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LETTER TO THE EDITOR

Semen Analysis

Seminal HPV detection: a pilot study comparing the preservation effectiveness and cost between a methanol-based solution and cryopreservation with liquid nitrogen

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Dear Editor,

Human papillomavirus (HPV) is the most common sexually transmitted virus worldwide, and high-risk HPV infection is the most important risk factor for cervical cancer.[1] However, few conclusive data are available on the prevalence of the male HPV infection and its health consequences. It has been found that the prevalence of seminal HPV infection is higher in men with unexplained infertility, ranging between 10% and 35% compared with the general population (range: 2% to 11%).[2–4] In three recent systematic reviews, it was seen that HPV sperm infection may be associated with impaired sperm function that could lead to male infertility.[2,4,5] HPV sperm infection with high-risk HPV among infertile men was shown to be associated with lower progressive sperm motility and higher DNA fragmentation index.[6] Moreover, a high rate of pregnancy loss has been found among couples with HPV sperm infection after *in vitro* fertilization (IVF).[7] In the majority of the studies performed to assess seminal HPV infection, samples were preserved in liquid nitrogen until analysis with polymerase chain reaction (PCR). Liquid nitrogen is used to store cells at low temperatures; however, its handling and laboratory safety requires special infrastructure. An alternative, not requiring cryopreservation, is the methanol-based ThinPrep PreservCyt Solution (Hologic, Marlborough, MA, USA). It is a low-cost and safe reagent that serves as a collection, transport, and preservative medium widely used in Sweden for cervical HPV detection.

The purpose of this study was to compare the preservation effectiveness and resources needed, between the methanol-based solution and cryopreservation with a cryoprotectant in liquid nitrogen, for the detection of seminal HPV by TaqMan Real-time PCR (Applied Biosystems-7300 Real-time PCR system, Thermo Fisher Scientific Inc., Waltham, MA, USA).

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A total of 18 men undergoing infertility workup between February 2021 and April 2021 at Sahlgrenska University Hospital, Gothenburg, Sweden, were included in the pilot study. No extra samples were collected, but the sample collected for the infertility workup was used for the study. All participants signed an informed consent form before inclusion. The study was approved by the Swedish Ethical Review Authority (Dnr. 2020-06127).

After collection, the semen sample was prepared for analysis by gradient centrifugation (PureSperm, Nidacon International AB, Mölndal, Sweden) at 300*g* for 20 min according to the clinic's routines. The upper layer with seminal plasma was pipetted off and kept. A volume of 0.2 ml of the prepared sample was used for the routine analysis. The remaining part was washed with buffer (PureSperm Wash, Nidacon International AB) and then mixed with the seminal plasma. The reason for this re-mixture was to enable the HPV analysis on a full ejaculate including both sperm cells and seminal plasma, while at the same time, not compromising the routine workup analysis by splitting the sample. Half of the samples ($n = 9$) were then diluted with the methanol-based solution (1:2) and stored at room temperature, and the other half ($n = 9$) was diluted with cryoprotectant (1:2) CryoProTec (Nidacon International AB), directly frozen in liquid nitrogen and stored at −20°C.

In order to assess whether HPV DNA could be detected in the sperm samples by TaqMan Real-time PCR, a plasmid mixture including a total of 1 million copies of HPV 6, HPV 11, HPV 16, and HPV 18 genomes cloned into plasmid vectors was added per positive control sample (**Figure 1**). All samples were then analyzed for HPV 6, HPV 11, HPV 16, and HPV 18. β-globulin was used as a quality indicator of the samples that contained spermatozoa. Every HPV subtype contained in the plasmid mixture that was added to the samples used as positive controls was detected by TaqMan Real-time PCR. All native sperm samples were negative for HPV and positive for β-globulin. Both of the tested sperm sample preservation methods resulted in equivalent results for the detection of seminal HPV presence, validated through the comparison of the PCR cycle threshold (CT) values from all analyses (ThinPrep PreservCyt Solution mean CT value of 26.16 *vs* cryoprotectant and liquid nitrogen mean CT value

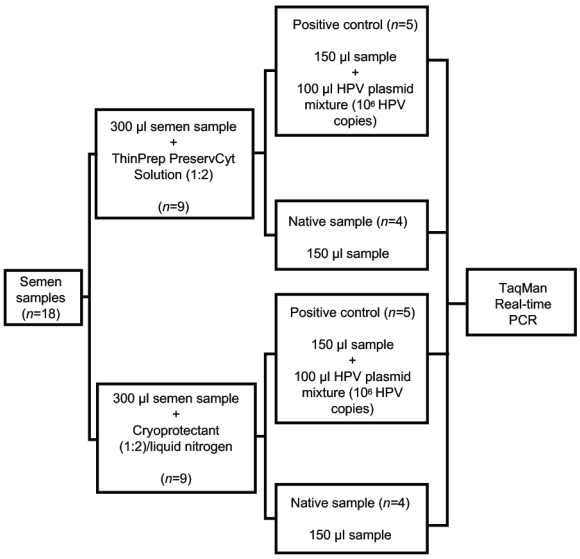

Figure 1: Flowchart of the study. Comparison of sperm sample preservation for the detection of HPV by TaqMan Real-time PCR. Comparison between preservation with a methanol-based solution and with cryoprotectant and liquid nitrogen. HPV: human papillomavirus; PCR: polymerase chain reaction.

of 26). However, the cryopreservation method requires specialized equipment such as liquid nitrogen, cryotubes, and freezing devices, as well as cryo-storage space. Thus, after comparing the necessary time and costs involved to preserve the sperm samples between the two methods, and considering their equal quality and effectiveness of seminal HPV detection, ThinPrep PreservCyt Solution came out as the most optimal alternative.

In conclusion, sperm sample preservation in a methanol-based (ThinPrep PreservCyt) solution and analysis by TaqMan Real-time PCR can be an effective, low-cost, and safe method of seminal HPV detection.

AUTHOR CONTRIBUTIONS

PT conceived the study. PT, JF, CK, JH, ML, SG, ATK, KL, and RA participated in the design of the study. PT, JF, and KL participated in the coordination of the study and helped draft the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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