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A Comprehensive Study on Forensic Analysis of Semen: Issues Related to Integration, Quantification and Quality Assessment

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A B S T R A C T

Semen analysis is vital in sexual assault investigations, paternity testing, and other medico-legal matters involving sexual crimes. This extensive research examines forensic semen integration, quantification, and quality evaluation difficulties. Semen analysis entails systematically examining and collecting evidence including clothes, bedding, and swabs that may contain semen. The research examines semen stain identification and recovery issues, including staining patterns, ambient conditions, and biological elements. It also investigates semen sample preservation methods for travel and storage. Forensic analysis relies on semen quantification to determine spermatozoa levels. Microscopic sperm counts, biochemical indicators, and genetic methodologies are examined in this research. It addresses their benefits, weaknesses, and probable errors, emphasizing the need of correct quantification in determining semen presence and sexual assault instances. Forensic analysis also requires semen quality evaluation. The research examines sperm vitality, motility, morphology, and DNA integrity. Microscopy, staining, and sophisticated molecular biology are used to analyze these characteristics. The research also covers semen sample degradation and its effects on analysis and interpretation. This extensive research also emphasizes the necessity for defined techniques, quality control, and proficiency testing in forensic semen analysis. To improve semen analysis findings, forensic scientists, geneticists, pathologists, and other professionals should collaborate.

Keywords: Semen, DNA fingerprinting, Contamination, Degradation.

1. INTRODUCTION

Semen analysis for forensic purposes is essential in criminal investigations, particularly those involving cases of sexual assault. The fluid generated by the male reproductive system, known as sperm, also includes additional substances in addition to spermatozoa [1]. Its presence or absence, as well as the identification and quantification of spermatozoa, can offer crucial information for figuring out the timing and identity of sexual activity. Semen can be used as evidence in sexual assault cases to establish criminal charges and substantiate the victim's statement [2]. By comparing the DNA profiles from semen samples to those of known people or DNA databases, semen analysis can also aid in the identification of prospective suspects. Additionally, paternity testing relies heavily on semen analysis to determine biological ties between people for both legal and private reasons [1,3,4**].** Semen is the biological organic fluid found in males also known as seminal fluid containing spermatozoa. In forensics, semen is common biological evidence found in sexual assault or rape cases. Mainly dried seminal stains are detected on mattresses, undergarments, sheets, condom and private parts [5]. It is a primary role of forensic examinations in sexual offences to analyze the semen stains taken from the victim cloths and to link them with the suspect(s) and crime scene [6]. It is not an easy task to sure that particular stain is semen without any examination because many stains look identical in color or appearance to other stains or fluids [7]. The major issue is that in most of the assault cases, the quantity of the sample is less therefore, it makes it challenging for analysis of spermatozoa [8] as well as it has high probability of getting contaminated. Avoid tampering with the evidence. Semen or seminal stains in extremely small quantities might be used as proof. Therefore, additional care must be taken when gathering and conserving the semen or seminal stains for forensic investigation due to contamination concerns. In forensic casework, the biological sample's integrity is crucial. Particularly when dealing with trace evidence, biological contamination of evidence from another source is a very real risk [9]. Wearing of disposal gloves, washing of apparatus, avoid contamination while handling [10]. Refrain from speaking, sneezing, or coughing near the evidence. Thoroughly air-dry any evidence before packaging. Do not place evidence in plastic bags, but rather in fresh paper bags or envelopes. Avoid using staples [11]. Semen, commonly referred to as seminal fluid, is a sophisticated biological fluid generated during ejaculation by the male reproductive system. It is made up of different parts that are crucial for reproduction. Forensic analysis demands an understanding of seminal fluid composition. The main elements and their roles are listed below:

1.1 Spermatozoa

The reproductive cells seen in seminal fluid are known as spermatozoa. Their main job during sexual reproduction is to fertilize the female egg. Spermatozoa have distinctive physical characteristics that can be utilized for personal identification and convey genetic information

1.2 Seminal plasma

The majority of the volume of the seminal fluid is made up of seminal plasma. It is a complicated mixture of ions, hormones, proteins, enzymes, and other bioactive substances. Spermatozoa are given nourishment, defense and motility factors by seminal plasma, ensuring their survival and ability to enter the female reproductive system.

1.3 Prostatic Fluid

The prostate gland secretes prostatic fluid. It helps semen's alkaline pH balance out the acidic environment of the female reproductive system. Proteins and enzymes found in prostate fluid help sperm move around and liquefy semen following ejaculation. Fluid produced by seminal vesicles is known as seminal vesicle fluid. It increases the fructose level of semen, giving spermatozoa a source of energy. Prostaglandins, which encourage uterine contractions and aid sperm motility inside the female reproductive system, are also present in seminal vesicle fluid.

