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Forensic Identification of Seminal Stains as Evidence in Sexual Assault Investigations

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ABSTRACT

The detection of human seminal stains is a critical aspect of forensic investigations, especially in sexual assault cases. Various methods are utilized to identify and confirm the presence of semen, ranging from visual inspection to biochemical and molecular techniques. Presumptive tests, such as the Acid Phosphatase (AP) test, help in locating potential stains but require further verification. Other methods, including microscopic examination for sperm cells and the detection of prostate-specific antigen (PSA), provide more definitive results. Advanced DNA profiling techniques, such as Short Tandem Repeat (STR) and Y-STR analysis, enable individual identification, strengthening forensic evidence. Some more advanced techniques such as CASA (Computer Assisted Sperm Analyser), Flow cytometry, etc., now being more commonly utilized for the identification of semen in forensic science laboratories mostly in cases of sexual assault. The analysis of seminal fluid provides crucial information about personal identification related to crime scene investigation. Fructose is also a significant biochemical indicator used in this process. It is a type of sugar found naturally in seminal fluid, which is secreted mainly by the seminal vesicles. Notably, vaginal secretions do not contain fructose, making this difference vital in forensic analysis, it provides strong evidence of seminal fluid deposition, suggesting recent ejaculation and supporting allegations of sexual contact or assault. Thus, the detection and analysis of human seminal stains play a vital role in forensic investigations of sexual assault cases. By employing a combination of presumptive, confirmatory, and advanced molecular techniques, presence of semen can be identified and link to the potential suspects. These methods not only aid in uncovering the truth but also support the pursuit of justice in sensitive criminal cases.

Keywords: CASA, Acid Phosphate test, DNA profiling, STR analysis.

1. INTRODUCTION

Seminal fluid, commonly referred to as semen, is a complex biological substance produced by the male reproductive system. It plays a crucial role in human reproduction by facilitating sperm transport, providing essential nutrients, and creating a protective environment for spermatozoa [1]. Seminal fluid is composed of a mixture of secretions from the seminal vesicles (which contribute approximately 60-70% of the fluid), the prostate gland (which contributes enzymes and proteins), and the bulbourethral glands (which secrete mucus for lubrication) [2]. The presence of various biomolecules, including fructose, citric acid, proteins, enzymes, and hormones, ensures sperm motility and fertilization capability

[3]. Apart from its physiological significance, seminal fluid is of great importance in forensic investigations, especially in cases involving sexual offenses. identification of seminal stains on physical evidence can provide crucial leads in criminal cases, linking suspects to crime scenes or victims. Forensic techniques used to detect semen include different tests such as the Acid Phosphatase (AP) test, prostate-specific antigen (PSA) test , microscopic identification of spermatozoa, and molecular analysis including the detection of DNA profiling [4]. These methods help forensic experts in the analysis of biological evidence, strengthening legal cases and ensuring justice. Despite advancements in forensic science, the identification of seminal stains poses certain challenges. Factors such as azoospermia (absence of sperm in semen), environmental degradation of stains, and contamination can affect forensic results [5]. The continuous development of advanced techniques, such as Y-STR analysis and proteomic approaches, has improved the accuracy of seminal fluid identification in forensic investigations.

2. COMPOSITION OF SEMEN

Semen is a heterogeneous biological fluid produced by the male reproductive system, primarily composed of seminal plasma and spermatozoa. It serves a crucial role in human reproduction by transporting, nourishing, and protecting sperm cells during their journey to fertilize an ovum. Semen is secreted by the testes, seminal vesicles, prostate gland, and bulbourethral glands, each contributing distinct components essential for sperm viability and function [6].

Cellular and Non-Cellular Components of Semen

Semen consists of cellular components (spermatozoa and epithelial cells) and non- cellular components (seminal plasma, enzymes, lipids, proteins, and ions) [7].

Cellular Components

Spermatozoa – Highly specialized haploid cells responsible for fertilization, produced in the seminiferous tubules of the testes.

Round Cells – Immature sperm cells and epithelial cells shed from the reproductive tract.

Leukocytes (White Blood Cells) – Present in low concentrations; excessive leukocytes may indicate infection or inflammation.

Non- cellular components

Table : Non- Cellular components [8]

Component	Function	Source
Carbohydrates & Energy Sources	Provide energy for sperm motility	Seminal vesicles
Fructose	Primary energy source for sperm metabolism	Seminal vesicles

Glucose & Sorbitol	Alternative energy sources	Seminal vesicles
Proteins & Enzymes	Regulate seminal fluid properties	Seminal vesicles, prostate gland
Semenogelin I & II	Forms gel-like coagulum to protect sperm	Seminal vesicles
Prostate-Specific Antigen (PSA)	Liquefies coagulated semen to enhance sperm motility	Prostate gland
Acid Phosphatase (AP)	Forensic marker of semen presence	Prostate gland
Lipids & Hormones	Support sperm membrane integrity and function	Seminal vesicles, prostate gland
Cholesterol	Maintains sperm membrane stability	Seminal plasma
Prostaglandins	Induce uterine contractions to aid sperm transport	Seminal vesicles
Ions & pH Regulators	Maintain optimal environment for sperm function	Seminal vesicles, prostate gland
Zinc (Zn^{2+})	Stabilizes sperm DNA and enhances fertility	Prostate gland
Calcium (Ca^{2+})	Essential for sperm activation and motility	Prostate gland
Magnesium (Mg^{2+})	Regulates sperm metabolism	Seminal plasma
pH (7.2 - 8.0)	Creates a favorable environment for sperm survival	Seminal plasma

Variability in Semen Composition

The total semen volume per ejaculation typically ranges from 1.5 to 6 mL, depending on factors such as age, frequency of ejaculation, and overall health [9]. The sperm concentration and composition of seminal plasma can also vary due to lifestyle, diet, environmental exposure, and medical conditions [10].

