Fundamental Issues in Forensic Semen Detection

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ABSTRACT

Sperm detection can be an important factor in confirming sexual assault in cases of rape. A large number of cases received in a forensic laboratory involve sexual offenses, making it necessary to examine exhibits for the presence of seminal stains. The objective of this paper is to provide an overview of the most important methods and tests used in the identification of spermatozoa or constituents of seminal fluid during the investigation of alleged sexual assault cases in forensic medical practice. Furthermore, this paper focusses on the basic knowledge that is necessary to the graduate students who wish to specialize in forensic sciences.

Keywords: Semen detection, seminal stains, sexual assault, rape

Problemas Fundamentales en la Detección Forense de Semen

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RESUMEN

La detección de esperma puede ser un factor importante a la hora de confirmar un ataque sexual en los casos de violación. Un gran número de los casos recibidos en el laboratorio forense tienen relación con ofensas sexuales, lo cual hace necesario examinar muestras de presencia de manchas seminales. El objetivo de este trabajo es proporcionar una apreciación global de los métodos y pruebas más importantes usados en la identificación de espermatozoos o constituyentes del fluido seminal durante la investigación de supuestos casos de ataque sexual, en la práctica médica forense. Además, este trabajo presta atención al conocimiento básico necesario para los estudiantes graduados que desean especializarse en las ciencias forenses.

Palabras claves: Manchas seminales, detección de semen, ataque sexual, violación

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INTRODUCTION

The identification of seminal stains is frequently of great value in medicolegal practice, particularly in cases of alleged rape, sexual assault, sexual homicide or even adultery. One of the primary aims of the forensic laboratory sexual offence investigations is to sample and examine smears or other biological material taken from the assailant or the victim or stains found on cloths or linen or any other evidence concerning the assault for the presence of semen, with the potential to link them (1, 2).

In cases of sexual assault, the forensic examination of evidence for seminal stains can actually be considered a two-step process. Firstly, the stain must be located. Secondly, the stain will be examined to prove its identity; possibly it may even be tested for the blood type of the individual from whom it originated. Furthermore, forensic scientists can successfully link seminal material to an individual suspect by DNA typing (2–4).

In rape cases, the condom plays an important role, since if it is found at the rape scene, it will be useful for the identification of the assailant. Semen of the rapist may be found on one side of the condom while blood found on the other side of the condom usually originate from the victim. Pubic hairs recovered from the condom usually match those of the victim and not those of the suspect. In the laboratory, efforts will be made to link seminal material to donors using DNA typing. The DNA analysis of the condom gives the profile of the victim from the blood and the profile of the suspect from the semen (5, 6).

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SEMEN APPRAISAL

Location of semen with macroscopic examination and fluorescence techniques

The first step in semen investigation focus on the careful examination of clothing and linen or other objects upon which the assault took place for semen analysis. Items suspected of containing seminal stains must be handled carefully and must be submitted for laboratory processing. Common methods that have been employed are visual (examination of items for stains), fluorescence (the use of ultraviolet light to exploit the fluorescent properties of semen) and chemical (detection of acid phosphatase). On fabrics, seminal stains commonly show a slight degree of viscosity and they are readily visible, because they exhibit a stiff, crusty appearance. However, confidence on such appearance for locating the stain is unreliable and is useful to criminalist only when the stain is present in a rather obvious area. Certainly, if the fabric has been washed or contains only minute quantities of semen, visual examination of the article will offer little chance of detecting the stain (4, 7).

The Wood's lamp emits ultraviolet light and has been established as a useful evaluation in rape cases because it is purported to cause semen to fluoresce (3, 4). Another light source based on a xenon arc lamp is also used (Polilight lamp) (8). An alternative light source is also used to detect semen on both inanimate surfaces and on human skin, with an excitation filter of 450 nm and barrier filter goggles (2). The broad excitation spectrum of semen allows the fluorescence to be generated at a range of wavelengths. This permits the excitation and emission conditions to be selected so that interference is minimized from background fluorescence of the fabric and thereby the contrast between the fabric and the stain is optimized. Furthermore, the fluorescence from semen stains is significantly enhanced using appropriate interference filters (2, 7).

Detection of semen with microscopic examination

The second step in the forensic examination of evidence is the detection of semen. Semen can be unequivocally identified by the presence of spermatozoa, that is, the current criterion confirming sexual intercourse. This biological evidence is accepted both by the medical forensic teams and by the judicial authorities (9, 10).

The victims of sexual assault may be adult females, pre-adolescent females and males. In the last case, the point of penetration is either the anus (sodomy) or the mouth (fellatio). In the rape of a child or virgin, there is usually ample evidence in the form of haemorrhage and laceration of tissue. Further evidence may be obtained by looking for traces of semen either on the genitalia, the thighs and the pubic region or in the vagina of the living victim as soon as possible after the assault.

