

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.

For more information visit www.intechopen.com



Human Papillomavirus in Donor Semen in Belgium

K.W.M. D'Hauwers¹, W.A.A. Tjalma², U. Punjabi³ and C.E. Depuydt⁴

¹*Radboud University Nijmegen Medical Center Department of Urology Nijmegen*

²*University Hospital of Antwerp Department of Gynaecological Oncology Antwerp*

³*University Hospital of Antwerp Department of Fertility*

⁴*Laboratory for Molecular Pathology, Labo Lokeren Campus RIATOL, Sonic Healthcare*

Benelux, Antwerp

¹*Netherlands*

^{2,3,4}*Belgium*

1. Introduction

1.1 Epidemiology of HPV

Human papillomavirus (HPV) is the most common sexually transmitted infection (STI) worldwide that affects women as well as men. Around 75% of sexually active people will have an HPV infection at some point of life [1].

HPV's are small, non-enveloped DNA viruses and infect both cutaneous and mucosal squamous epithelia. They have been categorized as either low-risk types (lrHPV) or high-risk types (hrHPV) depending on their oncogenic potential [2]. Following HPV types are considered as high risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82. Types 26, 53, and 66 are considered as probable high risk (phr). The category low-risk HPV types are types 6, 11, 40, and 42. HPV type 67 is of undetermined risk. The most commonly detected types of HPV in cancers are 16 and 18 [1]. The most commonly detected HPV HPV in cancers are 16, 18 and 45.

Most HPV infections are subclinical and transient in nature. A persistent oncogenic HPV infection has been found to be a necessary but insufficient risk factor in almost all cervical cancer cases [3] and an important risk factor in a subset of penile [4], vulvar, vaginal, and anal cancers [5], anogenital warts [6], recurrent respiratory papillomatosis [7, 8], and a fraction of head, and neck tumours [9, 10]. Table 1 [11].

Men are considered to carry the virus on them, function as a reservoir and act as transmitters of the virus. HPV DNA has been detected in samples of internal and external anogenital sites.

Rintala found HPV in the vas deferens (18.5%): five of 27 vas deferens samples (of vasectomized men) contained HPV 6, 11, or 16 [12].

Svec found HPV in the epididymis: lrHPV 6 and hrHPV 16, 33, 35, 55 and 73 were detected in the epididymis of 7/17 patients, treated with epididymectomy because of nontuberculous epididymitis [13].

| Site and histological type | Incidence (per 100,000) | HPV DNA range (%) |
|---------------------------------|--------------------------------|-------------------|
| CERVIX | 8-10 | 76-97 |
| VULVA | 0.0-3.5 | |
| VIN3 | | 72-100 |
| Vulva warty-basaloid | | 75-100 |
| Vulva squamous | | 2-23 |
| VAGINA | 0.0-1.5 | |
| VAIN | | 82-100 |
| Vagina squamous | | 64-91 |
| PENIS | 0.0-3.7 | |
| PIN | | 90 |
| Penis warty-basaloid | | 46-100 |
| Penis squamous verrucous | | 31-35 |
| ANUS | 0.1-2.8 males/0.0-2.2 females | |
| AIN | | NA |
| Anus squamous | | >80 |
| OROPHARYNX & TONSILS | 0.3-21.5 males/0.0-2.8 females | 33-72 |

VIN: vulvar intraepithelial neoplasia

VAIN: vaginal intraepithelial neoplasia

PIN: penile intraepithelial neoplasia

AIN: anal intraepithelial neoplasia

Table 1. Epidemiological traits of HPV and cancers.

Martorell found HPV DNA in 12 testicular biopsies of 185 infertile men (6.5%). The HPV DNA was found in Leydig cells, in Sertoli cells, and probably in germinal cells [14]. Table 2

Also the external anogenital areas have been extensively brushed to examine if these samples can be used to measure the presence of HPV DNA in a general population. Often they were combined with semen and urine samples, which are easy and painless to obtain.

Furthermore, it was assumed that HPV DNA positivity in urine of semen reflected the presence of an "internal" HPV reservoir.

HPV DNA is found on the foreskin, glans/coronal sulcus, penile shaft, scrotum, perianal region, urine, and urethra. Table 2

Weaver found most HPV on the foreskin [15]. Giovannelli used penile brushes (PB), urethral brushes (UB), and semen (SE). The HPV DNA detection rate in PB, UB, SE, PB and UB, and PB and SE were 88.9%, 50.0%, 33.3%, 100% and 97.2%, respectively. He concluded that the use of PB and UB appeared to be the most accurate method to screen; as an alternative to UB (which is a rather painful swab), the use of SE with PB could be used to improve the detection rate [16]. Giuliano [17] examined penile shaft, glans penis/coronal sulcus, scrotum, urethra and semen and concluded that urethral swabs and seminal samples were adequate swabs, which contained sufficient human DNA and beta-Globin, but that HPV DNA presence was very low. She concluded that these two samples did not contribute to optimal sampling: exclusion of urethra, semen, scrotum and perianal region resulted in a < 5% reduction in prevalence. Nielson [18] did extensive sampling of the anogenital region in healthy, heterosexual men and concluded that the more complete the sampling was, the more HPV DNA was found.

