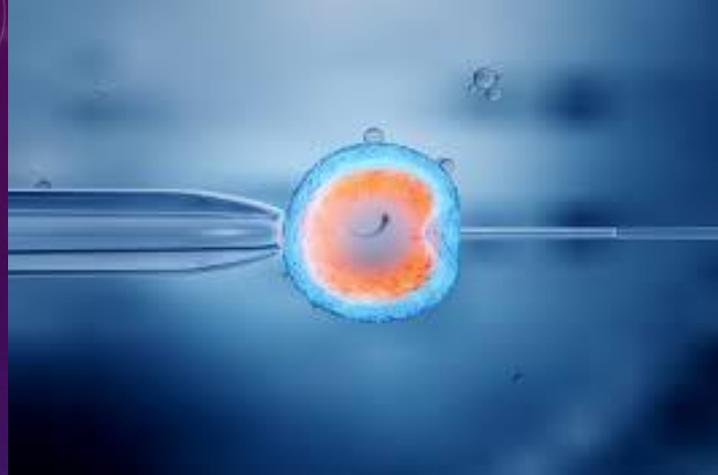
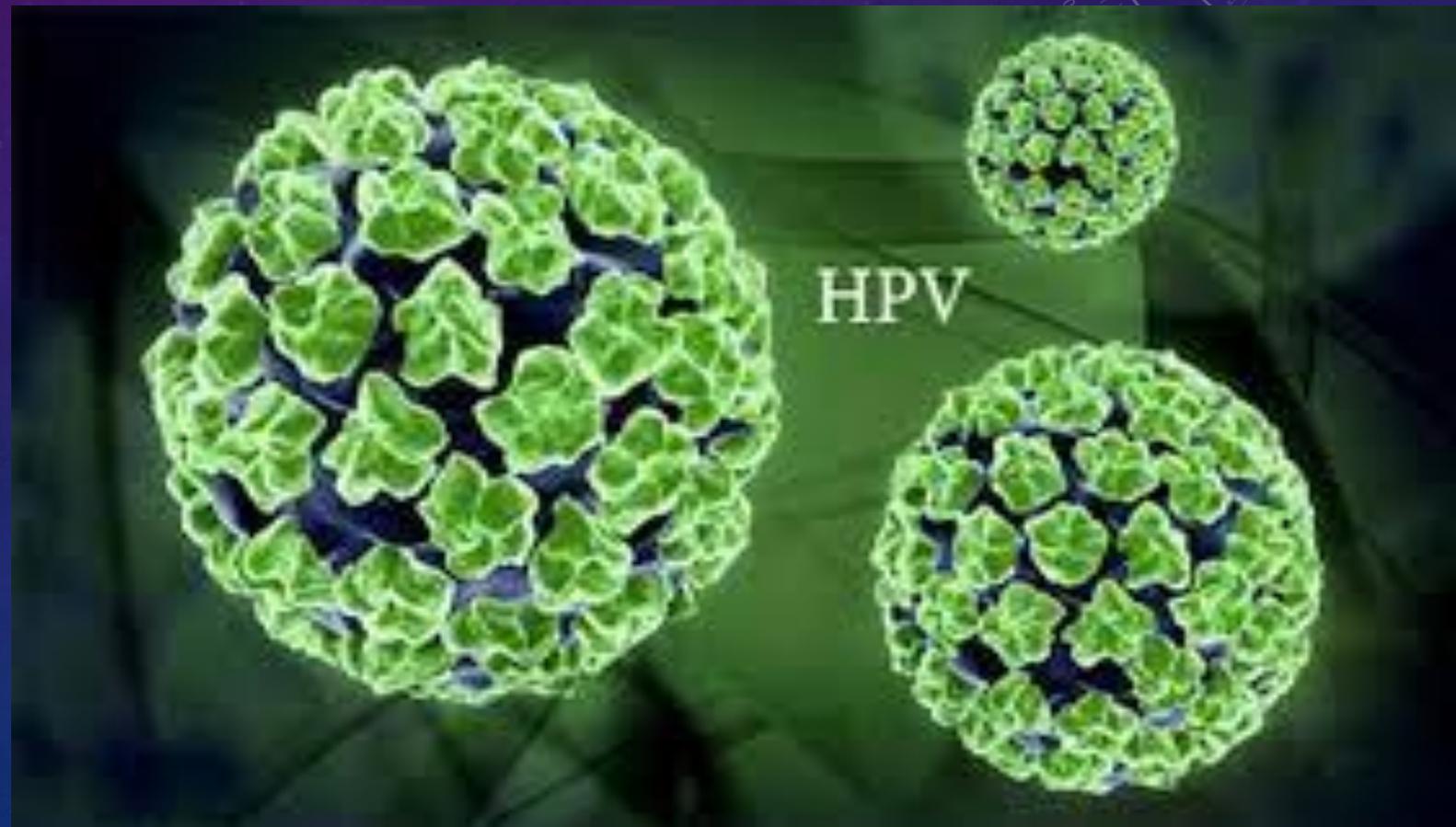