Spermatozoa

A normal spermatozoon consists of three main corridors, videlicet, head, middle part and tail. Head consists of nexus containing a haploid set of chromosomes. It also possesses a bitsy clerk cap like structure called acrosome. Middle piece harbors mitochondria which give energy to the sperm to sustain stir. Flagellum is the name of the last member, the tail. Tail provides the capability to pass through the vaginal depression towards the eggs. Without flagellum, the sperm becomes non motile. Nature has dashingly evolved multi-cellular organisms by secerning cells in agreement to their specific

tasks. The sole motive of a sperm cell is to transfer the inheritable material to the egg cell. Hence sperm cells do n't include other cellular organelles like Golgi bodies, ribosomes, etc. These organelles feel insignificant for this task and thus nature has not included them in the sperms. analogous analogy is also seen in mature red blood cells, where the nexus is absent so as to incorporate haemoglobin patch. So, a sperm cell is composed of Head, mid-piece and tail. The tip of the sperm cell is known as acrosomal cap or acrosomal vesicle. Acrosomal vesicle containing hydrolytic enzyme grease the penetration of sperm into the egg. When a sperm comes in contact of an egg, the contents of the vesicle are released by exocytosis.

Presumptive Tests for Seminal Stains

Presumptive tests are the initial screening methods used to identify the possible presence of seminal fluid. These tests are rapid and sensitive but require confirmatory analysis to avoid false positives [11].

Acid Phosphatase (AP) Test

The Acid Phosphatase test is one of the most commonly used presumptive tests for semen detection. Acid phosphatase is an enzyme found in high concentrations in seminal fluid. The test involves the application of a substrate that reacts with the enzyme, producing a color change within seconds. Despite its effectiveness, the AP test can yield false positives from other bodily fluids, necessitating further verification [12]

Fluorescence-Based Techniques

Certain components in semen exhibit fluorescence under ultraviolet (UV) or alternate light sources (ALS). This method allows forensic investigators to locate potential stains on evidence. While useful for screening, fluorescence is not confirmatory, as other substances such as detergents and lotions can also fluoresce [13].

Confirmatory Test

Confirmatory tests are essential to establish the presence of semen definitively. These methods provide strong forensic evidence and are admissible in legal proceedings [14].

Microscopic Identification of Spermatozoa

The most definitive confirmatory test involves the microscopic examination of sperm cells using stains such as the Christmas Tree Stain, which highlights sperm heads and tails distinctly. This method is reliable but may not be effective in cases involving azoospermic or vasectomized individuals [15].

Semenogelin Test-

Semenogelin is a protein responsible for semen coagulation. It serves as another confirmatory marker, detected using immunochromatographic tests. This test complements PSA analysis and enhances the specificity of semen identification [16].

Molecular Analysis and DNA Profiling

Modern forensic science employs DNA analysis to confirm the presence of seminal fluid and establish individual identity [17].

Short Tandem Repeat (STR) Analysis

STR profiling is the gold standard for forensic DNA analysis. This technique amplifies specific DNA regions, allowing forensic scientists to obtain a unique genetic profile. STR analysis is highly sensitive and can generate results from minute samples [18].

Y-Chromosome STR (Y-STR) Analysis

In cases where male DNA must be isolated from female DNA, Y-STR analysis targets male- specific markers on the Y chromosome. This method is particularly useful in mixed samples and sexual assault cases [19].

Computer-Assisted Sperm Analysis (CASA) and Flow Cytometry

Advanced methods such as Computer-Assisted Sperm Analysis (CASA) and Flow Cytometry are now being more commonly utilized in forensic science labs to detect semen in sexual assault investigations. These modern techniques offer greater accuracy and objectivity compared to traditional approaches. They allow for the detailed assessment of sperm characteristics like shape (morphology), movement (motility), and DNA content, which helps in the effective identification and examination of semen evidence[20 ,21].

RNA-Based Identification

Emerging forensic techniques involve the detection of semen-specific RNA markers, such as semenogelin and PRM1. RNA profiling offers a novel approach to identifying seminal stains, even when DNA is degraded [22].

Forensic importance of Seminal Stains

Seminal stains hold immense forensic significance as they can provide crucial evidence in criminal investigations. Their detection is particularly relevant in cases of sexual offenses, where the presence of semen can establish contact between the suspect and the victim. The forensic importance of seminal stains includes:

Connecting Suspects to the Scene of the Crime

DNA obtained from semen can be used to generate a genetic profile, which can then be compared with that of potential suspects to either confirm or rule out their connection to a crime.

Supporting Victim Statements

In cases of sexual assault, identifying seminal stains can corroborate the victim's testimony and assist in piecing together the timeline and circumstances of the incident.

Proving Innocence of Unjustly Accused Individuals

Genetic profiling of seminal stains can help eliminate individuals who are not involved, thereby reducing the risk of wrongful convictions.

Analysis of Time-Dependent Degradation: Analyzing seminal stains in forensic investigations can offer information about how much time has passed since their deposition, aiding in the reconstruction of crime timelines.

Identification of Unknown Individuals: In cases where a suspect is not initially known, DNA databases can help identify perpetrators through stored genetic information [23].

3. CONCLUSION-

The detection and analysis of human seminal stains play a vital role in forensic investigations of sexual assault cases. By employing a combination of presumptive, confirmatory, and advanced molecular techniques, presence of semen can be identified and link to the potential suspects . The use of biochemical markers like fructose, along with modern technologies such as STR analysis and CASA, enhances the reliability and precision of forensic evidence. These methods not only aid in uncovering the truth but also support the pursuit of justice in sensitive criminal cases.

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