform and counsel the patient
- to gain insight into the implications for patient and (unborn) child
- to reflect about decision making in medically assisted reproduction with regard to chronic viral diseases

Screening

- a strategy used to detect disease in individuals without signs or symptoms of that disease
- with the intention to identify disease in the individual and/or the community enabling early intervention and management in the hope to reduce morbidity and mortality
- the quality of a screening test depends on its sensitivity and specificity
- potential pitfalls of screening are: overdiagnosis, misdiagnosis or creating a false sense of security


PRINCIPLES AND PRACTICE
OF SCREENING FOR
DISEASE

J. M. G. WILSON

G. JUNGNER

WORLD HEALTH ORGANIZATION
GENEVA

Screening in practice

- the World Health Organization published screening guidelines in 1968:
  - the condition should be an important health problem
  - there should be a treatment for the condition
  - facilities for diagnosis and treatment should be available
  - there should be a latent stage of the disease
  - there should be a test or examination for the condition
  - the test should be acceptable to the population
  - the natural history of the disease should be adequately understood
  - there should be an agreed policy on who to treat
  - the total cost of finding a case should be economically balanced in relation to medical expenditure as a whole

Screening in assisted reproduction

- "what must prevail in the medical decision is a balance between the importance of the message advising against pregnancy and the benefit for patients of being assisted in their plans to have a child"

Englert Y et al. Hum Reprod 2001; 16: 1309-15

Outweighing decisions in assisted procreation

- "do not become pregnant"
  - unprotected intercourse
  - contamination of the child
  - short life expectancy of the parent(s)

versus

- "seek for medically assisted procreation"

The position of the health care worker

- opposing the patient's desire to procreate
  - patient's autonomy?
  - doctor's authorization to have unprotected intercourse?

or

- advising the patient desiring to procreate
  - positively =a non-collaboration attitude
  - negatively =providing medical assistance

Providing medical assistance

- attitude of non-rejection
- minimalisation of contamination risk
  - the couple
  - the (unborn) child
  - other patients
  - the medical team members

Contamination risk

- suspicion of infectious agents
- detection of infectious agents

SCREENING

Screening for infectious diseases

- arguments pro
  - information (patient / physician)
  - safe sex promotion
  - revision of the vaccination status
  - counseling about the child's health
- arguments contra
  - accepted criteria not fulfilled
  - ethics?

→informed consent

Informed consent and screening

- opt-out strategy
  - testing is routinely done
  - unless the patient chooses not to be tested
- opt-in strategy
  - testing is offered and done
  - only after formally obtained informed consent

Interpretation of screening tests

- what condition is tested for?
- what's the prevalence of the condition?
- what is the nature of the test?
  - type of the test
  - sensitivity
    =proportion of those who have the condition with an abnormal test result
  - specificity
    =proportion of those who don't have the condition with a normal test result
- what does an abnormal test result mean?
  - positive predictive value
    - chance that a positive test result will be correct
  - negative predictive value
    - chance that a negative result will be correct
  - positive/negative predictive values change if the prevalence of the disease changes
- will the knowledge of the test result be useful?

Hepatitis C

- prevalence
  - worldwide prevalence 3%
  - vertical transmission risk 3-5%
- natural evolution
  - infection of the host
  - acute hepatitis
  - viral clearance or chronic carrier state
  - asymptomatic state or chronic liver disease
- laboratory diagnosis
  - elevated liver enzymes
  - direct measurement of HCV
    - HCV-RNA (PCR)
  - anti-HCV antibodies
    - EIA
    - RIBA

Hepatitis C and the neonate

- risk of perinatal transmission 5%
- little information on possible interventions during pregnancy
- limited experience with antiviral therapy during pregnancy
- protective effect of cesarean delivery?
- breast feeding not considered a risk factor for transmission (unless hiv co-infection)
- high sensitivity of available tests (97-99%)
- little information on the cost-effectiveness of universal screening

Hepatitis B

- prevalence
  - worldwide prevalence 2-20 %
  - vertical transmission risk 5-90%
- natural evolution
  - infection of the host
  - acute hepatitis
  - viral clearance or chronic carrier state
  - asymptomatic state or chronic liver disease
- laboratory diagnosis
  - elevated liver enzymes
  - direct measurement of HBV
    - HBsAg, HBeAg
    - HBV-DNA (PCR / quantitative or qualitative)
  - anti-HBV antibodies
    - anti-HBs
    - anti-HBc
    - anti-HBe

Hepatitis B and the neonate

- risk of perinatal transmission 5 - 90%
- treatment during pregnancy may reduce the risk of vertical transmission
- newborns of infected mothers should receive active (and passive) immunisation
- breast feeding not considered a risk factor for transmission
- household contacts and sexual partners should be vaccinated if not immune
- high sensitivity and specificity of available tests (99-100%)
- screening of pregnant women estimated cost-effective if prevalence of HBs-Ag at least 0.06%

Human immunodeficiency virus

- prevalence
  - worldwide prevalence highly variable (0.1–30%)
  - vertical transmission risk 2–25%
- natural evolution
  - infection of the host
  - asymptomatic phase
  - disease
- laboratory diagnosis
  - anti-hiv antibodies = “hiv test”
    - ELISA
    - Western blot
  - direct measurement of hiv
    - p24 antigen (ELISA)
    - HIV-RNA (PCR / primarily used for follow-up after diagnosis)

Hiv and the neonate

- risk of perinatal transmission < 5 % …
- ...if access to adequate “MCT* treatment protocol”
- breast feeding is a risk factor for transmission
- high sensitivity and specificity of available tests (99%)
- screening and MCT prevention estimated cost-beneficial

*MCT= mother-to-child transmission

Conclusions

- there is increasing interest in the reproductive desire of persons who carry chronic viral diseases
- the progress made in prevention and treatment of these diseases has caused a movement in the direction of intervention by medical teams for assisted reproduction
- in this context screening -after informed consent has been obtained- is desirable and legitimate
- there is medical and ethical agreement that screening should be coupled to counseling
- the ultimate goal of the involved health care workers should be the physical and psychological well-being of individual (future) parents and newborns in the broader scope of a professional commitment of contributing to a healthier society

Literature

- A review of hepatitis C (HCV) vertical transmission: risks of transmission to infants born to mothers with and without HCV viraemia or human immunodeficiency virus infection. *SL.Thomas et al. International Journal of Epidemiology 1998; 27: 108-117*.
- Understanding sensitivity and specificity with the right side of the brain. *TW.Loong. British Medical Journal 2003; 327: 716-719*.
- Medically assisted reproduction in the presence of chronic viral diseases. *Y.Englert et al. Human Reproduction Update 2004; 10: 149-162*.
- Accuracy of HCV-RNA PCR tests for diagnosis or exclusion of vertically acquired HCV infection. *European Paediatric Hepatitis C virus network. Journal of Medical Virology 2006; 78: 305-310*.
- When to screen in obstetrics and gynecology. *HIJ.Wildschut, CP.Weiner, TJ.Peters. Second Edition, Saunders Elsevier 2006*.

The most appropriate treatment for positive patients

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Learning objectives

- Being able to counsel adequately positive (discordant) couples regarding their possibility of having a child minimizing the risk of infection of the partner and future child
- Having a basic knowledge of the criteria that are used for inclusion of discordant couples in ART programs
- Having a basic knowledge of which ART treatment could be offered in serodiscordant couples
- Having a basic knowledge of the precautions that should be taken in the operating theater and laboratory when infected patients are treated

Why ART treatment in positive patients?