HUMAN PAPILLOMA VIRUS



& ART

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HUMAN PAPILLOMA VIRUS (HPV)

Prevalence

Impact on fertility

Diagnosis before ART

Prevention before and during ART

Impacts on ART outcomes

PREVALENCE

- HPV is the most common sexually transmitted infection .
- HPV infection is generally asymptomatic, with the worldwide rate in women with normal cytology of **11.4%** .
- Globally, prevalence of genital HPV infection in men is higher than in women.
- HPV is more prevalent among young women, with a rate of 24% in younger than 25.
- About 90% of HPV infections cause no symptoms and resolve spontaneously within two year.
- The most common types of HPV worldwide are **HPV16** (3.2%), **HPV18** (1.4%), **HPV52** (0.9%), **HPV31** (0.8%), and **HPV58** (0.7%).
- HPVs can be divided into high-risk and low-risk groups according to their ability to induce cellular transformation and carcinogenesis.
- High-risk HPV infection has been identified be involved in: cervix, oropharynx, penis, vulva, vagina and anus cancers
- High-risk, oncogenic HPV types, such as **HPV16/18 are cumulatively associated with over 99% of all cervical cancer.**

PREVALENCE ...

- High-risk HPVs cause about **5% of all cancers worldwide**, with an estimated **570,000** women and an estimated **60,000** men getting an HPV-related **cancer** each year.
- Over **45,000** HPV-associated **cancers** occur in the **United States each year**: about 25,400 among women, and about 19,900 among men.
- among men, HPV rate is **highest at the penis and lowest at the urethra**; among women, HPV prevalence is **highest at the cervix and vagina and lower at the vulvar epithelium**
- **Cervical cancer is the most common HPV-associated cancer among women, while oropharyngeal cancers are the most common among men.**
- In general, HPV has been related to more **than 90% of anal and cervical cancers, about 70% of vaginal and vulvar cancers, and more than 60% of penile cancers.**

IMPACT ON FERTILITY

- The role of HPV in infertility remains to be elucidated.
- Determining the origin of HPV DNA is the key to predict the impact of the HPV infection.
- When the detected viral HPV DNA originates from a **dividing cell**, the detected HPV DNA is never infectious (dividing cells do not support virion production) and does not affect fertility, but the viral DNA can **transform** the dividing cell which could in time lead to pre-cancer and cancer. High-risk HPV types have oncogenes that produce oncogenic proteins that can transform dividing cells or even leads to blocking of the cell division of the early embryo. It always leads to a self-limiting HPV infection since no progeny virions can be made and the HPV life cycle ends.
- When the detected viral HPV DNA originates from **non-dividing differentiated cells** or from **free virions**, it is infectious and exerts its deleterious effects thru **weakening** or **incapacitating** the cells it resides in.
- For the already dying epithelial cells the new virions are released when the cells desquamate and allow to restart the HPV life cycle.

IMPACT ON FERTILITY...

- In embryos the accumulation of newly formed virions in the syncytiotrophoblasts results in the **weakening of the implantation** bond with the endometrium and diminished energy uptake leads to **miscarriage**.
- For spermatozoa the binding or internalization of viral DNA also leads to a decrease in **functionality of sperm** (lower motility, DNA damage).
- Although in the majority of cases only one of the two HPV induced pathways is present, in almost one fifth of HPV infected women (18%) both the virion producing and HPV transforming pathway occurs at the same time.
- In most cases multiple HPV types are present but it is also possible that only one HPV type is detected .

TESTING ?

- the current standard screening test for cervical cancer and CIN lesions is a cytological staining based technique, known as the **pap-smear** test.
- The actual gold standard for HPV detection is Nucleic Acid Tests (NAT), that allow also for the genotyping of the virus.
- The NAT currently uses PCR techniques, such as **qPCR** and **ddPCR** (droplet digital PCR) as well as blotting tests, such as: (i) **Line blot assay**, (ii) **Linear Array** and (iii) **Dot-blot hybridization**.
- qPCR has several intrinsic limitations, including: (i) low sensitivity in quantifying the amount of viral DNA when present in a low-copy number (ii) lack of precision in estimating small differences in copy number among samples (iii) the need for calibration curves, represented by plasmid vectors carrying viral DNAs, thereby increasing the risk of false-positive results.
- Previous studies also reported on HPV DNA detection/quantification employing ddPCR methods. These studies are addressed to the identification of a single specific HPV type.

TESTING?

