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RESEARCH

Pre-ejaculate – an overlooked body fluid with high amounts of DNA

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Abstract

Pre-ejaculate is a clear, sperm-free liquid secreted by the male bulbourethral glands. Since it is currently not a subject of routine forensic testing, pre-ejaculate is often overlooked. The aim of this study was to characterize the forensic value of pre-ejaculate: Pre-ejaculate from 58 healthy male donors (aged 20–59) was analyzed with respect to DNA yield and the reactivity of various tests specific for seminal fluid, semen and sperm, respectively. DNA was subsequently extracted, quantified, and subjected to routine forensic STR analysis. We found that the tests for the presence of seminal fluid showed varying reactivity for pre-ejaculate: While the test for semenogelin returned negative in all samples, the test for PSA showed a positive reaction in 84.5% of the samples. The acid phosphatase test returned positive in 29.3% of the samples. Furthermore, ≤ 436 ng DNA were detected, and full STR profiles were obtained in 49 of the 58 analyzed samples. Taken together, these findings show that pre-ejaculate contains high levels of DNA and, thus represents a relevant body fluid in the forensic investigation of cases of sexual assault. The varying sensitivity of tests for seminal fluid implies that positive results may originate from pre-ejaculate even in the absence of seminal fluid or semen. Conversely, negative results may lead to the exclusion of samples from further STR analysis, with the risk of missing crucial case information. Consequently, additional studies are required to confirm the presence of this body fluid and to enable a reliable differentiation between pre-ejaculate and semen.

Keywords Pre-ejaculate · Pre-ejaculatory fluid · Body fluid identification · Sexual assault cases

Introduction

The identification of forensically relevant body fluids serves as highly valuable evidence in the investigation of sexual offenses. Various methods for identifying peripheral and menstrual blood, saliva, semen, sweat, urine, and vaginal fluid have been established in the forensic community [1, 2]. The detection of seminal fluid and semen is of special significance in the context of sexual crime, as it can provide clear evidence that sexual activity has taken place. Conversely, however, the absence of semen—i.e., the absence of ejaculation—does not imply that no (potentially criminal) sexual activity has occurred. In an alleged case of rape, the 8-year-old male victim reported that anal penetration had occurred and intimate swabs were subjected to forensic testing. Forensic Short Tandem Repeat (STR) analysis revealed a mixture with an unknown major component matching the suspect's DNA profile. Tests for the presence of semen, saliva, and blood showed negative results. Skin contact was considered an unlikely source for the high amount of DNA observed and pre-ejaculate was suggested as a potential source of DNA.

Pre-ejaculate, commonly referred to as "pre-cum", is a body fluid that has received little attention in the forensic investigation of sexual offences to date, especially in cases where no ejaculation is reported. Secreted by the

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bulbourethral glands, that are located below the prostate within the male pelvic floor muscles, this fluid plays a distinct role in male sexual physiology. It is released during sexual arousal before ejaculation, serving as a natural lubricant and preparing the urethra for ejaculation by creating a sperm-friendly pH environment [3]. Studies show that the amount of secretion of pre-ejaculate varies greatly between individuals and generally does not contain spermatozoa [4]. It is known that pre-ejaculate exhibits a heterogeneous composition, containing glucose, leukocytes, individual epithelial cells, enzymes, and glycoproteins [4–8]. This body fluid is not only secreted prior to ejaculation, but also during the ejaculatory phase, which can cause a mixture of pre-ejaculate and semen [9–11]. Previous studies demonstrate different findings on the presence of spermatozoa in pre-ejaculate: While some studies show the presence of spermatozoa in pre-ejaculate after sample collection, others conclude their absence [4, 8, 12–14]. Ejaculation prior to sample collection might explain the presence of seminal fluid and spermatozoa in the urethra, which might be washed out by pre-ejaculate resulting in a mixture of these body fluids.

These findings show that the current state of research on pre-ejaculate does not yet reveal all information on its composition.

