A32 Searching for the Elusive Spermatozoa: Revisiting Seminal Fluid

Kelsey R. Sakaida, Kyle A. Williston, Kimberly A. Strong, and Reena Roy, PhD, Penn State Univ, Forensic Science Program, 107 Whitmore Laboratory, University Park, PA 16802*

After attending this presentation, attendees will understand how to find rare human spermatozoa on a low budget without resorting to expensive equipment and software.

This presentation will impact the forensic science community by teaching the practicing biologist how to detect human spermatozoa when they are extremely rare in a specimen and also mixed with spermatozoa of other animals and other human body fluids.

Forensic crime laboratories analyze evidence from various types of sexual assault cases. The most likely source of male DNA in sexual assault cases comes from semen, which contains male cells commonly known as spermatozoa. Although most of the sexual assault cases involve humans, forensic bestiality cases are also common, and differentiating human male cells from animal spermatozoa becomes a necessity. In such cases, it is important to confirm the presence of human spermatozoa for the investigators and attorneys to bring charges against an assailant.

Examining such evidence to identify spermatozoa requires a great deal of time and effort using stains such as Kernechtrot-Picroindigocarmine (KPIC) staining, commonly used in the crime laboratories for the visualization of sperm. In some instances, because of the rarity of the spermatozoa in the evidence, an analyst may not be able to detect the KPIC-stained heads of the spermatozoa.

The goal of this research was to manually identify human spermatozoa where mixtures of human and animal spermatozoa exist. Some of the simulated evidence samples contained body fluids such as blood and saliva as well as seminal fluids from humans and animals. Another objective was to detect rare human spermatozoa from slides that have been prepared from vaginal swabs collected several days after coitus and where the cells have been dyed with dyes commonly used in the crime laboratories. The project included re-staining these KPIC-stained slides with immunofluorescent dyes to enhance the detection of the male cells.

SPERM HY-LITER™ technology in conjunction with fluorescent microscopy and specific computer software to detect spermatozoa is a novel method in the forensic community. This immunofluorescence staining technique is specific for human sperm cells since it does not stain animal spermatozoa, human epithelial cells, or other types of body cells that may be present in the sample. In some instances, where the victim does not report the crime for several days and spermatozoa may become rare and difficult to detect, this immunofluorescent technology can detect rare spermatozoa among many other non-human spermatozoa and human cells. This technique also allows the detection of rare spermatozoa present as one of the components in a complex mixture of other body fluids. The microcopy and the software to search for the stained spermatozoa are expensive, and budgetary constraints may not allow a laboratory to use such tools.

In this research, SPERM HY-LITER™ was used to stain human and animal spermatozoa. Once stained, these cells were analyzed manually by a fluorescent microscope which did not have the expensive computer software necessary for the automatic detection of stained human spermatozoa. The goal of this research was to identify human spermatozoa where a mixture of human and animal spermatozoa may exist. Another objective was to be able to detect rare spermatozoa even after obtaining vaginal swabs several hours after coitus. In one of the aspects of the study, slides were prepared from vaginal swabs and stained with KPIC. Once the spermatozoa were visualized, the cells were re-stained with fluorescent dye. In all of the instances, human spermatozoa were identified from various samples analyzed by this method.

The results obtained from this research would benefit the other forensic biology laboratories as they can use this technology accurately and efficiently for identification of spermatozoa without straining the budget.

Spermatozoa, Animal Semen, Human Body Fluid

 ** Presenting Author*