Secondly, she stated that anogenital HPV prevalence in asymptomatic men is higher than expected (previously). The penile shaft was most likely to be positive for HPV.

| Author | Site | HPV DNA (%) | Types | |
|-------------------|--------------------|-------------|-----------------------|--------------------------------------|
| Rintala, 2002 | Vas deferens | 18.5 | 6, 11, 16 | Post-vasectomy samples |
| Svec, 2003 | Epididymis | 41.2 | 6, 16, 33, 35, 55, 73 | Nontuberculous epididymitis |
| Martorell, 2005 | Testis | 6.5 | | Testicular biopsies of infertile men |
| Weaver, 2004 | Penile shaft | 24 | | Men attending STD clinic |
| | Glans | 16 | | |
| | Foreskin | 28 | | |
| | Scrotum | 17 | | |
| | Urine | 6 | | |
| Giovannelli, 2007 | Penile brushing | 88.9 | | Partners of HPV positive women |
| | Urethral brushing | 50.0 | | |
| | Semen | 33.3 | | |
| Giuliano, 2007 | Penile shaft | 49.9 | | Heterosexual men |
| | Glans penis/sulcus | 35.8 | | |
| | Scrotum | 34.2 | | |
| | Urethra | 10.1 | | |
| | Semen | 5.3 | | |

Table 2. Anogenital sites and prevalence of HPV DNA

2. HPV, semen and transport

2.1 Interaction of semen and HPV

The idea that sperm and seminal fluid could act as a vector for HPV transportation is not a new one. In 1979, epidemiological evidence had already suggested a correlation between some male penile cancers and female partner cervical carcinomas [19]. A few years later there was proof that male sexual partners of women with various benign or premalignant cervical lesions were at high risk for having penile lesions [20]. The incidence of HPV DNA in men is lower than in women, so questions rose about the possible modes of sexual transmission of HPV. Possibilities are that HPV DNA is present as free virus particles (virions) or is integrated in shed cells. Corollary questions are: 1) if the virus, once integrated in the cell's DNA, can also express certain genes: 2) if the infected sperm cell can transfer HPV DNA into an embryo or to a sexual partner.

To explore these questions Ostrow et al. (1986) examined the semen of patients with epidermodysplasia verruciformis and chronic lymphatic leukemia and found HPV 2 and HPV 5 [21]. About 95% of HPV DNA was found associated with extracts of the washes and not with the sperm pellet. They concluded that HPV DNA is not associated with the sperm itself, but is present as free HPV DNA or free virus particles. Green et al. (1989, 1991) demonstrated the presence of HPV DNA in the semen of patients with intrameatal and penile warts. They concluded that HPV DNA transmission occurred from warts from which surface epithelial cells are shed during ejaculation [22, 23]. They found HPV DNA in the

pellet fraction and suggested that HPV DNA is associated with cellular material since virions are supposed to be found in the supernatant fraction of the semen. More recent studies confirmed convincingly the presence of HPV DNA in semen [16, 17, 18, 24].

Question 1 was approached by Lai et al. (1996): they wanted to examine the presence and expression of HPV in human plasma and sperm cells. They examined semen of 24 randomly selected patients who attended the Fertility Clinic. Type 16 E6-E7 DNA and RNA were found in 8.3 and zero % of seminal plasma specimens, respectively, and in 25 and 8.3% of sperm cells specimens, respectively. DNA and RNA sequences of HPV type 18 were found in 33.3 and 8.3% of seminal plasma specimens and in 45.8 and 20.8% of sperm cells specimens, respectively. They suggested that HPVs not only infect human sperm cells, but also succeed in expressing certain genes in the infected sperm cells [25].

The second question was addressed by Chan et al. [26]. They developed an in vitro model that allowed sperm cells, carrying DNA fragments from HPV 16, 18, 31, and 33, from one end of an artificial reproductive tube and to come in contact with hatching mouse blastocysts at the other end of the tube. After washing the blastocysts were analyzed for the presence of foreign DNA fragments. Especially transference of DNA HPV type 18 to the blastocyst was shown. Not all DNA fragments were transferred equally. These results seemed to suggest that sperm can serve as a non-invasive gene delivery system to transport gene fragments into pre-implantation embryos. The fact that some parts of DNA were more easy to deliver, supports the assumption that a variety of factors and mechanisms are involved in transporting HPV DNA.

Pao et al. (1996) examined the take up or retaining of different regions of HPV 18 by sperm cells [27]. They collected sperm samples of 23 subfertile men and found that the oncogenic regions of the viral genome were preferentially retained: 30% (E6) and 83% (E7), vs. 17% (upstream regulatory region), 22% (E1), and 4% (L1).

In 2009, Perez-Andino et al. [28] compared the adsorption of HPV 16 to live human sperm cells in freshly ejaculated, undiluted human semen and in conditions that resemble the female genital tract. Fluorescent HPV 16 capsids were added to semen (concentration 80 microg/l) and the mixture was incubated at 37°C. Even after several hours of incubation, no HPV 16 capsids were detected on the surface of sperm. When the vaginal environment (with a more acidic pH) was mimicked, viral binding was observed on 52% (pH 8.6) and on 72% (pH 7.4) of live sperm. Their conclusion was that association of HPV16 with sperm will probably not occur in neat semen, but may happen in the female genital tract, at low pH, following the dilution of the sperm. Furthermore they found that the HPV 16 capsids bind to two specific sites at the equatorial region of the sperm head surface. They suggested that by means of competitive binding on the virus, attachment to the sperm head may be inhibited. If applicable, this would be a way of protection of sperm cells, and consequently of blastocysts, of embryos, and of sexual partners.

