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International Journal of Forensic Sciences
ISSN: 2573-1734

# Superabsorbent Sanitary Pads as Evidence in Sexual Aggression Cases

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**Mini Review**

**Volume 5 Issue 4**

**Received Date:** November 25, 2020

**Published Date:** December 17, 2020

DOI: 10.23880/ijfsc-16000214

## Abstract

Bodily fluids recovered from sexual assault cases are key evidence in forensic investigations. Through the detection and extraction of bodily fluids, it may be possible to recover male-cell DNA and obtain a genetic profile which might help identify the perpetrator. Nowadays, the recovery of bodily fluids as evidence for posterior genetic profiling still represents a difficult challenge.

Firstly, the usual methods for bodily fluid identification can be partially destructive, using a portion of the sample for analysis. The difficulty increases when bodily fluids, mainly semen, must be extracted from substrates like superabsorbent sanitary pads, which provide intimate and recent evidence. Superabsorbent sanitary pads are composed of several layers with different compositions and absorbing capabilities. The lower layer is the most absorbent, composed of superabsorbent polymers (SAPs). Currently, only the upper SAP-free layers of superabsorbent pads are used for extracting bodily fluids after incubation with deionised water, since SAPs in the lower layer form a hydrogel when in contact with fluids. Thus, the validation and implementation of semen extraction procedures are currently needed to recover higher quantities of retained bodily fluids within the lower cores. Recently, some authors have suggested different strategies for the extraction of semen and male-cell DNA from SAPs, by both a chemical and physical treatment approach, to promote the dewaterisation and a physical separation of the SAPs from the biological material. The present work is a review of the up-to-date research on superabsorbent substrates like sanitary pads as an important evidence of sexual aggression cases at the forensic laboratory.

**Keywords:** Superabsorbent Sanitary Pads; Bodily Fluids; Semen Extraction; Forensic Evidence; Sexual Aggression

## Introduction

The finding of relevant bodily fluids in sexual assault samples is of extreme importance for the forensic investigation. Their detection and recovery allow the characterisation of the perpetrator's genetic profile, helping to associate or dismiss a suspect from involvement [1]. Swabs taken from the victim's body are commonly used to collect the bodily fluids, as well as other samples like the victim's underwear and other substrates – surfaces, sheets, and clothing - present during the aggression [2]. Similarly, superabsorbent sanitary pads or diapers used during and

after the sexual assault might be collected for analysis. Immediately after sexual intercourse most of the semen flows back out. However, the remaining will be slowly expelled during the following several days to the underwear or superabsorbent sanitary pads [3,4]. Superabsorbent sanitary pads are an important evidence when the samples collection is performed, within the 72 hours after the presumptive abuse, because they are likely to be used during or after the aggression [5,6], so they may contain remains of the aggressor's semen. Nevertheless, obtaining bodily fluids from this support material, for posterior genetic profiling, is still a challenge. Currently, the methods used for sperm cells recovery retained in pads do not consider their lower core, where more quantity of bodily fluids is retained within the superabsorbent polymers (SAPs) [7,8].

## Superabsorbent Sanitary Pads

Superabsorbent sanitary pads are composed of different layers with various compositions. According to literature and the manufacturers' information [9,10], the first layer is porous and made of hydrophobic polypropylene and polyethylene; and the lower layer, the absorbent core, is composed of cellulose and sodium polyacrylate salts. The sodium polyacrylate salts are crosslinked hydrophilic polymers and are called superabsorbent polymers (SAPs), as they are designed to swell, absorb and retain large volumes of water or other fluids in a ratio highly superior to their dried weight [11-15] (Figure 1). Thus, bodily fluids will be absorbed in the core by the cellulose and the SAPs, forming hydrogels. The aim of the hydrogel is to retain the fluidic materials, preventing a flow back into the surface of the pad, with a low dewaterisation capability [14,16].

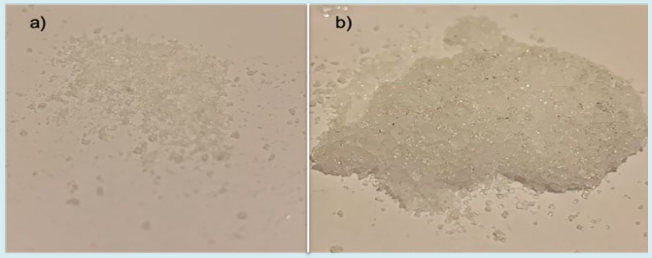


**Figure 1:** Superabsorbent polymers: a) polyacrylate salts before waterisation; b) hydrogel generated after waterisation.