The aim of this study was to investigate the potential significance of pre-ejaculate in forensic case work. Reactivity of commonly used tests specific for seminal fluid, semen and sperm to pre-ejaculate samples was analyzed, DNA yield was determined and the possibility of generating STR profiles was explored.

Materials and methods

Sample preparation

Pre-ejaculate samples were donated by a total of 94 male participants of reproductive age, aged 20 to 59, with informed consent using procedures approved by the local ethical committee (Ethik-Kommission der Ärztekammer Westfalen-Lippe und der Universität Münster).

Samples were collected using Copan FLOQSwabs™ at the participant's home and transported in thermal envelopes. The samples were frozen immediately upon arrival for storage at −20 °C.

Microscopy

Since we propose that the presence of spermatozoa in pre-ejaculate reported in previous studies was due to a mixture with semen, all samples were first analyzed using the HY-LITER™ Express (Galantos Genetics GmbH, Mainz, Germany) kit, a human-specific monoclonal antibody-based fluorescence staining kit to visualize nuclei and sperm heads with corresponding filters according to protocol [15]. In order to minimize sample loss, swabs were directly smeared onto microscope slides without prior dissolution in water. Microscopic analysis was performed using a Zeiss™ Axioscope 5 fluorescence microscope and results were captured by the Axiocam 305 color camera (Carl Zeiss AG, Oberkochen, Germany). Samples containing spermatozoa were excluded from further analysis. After histological analysis, 58 samples remained for further analysis.

Body fluid tests

Swabs were thawed and gently rubbed onto three 1 cm^2 filter papers. Three tests for the detection of seminal fluid and semen were used: RSID™-Semen (Galantos Genetics GmbH, Mainz, Germany), Seratec® PSA-Semiquant (Seratec GmbH, Göttingen, Germany), and Phosphatesmo KM (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany).

Both the RSID™-Semen test for human seminal fluid, detecting semenogelin, and the Seratec® PSA-Semiquant tests for detection of human seminal fluid through prostate-specific antigen were used according to protocol. The remaining sample buffer solutions were stored at 6 °C for subsequent DNA analysis.

For the Phosphatesmo KM, a test for acid phosphatase, a filter paper was moisturized with 20 µL nuclease free water in an Eppendorf tube. After one minute of soaking in nuclease free water, the filter paper was placed onto the test field. The results of the test were indicated by a color shift and recorded after a one-minute incubation time.

DNA analysis

DNA was extracted from the pad underneath the sample well of the Seratec® PSA-Semiquant test cassette and the remaining sample buffer solutions according to a previously described protocol [2], using the Maxwell® instrument (Promega, Mannheim, Germany) with the Maxwell® FSC DNA IQ™ Casework Kit (Promega, Walldorf, Germany).

DNA was quantified using the PowerQuant® System (Promega, Walldorf, Germany) with the 7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, USA), and amplified using the PowerPlex® ESX/ESI 17 Pro System (Promega, Walldorf, Germany). Samples were subsequently analyzed using the 3500 Genetic Analyzer with

the GeneMapper® ID software v1.7.3.1 (Thermo Fisher Scientific, Waltham, USA).

Statistical evaluation

The results were statistically evaluated using a descriptive analysis of the percentage distribution of the test results.

The data were evaluated for three test systems (Acid phosphatase marker using Phosphatesmo KM, Semenogelin marker using RSID™-Semen, and Prostate specific antigen marker using Seratec® PSA-Semiquant) with the categories positive, negative, and ambiguous. The results were analyzed using R (Version 4.5.2) and summarized in bar plots using ggplot2 package.

DNA yields (ng) were determined for each sample and presented graphically as a bar chart. To assess sample quality, STR profiling was classified into three categories: Complete profile, partial profile, and no profile. The analysis and visualization were performed using R (version 4.3.1) with the ggplot2 package.