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

- HPV testing of couples pursuing MAR treatment is not included in the European Tissue and Cells directory, however, this is a **rapidly developing area** and national guidelines (focussed research is urgently needed) **need to be followed**.
- Testing for HPV is complicated and its importance still needs to be clarified in the field of medically assisted reproduction.
- In couples **attempting IUI**, it may be **beneficial** to test for the presence of HPV.
- The results of the testing could be informative for the cause of **unexplained infertility** since male HPV positivity contributes to the risk of **male infertility**.
- The role of HPV infection **in females** undergoing assisted reproduction is **still controversial**.

PREVENTION

- HPV transmission occurs during vaginal intercourse and the risk of transmission increases with the **number of partners**.
- The use of condoms **does not** completely prevent HPV infection as it can be transmitted via skin-to-skin contact, however, it can lower the risk of HPV transmission

Recommendation

As human papilloma virus (HPV) infection has no known cure to date, prevention through vaccination is recommended for both partners.

GPP

The use of barrier contraception during sexual intercourse is advised to lower the risk of HPV transmission.

GPP

- A small study including 15 pregnant women reported that transmitters had a significant higher human papilloma virus (HPV)-16 viral load compared to non-transmitters .The viral copy number was also significantly higher in transmitters versus non-transmitters
- There is no evidence that there is a specific HPV DNA copy number threshold below which (horizontal or vertical) transmission is unlikely.

WHICH TECHNIQUE (IUI ,IVF.ICSI) IN COUPLES WITH HPV?

- Women infected with HPV should be informed that MAR does not **eliminate** the risk of **vertical transmission**.

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

CAN HPV DNA BE DETECTED IN OOCYTES/ SPERM?

- Specimens of semen were collected from 308 male partners of couples undergoing IVF to detect the presence of HPV DNA. HPV DNA was found in **24/308 semen samples**, and ISH showed a clear localization of HPV at the **equatorial region** of the **sperm head** in infected samples.
- The presence of 35 types of HPV was examined **on DNA** from semen samples of 188 Danish sperm donors using a sensitive HPV array. Characteristic **protrusions** at or near the **equatorial segment** of **the sperm head** were found.
- Similar findings were reported by Foresta et al. (2011). In sperm cells of a HPV-16 positive male, **FISH analysis** showed that HPV localizes at the equatorial region of the sperm head. Furthermore, they showed that sperm exposed to HPV can transfer the virus **into oocytes**.

CAN HPV DNA BE DETECTED IN PLACENTA?

- A prospective cohort study, including **72 pregnant HPV positive** women, reported a vertical transmission rate of **20.8%** (15 neonates). However, all placenta and cord blood samples were negative for HPV by both PCR and IHC.
- A prospective cohort study investigated the vertical transmission of HPV in **329 pregnant** women and their newborns. HPV DNA was detected **in 4.2%** (13/306) **of placental** samples and in **3.5%** (11/311) of **cord blood** samples. Oral HPV carriage in newborns was most significantly associated with the detection of HPV in the placenta .
- Another prospective cohort study investigated the trans placental transmission of HPV and included **49 HPV positive** pregnant women at delivery. Twelve out of 49 **placentas (24.5%)** tested positive for HPV DNA, of which 5 on the fetal side of the placenta, 2 on the maternal side and 5 on both sides of the placenta. **Eleven newborns** tested positive for HPV; in 5 cases (10.2%) there was HPV type-specific agreement between genital/placenta/newborn samples.

DOES HPV IMPACT THE OUTCOME OF MEDICALLY ASSISTED REPRODUCTION?

- Male infected :
- In a prospective multicenter study .the clinical pregnancy rate of **732** couples undergoing 1713 IUI ,was significantly lower in women inseminated with HPV positive semen (**2.9 %** per cycle) versus IUI with HPV negative semen (**11.1 %** per cycle). Above a ratio of 0.66 HPV virions/spermatozoon **no pregnancies** occurred (Depuydt et al., 2019).
- In an observational prospective cohort study of **226** infertile couples ,in the IUI treated group the clinical pregnancy rate was **20 %** (12/60) for HPV negative and **9.5 %** (2/21) for HPV positive men. In the ICSI treated group, the clinical pregnancy rate was 40/98 (**40.8%**) for HPV negative and 6/33 (**18.2%**) for the HPV positive patients. The cumulative pregnancy rate (IUI and ICSI) for HPV positive men was **14.2 %** (5/54) compared to **38.4 %** (66/172) for HPV negative men while the **miscarriage** rate was significantly higher in HPV positive versus HPV negative men (**62.5 % vs 16.7 %**) (Garolla et al., 2016).

