H7 A New Approach to Collecting, Fixing, and Preparing Samples for Sperm Cells in Cases of Alleged Rape

Helga Haahr-Lillevang, MD, Institute of Forensic Medicine, Brendstrupgårdsvej 100, Aarhus N 8200, DENMARK; Maria Pihlmann, MD, Institute of Forensic Medicine, Brendstrupgaardsvej 100, Aarhus N 8200, DENMARK; Anette M. D. Funder, Institute of Forensic Medicine, Brendstrupgårdsvej 100, Aarhus N 8200, DENMARK; Marianne S. Martiny, MA, Institute of Pathology, Aarhus University Hospital, Aarhus C, DENMARK; Tine H. Meyer, MA, Institute of Pathology, Aarhus University Hospital, Aarhus C, DENMARK; and Iana Lesnikova, MD, PhD, Havkaertoften 14, Tilst 8381, DENMARK*

After attending this presentation, attendees will be introduced to a new approach of collection, Alcohol-Based Fixation (ABF), and preparation of samples suitable for both Hematoxylin-Eosin (HE) and immunohistochemical detection of sperm cells.

This presentation will impact the forensic science community by introducing the ABF technique, allowing a faster and easier visualization of sperm cells in cases of alleged rape.

In cases of alleged rape, the early detection of sperm cells has great importance, since it strongly indicates that a sexual act has taken place. Like most forensic laboratories, the laboratory of the Institute of Forensic Medicine, University of Aarhus, Denmark, in all cases of alleged rape and suspected sexual assault over the years has routinely performed the light microscopy of HE-stained cytological smears. Samples have been collected using dry cotton swabs, spread over glass slides, air dried, and then proceeded to HE staining and light microscopy. This method has clear benefits: it is quick to perform, very inexpensive, and does not need any advanced laboratory facilities. The major limitation is that there is only a single glass slide available and there is no possibility for additional stains. Another known limitation of this method is a relatively large amount of time spent in evaluation of slides due to difficulty in identification of sperm cells covered by layers of overlaying cells, crush artifacts, and difficulty in distinguishing sperm cells from cellular debris, lymphocytes, and clusters of bacteria and fungi.

Test sample series and one control sample were obtained from four anonymous healthy female volunteers of childbearing age. Samples were taken after intercourse and for the following three days. Vaginal fluid samples were obtained by the volunteer using a vat tip swab. Swabs were briefly soaked in a tube containing 1ml of fixing solution and were then disposed. The corresponding conventional, air-dried smear samples were also obtained. Two alcohol-based fixing solutions (58% ethanol solution and commercially available methanol-based buffered CytoLyt® solution) were tested. Sample slides were prepared using the Cytospin technique, which is a well-established cytology method that is specifically designed to concentrate cells such as those that are found in small numbers. HE and Immunohistochemical (IHC) stains using a SPERM HY-LITER™ kit were performed and evaluated at light microscopy.

The sperm cells were better preserved in CytoLyt® fixed samples, but the difference was negligible. In all ABF and conventional smears taken less than 24 hours after intercourse, sperm cells with tails were found. In two ABF samples (taken within 48-72 hours and within 72-96 hours after intercourse, respectively), sperm cells were found only in conventional slides but not in ABF samples. In one sample, taken within 24-48 hours after intercourse, a few sperm cells without tails were in the ABF sample but not in conventional slide. The IHC staining was unproblematic in all ABF samples. The evaluation time was significantly lower in ABF samples.

This result showed the ABF technique is suitable with both HE and IHC staining procedures. The level of detection of sperm cells in ABF-based slides seemed to be similar to conventional smears, but the ABF technique has provided faster, easier visualization of sperm cells.

Forensics, Sperm Cells, Cytology

*Presenting Author