1.4 Secretion from the Cowper's gland

The Cowper's gland, also known as the bulbourethral gland, secretes a tiny amount of fluid that lubricates the urethra and neutralizes any leftover pee, fostering the ideal conditions for sperm transmission. The most crucial part of semen for forensic investigation is spermatozoa because they include genetic data that can be used for identification and individualization. Semen analysis requires an understanding of the makeup and traits of spermatozoa. The following are important factors: Spermatozoa are tiny, elongated cells with a head, middle section, and tail. The genetic material is kept in the nucleus, which is located in the head. There are many mitochondria in the middle piece, which give sperm movement energy. The spermatozoon's tail, also known as the flagellum, acts as its propulsion [12]. Spermatozoa are essential in forensic examinations of sexual assault, according to forensic analysis. Spermatozoa can be identified and analyzed to prove sexual intercourse and show the presence of the offender. Spermatozoa can be found, seen, and examined in forensic samples using methods like microscopy, immunohistochemistry, and DNA profiling.

1.5 DNA profiling

: Spermatozoa are useful for DNA profiling because they include paternal DNA. Spermatozoa can be subjected to forensic DNA analysis to provide DNA profiles, which can then be compared to suspects or DNA databases for identification reasons. Forensic examination of semen greatly depends on the seminal fluid's composition as well as the shape and traits of spermatozoa. For effective semen detection, identification, and quantification as well as DNA profiling for individualization and identification in criminal investigations and paternity tests, it is essential to comprehend these components [13].

1.6 File naming and delivery

Visual inspection: To find any probable semen stains, forensic experts visually examine the sample under the proper lighting conditions, such as ultraviolet (UV) light. On fabric or other surfaces, semen stains can look yellowish or white. Visual inspection, however, is insufficient to identify spermatozoa or conclusively confirm the presence of semen [14]. Analysis with a microscope: Microscopy is frequently used to confirm the presence of spermatozoa in samples of suspected semen. An examination of the stain or sample under a compound microscope is done on a small area of it. To confirm the presence of spermatozoa, the morphological characteristics of spermatozoa, such as head shape, tail structure, and motility are investigated. Compared to eye inspection alone, this method enables a more precise identification of spermatozoa [15].

2. METHODS AND TECHNIQUES

2.1. Immunological techniques

Antibodies are used in immunological procedures to precisely detect and identify semen components, such as spermatozoa or seminal plasma proteins. These methods are based on the idea of interactions between antigens and antibodies [16]. Here are a few often employed immunological methods:

IHC stands for immunohistochemistry. In IHC, certain antibodies are used that bind to antigenic regions on spermatozoa or seminal fluid constituents. A visible marker, such as a fluorescent or enzyme-linked tag, is applied to the antibodies. The target antigen is recognized by the antibodies when they are administered to a sample, allowing for either color development or microscopic visualization of the antigen under a microscope [17].

Examining with an alternate light source (ALS) improves the visibility of semen stains by using particular light wavelengths. Flavins, a naturally fluorescent substance found in seminal fluid, exhibit fluorescence when exposed to specific light wavelengths. The sensitivity of detection can be increased by using ALS to help locate and identify probable semen stains.

2.2 Molecular techniques (PCR-based methods)

Semen detection and identification in forensic science have been transformed by molecular techniques, particularly those based on polymerase chain reaction (PCR). These methods depend on the identification of particular DNA sequences found in semen.

Analysis of short tandem repeats (STR) requires amplifying particular microsatellite-containing sections of DNA. Repeated DNA sequences with different lengths can be found in these locations. Microsatellite marker lengths can be used to identify individuals and determine the presence of semen by comparing samples to well-known reference samples.

Y-chromosomal analysis: The Y chromosome is distinctive to males, and it can be used to identify the presence of male DNA in a sample by focusing on certain markers on the Y chromosome. This technique can establish the existence of semen and ascertain the genetic data of the male contributor, assisting in the identification of possible offenders [18].

Quantitative PCR (qPCR): This technique measures the amplification of particular target sequences to calculate the amount of male DNA contained in a sample. It can be used to quantify semen and offers an estimate of the quantity of spermatozoa in a sample. These immunological and molecular methods greatly improve the specificity and sensitivity of semen detection and identification, enabling more precise forensic analysis in paternity cases and sexual assault investigations. They give forensic scientists and investigators useful instruments for identifying the existence of semen and establishing the genetic data connected to it [19].