In 2011, Foresta et al. confirmed the binding of the virus at the equatorial region of the sperm head and demonstrated that this happened through interaction between the HPV capsid protein L1 and the receptor syndecan-1. Furthermore, they showed (using hamster egg-human sperm penetration test) that sperm transfected with HPV E6/E7 genes and sperm exposed to HPV L1 capsid protein are capable to penetrate the oocyte and transfer the virus into oocytes. Inside the oocytes, viral genes are activated and transcribed [29].

2.2 Effect of sperm washing

Fertility clinics use washing methods to clean the sperm cells from unwanted viruses. However, HPV seems resistant and sperm washing does not eliminate HPV DNA.

Olatunbosun [24] performed routine “swim up” washing of 27 semen samples that contained HPV DNA: 21 samples were from men with genital lesions, six from sperm donors without prior or current HPV infection. Dodson[30] on the other hand, separated sperm cells by percoll gradient centrifugation in a 1ml aliquot and washed four times with sterile phosphate-buffered saline. All samples, coming from men with genital lesions, still contained HPV DNA after the washing procedure (100%). In six men, without genital lesions, the procedure reduced HPV DNA below detectable levels in only two (33.3%).

Czegly [31] washed sperm cells in Percoll [Pharmacia] or in Sperm Rinse™ [Vitrolife] and found no change in HPV status.

In 2011, Foresta and al. [32] performed semen analysis and in situ hybridization for HPV detection before and after sperm washing, discontinuous gradients, and swim-up protocols. Sperm washing centrifugation did not change the presence of HPV DNA; Ficoll density gradients and swim-ups brought about only a slight reduction.

3. HPV, semen and fertility

3.1 Effect on the sperm parameters

The actual significance of HPV infection in sperm might be poorly understood and reports are conflicting, but it is a concern for those working in the field of reproductive medicine. Recent articles mention a negative influence on the quality of the sperm and on the pregnancy outcome.

Connelly et al. [33] examined sperm samples of six subfertile patients. He found that specific sperm DNA fragmentation only occurred after exposure of the sperm to DNA of HPV types 16 and 31. These viruses caused breakage characteristics of apoptotic but not necrotic sperm. His data suggest that these viruses do adversely affect subsequent embryonic development after fertilization. HPV DNA of types 6, 11, 18, and 33 did not compromise sperm DNA integrity: apparently sperm DNA is able to resist these types, or repairing mechanisms occur.

In 1997 Lai [34] examined 24 samples of subfertile men for the presence of HPV DNA and RNA. HPV 16 DNA and RNA were found in 25% and 2% of sperm samples, respectively. HPV 18 DNA and RNA in 46% and 21%, respectively. The incidence of asthenozoospermia among patients infected with either HPV was significantly higher than in those without HPV in their sperm cells (75% vs. 8%). Performance of curvilinear velocity, straight-line velocity, and mean amplitude of lateral head displacement was significantly lower in HPV-infected specimens; the differences of linearity, beat cross frequency, and straightness were not statistically significant. Lai concluded: 1) that certain HPV-specific genes are actively transcribed; 2) that the presence of HPV in sperm cells may affect sperm motility parameters; 3) and that asthenozoospermia may be associated with sperm HPV infection.

Recent studies (2009, 2010) by Foresta and al. confirmed these findings [35, 36].

In the first study he examined 200 samples from healthy, young volunteers. Ten persons had HPV DNA positive samples, which was associated with reduced sperm motility [35].

In the second study he collected 290 sperm samples from varying populations: patients with genital warts (n=26), HPV positive partners (n=66), infertile patients (n=108), and fertile controls (n=90). HPV semen infection in these groups was as follows: 53.8%, 40.9%, 10.2%, and 2.2%. The infertile patients had a higher prevalence of HPV DNA in their sperm samples than the other groups. Comparison of sperm parameters showed a more frequent reduction of sperm motility in infected samples, especially when the infection was present in the sperm itself [36].

Rintala [37] examined sperm samples of 65 fathers-to-be; no assisted reproductive techniques (ART) were involved in the pregnancies. Ten men (15.4%) had seminal hrHPV DNA, without any affect on semen parameters such as semen volume, sperm concentration, motility and vitality of spermatozoa. No oligo- or asthenozoospermia was associated with seminal HPV DNA. However, there was a lowering of sperm pH in HPV DNA positive samples, with borderline statistical significance (7.4 vs. 7.5). Ejaculate acts as a potential alkaline buffer (pH 7.2-7.8) neutralizing vaginal acidity (pH 4.0-4.5) within seconds after ejaculation, keeping the vagina neutralized (pH 6.0-7.0) and semen motile. At pH 4.0, sperm cells are immobilized within one minute and are irreversibly immobilized and lose their vitality within 10 minutes [37].

3.2 Effect on pregnancy

Nothing is known about the exact interference of an HPV positive sperm cell, injected in the oocyte cytoplasm (like during the ICSI procedure), with embryo development. Some studies mention an influence on the pregnancy outcome, while others do not find any different rates of spontaneous abortions and major birth defects in an HPV-exposed vs. an HPV-unexposed population [38].

Perino assessed the relation ship between HPV infection in 199 infertile couples and the outcome of ART. He found a significant correlation between pregnancy loss rate and positive HPV DNA testing in the male partner of infertile couples, compared with HPV negative male partners [39].