Results

Microscopy

Histological examination identified spermatozoa in 35 of the 94 samples, leading to their exclusion from further analysis, even when only a single spermatozoon or very few spermatozoa were observed (Fig. 1a). In 58 samples no spermatozoa were detected.

In 23 pre-ejaculate samples without detectable spermatozoa, microscopic examination indicated the presence of isolated cells or cell-like structures (Fig. 1b), while in the remaining 35 samples findings were inconclusive regarding the presence of cells, taking into account the possibility of background interference (Fig. 1c).

Body fluid tests

The test results varied across the 58 pre-ejaculate samples (Fig. 2). All samples tested negative for the presence of

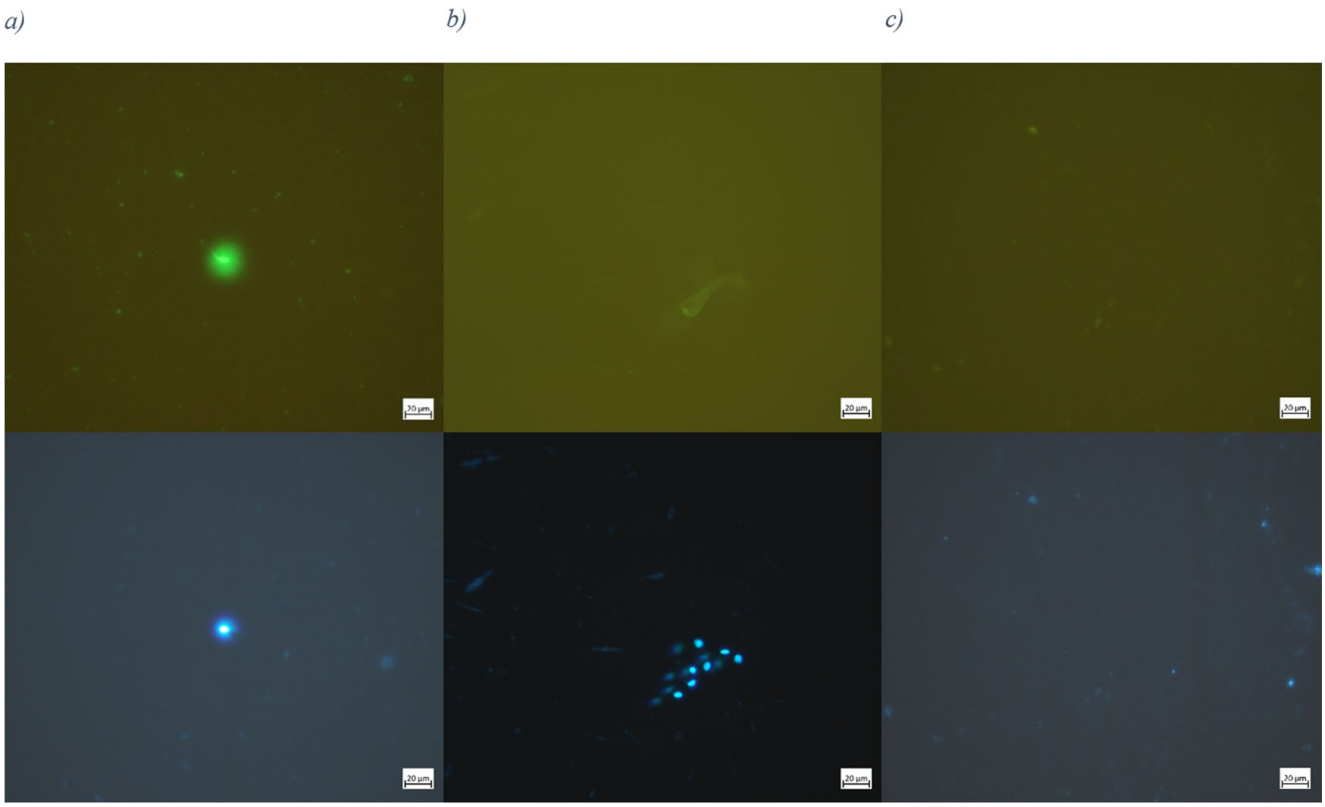

Fig. 1 Examples of Sperm Hy-Liter™ staining results using pre-ejaculate collected with Copan FLOQSwabs™. Slides were examined using a 40× objective on an Axioscope 5 fluorescence microscope with according filters for sperm head staining (green; upper images) and nuclei staining (blue; lower images). Images were acquired using an Axiocam 305 color camera (ZEISS AG). (**a**) The presence of a single spermatozoon was detected, leading to the exclusion of the sample from further analysis. (**b**) No spermatozoa were detected; however, a small number of nuclei were observed. Therefore, the sample was included for further analysis. (**c**) Neither spermatozoa nor cellular material was detected; consequently, the sample was included for further analysis

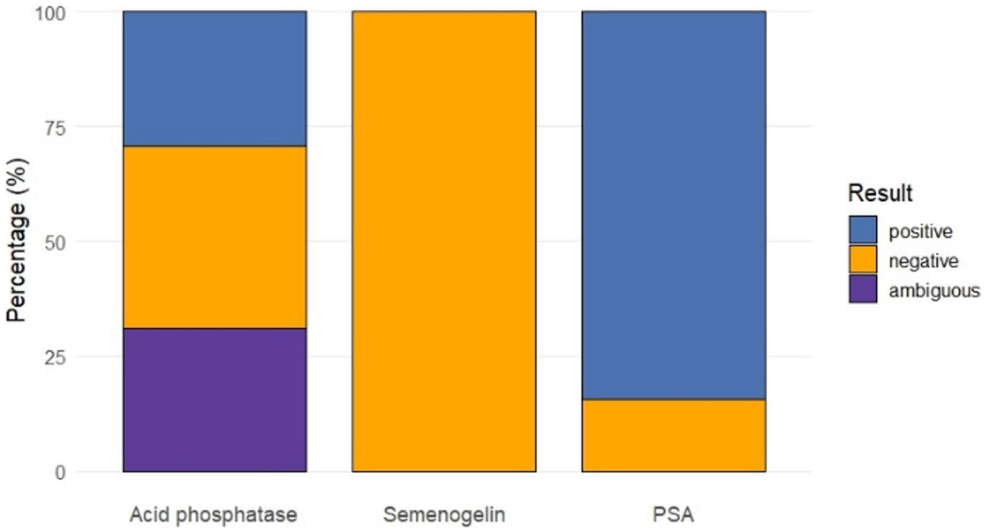

