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# Maximizing semen extraction from sanitary pads by chemical and shredding treatments

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**KEYWORDS**

Superabsorbent polymers; Semen extraction; Sanitary pads; Male cell DNA; Chemical incubation; Shredding treatment

**ABSTRACT**

Evidence of sexual aggression may be obtained from superabsorbent polymer (SAP) sanitary pads, which are used by forensic laboratories for semen evaluation. Semen can be extracted from their upper layers, which are free of SAPs. However, our previous results showed a need to optimize the protocol for semen analysis by considering its extraction from the lower core, often composed of sodium polyacrylate SAPs. SAPs generate a hydrogel, which traps the cellular components, hindering the possibility of obtaining cells and hence their ge- netic material. Simple filtration has been tried previously, but further maximization by application of a treat- ment has never been

attempted. In this paper, we compare both chemical and physical shredding treatments for maximizing gel-trapped sperm and male cell DNA recaptures from hygienic superabsorbent substrates in sanitary pads, panty-liners or diapers. Our findings suggest that the lower core should be treated to induce a dewater- isation of the SAP hydrogels in order to maximize the extraction of bodily fluids.

## INTRODUCTION

Evidence of sexual aggression may be found in sanitary pads re- quired for semen detection and male cell DNA profiling in forensic laboratories. Current protocols employed for these samples include ana- lysis of their upper layers which are free of superabsorbent polymers (SAPs) [1,2]. Because of their design and composition for retaining fluids [3,4], cells and biological fluids are repelled by the hydrophobic upper layers, and become enmeshed within the SAPs in the super- absorbent lower core, forming a hydrogel [5–7]. SAPs are cross-linked polymeric materials designed to absorb fluid up to 100% of their volume [3] and to swell thermodynamically without dissolving, reaching equilibrium as a hydrogel [4,5,8]. As defined by Zohuriaan-Mehr and Kabiri [3], SAP materials should desirably have the highest absorption and the lowest rewetting rates possible, in order to take in the fluids without releasing them. Bialasiewicz et al. employed SAPs for transport of urine, forming a hydrogel to avoid leakage, whereupon the bodily fluid had to be extracted for posterior analysis [9]. This process makes it hard to extract semen from the embedding hydrogels and risks losing biological information, which could lead to an incomplete genetic profile of the suspect. Besides, sexual aggression samples are often mixtures of the victim's and suspect's bodily fluids, with a higher concentration of the female (victim's) fluids [10,11]. Consequently, there is a need to improve the method of extracting bodily fluids from sanitary pads, by taking their lower layers into account.

Hulme et al. was the first author to attempt to extract semen/DNA from the SAP substrate of a sanitary napkin by water elution and Sperm Elution Cellmark incubations, and neither procedure worked [12]. Camarena et al. demonstrated that the presence of SAPs without a filtra- tion step blocks the creation of the genetic profile because of the difficulties involved in cleaning and transferring evidence [6]. In this way, it is necessary to investigate possible methods for SAPs-free extraction of bodily fluids. Previous research referred to the positive impact on fluid extraction achieved by cutting the fabric into small pieces and filtering it with a nylon mesh [6,13] or by incubating the gel with isopropanol for urine extraction

[9]. It is also known that lowering the pH or increasing sodium chloride concentration improves the dewa- terisation of SAPs [14,15]. Some authors have suggested that applying pressure could help to liberate fluids from the SAP mesh [1,3,16]. Carson et al. recently presented the idea of applying pressure prior to the DNA extraction procedure, to improve the differential lysis of vaginal-epithelial cells and sperm cells [17]. Our aim in the present study was to combine both chemical and physical treatments to maximize the extraction of semen fluid and sperm cells from the complex structure and composition of superabsorbent pads, allowing for a more accurate fluid diagnosis and consequently better-quality extraction of male cell DNA to obtain optimised genetic profiles.

## MATERIALS AND METHODS

### *SAMPLES*

The superabsorbent pads studied herein were selected as typical examples of common commercial hygienic sanitary pads found in the market or at the forensic laboratory from sexual violence cases, and are categorised as: thick and thin-type sanitary pads/napkins (Bonté and Evax; DIA Corporate, Procter & Gamble, USA, respectively), panty-liners (Bonté, DIA Corporate) and diapers (Dodot, Procter & Gamble, USA). Thus, four types of sanitary pads, which are commonly analysed in sexual aggression cases, were subjected to the study: two types with a thin lower core mostly composed of SAPs (thin sanitary pads/napkins and panty-liners); and two types with a thick lower core, composed of cellulose fluff and SAPs (thick sanitary pads/napkins and diapers).