- Better prognosis and reduced vertical transmission risk in developed countries in patients with HIV, Hepatitis B or hepatitis C
- The ethical debate is still ongoing *(Ethics Committee of the ASRM, 2002)*
- Many European countries now find that these couples should have the possibility of having children safely
- Priority: Preventing the uninfected partner or future child

HIV

HIV

- Patients with HAART: the course of HIV shifted from a lethal to a chronic disease *(Yeni et al., 2004)*
- Most patients are in reproductive age and consider having offspring *(Frodsham et al., 2006)*
- Aim of the ART treatment: transmission reduction or treatment of co-existing subfertility or a combination of both

HIV in the male

- HIV-1 in semen: free HIV-1 RNA particles in seminal plasma and cell associated virus in non spermatozoal cells (lymphocytes and macrophages) *(Lowe et al., 2004)*
- It seems unlikely that spermatozoa are directly infected with HIV-1 *(Pudney et al., 1999)*
- Most HIV-1 RNA originate from seminal vesicles and prostate *(Krieger et al., 1998)*

HIV in the male

- Untreated HIV-1: conc of HIV-1 RNA in semen is ~ 10 fold lower than in blood plasma. But, in some individuals, HIV-1 RNA conc in seminal plasma is higher than in blood plasma *(Lowe et al., 2004)*
- Most antivirals penetrate well into the male genital tract, except for some protease inhibitors *(Lowe et al., 2004)*
- In general, HIV RNA conc in blood and seminal plasma show a parallel decrease in response to HAART *(Leruez-Ville et al., 2002a)*
- However, intermittent shedding in semen leads to occasional discrepancies between HIV1 RNA in blood and seminal plasma

Fertility of the HIV positive male

- In general, semen parameters are NOT impaired by asymptomatic HIV infection *(Muller et al., 1998)*
- Occasionally a reduction in sperm motility and morphology has been observed (due to the HIV1 infection itself or the antiretroviral therapy?) *(Nicopoullos et al., 2004)*
- In advanced cases (especially if CD4 < 200 cells/mm^3): increase of alterations of number, motility and leucospermia has been observed

ART if the male is HIV positive

- High-technology ART is necessary to prevent sexual transmission
- **Semen processing**: goal to obtain an HIV-1 free spermatozoal fraction by separating spermatozoa from all other semen components
- Afterwards, the spermatozoal fraction is **tested for the presence of HIV-1 by PCR based methods**
- Successful semen processing: sufficient spermatozoa with a negative (undetectable), valid HIV-1 test
- HIV-1 could not be detected by PCR in the spermatozoal fraction in 98% of samples of men using HAART and in 82% of men without antiretroviral treatment after semen processing *(Leruez-Ville et al., 2002b)*
- So, semen processing seems to be more effective in men with HAART but even if full suppression of HIV-1 RNA in blood, HIV-1 RNA has been measured in the spermatozoal fraction after semen processing!

ART if the male HIV positive

- **IUI**: favoured ART in many countries. No seroconversions have been reported but very large numbers are necessary to prove the ultimate safety of this technique
- **ICSI**: Some groups advocate the use of ICSI instead of IUI. Argue: because of the amount of semen exposed to the oocyte is extremely low, that is one spermatozoon. However, it is unknown what will happen if one accidentally injects a viral particle directly into a human oocyte
- All couples should practice safe sex while being treated. After a condom accident, ART should be delayed for 6 months *(Panlilio et al., 2005)*
- Women should have HIV testing after unsuccessful ART and at 4, 12 and 24 weeks amenorrhea, to detect iatrogenic infection

HIV in the female

- HIV-1 can be detected in vaginal and cervical secretions as a cell free virus an as a cell associated virus *(Mostad and Kreiss, 1996)*
- Blood plasma concentration is the most important predictor for HIV-1 genital shedding but there is an increased vaginal and cervical shedding in case of: use of oral contraception, vitamin A deficiency, candida albicans infection, gonorrhoea cervicitis *(Mostad et al., 1997)*
- HAART results in decreased shedding of HIV-1 in the female genital tract. But, HIV-1 RNA was still detected in the genital secretions of 33% of women with blood plasma RNA of < 500 copies/ml and in 25% with blood of < 50 copies /ml *(Kovacs et al., 2001)*
- So, even with successful HAART, unprotected intercourse should be discouraged at all times

Fertility of the HIV positive female

- Cycle disturbances are equally prevalent in HIV-1 infected women or negative controls, although more advanced immunodeficiency is associated with menstrual dysfunction *(Harlow et al., 2000)*
- High prevalence of STD's in HIV-1 infected women and therefore at risk for tubal infertility *(Coll et al., 2007).* Results of ovarian reserve are conflicting *(Martinet et al., 2006; Englert et al., 2004)*
- Pregnancy rate in HIV-1 infected women: lower when compared with women without HIV-1 infection *(Lo and Schambelan, 2001).* Progression of HIV disease resulted in a dramatic decline in pregnancy and live birth rate *(Sedgh et al., 2005).*
- Data on HAART and fertility in women are limited to one case report. NO conclusions are present at the moment *(Vigano et al., 2003)*

ART if the female is HIV positive

- **Inclusion criteria for ART**: NO consensus!

  Evaluation by specialist necessary and only patients with no advanced disease should be accepted

  IVI: CV undetectable and CD4>200 cells/mm3
- **Which treatment?**
  - Self inseminations at home around the time of ovulation
  - If no conception: IUI, IVF, ICSI
- IUI and IVF seem safe to perform in these women
- Can the ICSI procedure itself increases vertical transmission rates? Receptors for HIV-1 have not been demonstrated on the surface of the oocyte itself but HIV-1 has been detected in ovarian follicles. Theoretically, a viral particle could be injected into a human oocyte during the ICSI

ART if the female is HIV positive

- Few data on success rates of IVF/ICSI in HIV-1 infected women: no ultimate conclusion can be drawn *(Ohl et al., 2003; Coll et al., 2006)*
- Need to avoid multiple gestations. The prematurity could increase the risk of transmission vertical. Increase in viral load after the use of gonadotrophins compared with natural cycles: need to use “soft stimulations” *(Weigel et al., 2001)*
- All couples should practice safe sex while being treated

Male and female are HIV positive

- ESHRE advised AGAINST ART if both infected (because of the risk of death and leaving an orphaned child)
- **Inclusion criteria for ART**: see before
- **Which ART treatment?**
- Self inseminations for most seroconcordant couples but HIV superinfection of the women can occur and can possibly enhance the disease progression *(van der Kuyl et al., 2005)*
- Self inseminations instead of unprotected intercourse because it eliminates the risk of superinfection of the man
- ART (sperm washing and PCR) for prevention of transmission of discordant HIV-1 stains or subfertility after unsuccessful attempts to conceive naturally
- Semen processing is always advised when resistant virus is present

Hepatitis C

Hepatitis C in the male

- Risk of sexual transmission is estimated at 5% *(Keck et al., 1998)* and HCV is present in semen of positive men in 5-30% of the cases *(Levy et al., 2000)*
- Although transmission through intercourse is still controversial, safe sexual practice should be encouraged
- Ribavirine: should be discourage during the ART treatment because of the possible negative effects on germinal cells (induces morphologic anomalies in rat germ cells) *(Narayana et al., 2002)*
- A pregnancy is possible 6 months after the treatment with interferon and/or Ribavirine has been stopped