3.3 Effect on offspring

Czegledy used six HPV positive sperm samples for in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Three washed samples carried hrHPV 16. She tested the native sperm, the washed sperm cells, and the connected blastocysts for HPV sequences. All expectant mothers were HPV negative (negative PAP, negative colposcopy, negative HPV test). She found that (1) the washing procedures (Percoll [Pharmacatia] or Sperm Rinse™ [Vitrolife]) did not change the HPV status of the sperm cells; (2) that HPV-positive spermatozoa kept their procreative capacity (gestation); (3) that HPV positive sperm cells create healthy offspring (follow-up 5 years) [31].

3.4 (Cryo)banking

In an Italian cryobank, in 6/98 thawed samples of oncologic patients and in 2/60 samples of controls, HPV DNA was found. Seven samples carried hrHPV's 16, 18, 53 and 61. Only the

sperm head was infected by the virus [40]. Besides the possible effect of the presence of the virus on the sperm parameters, or on the offspring, or on the partners, another question was if HPV-infected sperm is able to cross-contaminate cryovials and impair also the outcome of ART of other couples or infect other partners.

4. HPV, semen and cervical cancer

Cervical cancer is the second most common malignancy among sexually active women worldwide. The pivotal, though insufficient, role of persistent oncogenic HPV infection in almost all cancer cases (99.7%) is well established. Factors in the acquisition of HPV are related to sexual behavior: young age at sexual debut and multiple sexual relations. Still, it usually takes several decades between the initial HPV infection and the onset of cervical cancer.

Wang (2010) postulated that human semen may play a role in genital transmission of HPV and in cervical carcinogenesis itself, based on the following arguments [41].

- Intact genital epithelium is resistant to HPV infection. Tiny tears are necessary to make passage of HPV particles to the basal epithelial cell surface possible, and to initiate infection. Besides spermatozoa semen also contains various chemical components, which may be able to disrupt the normal architecture of cervical epithelium [42].
- Secondly, the immunosuppression of human semen allows interaction with the female and the zygotes. The cell-mediated immunity is considered as the major protection against HPV infection [43]. So, it is possible that semen-mediated immunosuppression may facilitate HPV transmission and/or may reactivate latent HPV infection.
- Chronic inflammation is generally considered a major risk factor for most cancers [44]. Prostaglandins are present in semen at 10,000-fold higher concentrations than those detected at the site of inflammation with PGE2 being the predominant type [45]. Up-regulated PGE2-synthesis is regarded as a possible promoter of cervical carcinogenesis [46]. Consequently, in sexual active women, repeated exposure of HPV infected cervical epithelial cells to high concentrations of PGE2, may be seen as a paracrine modulation of cervical carcinogenesis.
- Degradation of the extracellular matrix by matrix metalloproteinases (MMP's) is essential to tumor invasion and metastasis [47]. Semen has the capacity to stimulate the production of messenger ribonucleic acid (m-rna) for MMP-9 [48], which is correlated with the invasive behavior of cervical cancer [49].
- Furthermore, as mentioned above, HPV capsids could adsorb to spermatozoa, which are highly motile to traverse the thick mucus layer in the female genital tract [28].

However, this hypothesis is in conflict with the first results published on this issue by Nieminen et al. They did not find (dot blot DNA hybridization) any HPV DNA in the sexual partners of 27 women positive for HPV DNA [50]. The method used for the processing of the samples can be an explanation.

5. Study in the Fertility Clinic of the University Hospital of Antwerp

From the above observations, it is clear that sperm cells may be necessary co-promoters of cervical carcinogenesis and that they compromise male fertility.

Furthermore, we wanted to objectify the presence of HPV in sperm samples of subfertile men and of sperm donors.

5.1 Materials and methods

The study protocol was approved by the local Ethics Committee (UA A08 22).

Written informed consent was obtained from all couples/sperm donors.

5.2 Semen samples

Samples were obtained from 41 subfertile men and 21 sperm donors. All samples had been approved for IVF/IUI and ICSI procedures. They were submitted to real time-PCR to determine the presence of 18 HPV types.

5.3 Semen collection and analysis

Samples were collected after 3-6 days of sexual abstinence by masturbation. After liquefaction, a basic semen analysis was performed and scored according to World Health Organisation (WHO 1992) guidelines. These guidelines were adopted after successful training of lab technicians via ESHRE (European Society for Human Reproduction and Embryology) Basic Sample Analysis Courses. Quality assurance was guaranteed by applying standardized methods accompanied with regular internal and external quality controls.

5.4 Semen processing

A part of the semen sample was treated with a two-step discontinuous density gradient [51] using Puresperm® (Nidacn, International AB, Gothenburg, Sweden). Briefly, 40% and 80% Puresperm® density gradient were prepared using 1.0ml of each suspension. Semen was layered on the top of each gradient and centrifuged for 15 mins at 300g. After which the upper layer seminal plasma and the 40 - 80% interface were discarded and the remaining spermatozoa in the 80% pellet was collected from the bottom of the tube and washed once with Earle's Balanced Salt solution (EBSS, Life Technologies, Paisley, Scotland) supplemented with sodium pyruvate (0.011 g/l) and penicillin-streptomycin (50,000 units/l and 50,000 µg/l, Life Technologies, Paisley, Scotland). Samples were kept at -20°C.