The rape victim must undergo a thorough medical examination as soon as possible after the assault since evidence of semen can play a crucial role in corroborating the victim's allegations. Sperm may be detected in the vagina, rectum or mouth as well. Preservation of sperm in the mouth is usually not as good as in the vagina, since salivation, drinking and expectoration tend to cleanse the oral cavity. Defecation may well rid the rectum of sperm and seminal fluid (10, 11).

The forensic scientist must focus on the careful collection of smears and other biological material from the victim according to the routine sampling protocol. In particular, forensic sampling from all surfaces of the external genitalia, especially the labia can be difficult even when special care is taken. When sampling from areas of skin which are larger, such as the inner thigh, routine swabbing with a cotton swab will not necessarily make contact with all inner thigh skin, even with the best intentions of the clinician. The pressure used when placing the swab onto the skin and thus the likelihood of collecting material, may also differ when swabbing a large area "blindly" as opposed to swabbing a smaller defined fluorescent area (2).

In the forensic laboratory, the usual tests in cases of alleged rape are the examination of the vaginal, oral and anal smears. These smears must be obtained on slides at the earliest opportunity for subsequent laboratory examination. The finding of seminal constituents on a rape victim is important evidence for substantiating the fact that sexual intercourse or any other sexual assault has taken place, but their absence does not necessarily mean that a rape did not occur (4, 12).

In charges of rape, the persistence of seminal constituents in the vagina may become a factor when trying to ascertain the time of the alleged sexual attack, since inquiry often is made whether the identified seminal fluid components were deposited during a previous voluntary intercourse or during the alleged event (10).

When spermatozoa are located through a microscopic examination, the stain is definitely identified as having been derived from semen. In recent specimens, motile spermatozoa may be found and to identify them absolutely, it is necessary to find perfect bodies with head and tail complete. However, this is not always feasible, since spermatozoa are extremely brittle when dry and easily disintegrate. There are many factors that lead to the disintegration of spermatozoa making their available numbers in a stain very low or absent altogether. Furthermore, sexual crimes may involve males with oligospermia who have an abnormally low sperm count or they may involve individuals who have no spermatozoa at all in their seminal fluid (aspermia); or finally they may involve healthy young men with seminal fluid completely devoid of spermatozoa if they have experienced numerous ejaculations over a relatively short period of time. Significantly, aspermatic individuals are increasing in numbers (10% of the male population) due to the growing popularity of vasectomies (4, 13, 14). In these cases, spermatozoa count is not a contributory factor in cases of rape, but it may be of value in detecting cases of azoospermia where spermatozoa

are absent. Seminal stains are detected definitely by finding spermatozoa under the microscope, without or with staining (10).

The time since intercourse is of major importance in spermatozoa detection. While the presence of spermatozoa in the vaginal cavity provides evidence of intercourse, important information regarding the time of sexual activity can be obtained from the knowledge that motile or non-motile living sperm may generally survive up to four to six hours in the vaginal cavity of a living person, while other authors report that spermatozoa are found as long as three days after intercourse, and occasionally up to six days. However, intact sperm (sperm with tails) are not normally found 16 hours after intercourse, but have been found as late as 72 hours after intercourse (15, 16).

Although this is true in rape cases where the victim is alive, in those cases in which death has occurred, spermatozoa apparently may not live very long in the vagina. However, a successful search for motile sperm requires that a microscopic examination of a vaginal smear be conducted immediately after it is taken from the victim. Material is obtained from other body orifices, such as the oral and anal cavities by means of dry cotton or synthetic swabs. Spermatozoa may be found in the oral cavity as long as 12 hours after ejaculation, although their investigation is usually negative, while spermatozoa in the anal cavity may be found as long as 24 hours after the ejaculation (17). Thus, the pathologist must exert great care in the interpretation of these slides for possible identification of the heads of spermatozoa since a number of other contaminating substances, such as fungi in oral smears and fecal material in rectal smears, can simulate such structures (4, 18). A more extensive examination of all smears is later made at a forensic laboratory (15, 16).

In the absence of identifiable spermatozoa, the forensic serologist must rely on other tests to identify seminal stains. These tests are directed toward components of semen which are either unique to semen or are found in high levels in semen.

Detection of semen with acid phosphatase test

One important test includes the detection or quantitation or both of prostatic acid phosphatase. This test is the best way to locate and at the same time characterize a seminal stain.