2.3 Microscopic sperm count

Traditional techniques for estimating the number of spermatozoa in semen samples include microscopic sperm counts. Under a microscope, spermatozoa are visually counted using a hemocytometer or specialized counting chamber. Here is a characterization of this approach:

Sample preparation: To enable accurate visualization and spermatozoa counting, the semen sample is diluted and combined with a solution. Depending on the original spermatozoa concentration in the sample, several dilution factors could be employed.

Microscopic counting: The diluted semen sample is placed on a hemocytometer slide or a counting chamber with a known volume. Under a microscope, the spermatozoa in a specific region of the counting chamber are counted. The count is extrapolated to determine the approximate quantity of spermatozoa in the original semen sample [20].

2.4 Alternative methods for sperm quantification

Although the microscopic sperm count is a common technique, several approaches have been developed to increase the precision, efficacy, and accuracy of sperm measurement [21]. Here are a few other approaches:

Using laser-based technology: flow cytometry measures the optical and physical characteristics of each individual spermatozoa in a sample. It can offer thorough details on sperm quantity, morphology, DNA content and other traits. Numerous spermatozoa can be quickly and automatically analyzed using flow cytometry [22].

Technologies for digital imaging: Computer-assisted sperm analysis (CASA) technologies capture and examine sperm motility and morphology using digital imagery and sophisticated software algorithms. Measurements of numerous sperm characteristics, including as concentration, motility, velocity and morphology are provided by these devices in an objective and standardized manner.

2.5 Quality Assessment of Semen

2.5.1 Sperm motility analysis

Analyzing sperm motility is an essential part of evaluating semen quality. It entails assessing the spermatozoa's mobility and forward progress. An outline of this analysis is given below:

Sperm motility analysis evaluates a number of factors, such as the proportion of moving spermatozoa, or progressive motility (the percentage of spermatozoa that are moving forward), velocity, and linearity of movement. These variables shed light on the spermatozoa's functional capacity and reproductive potential [23].

Motility analysis techniques include manual approaches like visual inspection under a microscope and automated approaches like computer-assisted sperm analysis (CASA). CASA systems use cutting-edge tracking and image processing algorithms to deliver accurate measurements of sperm motility characteristics.

2.5.2 DNA integrity and viability assessment

It is essential to evaluate the spermatozoa's DNA integrity and viability in order to determine their reproductive potential and conduct forensic investigation. Here is a summary of these evaluations:

DNA integrity: Spermatozoa with DNA damage may have reduced fertility and may have produced kids with genetic defects. By detecting DNA fragmentation and structural anomalies, many methods, including the sperm chromatin structure assay (SCSA), comet test, and TUNEL assay are utilized to evaluate DNA integrity.

Viability evaluation: Viability evaluation establishes the percentage of metabolically active, living spermatozoa in a sample of semen. Based on membrane integrity and metabolic activity, methods like the hypo-osmotic swelling test (HOST) and staining techniques using eosin-nigrosin or fluorescent dyes (such as propidium iodide) can distinguish between live and dead spermatozoa [24].

2.5.3 Assessment of semen age and degradation

In order to establish the duration since ejaculation and guarantee the accuracy of the results, forensic analysis must evaluate the age and degradation of semen samples. This assessment takes into account several criteria, including:

Sperm morphology: Spermatozoa go through structural changes throughout time, such as losing cytoplasmic droplets or changing the form of their heads. The age of the semen sample can be determined by analyzing the morphology of the sperm.

Liquefaction: When semen samples are naturally liquefied, the coagulated semen changes from a solid condition to a liquid one. The length of time needed for complete liquefaction can reveal details about the sample's age.

Biochemical markers: The degradation and stability of semen samples can be evaluated via the analysis of particular biochemical markers, such as acid phosphatase or prostate-specific antigen (PSA). The age of the sample can be determined by changes in the amounts of these markers throughout time.

It is significant to highlight that the assessment of semen age and degradation offers estimation based on the information at hand and the characteristics of the sample rather than an exact determination. In forensic analysis and reproductive medicine, integrating several characteristics and using approved techniques can improve the accuracy and reliability of these assessments [25].

2.6 Sperm motility analysis

Semen quality assessment must include sperm motility analysis since it offers crucial data on spermatozoa's functional competency and reproductive potential. An overview of sperm motility analysis methods is given below:

Manual assessment: Sperm motility can be assessed by looking at a semen sample under a microscope and grading the proportion of moving spermatozoa based on how quickly they advance. This technique depends on the examiner's knowledge and could be vulnerable to inter-observer variability.