5.5 DNA extraction

DNA is bound to the surface of carboxylated magnetic particles under conditions of high polyethylene glycol and salt concentrations (Magnetic Beads extraction Abott M 2000), following the manufacturer's instructions [52]. Briefly, Abbott RealTime HR HPV test procedure consists of sample preparation, reaction assembly, real-time PCR, and result reporting. During sample preparation, 0.4mL of sample is processed using the Abbott mSample Preparation System DNA where sample is lysed with chaotropic reagents, and DNA is captured with magnetic microparticle technology. Unbound sample components and inhibitors are washed away and the bound purified DNA is eluted and is ready for amplification. Abbott RealTime HR HPV is currently validated for use with cervical specimens collected in PreservCyt solution (Hologic Inc., Marlborough, MA, USA).

Specimens can be transported at room temperature or refrigerated, and may be stored up to 4 months at room temperature or up to 6 months refrigerated or at 10°C or colder following collection. At the completion of sample preparation, an amplification master mix is created with AmpliTaq Gold enzyme (Roche Molecular Systems, Inc. Branchburg, NJ, USA), magnesium chloride, and oligonucleotide reagent containing primers, probes and dNTPs. As a preparation method we used the fully automated m2000sp for medium-to-high test volume that processes up to 96 samples in a run..

5.6 Real time quantitative PCR analysis of HPV DNA

Presence of 18 different HPV geno-types was determined using TaqMan-based real-time quantitative PCR's targeting type specific sequences of viral genes: 6 E6, 11 E6, 16 E7, 18 E7, 31 E6, 33 E6, 35 E6, 39 E7, 45 E7, 51 E6, 52 E7, 53 E6, 56 E7, 58 E6, 59 E7, 66 E6, 67 L1 and 68 E7. Analytical sensitivity of the different PCR assays ranged from 1-100 copies and was calculated using standard curves for 16 type-specific PCRs constructed with plasmids containing the entire genome of the different HPV types [53]. Real-time quantitative PCR for β -globin was always performed and was used to verify the quality of DNA in the sample and to measure the amount of input DNA [54]. The amount of β -globin DNA (in nanograms) present in each sample was divided by the weight of 1 genome equivalent (i.e., 6.6 pg/cell) and a factor of 2 (since there are 2 copies of β -globin DNA / cell) to obtain the number of genome equivalents in the sample. Viral loads in each specimen were expressed as the number of HPV copies / cell. The 18 HPV types we tested for were divided according to Munoz [55]

5.7 Questionnaire and screening

Standard questionnaire with questions about:

- Life style: use of tobacco, alcohol, and drugs
- Sexual history: Number of partners during the past 6 months, homosexual partners, anal sexual activities, SOI.
- Motivation to donate sperm.

Routine:

- screening for HIV, Hepatitis B, Hepatitis C, Syphilis, Chlamydia trachomatis, Neisseria gonorrhoea.
- HBA1C, FSH, Testosteron, SHBG, Inhibine B

6. Results

All samples were adequate and contained sufficient beta-Globin. In the native sperm of two patients high risk HPV type 39 was found: one in a sample of a man visiting the fertility clinic, one in a sample of a sperm donor. In the gradient specimens, no HPV DNA was found.

7. Discussion

Investigation of sperm in our fertility clinic resulted in only 2 HPV DNA positive samples, out of 82. Our samples came from subfertile, but healthy men and sperm donors. Foresta [40] found a significant number of HPV DNA in thawed sperm samples from patients with

testicular cancer (6.1%), when compared to the samples of controls (93.3%). The HPV types involved in the patients were 4x hrHPV, 1 medium-risk HPV, and 1 lrHPV.

Questions concerning HPV DNA in a cryobank are whether the virus can cross-contaminate other vials and if contamination has clinical consequences for the outcome of the ART, and for the health of the offspring and the receiving partner. To answer these questions, more research is needed to understand more about the exact interaction of the human papillomavirus with sperm cells and the clinical consequences of (the use of) HPV positive sperm cells.

We feel that, at this moment, routine screening of sperm for HPV, will not contribute to the safety of the procedure.

8. Overall conclusion

Research on the prevalence of HPV DNA in semen and its effect on sperm quality and pregnancy outcome is growing.

It seems that the lower pH in the vaginal environment has a double effect: it increases the capacities of the virus to adsorb to sperm cells. Consequently it affects capacities of the sperm cells in a negative way, especially the motility. Other studies mention consequent problems with embryogenic development and a worse outcome of pregnancies [39]. Therefore, it has been suggested to discourage the use of fresh semen for artificial donor insemination program, until accurate, rapid diagnostic tests are available to exclude the presence of HPV infection, and to use only frozen semen that has been appropriately screened [24].

Some author's hypothesized that the presence of HPV in semen may be a co-risk factor for women to develop, cervical cancer, later on in life. Therefore, they promote the use of condoms to prevent genital transmission of HPV and reduce the incidence of cervical cancer [41]. This advice is difficult to follow for couples with a long-lasting, monogamous sexual relationship.

The conclusions of the different studies are not unanimous and further research has to focus on the mechanisms involved, the clinical consequences, and on the prevalence of these problems.

Furthermore, the number of women vaccinated with one of the prophylactic vaccines is growing. This protection may reduce the possible risks, caused by the presence of HPV DNA in semen. Wise examined the effect of the prophylactic quadrivalent vaccine on reproductive organs and semen. He vaccinated male and female rats. All rats had a specific antibody response to the four types after each injection. There were no effects on the reproductive parameters of cohabited male rats: they had normal sperm count and sperm motility. Also the histomorphology of testis and epididymis were unchanged [56].

The vaccine will probably cause no harm to the fertility of its users, and will in addition, provide protection for the development of cervical lesions [57].