Fertility of the HCV positive male

- The impact of HCV infection on male fertility is still under debate
- In general: viral infections have been shown to contribute to male infertility (direct toxic effects, indirectly causing local inflammatory or immunological reaction) *(Keck et al., 1998)*

ART if the male is HCV positive

- Information on transmission of HCV in IUI samples is limited and separation and removal of the infective fraction of the ejaculate has not been well studied
- **Semen processing:**

  HCV RNA was detected in 5% of the semen samples but in 0% of the samples after density gradient preparation *(Levy et al., 2000)*
- Afterwards, the spermatozoal fraction is **tested for the presence of HCV by PCR based methods** *(Bourlet et al., 2003)*
- IUI or IVF
- NO necessity to determine the viral load in blood neither to treat the men before realizing the sperm processing

Hepatitis C in the female

- **Inclusion criteria for ART:**
- Depending on the degree of hepatic damage (evaluation by a specialist is mandatory: evaluation of the necessity of treatment and vaccination against HBV and HAV)
- Depending on the risk of vertical transmission

  Correlation between risk of vertical transmission and maternal titres of HCV RNA *(Alter et al., 1995)*

  If RNA negative: risk of vertical transmission < 1%

  If ARN positive: 5-10%
- Patients needing treatment need to start it before pregnancy in order to diminished the viral load and stop the medication (Interferon and/or Ribavirin) 6 months before conception

ART if the female HCV positive

- **Which treatment?**
  - Self inseminations at home around the time of ovulation
  - If no conception: IUI, IVF, ICSI

Male and female are HCV positive

- Idem female infected
- Also semen processing and PCR if male and female are infected by different stains

Hepatitis B

Hepatitis B in the male

- VHB is present in the ejaculate of the men with a chronic infection *(Hadchouel et al., 1985)*
- Only precaution: vaccinate the female partner and advise to use a condom till immunity is acquired
- NO necessity of additional treatments (as sperm washing) unless the female has failed to be effectively vaccinated *(Practice Committee of the ASRM, 2004)*

Hepatitis B in the female

- Important to vaccinate the male partner and to advise to use a condom till immunity is acquired
- **Inclusion criteria for ART:**
- Evaluation of the hepatic function and necessity of treatment with interferon by specialist. ART only if the no advanced hepatopathy
- The risk of vertical transmission is depending of the viral load *(Michielsen et al., 1999)*
- Levels of viral ADN should be measured and allow pregnancy if viral load is low (if HBeAg negative and $< 10^6$ copies/ml)

ART if the female is HBV positive

- **Which treatment?**
  - Spontaneous conception (when immunity of the male partner is acquired)
  - If no conception: IUI, IVF, ICSI

Laboratory safety for positive patients

Laboratory safety for positive patients

- Few publications on guidelines on lab procedures *(Gilling-Smith et al., 2005)*
- Screening before procedures: HIV, HBV, HCV (done nearly everywhere) *(Van den Eede B, 1995)*
- Risk for health workers and other patients
- For health co workers:
  - Main risk through needle stick or splash injuries
  - HBV prevention: a vaccine which is 90% immunogenic is available *(Bonanni and Bonaccorsi, 2001)*
  - If injury with HIV: period of anti retroviral treatment
  - If injury with Hep B: anti HBV immunoglobulin if health co worker failed to develop immunity

Laboratory safety - risk of cross contamination

- In IVF setting, the main concern is transmission to uninfected gametes and embryos during laboratory procedures
- Great concerns since the publication:
  - of transmission of HCV from an infected patient undergoing IVF to 2 non infected patients undergoing IVF within the same clinic during the same time period *(Lesourd et al., 2000)*
  - of the transmission of HBV from HBV contaminated cryopreserved bone marrow samples to HBV negative cryopreserved bone marrow samples

Laboratory safety - risk of cross contamination

- Blood contamination of the follicular fluid samples
  - HIV: HIV only detected in the follicular fluid of a patient with a detectable viral load *(Bertrand, 2004).* But, in another series HIV were detectable in follicular fluid irrespective of the viral load or antiretroviral therapy *(Frodsham et al., 2004)*
  - HCV: HCV RNA was detected in 89% of the follicular fluids and in 25% of the culture media at day 1 from 22 IVF trials of HCV patients *(Devaux et al., 2003)*
- Cross contamination in tanks storing biological material *(Clarke, 1999)*

Laboratory safety - Precautions

- Sanitization and sterilization (like in any patient)
- All non disposable material: sterilized with Virkon and ethanol
- Ultrasound probe: protective sheath and wipe the probe with germicidal impregnated tissue before and after each patient *(Milki and Fish, 1998)*
- Working surfaces and equipment need to be cleaned with additional disinfecting agents, e.g. Virkon, to further minimize potential cross-contamination risk. But, these are potentially embryotoxic: wait > 30 min to evaporate
- Put positive patient the last on the list

Laboratory safety - Precautions for the laboratory

- Ideal: separate laboratory
- In France: the law oblige to separate the infected/non infected patients in space or time
- Separation in time does not solve the problem of cryopreservation of infected samples
- No necessity for the use of separate incubadores separados (but recommended)

Laboratory safety - Precautions for the laboratory

- **Cryopreservation**
  - Recommendations by HFEA in UK *(Tedder et al., 1995)*: freeze samples from known infected sample in a separate tank for each infection and infection combination: this is nearly not feasible
  - Other option: use of heat-sealed high security straws made from shatterproof ionomeric resin *(Clarke, 1999)*
  - Vapour phase storage in case of known infected samples would offer more security then liquid storage phase *(Tomlinson and Sakkas, 2000)*
  - Use of sperm "washing" techniques to decrease the viral load before freezing the samples

References

•Gilling-Smith C, Emiliani S, Almeida P et al. Laboratory safety during assisted reproduction in patients with blood-borne viruses.Hum Reprod. 2005 20:1433-8

•Ethics Committee of the American Society for Reproductive Medicine (2002) Human immunodeficiency virus and infertility treatment. Fertil Steril 77,212-222

•Lowe SH, Sankatsing SU, Repping S, et al. AIDS. 2004, 21353-62. Is the male genital tract really a sanctuary site for HIV? Arguments that it is not.

•Pudney J, Nguyen H, Xu C, Anderson DJ. Microscopic evidence against HIV-1 infection of germ cells or attachment to sperm.J Reprod Immunol. 1999, 44:57-77.

•Krieger JN, Nirapathpongporn A, Chaiyaporn et al. Vasectomy and human immunodeficiency virus type 1 in semen.J Urol. 1998 Mar;159(3):820-5;

•Yeni PG, Hammer SM, Carpenter CC et al. Antiretroviral treatment for adult HIV infection in 2002: updated recommendations of the International AIDS Society-USA Panel.JAMA. 2002 Jul 10;288(2):222-35.

•Frodsham LC, Boag F, Barton S, Gilling-Smith C. Human immunodeficiency virus infection and fertility care in the United Kingdom: demand and supply. Fertil Steril. 2006 Feb;85(2):285-9

•Leruez-Ville M, de Almeida M, Tachet A et al.Assisted reproduction in HIV-1-serodifferent couples: the need for viral validation of processed semen. AIDS. 2002a, 22;16:2267-73.

•Muller CH, Coombs RW, Krieger JN. Effects of clinical stage and immunological status on semen analysis results in human immunodeficiency virus type 1-seropositive men. Andrologia. 1998;30 Suppl 1:15-22.