DOES HPV IMPACT THE OUTCOME OF MEDICALLY ASSISTED REPRODUCTION

- Female infected:
- A systematic review and meta-analysis including **7 cohort studies** (4 prospective and 3 retrospective) with a total of **1,390** participants, investigated the **impact of HPV infection** on the risk of clinical pregnancy rate of MAR.
- The pooled result **indicated no significant association** between HPV infection and clinical pregnancy rate in MAR, with a pooled RR of 1.04 (95% CI 0.64–1.70).
- Three studies reported infection with a high-risk HPV alone with a pooled RR of 1.66 (95% CI 0.29–9.63).
- The other four studies reported mixed-type infection with high risk/low risk-HPV with a pooled RR of 0.85 (95% CI 0.66–1.09).
- The risk ratio of spontaneous abortion of MAR pregnancy was 1.47 (95% CI 0.86-2.50) (Xiong et al., 2018).
- In a study cohort of **1044** Chinese women undergoing IVF for tubal infertility or male subfertility, **no association** was found between **IVF-ET outcome** and cervical infection, cytopathologic result, HPV detection, or result from the colposcopy or biopsy (Wang et al., 2008).

RECOMMENDATIONS

- There are **over 200 HPV** subtypes **and most people** will test positive for a HPV type at some point during life.
- It currently however not clear which types of HPV are responsible for the observed effects, making it **impossible to recommend routine testing of HPV outside research settings**.
- Furthermore, HPV is a transient infection which most often clears spontaneously. It is, however, not clear at this stage, how fast infectious HPV is cleared in males and females.
- Emerging evidence indicates that HPV infection in males affects sperm parameters and may cause reduced pregnancy and increase miscarriage rates. For this reason and in the case of appropriate research settings, couples may be advised to proceed with **HPV testing prior to IUI**.

Recommendation	
The possibility of HPV testing could be discussed with couples undergoing IUI.	Research only
Couples with a known positive HPV test should be advised that HPV is a transient infection, and postponing MAR treatment is an option depending on the individual circumstances.	GPP

PREVENTION/REDUCTION OF TRANSMISSION DURING ASSISTED REPRODUCTION

VACCINATION:

A retrospective analysis was performed on **151** infertile couples with detection of **HPV in semen**. Patients were counseled to receive adjuvant HPV vaccination. Seventy-nine accepted vaccination (vaccine group) whilst 72 did not (control group). Forty-one pregnancies, 11 in the control group and 30 in the vaccine group, were recorded (respectively 15% and 38,9%,) and resulted into 4 deliveries and 7 miscarriages (control group) and 29 deliveries and one miscarriage (vaccine group)

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

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

PREVENTION/REDUCTION OF TRANSMISSION DURING ASSISTED REPRODUCTION

- SEMEN PROCESSING:
- **Simple semen processing** does not clear HPV in sperm.
- Direct swim-up reduces the number of HPV-infected sperm by **~24%** ($P < 0.01$), while modified swim-up (with added Heparinase-III) is able to remove **completely** HPV DNA both from naturally and artificially infected sperm (Garolla et al., 2012)

Recommendation

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

GPP

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

- **CAESAREAN SECTION :**

- A systematic review and meta-analysis including **9 studies investigated** the risk of transmission between caesarean and vaginal delivery. There was **no statistically significant** difference in risk of vertical transmission between caesarean and vaginal delivery. (Zouridis et al., 2018).
- An older systematic review and meta-analysis including 8 cohort studies, also investigated the prevalence of HPV in the offspring of HPV-infected women in association to their mode of delivery. They concluded that caesarean section is associated **with significantly lower rates** of HPV transmission than vaginal birth (14.9% vs. 28.2%,)The number of caesarean sections needed to prevent one case of perinatal infection would be **7.5 (NNT)** (Chatzistamatiou et al., 2016).
- The CDC guidelines on HPV state that caesarean section is indicated for women with anogenital warts if the pelvic outlet is obstructed or if vaginal delivery would result in **excessive bleeding** (Workowski and Bolan, 2015).

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

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

- BREASTFEEDING:
- Louvante et al. (2017) performed a study including 308 breast feeding mothers. 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. Breast milk HPV persisted among 5.5% (9/164) of the lactating mothers .
- According to their results breast milk is a potential vehicle for HPV transmission to oral mucosa of the spouse but not of the offspring.
- Transmission of HPV to the offspring by breastfeeding is very rare. To date there is no evidence of harm to the new-born by vertical transmission of HPV.

Conclusion

Breastfeeding is not contra-indicated in HPV positive women.

Conditional ⊕○○○

Summary

HPV	Male testing positive 	Female testing positive 	Couple testing positive 
BEFORE MAR	Vaccinate unaffected partner		
	Discuss: <ul style="list-style-type: none"> - Possibility of postponing MAR (transient infection) - Risk of viral horizontal transmission (not eliminated by MAR) 		
DURING MAR	IUI, IVF or ICSI depending on infertility work-up		
	Routine semen processing		
AFTER MAR	Caesarean section not recommended		
	Breastfeeding not contra-indicated		



از توجه شما سپاسگزارم