9. References

- [1] Tjalma WAA, on behalf of the HERACLES/SCALEstudy group. Human papillomavirus type distribution in cervical neoplasia: results from two parallel epidemiological

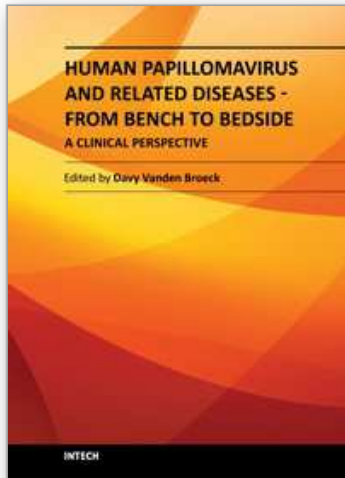
- studies in women with high-grade cervical intraepithelial neoplasia and invasive cervical cancer in Europe. In: IPvC 2010. Montreal, Canada, 8 February 2010. Abstract. (article submitted and expected to be in press)
- [2] Munoz N, Castellsague X, de Gonzalez AB, ... Chapter 1: HPV in the etiology of human cancer. *Vaccine* 2006; 24S3: S1-S10
- [3] Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Munoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol*. 1999; 189(1): 12-9
- [4] Palefsky JM. HPV infection in men. *Dis Markers* 2007; 23: 261-72
- [5] Carter JJ, Madeleine MM, Shera K, Schwartz SM, Cushing-Haugen KL, Wipf GC, Porter P, Daling JR, McDougall JK, Galloway DA. Human papillomavirus 16 and 18 L1 serology compared across anogenital cancer sites. *Cancer Res*. 2001; 61(5): 1934-40
- [6] Brown DR, Schroeder JM, Bryan JT, Stoler MH, Fife KH. Detection of multiple human papillomavirus types in Condylomata acuminata lesions from otherwise healthy and immunosuppressed patients. *J Clin Microbiol*. 1999; 37(10): 3316-22
- [7] Pou AM, Rimell FL, Jordan JA, Shoemaker DL, Johnson JT, Barua P, Post JC, Ehrlich GD. Adult respiratory papillomatosis: human papillomavirus type and viral coinfections as predictors of prognosis. *Ann Otol Rhinol Laryngol*. 1995; 104(10 Pt 1): 758-62
- [8] Rimell FL, Shoemaker DL, Pou AM, Jordan JA, Post JC, Ehrlich GD. Pediatric respiratory papillomatosis: prognostic role of viral typing and cofactors. *Laryngoscope*. 1997; 107(7): 915-8
- [9] Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev*. 2005; 14(2): 467-75
- [10] Shah KV, Westra WH. Genital HPVs in the aerodigestive tract: etiologic association with a subset of oropharyngeal/tonsillar cancers and with recurrent respiratory papillomatosis. *Dis Markers* 2007; 23: 235-45
- [11] Giuliano AR, Tortolero-Luna G, Ferrer E, Burchell AN, de Sanjose S, Kjaer SK, Munoz N, Schiffman M, Bosch FX. Epidemiology of human papillomavirus infection in men, cancers other than cervical and benign conditions. *Vaccine*. 2008; 26 Suppl 10: K17-28
- [12] Rintala MA, Pöllänen PP, Nikkanen VP, Grénman SE, Syrjänen SM. Human papillomavirus DNA is found in the vas deferens. *J Infect Dis*. 2002; 185(11): 1664-7
- [13] Svec A, Mikyskova I, Hes O, Tachezy R. Human papillomavirus infection of the epididymis and ductus deferens: an evaluation by nested polymerase chain reaction. *Arch Pathol Lab Med* 2003; 127(11): 1471-4
- [14] Martorell M, Gil-Salom M, Pérez-Vallés A, Garcia JA, Rausell N, Senpere A. Presence of human papillomavirus DNA in testicular biopsies from nonobstructive azoospermic men. *Arch Pathol Lab Med*. 2005; 129(9): 1132-6
- [15] Weaver BA, Feng Q, Holmes KK, Kiviat N, Lee SK, Meyer C, Stern M, Koutsky LA. Evaluation of genital sites and sampling techniques for detection of human papillomavirus DNA in men. *J Infect Dis*. 2004; 189(4): 677-85
- [16] Giovannelli L, Migliore MC, Capra G, Caleca MP, Bellavia C, Perino A, Viviano E, Matranga D, Ammatuna P. Penile, urethral, and seminal sampling for diagnosis of human papillomavirus infection in men. *J Clin Microbiol*. 2007; 45(1): 248-51