•Nicopoullos JD, Almeida PA, Ramsay JW et al. The effect of human immunodeficiency virus on sperm parameters and the outcome of intrauterine insemination following sperm washing.Hum Reprod. 2004,19:2289-97.

•Panlilio AL, Cardo DM, Grohskopf LA, et al.,Updated U.S. Public Health Service guidelines for the management of occupational exposures to HIV and recommendations for postexposure prophylaxis.MMWR Recomm Rep. 2005 Sep 30;54(RR-9):1-17.

•Leruez-Ville M, Dulioust E, Costabliola D et al. Decrease in HIV-1 seminal shedding in men receiving highly active antiretroviral therapy: an 18 month longitudinal study (ANRS EP012).AIDS. 2002b Feb 15;16(3):486-8.

•Mostad SB, Kreiss JK. Shedding of HIV-1 in the genital tract. AIDS. 1996,10:1305-15.

•Mostad SB, Overbaugh J, DeVange DM, et al. Hormonal contraception, vitamin A deficiency, and other risk factors for shedding of HIV-1 infected cells from the cervix and vagina. Lancet. 1997,7;350(9082):922-7.

•Kovacs A, Wasserman SS, Burns D et al.; DATRI Study Group; WIHS Study Group.Determinants of HIV-1 shedding in the genital tract of women.Lancet. 2001 Nov 10;358(9293):1593-601.

•Harlow SD, Schuman P, Cohen M et al.,Effect of HIV infection on menstrual cycle length.J Acquir Immune Defic Syndr. 2000 May 1;24(1):68-75.

References

- Coll O, Lopez M, Vidal R et al,Fertility assessment in non-infertile HIV-infected women and their partners.Reprod Biomed Online. 2007,14:488-94.
- Martinet V, Manigart Y, Rozenberg S et al. Ovarian response to stimulation of HIV-positive patients during IVF treatment: a matched, controlled study. Hum Reprod 2006: 21(5):1212-7
- Englert Y, Lesage B, Van Vooren JP, et al. Medically assisted reproduction in the presence of chronic viral diseases.Hum Reprod Update. 2004;10(2):149-62.
- Lo JC, Schambelan M.Reproductive function in human immunodeficiency virus infection.J Clin Endocrinol Metab. 2001 Jun;86(6):2338-43.
- Sedgh G, Larsen U, Spiegelman D, et al. HIV-1 disease progression and fertility in Dar es Salaam, Tanzania.J Acquir Immune Defic Syndr. 2005 Aug 1;39(4):439-45.
- Vigan A, Manzoni P, Riva S, et al .Hyperinsulinemia induced by highly active antiretroviral therapy in an adolescent with polycystic ovary syndrome who was infected with human immunodeficiency virus.Fertil Steril. 2003 Feb;79(2):422-3.
- Ohl J, Partisani M, Wittemer C, et al. Assisted reproduction techniques for HIV serodiscordant couples: 18 months of experience.Hum Reprod. 2003 Jun;18(6):1244-9.
- Coll O, Suy A, Figueras F,et al.Decreased pregnancy rate after in-vitro fertilization in HIV-infected women receiving HAART.AIDS. 2006 Jan 2;20(1):121-3.
- Weigel MM, Gentili M, Beichert M, et al. Reproductive assistance to HIV-discordant couples--the German approach.Eur J Med Res. 2001 Jun 28;6(6):259-62.
- van der Kuyl AC, Kozaczynska K, van den Burg R, et al. Triple HIV-1 infection.N Engl J Med. 2005 Jun 16;352(24):2557-9.
- Keck C, Gerber-Sch er C, Clad A, et al. Seminal tract infections: impact on male fertility and treatment options. Hum Reprod Update. 1998 Nov-Dec;4(6):891-903.
- Levy R, Tardy JC, Bourlet T, et al. Transmission risk of hepatitis C virus in assisted reproductive techniques. Hum Reprod. 2000 Apr;15(4):810-6.
- Narayana K, D'Souza UJ, Seetharama Rao KP.Ribavirin-induced sperm shape abnormalities in Wistar rat.Mutat Res. 2002 Jan 15;513(1-2):193-6.
- Bourlet T, Levy R, Laporte S, et al.,Multicenter quality control for the detection of hepatitis C virus RNA in seminal plasma specimens.J Clin Microbiol. 2003 Feb;41(2):789-93.
- Alter MJ.Epidemiology of hepatitis C in the West.Semin Liver Dis. 1995 Feb;15(1):5-14
- Hadchouel M, Scotto J, Hurez JL, et al. Presence of HBV DNA in spermatozoa: a possible vertical transmission of HBV via the germ line.J Med Virol. 1985 May;16(1):61-6.

References

•Practice Committee of the American Society for Reproductive Medicine (2004) Hepatitis and Reproduction. Fertil Steril 82,1754-1764

•Michielsen PP, Van Damme P Viral hepatitis and pregnancy.Acta Gastroenterol Belg. 1999 Jan-Mar;62(1):21-9.

•Van den Eede B. Investigation and treatment of infertile couples: ESHRE guidelines for good clinical and laboratory practice. European Society of Human Reproduction and Embryology. Hum Reprod. 1995 May;10(5):1246-71.

•Bonanni P, Bonaccorsi G. Vaccination against hepatitis B in health care workers.Vaccine. 2001, 21;19:2389-94.

•Bertrand E, Zissis G, Marissens D, G et al. Presence of HIV-1 in follicular fluids, flushes and cumulus oophorus cells of HIV-1-seropositive women during assisted-reproduction technology.AIDS. 2004 Mar 26;18(5):823-5.

•Frodsham LC and Gilling-Smith C (2003) ART in HIV infected couples: UK provision of care. Hum Reprod 18,(Suppl) 0-096:30

•Devaux A, Soula V, Sifer C, et al. Hepatitis C virus detection in follicular fluid and culture media from HCV+ women, and viral risk during IVF procedures. Hum Reprod. 2003 Nov;18(11):2342-9.

•Clarke GN.Sperm cryopreservation: is there a significant risk of cross-contamination?Hum Reprod. 1999 Dec;14(12):294

•Milki AA, Fisch JD. Vaginal ultrasound probe cover leakage: implications for patient care.Fertil Steril. 1998 Mar;69(3):409-11.

•Tedder RS, Zuckerman MA, Goldstone AH, et al. Hepatitis B transmission from contaminated cryopreservation tank. Lancet. 1995, 15;346:137-40.

•Tomlinson M, Sakkas D.Is a review of standard procedures for cryopreservation needed?: safe and effective cryopreservation-should sperm banks and fertility centres move toward storage in nitrogen vapour?Hum Reprod. 2000 Dec;15(12):2460-3.

•Lesourd F, Izopet J, Mervan C, et al. Transmissions of hepatitis C virus during the ancillary procedures for assisted conception. Hum Reprod. 2000 May;15(5):1083-5.

•van Leeuwen E, Prins JM, Jurriaans S, et al. Reproduction and fertility in human immunodeficiency virus type-1 infection.Hum Reprod Update. 2007 Mar-Apr;13(2):197-206.