Fig. 2 Results of three specific tests for seminal fluid and semen, conducted on 58 samples of pre-ejaculate. The Phosphatesmo KM test for acid phosphatase yielded 39.7% negative, 29.3% positive, and 31.0% ambiguous results. RSID™-Semen tests for semenogelin showed that all samples tested negative. The Seratec® PSA Semiquant test produced 84.5% positive and 15.5% negative results

semenogelin using the RSID™-Semen assay. In contrat, 49 samples (8.5%) tested positiveusing the Seratec® PSA Semiquant test, while 9 samples (15.5%) tested negative. The Phosphatesmo KM test produced a substantial proportion ofambiguous results, ith 18 samples (31.0%) that could not be clearly classified as either positive or negative. Of the remaining samples, 17 samples (29.3%) tested positive and 23 samples (39.7%) tested negative.

DNA analysis

The majority of pre-ejaculate samples contained a high amount of DNA, reaching up to 436 ng (supplementary material, Table 1). From 49 of the 58 samples full STR profiles could be generated. They contained a mean total DNA amount of 23.616 ng and a median of 2.768 ng. Two samples showed a partial STR profile with ten fully typed STR markers, while possible allelic dropouts were observed in the remaining six systems. They contained 0.068 ng and 0.044 ng of DNA. Despite the allelic dropouts, both profiles would fulfil the criteria for submission to the national DNA database. No STR profiles could be generated from seven samples with a mean DNA yield of 0.027 ng and a median of 0.024 ng (Fig. 3).