Semen aliquots (100 µL), at 1:50 dilution in deionised water from a normospermic anonymous donor (approx. 46,900 spermatozoa/µl, IVI semen Bank, Spain), were applied to original pad substrates for the re-

plicates of each treatment. Samples (n = 224) were cut into uniform 1.5 to 2 cm-diameter circles with sterile scissors, and allowed to dry for 1 h at room temperature (RT). The exterior plastic protection was then removed from the pads and discarded using sterile tweezers, without

separating the layers, and the samples were cut into millimetric pieces prior to incubation.

#### *ASSEMBLY*

Before starting the treatments, two types of filter membranes, 10 µm-pore nylon and polyester (PETE) membranes (Sterlitech, WA), were assembled in *NAO®Baskets* vials (Copan Italy, Brescia), and the baskets were perforated with a sterile needle.

#### *CHEMICAL TREATMENT AND FILTRATION STEP*

Samples were placed within the NAO® baskets, on top of the filters, and were incubated for 30 min at RT in a horizontal agitator at 180 U/ min, with 1 ml of one of the following reagents, respectively: dH2O, 100% isopropanol as per Bialasiewicz et al. [9], a TNE buffer (0.01 M Tris, 0.1 M NaCl, 0.001 M EDTA) and a commercial pH4 buffer (Panreac AppliChem, GmbH). The samples were then filtered by centrifugation at 10,000 rpm for 10 min to separate the liquid volume from the hydrogel and the pad structure (Fig. 1). This assay was run in duplicate for the two types of membrane (nylon/PETE membranes), considering the four treatments and the double analysis for sperm micro-visualization and male cell DNA quantification.

#### *CHEMICAL AND PHYSICAL SHREDDING TREATMENT*

A second group of samples was treated with a combined chemical and physical shredding treatment (Fig. 1). The samples were placed inside polypropylene shredder tubes with either plastic or metal lysis disks (respectively known as *PS* and *PMS* tubes) (*I&L Biosystems*, *Lyon*). After the addition of 1 ml of either $dH_2O$, 100% isopropanol or TNE, respectively, the samples were incubated for 30 min with agitation (180 U/min) at RT. After incubation, the samples were shredded with a manual *Shredder SG3*™ (*I&L Biosystems*, *Lyon*) for 6 s at medium in- tensity. The device promoted a rapid, progressive breakdown of the substrate. During the application of pressure by shredding, the samples passed through the lysis disk into the retention chamber. The released solution was introduced by pipette into the NAO®Baskets previously fitted with the

nylon membrane filters and centrifuged at 10,000 rpm for 10 min. This assay was run in triplicate for each type of pad, considering the three treatments and the two types of shredder tubes, and the triple analysis for sperm microscopy and male cell DNA quantification.

### *SPERM/MALE CELL DNA ANALYSIS*

The recovered solutions obtained after the different incubations and filtrations were analysed for both sperm visualization and DNA quantification, one replicate for each analysis. Spermatozoa visualizations were quantified using a Nikon 50iEclipse at 200×/400× magnifications, after Christmas-tree staining of the resulting sediment (50 µL). A few samples could not be pipetted directly because of jellification, and in these cases a sterile spatula was used to aid deposition of the sedi- ment onto the slide. Negative controls were used to ensure that there was no presence of epithelial/sperm cells in the substrates.

In parallel, the sediment was digested with sodium acetate 0.2 M, K- proteinase (10 mg/ml), DTT and SDS (10%) at 56 ± 2 °C. DNA was extracted using the phenol, chloroform-isoamylic (25:24:1) method in a 1:1 proportion with the obtained digestion product. The aqueous phase was then purified and concentrated with *Amicon®ULTRA-4* (*SIGMA- Aldrich*) to maximize DNA recapture from any entangled polymer or chemical derivate and thus concentrate the template for higher opti- mization. DNA was quantified using the *Quantifiler™Trio DNA Quantification kit (Thermo Scientific™) on an ABI Prism 7500 instrument*