Computer-assisted sperm analysis (CASA) devices follow and examine the motion of spermatozoa seen in movies using sophisticated computer algorithms. These devices offer precise measurements of several motility parameters as lateral head displacement amplitude, linearity, and velocity.

Kinematic parameters: To evaluate the effectiveness of sperm motility, many kinematic parameters, such as curvilinear velocity (VCL), straight-line velocity (VSL), and average path velocity (VAP) can be assessed. These variables shed light on the linearity, directionality, and speed of sperm migration [26].

2.7 DNA integrity and viability assessment

Spermatozoa's DNA integrity and viability must be evaluated in order to assess their reproductive quality and potential. An overview of the methods for determining DNA integrity and viability is provided below:

DNA fragmentation assays: A number of assays, including the comet assay, terminal deoxynucleotidyl transferase nick-end labelling (TUNEL) assay, and sperm chromatin structure assay (SCSA), can be used to measure DNA fragmentation in spermatozoa. These tests look for DNA alterations and breaks that could affect the success of conception and pregnancy.

Viability staining: Based on the integrity of the membrane, staining methods can distinguish between live and dead spermatozoa, such as the eosin-nigrosine stain or fluorescent dyes (such as propidium iodide). Viable spermatozoa with intact membranes exclude the stain, whereas dead or injured spermatozoa with defective membranes take up the stain.

Acrosome integrity evaluation: The acrosome is a vital component of fertilization and is located on the head of spermatozoa. The integrity and functionality of the acrosome can be assessed using methods such as the peanut agglutinin (PNA) assay or the Pisum sativum agglutinin (PSA) assay [27].

2.8 Assessment of semen age and degradation

In forensic analysis and reproductive medicine, it's critical to evaluate the age and deterioration of semen samples. Here is a summary of the methods for determining semen age and degradation:

Evaluation at the microscopic level: Sperm morphology can reveal information about the age of a semen sample. Spermatozoa may alter structurally over time, such as losing cytoplasmic droplets or changing the form of their heads.

Evaluation of liquefaction: Following ejaculation, semen passes through a process known as liquefaction in which it changes from a gel-like condition to a liquid state. The length of time needed for complete liquefaction can reveal details about the sample's age.

Biochemical markers: The degradation and stability of semen samples can be evaluated via the analysis of particular biochemical markers, such as acid phosphatase or prostate-specific antigen (PSA). The age of the sample can be determined by changes in the amounts of these markers throughout time [28].

2.9 Methods of analysis of Semen Integration

2.9.1 Combined approach for comprehensive semen analysis

Examining the semen sample's volume, color, pH, and consistency is part of the macroscopic examination that starts the study. Initial insights on semen quality and probable anomalies can be gained from macroscopic results.

2.9.2 Microscopic examination

microscopic analysis entails evaluating spermatozoa under a microscope. Assessing sperm concentration, motility, and morphology is part of this process. The identification of spermatozoa and the detection of any abnormalities can be aided by visual inspection and staining procedures.

2.9.3 Immunological methods

Immunological techniques can be used to establish the presence of semen and determine its origin, such as the detection of prostate-specific antigen (PSA) or sarmentogenin. Spermatozoa can be seen and their existence in questionable stains or samples can be further confirmed using immunofluorescence or immunohistochemistry procedures.

Molecular approaches are extremely important in the examination of semen. Male DNA can be detected and sperm concentration can be measured using DNA-based methods like polymerase chain reaction (PCR). DNA damage and fragmentation can be measured using DNA integrity assays like the TUNEL assay and comet assay [29].

3. RESULT & DISCUSSIONS

In forensic investigations, reproductive medicine, and fertility evaluation, semen analysis is essential. Semen is typically detected and identified using methods like visual inspection, microscopic analysis, immunological procedures, and molecular techniques (PCR-based methods). Analysis of sperm motility reveals important details about spermatozoa's capacity for function. The motility characteristics can be measured objectively and consistently using computer-assisted sperm analysis (CASA) equipment. For the purpose of assessing sperm quality, DNA viability and integrity must be evaluated. For this aim, viability staining methods and DNA fragmentation assays are frequently used. By examining sperm morphology, liquefaction time, and particular biochemical indicators, semen age and degradation can be evaluated. Multiple semen analysis techniques can be used to provide a more thorough assessment of semen quality and reproductive potential. Due to the lack of standardized criteria, inter-individual variability, and the requirement for integrating various characteristics, result interpretation and reporting present difficulties. Further research is needed to determine the clinical importance of specific semen abnormalities and how they affect reproductive results. In the forensic setting, precise identification and legal requirements depend on clear and succinct reporting supported by the necessary paperwork [30].

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