- [17] Giuliano AR, Nielson CM, Flores R, Dunne EF, Abrahamsen M, Papenfuss MR, Markowitz LE, Smith D, Harris RB. The optimal anatomic sites for sampling heterosexual men for human papillomavirus (HPV) detection: the HPV detection in men study. *J Infect Dis.* 2007; 196(8): 1146-52
- [18] Nielson CM, Flores R, Harris RB, Abrahamsen M, Papenfuss MR, Dunne EF, Markowitz LE, Giuliano AR. Human papillomavirus prevalence and type distribution in male anogenital sites and semen. *Cancer Epidemiol Biomarkers Prev.* 2007; 16(6) : 1107-14
- [19] Graham S, Priore R, Graham M, Browne R, Burnett W, West D. Genital cancer in wives of penile cancer patients. *Cancer.* 1979; 44(5): 1870-4
- [20] Levine RU, Crum CP, Herman E, Silvers D, Ferenczy A, Richart RM. Cervical papillomavirus infection and intraepithelial neoplasia: a study of male sexual partners. *Obstet Gynecol.* 1984; 64(1): 16-20
- [21] Ostrow RS, Zachow KR, Niimura M, Okagaki T, Muller S, Bender M, Faras AJ. Detection of papillomavirus DNA in human semen. *Science.* 1986; 231(4739): 731-3
- [22] Green J, Monteiro E, Gibson P. Detection of human papillomavirus DNA in semen from patients with intrameatal penile warts. *Genitourin Med.* 1989; 65(6): 357-60
- [23] Green J, Monteiro E, Bolton VN, Sanders P, Gibson PE. Detection of human papillomavirus DNA by PCR in semen from patients with and without penile warts. *Genitourin Med.* 1991; 67(3): 207-10
- [24] Olatunbosun O, Deneer H, Pierson R. Human papillomavirus DNA detection in sperm using polymerase chain reaction. *Obstet Gynecol.* 2001; 97(3): 357-60
- [25] Lai YM, Yand FP, Pao cc. Human papillomavirus deoxyribonucleid acid and ribonucleic acid in seminal plasma and sperm cells. *Fertil Steril* 1996; 65 (5): 1026 – 30
- [26] Chan PJ, Kalugdan T, Su BC, Whitney EA, Perrott W, Tredway DR, King A. Sperm as a noninvasive gene delivery system for preimplantation embryos. *Fertil Steril.* 1995; 63(5): 1121-4
- [27] Pao CC, Yang FP, Lai YM. Preferential retention of the E6 and E7 regions of the human papilloma virus type genome by human sperm cells. *Fertil Steril* 1996; 66 (4): 630-3
- [28] Perez-Andino J, Buck CB, Ribbbeck K. Adsorption of human papillomavirus 16 to live human sperm. *PLoS* 2009; 4 (6): e5847
- [29] Foresta C, Patassini C, Bertoldo A, Menegazzo M, Francavilla F, Barzon L, Ferlin A. Mechanism of human papillomavirus binding to human spermatozoa and fertilizing ability of infected spermatozoa. *PLoS One.* 2011; 6(3): e15036
- [30] Dodson WC, Moessner J, Miller J, Legro RS, Gnatuk CL. A randomized comparison of the methods of sperm preparation for intrauterine insemination. *Fertil Steril.* 1998; 70(3): 574-5
- [31] Czeglédy J, Szarka K. Detection of high-risk HPV DNA in semen and its association with the quality of semen. *Comment on Int J STD AIDS.* 2004; 15(11): 740-3.
- [32] Foresta C, Pizzol D, Bertoldo A, Menegazzo M, Barzon L, Garolla A. Semen washing procedures do not eliminate human papilloma virus sperm infection in infertile patients. *Fertil Steril.* 2011 Apr 30.
- [33] Connelly DA, Chan PJ, Patton WC, King A. Human sperm deoxyribonucleic acid fragmentation by specific types of papillomavirus. *Am J Obstet Gynecol.* 2001; 184(6): 1068-70

- [34] Lai YM, Lee JF, Huang HY, Soong YK, Yang FP, Pao CC. The effect of human papillomavirus infection on sperm cell motility. *Fertil Steril*. 1997; 67(6): 1152-5
- [35] Foresta C, Garolla A, Zuccarello D, Pizzol D, Moretti A, Barzon L, Palù G. Human papillomavirus found in sperm head of young adult males affects the progressive motility. *Fertil Steril*. 2009; 93(3): 802-6
- [36] Foresta C, Pizzol D, Moretti A, Barzon L, Palù G, Garolla A. Clinical and prognostic significance of human papillomavirus DNA in the sperm or exfoliated cells of infertile patients and subjects with risk factors. *Fertil Steril*. 2010; 94(5): 1723-7
- [37] Rintala MA, Génman SE, Pøllänen PP, Suominen JJ, Syrjänen SM. Detection of high-risk HPV DNA in semen and its association with the quality of semen. *Int J STD AIDS*. 2004; 15(11): 740-3
- [38] Dana A, Buchanan KM, Goss MA, Seminack MM, Shields KE, Korn S, Cunningham ML, Haupt RM. Pregnancy outcomes from the pregnancy registry of a human papillomavirus type 6/11/16/18 vaccine. *Obstet Gynecol*. 2009; 114(6): 1170-8
- [39] Perino A, Giovannelli L, Schillaci R, Ruvolo G, Fiorentino FP, Alimondi P, Cefalù E, Ammatuna P. Human papillomavirus infection in couples undergoing in vitro fertilization procedures: impact on reproductive outcomes. *Fertil Steril*. 2011; 95(5): 1845-8
- [40] Foresta C, Ferlin A, Bertoldo A, Patassini C, Zuccarello D, Garolla A. Human papilloma virus in the sperm cryobank: an emerging problem? *Int J Androl*. 2011; 34(3): 242-6
- [41] Wang X, Zhuang J, Wu K, Xu R, Li M, Lu Y. Human semen: The biological basis of sexual behavior to promote human papillomavirus infection and cervical cancer. *Med Hypotheses* 2010; 74(6): 1015-6
- [42] Roberts JN, Buck CB, Thompsom CD, Kines R, Bernardo M, Choyke PL, et al. Genital transmission of HPV in a mouse model is potentiated by nonoxynol-9 and inhibited by carrageenan. *Nat. Med* 2007; 13(7): 857-61
- [43] Stanley M. Immunobiology of HPV and HPV vaccines. *Gynecol Oncol* 2008; 109(Suppl.2): S15-21
- [44] Baniyash M. Chronic inflammation, immunosuppression and cancer: new insights and outlooks. *Semin Cancer Biol* 2006; 16(1): 80-8
- [45] Sales KJ, Katz AA, Millar RP, Jabbour HN. Seminal plasma activates cyclooxygenase-2 and prostaglandin E2 receptor expression and signalling in cervical adenocarcinoma cells. *Mol Hum Reprod*. 2002; 8(12): 1065-70
- [46] Herfs M, Herman L, Hubert P, Minner F, Arafa M, Roncarati P, Henrotin Y, Boniver J, Delvenne P. High expression of PGE2 enzymatic pathways in cervical (pre)neoplastic lesions and functional consequences for antigen-presenting cells. *Cancer Immunol Immunother*. 2009; 58(4): 603-14
- [47] Coussens LM, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science*. 2002; 295(5564): 2387-92
- [48] Jeremias J, Witkin SS. Effect of human seminal fluid on production of messenger ribonucleic acid for metalloproteinase 2 and metalloproteinase 9 in cervical epithelial carcinoma cells. *Am J Obstet Gynecol*. 1999; 181(3): 591-5
- [49] Radilova H, Libra A, Holasova S, Safarova M, Viskova A, Kunc F, Buncek M. COX-1 is coupled with mPGES-1 and ABCC4 in human cervix cancer cells. *Mol Cell Biochem*. 2009; 330(1-2): 131-40