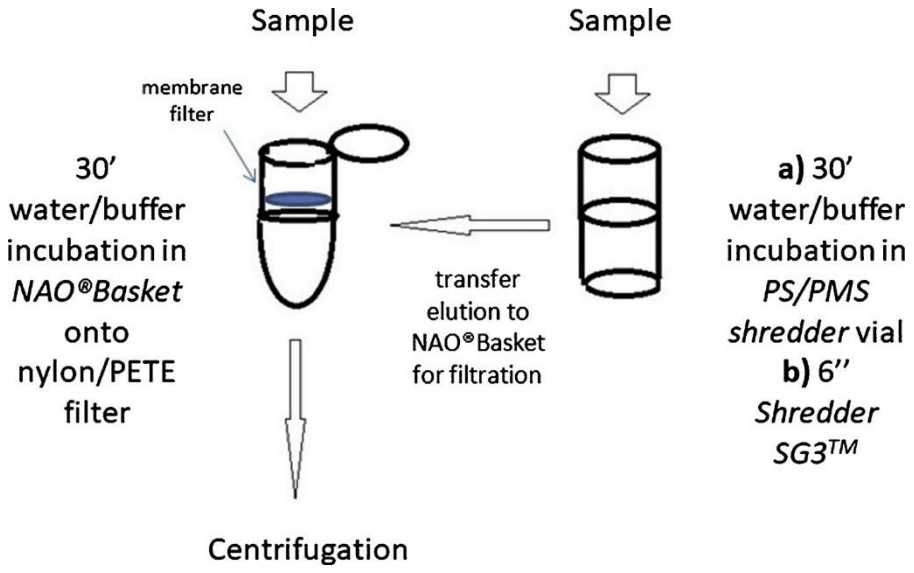


**Fig. 1.** Schematic diagram of incubation treatment for both chemical and chemical/ physical shredding procedures. Samples are set directly onto the nylon/PETE filters within the *NAO®Basket* vials for incubation and then centrifugation follows to obtain a filtered SAPs- free volume. In the chemical/shredding treatment, samples are set (a) within the PS/PMS shredding vials for incubation, following (b) pressure with the SG3™ mechanism and, lastly, they are transferred to the *NAO®Basket* prior to centrifugation.

(*Life Technologies*), per manufacturer's specifications. The data was analysed using *HID Real-Time PCR Analysis software v1.2*. Each assay (extraction and quantification) was carried out at least in duplicate for each treatment when the obtained volumes were SAP-free. For a quality assessment of the resulting bodily-fluid DNA, extracted from SAPs after the different treatments, a posterior DNA amplification (Short Tandem Repeat, STR- typing) was performed to determine that the contact of SAP and/or treatments did not inhibit DNA amplification, as inhibition by the SAPs and their effect on downstream DNA analyses was an area of concern [6]. A *PowerPlex® Fusion 6C kit* (*Promega*) on a *Gen- eAmp®PCR System 9700* (*Applied Biosystems*) was carried out, per the manufacturer's specifications. PCR products were separated and detected by capillary electrophoresis on an *8-capillary 3500 Genetic Ana- lyzer* (*ThermoFisher Scientific*) and analysed with *GeneMapper® ID soft- ware v3.2*, per manufacturer's specifications, with an analytical threshold value of 150 relative fluorescent units (RFUs). Even when the DNA yield was relatively low in some replicates, the DNA amounts for amplification were adjusted to 1 ng/µL of the DNA template, and the resulting PCR products were injected with a 2-µL volume, and run at 1.2 kV on the 3500 analyzer. STR typing followed general SWGDAM recommendations and analyses were performed using manufacturer- provided allelic ladders, bins and panels. Electropherograms showed complete, clean and balanced profiles (peak balance ratios > 60%), regardless of the type of treatment (water, isopropanol, TNE; Supp. Fig. 1) and for all the substrate pad types. Positive and negative controls were used for extraction, quantification, amplification and profile editing.

Spermatozoa and male-cell DNA recaptures from the original seeded aliquot were estimated in duplicate, as in similar experiments [12], knowing the volume, dilution, and average number of spermatozoa per millimetre of semen for the donor (Section 2.1). Thus, the number of spermatozoa and male-cell DNA concentration within original seeded aliquots (100 µL of sperm at 1:50 dilution) was first calculated (avg. 46.36 ng). Therefore, the percentages of recovery were estimated from

these values. The significance of variance among the variables and treatments was analysed. A one-way ANOVA was applied to determine whether differences among treatments were significant ($\alpha < 0.05$). Paired t-test and *CHI-SQUARE* comparisons were applied. Results were calculated as a recovery percentage, taking into account the sperma- tozoa count and male cell DNA quantification in the original semen aliquots.

## RESULTS AND DISCUSSION

### *CHEMICAL TREATMENTS*

Optimized centrifugation with 10 µm-pore filter membranes in- serted in *NAO®Baskets* allowed the separation of semen from the hy- drogels. The presented results are mean recapture values for all the types of superabsorbent pads tested after filtration of the chemically treated samples (Table 1). The use of either nylon or PETE filter membranes did not influence the results for sperm recapture ($F = 1.88$, $df = 30$, $p > 0.05$) and DNA quantification ($F = 1.19$, $df = 30$, $p > 0.1$). Therefore, since the nylon membranes are easier to handle when arranged in the baskets (as in the present work) [2], further studies were performed using the nylon filters. In this way, our study follows Camarena et al (*SEASHOLS-WILLIAMS S., pers. comm.* 2016 – *unpublished results*) [19], in which nylon filters were preferred because of their easier manipulation and adhesion to the bottom of the basket. It was possible to combine the results obtained for both membranes, and therefore, it can be stated that the assay was run in quadruplicate. Aside from this, on average, pH4-buffer incubations yielded the lowest semen recaptures (Table 1), and therefore, the other three types of solution – water, TNE and isopropanol – were continued with the single nylon filtering procedure, for comparison during the second step (combina- tion of chemical and shredding treatment).