Hepatitis B, C and HIV. Pregnancy, delivery and maternal care

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Objectives

- To understand the ways of transmission of Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV) in pregnancy, during delivery and during breastfeeding
- To know how to reduce vertical transmission of HBV, HCV and HIV during pregnancy, delivery and postpartum

HBV

- DNA virus (*Hepadnaviridae* family)
- Liver is primary site of HBV replication
- Asymptomatic or symptomatic infection
- Self-limiting infection versus chronic infection
- Adults: 30-50% symptomatic, 95% self-limiting
- Infants: usually asymptomatic infection, 90% chronic infection
- Chronic infection: 15-25% liver cirrhosis or liver cancer

TABLE 1. Typical interpretation of serologic test results for hepatitis B virus infection

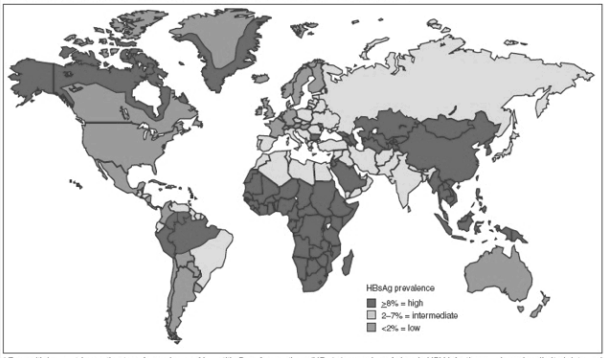
Serologic marker				
HBsAg*	Total anti-HBc†	IgM§ anti-HBc	Anti-HBs¶	Interpretation
–**	–	–	–	Never infected
+††§§	–	–	–	Early acute infection; transient (up to 18 days) after vaccination
+	+	+	–	Acute infection
–	+	+	–	Acute resolving infection
–	+	–	+	Recovered from past infection and immune
+	+	–	–	Chronic infection
–	+	–	–	False positive (i.e., susceptible); past infection; "low-level" chronic infection;¶¶ passive transfer to infant born to HBsAg-positive mother
–	–	–	+	Immune if concentration is ≥10 mIU/mL;*** passive transfer after hepatitis B immune globulin administration

CDC MMWR Recommendations and Reports Dec 23, 2005/54(RR16); 1-23

HBV

- HBV is detected in multiple body fluids, but only serum, semen and saliva are infectious.
- All HBsAg-positive persons are infectious, but HBeAg-positive persons are more infectious ~ high titers of HBV (HBV DNA levels of 10^6-10^9 IU/ml)

FIGURE 1. Geographic distribution of chronic hepatitis B virus (HBV) infection, 2005*



*For multiple countries, estimates of prevalence of hepatitis B surface antigen (HBsAg), a marker of chronic HBV infection, are based on limited data and might not reflect current prevalence in countries that have implemented routine childhood hepatitis B vaccination. In addition, HBsAg prevalence rates might vary within countries by subpopulation and locality.

CDC MMWR Recommendations and Reports Dec 23, 2005/54(RR16); 1-23

HBV

- No treatment for acute infection, only supportive care
- Chronic hepatitis ~ antiviral medication can achieve sustained suppression of HBV replication
  - In pregnancy lamivudine

HBV

- Acute HBV infection in pregnancy
  - ~non-pregnant population
  - No reason for delivery
  - Seldom severe infection DD HELLP, acute fatty liver
  - Risk of neonatal hepatitis when acute HBV infection in 1st, 2nd and 3th trim :<1%, 10%, 90%
  - In utero infection? Is possible when severe acute HBV infection in 3th trim
- Chronic carrier
  - Transmission with amniocentesis: rare
  - No increased risk of pregnancy complications or congenital abnormalities
  - HBsAg + and HBeAg - →10% vertical tranmission during delivery
  - HBsAg + and HBeAg + →90% vertical transmission during delivery

HBV

- All pregnant women should be tested for HBsAg (if high risk: repeat)
- When tested positive, appropriate counselling
  - Modes of transmission
  - Perinatal concerns (BREASTFEEDING IS NO PROBLEM)
  - Prevention of HBV to contacts
    - Importance of postexposure prophylaxis for the newborn
    - Importance of testing and hepatitis B vaccination for household, sexual and needle-sharing contacts
  - Other STDs
  - Medical evaluation and possible treatment of chronic hepatitis B

HBV

If mother HBsAg +
- Hepatitis B vaccine and hepatitis B immune globulin (HBIG) (0.5-1ml: 100-200IU) <12hours of birth

If status of mother unknown
- Hepatitis B vaccine
- Blood analysis asap: if HBsAg + → HBIG asap (within 1 week)

If mother HBsAg –
- Hepatitis B vaccine before hospital discharge (USA CDC)
- Hepatitis B vaccine at 2 months (Belgium)

Preterm babies: same strategy, but do not count the birth dose as part of the vaccination series

HBV

- Highly effective strategy
- Complete the vaccination series!
- Check for adequate immune response (HBsAl >10IU/ml)

HBV references

- CDC. MMWR. Recommendations and reports. Dec 23,2005/54(RR16); 1-23
- Gambarin-Gelwan M. Hepatitis B in pregnancy. Clin Liver Dis 2007; 11(4):545-63
- Ranger-Rogez S et al.Virus des hépatites: transmission mère-enfant. Pathologie Biologie 2002; 50: 568-575.
- Ranger-Rogez S et al. Hepatitis B mother-to-child transmission. Expert Rev Anti-infect Ther 2004; 2: 133-145.
- Tovo PA et al. Hepatitis B virus and hepatitis C virus infections in children. Curr Opin Infect Dis 2005; 18: 261-266.
- Boxall EH et al. Natural history of hepatitis B in perinatally infected carriers. Arch Dis Child Fetal Neonatal Ed 2004; 89: 456-460.
- Li XM et al. Interruption of HBV intrauterine transmission: A clinical study. World J Gastroenterol 2003; 9: 1501-1503.
- Euler GL et al. Antibody response to postexposure profylaxis in infants born to hepatitis B surface antigen-positive women. Pediatr Infect Dis J 2003; 22: 123-129.
- Hasanjani Roushan MR et al. Revaccination of non-responding infants delivered by HBsAg-positive mothers. Eur J Clin Microbiol Inf Dis 2005; 24: 434-435.

HCV

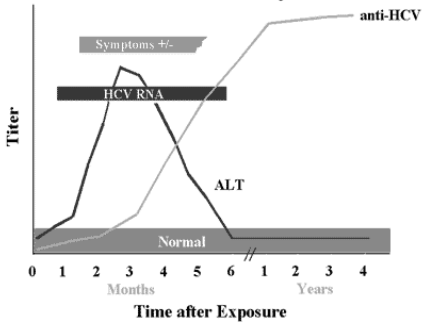
- RNA virus (*Flaviviridae* Family)
- Incubation period: Average 6-7 weeks (range 2-26)
- Acute illness:
  - asymptomatic or mild
  - Case fatality rate: Low
- Chronic infection*60%-85%
- Chronic hepatitis*10%-70%
- Cirrhosis*<5%-20%

*Age related

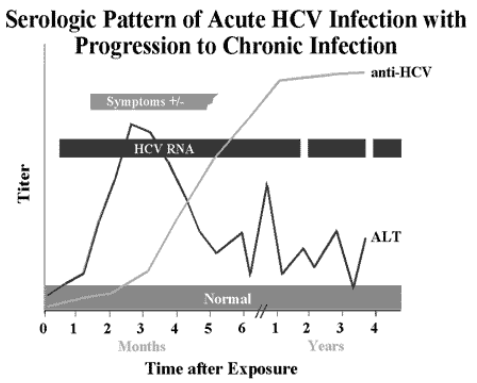
HCV

- Chronic Hepatitis C Factors Promoting Progression or Severity
  - Increased alcohol intake
  - Age>40 at time of infection
  - HIV co-infection
  - Male gender
  - Chronic HBV co-infection
- Transmission
  - >> blood: needle stick injury 3% risk of transmission
  - sexual contact
- Test
  - Anti-HCV
  - HCV RNA