Discussion

Pre-ejaculate has received little forensic attention despite its potential relevance. Although the DNA yield varied among samples, pre-ejaculate fluid generally contained high amounts of DNA, enabling the generation of STR profiles suitable for comparison with reference profiles. The amount of DNA observed in the test samples would be suitable to explain the findings of the rape case outlined above. In this case, two stains were detected in the area of the anal canal and in the perianal area with DNA concentrations of 1.272

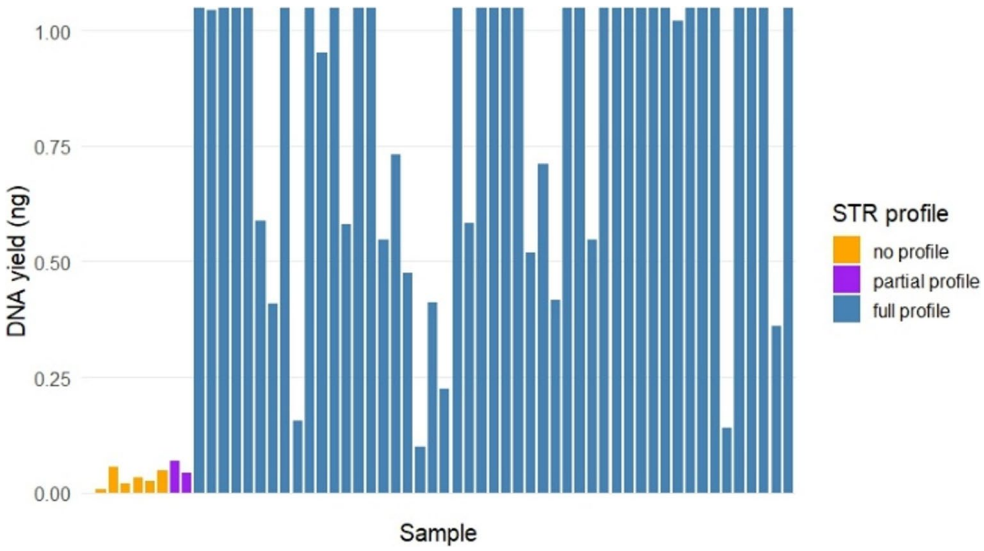

Fig. 3 STR profile distribution and DNA yield (ng) in 58 pre- -axis is restricted to 1.0 ng for visualization purposes, although measured DNA yields ranged up to *436* ng. Samples are coded by STR profile outcome. Full STR profiles were obtained from 49 samples (mean 23.616 ng, median 2.768 ng). Two samples produced partial profiles (0.068 ng and 0.044 ng), and seven samples yielded no profiles (mean 0.027 ng, median 0.024 ng)

ng and 6.712 ng, respectively. DNA typing revealed a mixed DNA profile of the accused and the victim for the anal canal swab and a single-source profile of the accused from the perianal swab. Presumptive and confirmatory testing as well as microscopic analysis was negative for the presence of seminal fluid and spermatozoa. While DNA of the accused was indeed found on the victim's anal area, the source of his DNA could not be determined and with the DNA report alone a sexual activity could not be proven. Hence, the detection of pre-ejaculate could have substantially contributed to reconstructing the course of events, indicating sexual activity.

Variation in DNA yield may be explained by inter-individual differences as well as the sampling process itself; it is important to note that some men produce little to no pre-ejaculate, whereas others produce comparatively large amounts [4].

Furthermore, the actual amount of pre-ejaculate material present on the swab could not be controlled in this study. Overall, due to the nature of sampling—with donors collecting it without supervision in the privacy of their homes—sampling cannot be standardized.