Acid phosphatase is an enzyme that is secreted by the prostate gland into seminal fluid and it is present in all portions of the ejaculate. Its concentrations in seminal fluid are up to 400 times greater than those found in any other body fluid (4). Acid phosphatase is also present in a very low level in the vaginal secretion, except after intercourse, where acid phosphatase levels are high but except in subjects whose partners used condoms. The likelihood of finding seminal acid phosphatase in the vaginal cavity markedly decreases with time following intercourse, with little chance of identifying this substance 48 hours after intercourse (15, 16).

Since in cases of oligospermia, aspermia or in cases of use of a condom, spermatozoa are not found, acid phosphatase testing would seem to be a more reliable assay for the characterization of a seminal stain in cases of alleged rape, because ejaculates in cases of aspermia give comparable high results of acid phosphatase (5). This test is valuable for the identification of seminal stains, in the absence of spermatozoa, since aspermatic individuals are increasing due to the popularity of vasectomies and since oligospermia and azoospermia are more prevalent among rapists than among adult males in general (4, 19).

Other methods of semen detection

The application and use of the following methods need a well organized forensic laboratory, in contrast with the above mentioned basic methodology that can be applied to any laboratory with poor equipment.

A significant indicator of recent sexual activity is the protein p30. Under the analytical conditions employed in forensic laboratories, p30 is unique to seminal plasma and it is measured with a crossed electrophoresis technique. This protein is a useful semen marker particularly in cases of azoospermia (20). At appropriate concentration levels, this protein usually is not found in the vaginal cavity beyond 8 hours following intercourse (4). Occasionally, p30 is positive in the face of a negative acid phosphatase (21).

Monoclonal antibodies against human seminal plasma were also produced and during screening procedures dissociation constants of the antigen/antibody complexes were determined. Monoclonal antibodies were used for the development of an express method for detection of human semen (22). Although all these methods have been used for many years, there are problems associated with each method (23, 24).

Prostate Specific Antigen (PSA) has been found to be present in high concentrations in semen. Simple, sensitive and reproducible methods have been developed for analysis of the presence of PSA, including the Tandem -E PSA Immunoenzymetric Assay. This method can be used to identify the presence of PSA that is of seminal origin in a biological stain in forensically significant specimens (24, 25). Traditionally, the finding of semen, that is, spermatozoa and acid phosphatase, in cervicovaginal specimens, has been considered as laboratory evidence needed to prove recent sexual contact. However, since not all cases of sexual assault result in the deposit of semen, recent research with Fluorescence In Situ Hybridization (FISH) has been found to be a very sensitive and specific method for detection of the Y chromosome from male cells in the absence of semen. This method demonstrates the presence of epithelial cells of male origin in the postcoital vaginal tract for extended amounts of time after sexual assault, using a commercially available probe. This can occur during penetration, ejaculation or from the saliva of the assailant. Y chromosome may be identified in intact epithelial cells on postcoital Days 1 through 4 and

perhaps up to Day 7. Additionally, Y chromosome positive epithelial cells may be identified in vaginal swabs obtained following intercourse with no ejaculation. The application of FISH for use in detecting the Y chromosome has been successful in many cases and could be considered a valuable method for proving sexual contact with a male. The FISH method is sensitive for the identification of the presence of male epithelial cells, non-spermatozoic, in the postcoital vagina in alleged sexual assault cases (9, 24, 26).

The FISH technique may also be employed on penile swabs to determine recent sexual activity and identify possible suspects. Once the presence of female cells is confirmed by FISH, the identity of the female can be confirmed by DNA analysis. Furthermore, blood or buccal swabs for DNA analysis are to be taken from any consensual partner having sex with the victim within 72 hours of the assault (11). Potentially, with such current molecular analyses, both the assailant and the victim can be positively identified.

Semenogelin is a protein originating in the seminal vesicles, it is a substrate for prostate specific antigen (PSA) and it is a useful marker for the identification of semen (27).

CONCLUSION

The forensic laboratory has numerous ways of detecting biological evidence left by a male individual following a sexual assault case. Ideally, to say conclusively that ejaculation has occurred, spermatozoa must be detected in the semen isolated from the victim. However, under some circumstances, such as vasectomy, sperm may be absent from the semen.

In the absence of spermatozoa, other methods can be used to detect the presence of semen. Some of them are simple, such as fluorescence techniques, microscopic examination and the acid phosphatase test, but unequivocally they are reliable assays for the characterization of a seminal stain in laboratories with poor equipment. Molecular biology methods, when available, lead forensic serologists to successfully link seminal material to an individual suspect by DNA typing and to identify both the assailant and the victim. Furthermore, it is important to ensure that those working in the field of sexual assault and forensic sciences in general are convinced that no positive finding in forensic tests does not mean that no attack occurred.

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