Serologic Pattern of Acute HCV Infection with Recovery



CDC Sept 25,2007 Hepatitis C. Slide sets



CDC Sept 25,2007 Hepatitis C. Slide sets

HCV

- 1-5% anti-HCV +; 60-70% with HCV RNA +
- Vertical transmission
  - Only if HCV RNA +
  - Risk 4-8%
  - HIV-HCV co-infection: risk 15-20%
- HCV not associated with negative pregnancy outcome or congenital abnormalities
- Screening of all pregnant women?
  - Most guidelines do not recommend universal screening

HCV

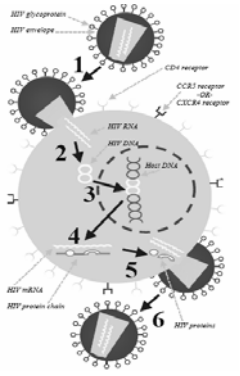
- Treatment
  - interferon and ribavirine
  - Contra-indicated in pregnancy
- Cesarean section to diminish vertical transmission??
  - Not enough evidence
- Transmission through breastfeeding??
  - American Academy of Pediatrics (AAP)/Centers for Disease Control (CDC): **no contra-indication for breastfeeding**; avoid if nipple sores…
- Infected infants generally do wel. Severe hepatitis is rare. Many unresolved questions…

HCV References

- Mariné-Barjoan E. et al. HCV/HIV co-infection, HCV viral load and mode of delivery: risk factors for mother-to-child transmission of hepatitis C virus? AIDS 2007; 21(13):1811-15
- Jain S. et al. Hepatitis C in pregnancy. Am J Perinatology 2007; 24(4):251-6
- Mast EE. Mother-to-infant hepatitis C virus transmission and breastfeeding. Adv Exp Med Biol 2004; 554: 211-6.
- Mok J. et al. When does mother to child transmission of hepatitis C virus occur? Arch Dis Child (N/F) 2005; 90: F156-F160.
- Roberts EA. et al. Maternal-infant transmission of hepatitis C virus infection. Hepatology. 2002; 36: S106-13.
- Memon MI. et al. Hepatitis C: an epidemiological review. J Vir Hep 2002; 9: 84-100.
- Tajiri H. et al. Prospective study of mother-to-infant transmission of hepatitis C virus. Ped Infect Dis J 2001; 20: 10-14.
- Ceci O. et al. Vertical transmission of hepatitis C virus in a cohort of 2,447 HIV-seronegative pregnant women: a 24-month prospective study. J Ped Gastroenterol Nutr 2001; 33: 570-5.
- Ackerman Z. et al. Intrafamilial transmission of hepatitis C virus: a systematic review. J Vir Hep 2000; 7: 93-103.
- Ceci O. et al. High rate of spontaneous viral clearance in a cohort of vertically infected hepatitis C virus infants: what lies behind? J Hepatol 2001; 35: 687-8.
- CDC MMWR. Recommendations for Prevention and Control of Hepatitis C Virus. 1998/vol.47/No RR-19

HIV

- RNA virus (*Retroviridae* family)
- Life cycle
  1. Binding and fusion
  2. Reverse Transcription
  3. Integration
  4. Transcription
  5. Assembly
  6. Budding

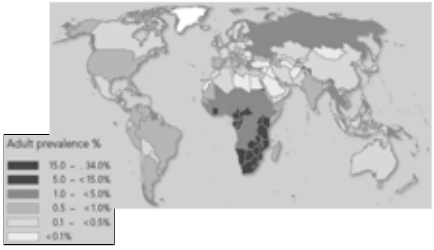


Aidsinfo.nih.gov

World Health Organization

A global view of HIV infection

38.6 million people [33.4 46.0 million] living with HIV, 2005



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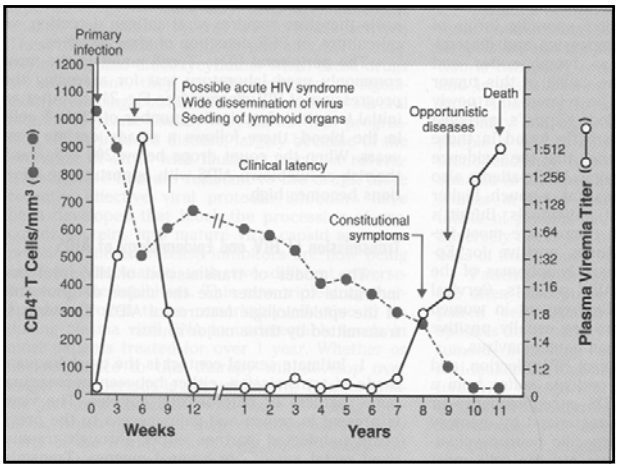


HIV

- Vertical transmission
  - 15-20% in non-breastfeeding population
  - 25-40% in breastfeeding population
- In non-breastfeeding population without intervention
  - 80% of HIV transmission late in 3th trim (>36w), labour and delivery
  - <2% in 1st and 2nd trim

HIV

- Risk factors for transmission
  - Advanced maternal HIV disease
  - Low CD4 count
  - **High plasma viral load = strongest predictor of transmission**
    - However, no threshold below which transmission never occurs
  - Vaginal delivery
  - Duration of membrane rupture
  - Chorio-amnionitis
  - Preterm delivery

HIV

- Testing

  Routine offer of HIV testing by health care providers
  - **C**onfidential
  - **C**ounselling
  - **C**onsent

  'Testing with patient notification and right of refusal' = opting-out method

  Recommended by most health authorities

HIV

- Partner notification

  *The woman's HIV diagnosis may be disclosed to a known sexual contact, in order to protect him from acquiring infection, where the woman has not informed him and cannot be persuaded to do so. The woman must be told of the disclosure and the clinician must be prepared to justify it. Information must not be disclosed to others. (*General Medical Council 1997. UK.)

  In Belgium: not allowed to disclose

HIV

- History
  - PACTG 076 trial in 1994
    - Oral ZDV from14-34 w
    - IV ZDV intrapartum
    - Oral ZDV to neonate during 6 weeks

  ⇒ Reduction of vertical transmission with 70%

  - ZDV
    - viral load ↓
    - Transplacentar passage and pre- and post exposure prophylaxis of foetus

HIV

- Management in pregnancy
  - Multidisciplinary team
    - HIV physician
    - Obstetrician
    - Midwife
    - Paediatrician
    - Psychologist, support groups, social service
  - Check for other STDs and bacterial vaginosis
  - High risk pregnancy
  - Ultrasound in beginning of pregnancy and at 20 weeks
  - Risk of amniocentesis/CVS? Start HAART?