In addition, swab handling was not standardized: Since one swab was used across all analyses to rule out the presence of spermatozoa as far as possible, we cannot confirm that the sample volume remained consistent. This lack of standardization may be a contributing factor to the strong variability in DNA concentrations observed, as some samples contained more material than others; consequently, DNA quantification provided an essential indication that biological material was present. Additionally, it should be noted that DNA was extracted from the sample pad of the immunochromatographic tests [2] rather than directly from the swabs, meaning that only a fraction of the material present on the swab was utilized.

Another limitation is the direct smearing of swabs onto microscopic slides and filter papers to minimize sample loss. Although positive (semen) and negative (water) controls were run with each batch to monitor detection reliability, they do not confirm sufficient material transfer in individual samples. DNA analysis did confirm biological material presence on the swabs but cannot rule out incomplete or non-representative transfer during smearing. It therefore cannot be ruled out that insufficient transfer in individual samples led to a false-negative result. In our study, about half of the samples contained spermatozoa and had to be excluded from further analyses. This finding is consistent with previous studies, some reporting the presence of spermatozoa in collected pre-ejaculate samples, others reporting their absence—highlighting the difficulties encountered in pre-ejaculate sample collection [4, 6, 8, 10, 12, 14]. Another aspect to consider is that this exclusion criterion cannot fully rule out that ejaculate from donors with azoospermia—who do not produce detectable spermatozoa—was misclassified as pre-ejaculate. This risk is somewhat reduced, though not eliminated, by recruiting donors of reproductive age and by written instructions specifying early-enough collection to avoid mixing with ejaculate.

Moreover, the absence of detectable cells in some samples despite sufficient DNA for STR typing may not solely be attributable to insufficient transfer during smearing, as discussed above; rather, the origin of DNA could not be determined conclusively, as the cellular composition of pre-ejaculate appears to be highly variable and currently remains poorly understood.

This is reflected in the study's Sperm Hy-Liter™ staining results, with some samples showing detectable cells, whereas others displayed no cells or cell-like structures despite sufficient DNA for full STR typing.

As it remains unclear whether the DNA in pre-ejaculate originates from urethral epithelial cells or from cell-free DNA, and given the ongoing debate about the molecular characteristics of this body fluid [4, 6, 7, 12, 14], interpreting the microscopic results proved challenging.

Our study, conducted on the basis of questions arising from a case, also demonstrated that body fluid tests reacted differently towards the presence of pre-ejaculate; inconsistent results were yielded, emphasizing the unknown molecular composition of this body fluid and the potential difficulties that complicate forensic interpretation in casework.

One possible explanation for these findings is that low quantities of PSA and acid phosphatase may be present in pre-ejaculate, leading to positive results in some samples but not in others. The review of Kelly et al. [9] summarized previous findings on pre-ejaculate and firstly highlighted its relevance in the forensic context. Consistent with our findings, studies summarized that semenogelin assays yield negative results for pre-ejaculate samples, as it is specific to seminal fluid [16], and reported the presence of PSA and acid phosphatase in pre-ejaculate [17].

Conclusion

The results of this study emphasize that pre-ejaculate represents a forensically relevant body fluid as it contains high amounts of DNA suitable for STR analysis. The presence of pre-ejaculate may support the assumption that a sexual encounter has occurred, even when no spermatozoa were found. Currently, pre-ejaculate may be overlooked during forensic investigations, especially when body fluid tests specific to seminal fluid return negative. To date, no validated method for its specific detection is available.

The exact cellular composition of pre-ejaculate remains unclear. Thus, further research is essential to characterize this body fluid and enable its reliable identification as a distinct body fluid.

This is important for police investigation as pre-ejaculate seems to be a key DNA source in sexual offences.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00414-026-04003-9.

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Data availability The original raw data are available and can be provided on request. Not published due to the significant proportions of the datasheet.

Declarations

Compliance with ethical standards Samples were collected with informed consent using procedures approved by the local ethical committee (Ethik-Kommission der Ärztekammer Westfalen-Lippe und der Universität Münster).

Competing interests The authors declare no competing interests.

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