Considering the fact that these materials are in contact with the intimate parts of the victim and their capability to retain bodily fluids and, thus, maintaining the integrity of these fluids and their DNA [17], this type of evidence is of great importance in forensic genetics related to sexual aggression cases.

## Detection of Bodily Fluids

Current analytical approaches for the bodily fluid detection at the forensic laboratory usually involve biochemical and immunological tests which require a portion of the sample. Specifically, to detect the presence of semen, forensic laboratories may perform the presumptive Acid Phosphatase assay, followed by the confirmatory observation of spermatozoa, by optical microscopy with Christmas-Tree staining, and the immunological tests of Prostate Specific Antigen (PSA) and Semenogelin antigen detection [18-20]. The mentioned assays require an initial elution of the sample in deionised water, which cannot be applied to superabsorbent pads. Thus, Gregório, et al. [21] proposed a non-destructive screening of bodily fluids, combining analytical vibrational spectroscopy and chemometric analysis of spectral data. This method is able to differentiate semen from other bodily fluids impregnated in superabsorbent pads, as well as in cotton fabric, and to identify spots with higher content of semen to improve the posterior male-cell DNA extraction.

## Extraction of Semen for Genetic Profiling

Regarding the extraction of bodily fluids, the current protocols only consider the elution of the upper layers of sanitary pads, because SAPs from the lower layers would block the routinely used elution [4,15]. Giusti, et al. [17] published the first article on the semen extraction considering all layers of the sanitary pads, by cutting the substrate into small pieces followed by an incubation with PBS and 2% Sarkosyl® at 4°C, and a filtration with a nylon membrane, which showed a minor recovery of male DNA. Later, Hulme, et al. [22] studied the semen/DNA recapture from the lower superabsorbent core of a panty-liner, eluting in water and a *Sperm Elution*® buffer, and the method resulted inefficient due to a hydrogel formation (partial STRs profile were obtained). More recently, Camarena, et al. [4] and Gregório, et al. [23] demonstrated that when considering solely the upper layers a significant lower sperm cells recovery is obtained, in comparison to a possible optimized elution protocol considering all layers of the substrate. Camarena, et al. [4] extracted semen from stained diapers through non-commercial fabric filters after an incubation in TNE buffer-salt solution, with a DNA yield recovery of 20-30% in the upper layers versus 40-50% considering the lower core with full STR profiles. The filtration step was necessary to separate SAPs to avoid hindering the sperm visualization and blocking the genetic profiling.

Gregório, et al. [24] compared the incubation results of different semen-embedded thick and thin sanitary napkins, panty-liners and diapers, considering the extraction from all layers after incubation in water, 0.1M NaCl, TNE buffer

and isopropanol. The elution was followed by a physical shredding treatment and filtration with commercial 10µm-pore nylon membranes inserted in NAO®basket vials, which obtained promising results with regard to both spermatozoa recovery and male-cell DNA profiling, obtaining full STR profiles regardless of the chemical treatment and type of pad. Results suggested an enhanced recapture when extracting the whole core of the semen-embedded substrates (up to 64% DNA yield) in comparison to the recovery from the upper SAPs-free layers alone (up to 8.4% DNA yield) [23]. Besides, no SAP inhibition was proved to occur when obtaining the STRs genetic profiles of semen control samples [24]. The incubation in water and 100% isopropanol obtained higher DNA yields. In that way, it was possible to evaluate the implementation of a protocol at the forensic laboratory.

In 2020, O'Connor, et al. [25] have proposed a *SAPSWash* method for the extraction of spermatozoa, similarly from the lower core of sanitary napkins, considering an incubation in a 0.5M calcium chloride solution, followed by centrifugation of the substrate within commercial spin baskets. The calcium ions interacted with the carboxyle groups of the hydrogel leading to dewaterisation. Complete STR profiles were obtained, although some samples needed a previous *Microcon* clean-up procedure. The two-latter articles [24,25] could extract semen after an incubation treatment and final commercial-filtering and showed protocols to either promote the dewaterisation of the hydrogels, altering their cross-linked network, or create a situation of molecular competition by increasing the water volume ration.

## Conclusion

The maximization of the elution of bodily fluids from all layers of the sanitary pads should be pursued; by considering the chemical and physical properties of SAPs. Forensic practitioners must take into account the effects of the dewaterisation method on the viability of cells and the quality of their DNA for further genetic profiling. An incubation/filtration protocol could be easily implemented at the forensic laboratory for an enhanced recovery of bodily fluids from SAPs-containing sanitary pads.

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