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Adeno-associated virus (AAV) in semen and testis : a role in infertility ? An overview

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ABSTRACT

In the past decade, infection of the female genital tract with the ubiquitous adeno-associated viruses (AAV) has been reported by a number of authors. Though the virus is thought to be non-pathogenic, there were hints on an association of AAV with miscarriage and problems in pregnancy. In more recent years it has been discovered that there is also a rather frequent infection of male genital tissues supporting the hypothesis that this virus may be sexually transmitted and may play a role in infertility.

Key words : *adeno-associated virus, semen, testis, infertility*

AAV is a small, non-enveloped, DNA virus belonging to the family of parvoviruses. In contrast to the so-called autonomous parvoviruses, AAV replicates only in the presence of helper viruses such as adenovirus, herpesviruses, vacciniavirus or papillomavirus or under specific cellular conditions (review in [4, 13]). The genome of AAV is a single-stranded DNA molecule of about 5kb consisting of two genes: The *rep* gene, coding for proteins which control viral replication, structural gene expression and integration of viral DNA in the host cell genome, and the *cap* gene, which codes for structural (capsid) proteins.

At least six sero-types of AAV (AAV type 1 [AAV-1] to AAV-6) have been described. AAV-2, the prototypical strain, as well as AAV-3 and AAV-5 have been isolated directly from human clinical specimens, AAV-5 having been isolated was from a penile flat condylomatous lesion [1] without apparent pathologies associated with infection.

In the absence of a helper virus, AAV DNA integrates into a specific site of the host genome (called *AAVS1* on chromosome 19 [19q 13-qter]) at a high frequency, at least in cell culture [9]. The virus is capable of infecting both dividing and non dividing cells and can induce cell differentiation, and, like other (animal) parvoviruses, it has anti-cancer activity (review in [12]). These properties render AAV a promising vector for gene transduction [6]. Though AAV infection of humans is yet thought to be non-pathogenic, there are hints on an association with early miscarriage and other problems in pregnancy [3, 8, 13, 14].

A frequent presence (50-80%) of AAV (DNA and/or infectious virus) was demonstrated first in samples from the female genital tract (cervix uteri, uterus, early abortion) [7, 14, 16, 17]. In addition, AAV DNA was found in gestational trophoblastic disease (about 40%; hydatiform mole, choriocarcinoma), amnion fluid and trophoblasts (about 40%) [3, 8, 15], demonstrating *in utero* infection with the virus. Moreover, in a mouse model, lethal effects of AAV infection on the embryos has been described [2].

More recently, AAV infection of the male genital tract was described [5, 11].

In analyses of semen, AAV DNA was detected by nested PCR, in about one third of ejaculates from infertile men. In 88% of the cases, AAV DNA was associated with the spermatozoa fraction of semen [11]. In 18% of the AAV DNA-positive semen samples, infectious virions could be isolated as well [Till et al., manuscript in preparation]. Interes-

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tingly, AAV DNA was in 38% of ejaculates from men with abnormal spermiogram and only in 4.6% of semen samples with normal spermiogram (association of AAV infection with pathological spermiogram: p=0.003; with oligoasthenospermia: p = 0.0006), (Figure 1) [5]. In contrast, DNA of helper viruses for AAV was detected less frequently (papillomavirus (HPV), 20%; cytomegalovirus (HCMV), 8%; herpes simplex virus (HSV), 1%) with rates not significantly different for normal and pathological semen samples [11]. However, in most AAV DNA-positive cases, DNA of helperviruses was not present (HPV DNA, 24%, HCMV, 8%, HSV 1%, in AAV-DNA containing samples). In urethral swabs, AAV was detected infrequently (17%).

These findings demonstrate infection of the male genital tract with AAV at a rather high frequency. The presence of AAV in ejaculates supports the hypothesis of sexual transmission of the virus, and the preferential detection of viral DNA in abnormal semen samples from infertile men hints to a possible role of AAV in male infertility.

Furthermore, AAV DNA was detected in 26% of testis biopsies from infertile (azoospermic) men [5]. Since in ejaculates AAV DNA was associated with the spermatozoa fraction, it is tempting to speculate that AAV might interfere with maturation of sperm cells, possibly leading to infertile spermatozoa.

To address the possible influence of AAV-2 on testis-specific cells, growth and proliferation kinetics of Leydig cells (mouse TM3 line) and Sertoli cells (mouse TM4 line) were analyzed. After infection with AAV-2, the Sertoli cell line showed reduced growth kinetics whereas no significant growth reduction of the Leydig cell line was observed. When tested for proliferative activity (MTT assay), AAV-2 infection was found to inhibit the proliferation rate of both cells lines by about 20% [Till et al., manuscript in preparation].

In cell culture, AAV was demonstrated to readily integrate its DNA into the host cell genome in the absence of a helper virus (see above). Therefore, we analyzed if AAV DNA detected in testis tissue was present in an integrated form. Using both *Walking Primer PCR* and *unidirectional PCR*, fragments indicating integrated AAV DNA could be detected in AAV DNA-positive testis samples [10]. Sequencing of cloned AAV-DNA/cellular-DNA junctions showed (at least two) integrations of AAV DNA in the *AAVS1* region on chromosome 19. Hence, at least in the testis samples analyzed, AAV DNA was found integrated in the specific chromosomal region described hitherto for cell culture. Recent, unpublished studies demonstrated, that the viral DNA seems to comprise the complete AAV genome [Hoecker et al., manuscript in preparation].

The clinical significance and consequences of this integration of viral DNA still remains to be elucidated (Table 1).

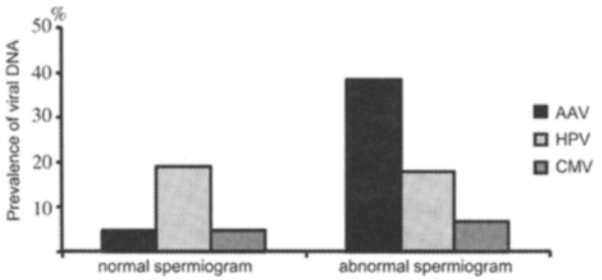

Figure 1 : Presence of viral DNA in semen : normal versus abnormal sperm analysis. *Association of AAV : - with abnormal sperm analysis : p = 0.003; with oligoasthenospermia : p = 0.0006; (p values are calculated as comparison with normal sperm analysis). AAV = Adeno-associated virus; HPV = papillomavirus; CMV = cytomegalovirus.*

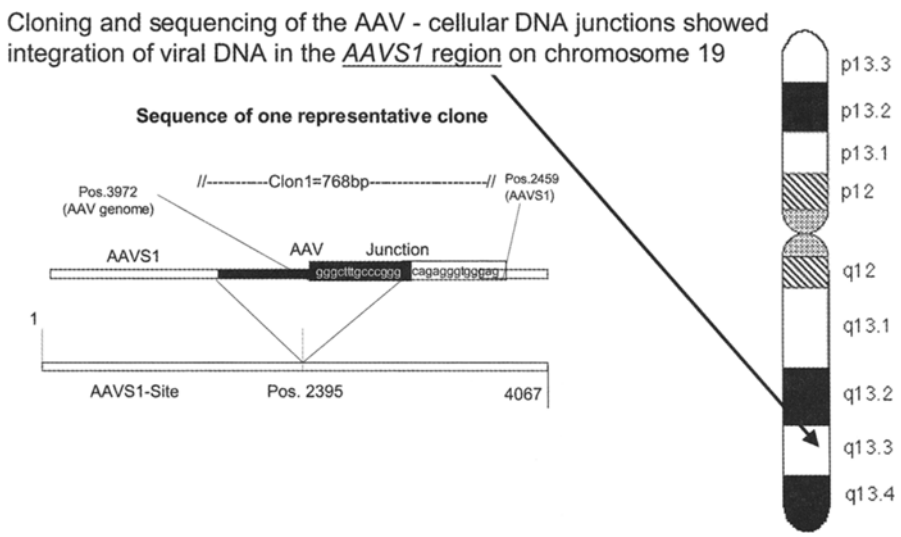

Figure 2 : Integration of AAV DNA in testis cells.

Table 1 : Possible significance of AAV infection of the male reproductive tract or semen.

- Sexual transmission of diseases / Spreading of virus.
- Infertility/sterility from alteration of testis cells.
- Incorporation of the viral genome into germ cell genome:
  - alteration of host cell genome;
  - risk of transmission to subsequent generations.
- AAV infection of ova and embryo:
  - miscarriage,
  - embryonic / fetal abnormalities.
- Immune response in the female genital tract.
- Problems with assisted reproduction (risk of transmission of infected sperm cells).

Further virological and molecular studies are required to assess whether AAV (and other viruses) may be a cause of infertility, whether it is possibly transmitted to the ovum, and whether infected sperm cells could influence the outcome of natural fecundation, or if transmission of virus may interfere with the success of assisted reproductive techniques.

Research is needed to identify the routes of entry of viruses in the male genital tract, the most susceptible target cells, and the consequences of virus infections on endocrine functions. The mode of virus replication and or persistence (latency) in the respective cells/tissues has to be characterized, the possible virus reservoirs (and latency) to be determined, and antiviral defense mechanisms of the reproductive tract to be elucidated.

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