HIV

- Management in pregnancy
  - Start HAART (highly active antiretroviral therapy)
    - ~viral load, CD4 count
    - antiretroviral drugs used before
    - If possible with ZDV
    - ! teratogenicity (Efavirenz)
    - ! Side effects
    - Importance of adherence
    - When to start? 14 weeks / 28 weeks

HIV

- Clinical scenario 1: Women who do not require HIV treatment for their own health
  - Start HAART at 14 weeks / 28 weeks
  - Check viral load before delivery
  - Stop treatment at delivery
  - Monotherapy with ZDV can be considered if viral load <1000 copies/ml

HIV

- Clinical scenario 2: Women who require HIV treatment for their own health
  - Start HAART if possible after 1st trim
  - Check viral load before delivery
  - Continue after delivery
  - Women who conceive while on HAART, should continue with HAART

HIV

- Maternal side effects of HAART
  - Anemia (ZDV)
  - Liver toxicity and rash (NVP especially when CD4 count >250 cells/mm^3)
  - Hyperglycemia (protease inhibitors)
  - Mitochondrial toxicity / Lactic acidosis (NRTI drugs)

  – Regular monitoring for complications of antiretroviral drugs and CD4 count/ viral load

- Pregnancy complications with HAART?
  - Preterm birth↑ preeclampsia↑ Conflicting data

HIV

- Foetal toxicity of HAART
  - Congenital abnormalities
    – Avoid EFV and other class D drugs
  - Mitochondral toxicity?
  - Long-term risk of neoplasia?
  - Long-term risk of organ system toxicities?

  – Short outcome generally good

HIV

- Mode of delivery
  - Cesarean section
    - Always when detectable viral load at 36 weeks
    - Always when monotherapy / no therapy
    - ZDV infusion
    - Antibiotic prophylaxis
    - Benefit in woman on HAART and undetectable viral load??
  - Vaginal delivery
    - ZDV infusion
    - Intact membranes as long as possible
    - Avoid use of scalp electrodes and fetal blood sampling
    - No ergometrine together with protease inhibitors

HIV

- Management of the neonate
  - No breastfeeding
  - 6 weeks of ZDV syrope
  - Triple therapy ?
  - Testing of neonate: with PCR at birth (day 5), 3 weeks, 6 weeks and 6 months
  - Negative HIV antibody test at 18 months is the definitive test!

HIV

- PROM and HIV
  - Study before HAART
    - Ruptured membranes > 4 hours doubles the risk of HIV transmission
    - 2% increase in transmission /4 hours up to 24 hours
- p-PROM and HIV
  - More risk of transmission because more chorioamnionitis and preterm infant
  - 2 small studies with patients with p-PROM on HAART→ no additional risk
  - In very preterm baby expectant management and short-term steroids may be the best option

HIV

- Data from Belgium
  - Vertical transmission rate dropped from 10% (1986-1993) to 4.7% (1999-2002)
  - Transmission rate 1.7% (HAART>1month before delivery) versus 13.3% (HAART<1 month before delivery)
  - Still high transmission rate in subgroup of women
    ~late diagnosis of HIV infection
    ~poor antenatal care

HIV References

- Kourtis A et al. Understanding the Timing of HIV Transmission from Mother to Infant. JAMA 2001; 285(6):709-712
- Public Health Service Task Force. NIH. Recommendations for Use of Antiretroviral Drugs in Pregnant HIV-Infected Women for Maternal Health and Interventions to Reduce Perinatal HIV Transmission in the United States. Nov 2,2007.
- RCOG. Management of HIV in pregnancy. RCOG guideline no. 39, April 2004.
- Williams CD et al. Reproduction in couples who are affected by human immunodeficiency virus: Medical, ethical, and legal considerations. Am J Obstet Gynecol 2003;189(2):333-41.
- Goetghebuer T et al. Vertical transmission of HIV in Belgium: a 1986–2002 retrospective analysis. Eur J Pediatr 2008 Apr 5 (epub ahead of print)
- ACOG. Human immunodeficiency Virus. ACOG Committee Opinion no. 389,Dec 2007.
- Aagaard-Tillery KM. Preterm Premature Rupture of Membranes in Human Immunodeficiency Virus-Infected Women: A Novel Case Series. Infect Dis Obstet Gynecol 2006; 2006:53234
- Alvarez J et al. Preterm premature rupture of membranes in pregnancies complicated by human immunodeficiency virus infection: A single center's five-year experience. J Matern Fetal Neonatal Med 2007; 20(12): 853-857
- UNAIDS/WHO policy statement on HIV testing. UNAIDS 2004.

HIV references

- International Perinatal HIV group. Duration of ruptured membranes and vertical transmission of HIV-1: a meta-analysis from 15 prospective cohort studies. AIDS 2001; 15: 537-68.

Safety of staff and risks of cross contaminaton

Josephine Lemmen, PhD,
Embryologist
Fertility Clinic, Rigshospitalet,
Copenhagen, Denmark

Learning objectives

- Where are the potential risks of cross contamination when treating patients with infectious diseases?
- What can we do to minimize risk of transmission to other patients and staff ?

Sexually transmitted pathogens

- Bacteria
  - Neisseria gonorrhoeae
  - Chlamydia trachomatis
  - Mycoplasma hominis
  - Ureaplasma urealyticum
  - Mycoplasma genitalium
  - Treponema pallidum
  - Haemophilus ducreyi
- others
  - Trichomonas vaginalis
  - Candida albicans
  - Trepanema pallidum

- Virus
  - **Human immunodeficiency virus 1**, 2
  - HTLV-1, 2
  - Herpes simplex virus 1, 2
  - Epstein-Barr virus
  - Human Herpesvirus 6, 8
  - Human papillomavirus
  - **Hepatitis** A, **B**, **C**, G virus
  - Cytomegalovirus

Identification of infected patients - Goals

- Protection of partner and child
- Protection of other patients
- Protection of staff
- New patient group – evaluation of procedures
- Procedures developed in close cooperation with hospital unit for hygiene and infection

Dilemma – cleaning vs. growth

Treat all patients/samples as infected

IVF clinics are set up to stimulate growth

Identification of infected patients is essential

HIV, HBV and HCV in the European population

0,1-0,6% of the European population is HIV-infected
Over 1% in parts of the former Soviet Union
(30% in south & central Africa) UNAIDS/WHO

0,1-7 % of the European population is HBV-infected
(10% in Asia, Africa & South America)

0,2-2,5 % of the European population is HCV-infected
5 million carriers (WHO-data)

Are we identifying new cases by screening our patients for HIV, HBV and HCV?

Since January 1st 2007 obligatory screening of infertile couples before treatment

In 2007 we found in our clinic (~ 2000 treatments/yr):

- No new HIV-positive cases
- 6 HBVc positive (but antigen negative)
- 1 HBVc and antigen positive man found
- 1 HCV antibody positive (but RNA negative)

Virus load prior to treatment

HBV/HCV: no specific rules

HIV: under 200 HIV-RNA copies/ml

but other clinics have different criteria

Infectious material – occupational infection HBV

Documented
- blood
- blood products

Not documented, but possible
- bloody fluids
- semen
- vaginal fluid
- saliva

Unlikely
- urine
- feces

Ref. Gerberding, 1995

Infectious material – occupational infection HCV

Documented
- blood

Not documented, but possible
- blood products
- bloody fluids
- semen
- vaginal fluid

Unlikely
- saliva
- urine
- feces

Ref. Gerberding, 1995

Infectious material – occupational infection HIV

Documented
- blood
- blood products
- bloody fluids

Not documented, but possible
- semen
- vaginal fluid
- follicular fluid
- cerebrospinal fluid
- breast milk
- saliva (at dentist)

Unlikely
- Saliva
- urine
- feces

Ref. Gerberding, 1995

Risk of transmission with needlestick injury

HBV: 2-40%

HCV: 3-10%

HIV: 0,2-0,5%

Ref. Gerberding, 1995

Risk of transmission with skin contact

HBV: not quantified, probably relatively higher than HCv and HIV

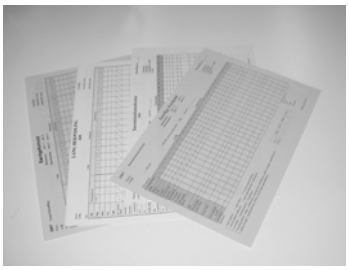
HCV: not documented, but plausible

HIV: documented

Ref. Gerberding, 1995

Separation of procedures

- Treatment-forms have a different colour



Where/when should we be cautious treating this patient group?

- **Scanning infected patients**
- Preparation of semensamples
- Inserting intravenous canula
- Performing the oocyte pickup
- Handling of follicular fluid
- Incubation of embryos
- Storage of surplus embryos/semen

Scanning infected patients

- Separation in time
- Appropriate cleaning of ultrasound scanner

- Condom over scanner
- Clean with detergent
- Clean with 70%alcohol

But..... Not all scanners tolerate alcohol

Special products exist

Where/when should we be cautious treating this patient group?

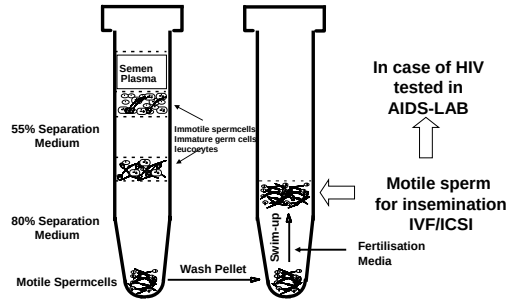
- Scanning infected patients
- **Preparation of semensamples**
- Inserting intravenous canula
- Performing the oocyte pickup
- Handling of follicular fluid
- Incubation of embryos
- Storage of surplus embryos/semen

Preparation of semensamples

Where is the virus localised?

	Semen plasma	White blood cells/ epithelial cells	Sperm cells
HIV-1	Yes	Yes	?/No
HBV	Yes	Yes	No
HCV	Yes	Yes	?/No

Gradient/Swimup Method



Semprini et al '96

Do these procedures protect HIV-negative women from getting infected?

- ~ 4.000 IUIs done worldwide
- Follow-up
- What is the risk of male-female infection with intercourse in this patientgroup?

Infection is dependent on other infections but estimated to be around 0,1-0,2% per intercourse

For HBV/HCV positive no post-test as intercourse is not contra-indicated

Where/when should we be cautious treating this patient group?

- Scanning infected patients
- Preparation of semensamples
- **Inserting intravenous canula**
- Performing the oocyte pickup
- Handling of follicular fluid
- Incubation of embryos
- Storage of surplus embryos/semen

Inserting intravenous canules

Use gloves when inserting intravenous canules

2 case reports HCV transmission (Lesourd, 2000)
Oocyte aspiration on same day as a HCV pos woman
Pickup in different rooms, other patients not infected

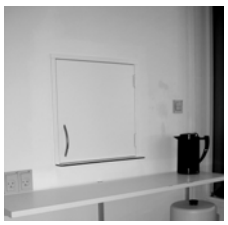
Other possibilitiy:
Inserting intravenous canule without changing gloves but using alcohol to disinfect them (doesn't work on HCV) (Schvarz et al, 1996)

Where/when should we be cautious treating this patient group?

- Scanning infected patients
- Preparation of semensamples
- Inserting intravenous canula
- **Performing the oocyte pickup**
- Handling of follicular fluid
- Incubation of embryos
- Storage of surplus embryos/semen

Performing the oocyte pickup

- if possible separate operating room or UV
- protective clothes
- use/cleaning of scanner
- discarding of material
- cleaning of room



Protective clothing

Nurse, doctor and laboratory staff wear

- Special coat
- Gloves
- Mouthcover
- Hat

Use/cleaning of scanner

The probe is covered by a transducercover (7 x 80cm)

After use cleaned with dry cloth and 70 % ethanol

Discarding/cleaning of used material

All disposables in high-risk garbage bag

Needlebox closed and put to destruction

Guidance set rinses through with hydrogenperoxide thereafter cleaning in washing machine drying and autoclaving

Cleaning of operating room after OPU

Before OPU fluidabsorbing material should be laid under the gynaecological 'leje'

Cleaning of scanner like with scanning

Room cleaning immediately after use

.....

Room should stand untouched in 20 minutes after cleaning

Where/when should we be cautious treating this patient group?

- Scanning infected patients
- Preparation of semensamples
- Inserting intravenous canula
- Performing the oocyte pickup
- **Handling of follicular fluid**
- **Incubation of embryos**
- **Storage of surplus embryos/semen**

Separate laboratory

- No mouth pipetting
- Use of plastics

Incubation of embryos

Dilemma: decontamination vs embryotoxicty

- cleaning lab surfaces

Separation of storage

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Fertility clinics 'risking HIV spread'

By Martin Hutchinson
BBC News Online health staff in Madrid

Many fertility clinics treating HIV positive patients are putting not only their partners, but other patients at risk, according to a UK survey.

Only a few have separate storage systems for sperm from HIV positive men - or make sure that treatments to

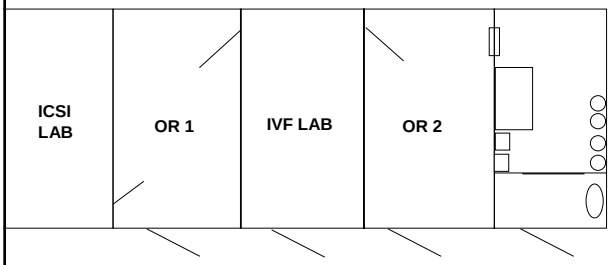
Samples were not kept apart

Separation of storage

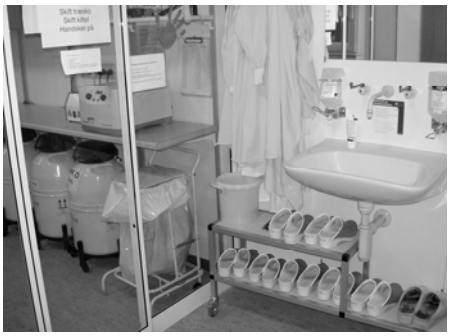
- Semen and embryos
- Separate cryotanks
- Sealing straws
- Nitrogen vapour

Separation of storage

Layout at Rigshopitalet



Separate laboratory



Conclusions

HBV: vaccination of staff, regular 5-yearly anti-HBV antibody checks or every 10 years a booster

All: handling and storage of high-risk samples in separate laboratory and cryo-tanks
Appropiate cleaning procedures

Conclusions

Resource demanding treatment

- different procedures, extra cleaning and staff
- special/extra instruments: laminar flow, incubators, freezer, cryotanks

Risk reducing not risk free treatment

Suggested reading

Y. Englert Medically assisted reproduction in the presence of chronic viral diseases. Human Reproduction Update 2004,10:149-162

J.L. Geberding Management of occupational exposures to blood-borne virusses. New England Journal of Medicine, 1995, 232: 444-451

C. Gilling-Smith et al. Laboratory safety during assisted reproduction in patients
with blood-borne viruses. Human Reproduction, 2005, 20: 1433–1438

R. Levy et al.Transmission risk of hepatitis C virus in assisted reproduction techniques. Human Reproduction 2000, 15: 810-816

S.R. Steyaert et al. Infections in IVF:review and guidelines. Human Reproduction Update, 2000, 6: 432-441

WHO