- [50] Nieminen P, Koskimies AI, Paavonen J. Human papillomavirus DNA is not transmitted by semen. *Int J STD AIDS*. 1991; 2(3): 207-8
- [51] Punjabi U, Gerris J, Van Bijlen J, Delbeke L, Gielis M, Buytaert Ph. Comparison between different pre-treatment techniques for sperm recovery prior to intrauterine insemination, GIFT or IVF. *Hum Reprod* 1990; 5: 75-83
- [52] Huang S, Tang N, Mak WB, Erickson B, Salituro J, Li Y, Krumpe E, Schneider G, Yu H, Robinson J, Abravaya K. Principles and analytical performance of Abbott RealTime High Risk HPV test. *J Clin Virol*. 2009; 45 Suppl 1: S13-7
- [53] Boulet GA, Horvath CA, Berghmans S, Moeneclaey LM, Duys IS, Arbyn M, et al. Cervical cytology biobanking: quality of DNA from archival cervical Pap-stained smears. *J Clin Pathol* 2008; 61(5): 637-41
- [54] Depuydt CE, Benoy IH, Bailleul EJ, Vandepitte J, Vereecken AJ, Bogers JJ. Improved endocervical sampling and HPV viral load detection by Cervex-Brush Combi. *Cytopathology* 2006; 17(6): 374-81
- [55] Munoz N. [Value of human papilloma virus testing in the diagnosis and screening of cervical neoplasia]. *Med Clin (Barc)* 2003; 121(12): 455-6
- [56] Wise LD, Pauley CJ, Michael B, Wolf JJ. Lack of effects on male fertility from a quadrivalent HPV vaccine in Sprague-Dawley rats. *Birth Defects Res B Dev Reprod Toxicol*. 2010; 89(5): 376-81
- [57] Joura EA, Kjaer SK, Wheeler CM, Sigurdsson K, Iversen OE, Hernandez-Avila M, Perez G, Brown DR, Koutsky LA, Tay EH, García P, Ault KA, Garland SM, Leodolter S, Olsson SE, Tang GW, Ferris DG, Paavonen J, Lehtinen M, Steben M, Bosch X, Dillner J, Kurman RJ, Majewski S, Muñoz N, Myers ER, Villa LL, Taddeo FJ, Roberts C, Tadesse A, Bryan J, Lupinacci LC, Giacoletti KE, Lu S, Vuocolo S, Hesley TM, Haupt RM, Barr E. HPV antibody levels and clinical efficacy following administration of a prophylactic quadrivalent HPV vaccine. *Vaccine*. 2008; 26(52): 6844-51

IntechOpen



Human Papillomavirus and Related Diseases - From Bench to Bedside - A Clinical Perspective

Edited by Dr. Davy Vanden Broeck

ISBN 978-953-307-860-1

Hard cover, 348 pages

Publisher InTech

Published online 20, January, 2012

Published in print edition January, 2012

Cervical cancer is the second most prevalent cancer among women worldwide, and infection with Human Papilloma Virus (HPV) has been identified as the causal agent for this condition. The natural history of cervical cancer is characterized by slow disease progression, rendering the condition, in essence, preventable and even treatable when diagnosed in early stages. Pap smear and the recently introduced prophylactic vaccines are the most prominent prevention options, but despite the availability of these primary and secondary screening tools, the global burden of disease is unfortunately still very high. This book will focus on the clinical aspects of HPV and related disease, highlighting the latest developments in this field.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

K.W.M. D'Hauwers, W.A.A. Tjalma, U. Punjabi and C.E. Depuydt (2012). Human Papillomavirus in Donor Semen in Belgium, Human Papillomavirus and Related Diseases - From Bench to Bedside - A Clinical Perspective, Dr. Davy Vanden Broeck (Ed.), ISBN: 978-953-307-860-1, InTech, Available from: <http://www.intechopen.com/books/human-papillomavirus-and-related-diseases-from-bench-to-bedside-a-clinical-perspective/human-papillomavirus-in-donor-semen-in-belgium>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen