Intracellular Viruses Identification in Sperm Assay of Patients with Fertility Problems

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Background: There are quite a number of viruses known that can infect the semen: human immunodeficiency virus, human papillomavirus, hepatitis B and hepatitis C viruses, herpes simplex virus (HSV), Epstein–Barr virus (EBV), and cytomegalovirus (CMV). For some of these viruses, intracellular localization in spermatozoa has been proven or discussed earlier. In our previous studies, we have detected intracellularly localized capsids of the Herpesviridae family viruses. But the role of vertical virus transmission for vertical dissemination of infection as well as for further abnormalities of embryo development is not clear up to now.

Main Questions: The main objectives of this study were to identify the viruses and to study the scale of viral infection in sperms from fertile patients and those from patients varying in infertility history.

Experimental Design: We examined semen of 75 fertile men (group I), 95 patients whose wives had a history of miscarriage (group II), and 67 patients with a complaint of no pregnancy for at least a year (group III). Patients with low spermatozoa quantity (less than ten million sperms/mL) were excluded from this study. We have undertaken transmission electron microscopy (TEM) analysis of spermatozoa with quantitative evaluation of the obtained data. DNA from human sperm samples with and without revealed by TEM viruses was isolated by a Chelex-100 method (Manuja et al. 2010). The viral DNA content was measured by quantitative SYBR Green PCR using three primer pairs specific for HSV1, CMV, or EBV DNAs. The results obtained were normalized by the dosage of a single-copy human gene (NOG)

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in the same sperm DNA samples. Since there is one copy of each single-copy gene per spermatozoid, these normalized values correspond to the average number of viral DNA copies per spermatozoid. Knowing the percentage of the spermatozoa containing viral particles from the TEM results, we could calculate the average viral content per infected spermatozoid.

Results: Sperm infection in group I was significantly lower than that of group II (3.12 ± 2.80 vs. 5.64 ± 3.65%, $p = 0.0004$) and lower than that of group III, but this difference was nonsignificant (4.33 ± 3.28%, $p = 0.05$). Detection rate of virus capsids was 24% (group I), 52% (group II), and 33% (group III). We did not find any difference in motility and sperm morphology between all examined groups. Viral capsids were detected in spermatozoa of normal morphology in each of the analyzed groups.

As concerning the results of viral DNA identification in sperm DNA samples investigated, we have observed the values from several copies to several 100 copies of CMV DNA per sperm, whereas HSV1 and EBV DNAs were never detected.

Conclusions: Quantities and detection rate of CMV revealed inside spermatozoa were higher among males whose wives had a history of miscarriage. The infected spermatozoa may be of normal morphology.

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Reference

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