**Table 1.** Average ( ± S.D.) recapture percentage of spermatozoa count and male-cell DNA quantification from semen-embedded pads (1–4) after filtration with nylon and PETE membranes and incubation treatments with (a) water, (b) isopropanol, (c) TNE and (d) pH4 buffer.

| Pads type | % sperm | % DNA yield | Treatment |
|---|---|---|---|
| (1) Thick sanitary napkin n = 32 | 0.57 ± 0.70* | 14.80 ± 0.04 | $H_2O$ |
| | 0.07 ± 0.02 | 0.60 ± 0.00 | Isopropanol |
| | 0.004 ± 0.006 | 6.80 ± 0.01* | TNE |
| | 0.003 ± 0.003 | 0.35 ± 0.004 | pH4 buffer |
| (2) Thin sanitary napkin n = 32 | 0.36 ± 0.20 | 64.00 ± 0.65* | $H_2O$ |
| | 0.05 ± 0.03 | 0.90 ± 0.00 | Isopropanol |
| | 0.21 ± 0.16 | 5.50 ± 0.02* | TNE |
| | 0.25 ± 0.17 | 1.70 ± 0.004 | pH4 buffer |
| (3) Panty-liner n = 32 | 1.71 ± 1.40* | 16.13 ± 0.12* | $H_2O$ |
| | 1.97 ± 2.40* | 8.15 ± 0.10 | Isopropanol |
| | 2.21 ± 1.00* | 18.96 ± 0.15* | TNE |
| | 0.15 ± 0.02 | 4.33 ± 0.02 | pH4 buffer |
| (4) Diaper n = 32 | 1.36 ± 0.10* | 9.28 ± 0.10* | $H_2O$ |
| | 0.30 ± 0.05 | 1.86 ± 0.005 | Isopropanol |
| | 2.10 ± 0.01* | 16.82 ± 0.01* | TNE |
| | 0.17 ± 0.02 | 1.40 ± 0.015 | pH4 buffer |
| Pad type | 0.16 ± 0.27 | 5.64 ± 6.70 | (1) Thick |
| | 0.22 ± 0.13 | 20.5 ± 30.00* | (2) Thin |
| | 1.51 ± 0.93* | 11.89 ± 6.80* | (3) Panty-liner |
| | 0.98 ± 0.92 | 7.34 ± 7.20 | (4) Diaper |
| Treatment | 1.00 ± 0.64* | 26.05 ± 25.47* | (a) $H_2O$ |
| | 0.59 ± 0.92 | 2.88 ± 3.56 | (b) Isopropanol |
| | 1.13 ± 1.19* | 12.02 ± 6.85* | (c) TNE |
| | 0.14 ± 0.10 | 1.95 ± 1.69 | (d) pH4 buffer |
| | | Pairwise comparison | |
| Overall Pad type | Overall treatment | $H_2O$ vs pH4 | % sperm: $t = 2.75$* |
| % sperm: $F = 4.82$* | % sperm: $F = 9.54$* | Iso vs TNE | % DNA: $t = -2.59$* |
| % DNA: $F = 4.65$* | % DNA: $F = 6.04$* | TNE vs pH4 | % DNA: $t = 3.24$** |

* $p < 0.05$.
** $0.001 < p < 0.01$.

The best overall recovery rate for all substrates was obtained with water elution (26.1 ± 25.5% male cell DNA, 1.00 ± 0.6% sperm), whereas the elution with pH4-buffer produced the lowest values (Table 1). Semen recaptures with TNE yielded a lower DNA percentage (12.02 ± 6.9%) and sperm results were similar to the water incubation (1.13 ± 1.2%). Thus, when comparing the treatments pairwise, water versus pH-4 buffer obtained significantly different sperm recaptures ($t = 2.75$, $df = 14$, $p = 0.016$). Meanwhile, TNE presented significant DNA yield differences compared to pH4-buffer ($t = 3.24$, $df = 14$, $p = 0.006$) and isopropanol ($t = 2.59$, $df = 14$, $p = 0.021$) (Table 1). Overall differences in the percentage of sperm/male-cell DNA quantification were significant for the treatment comparison ($F = 9.54$ *-sperm*; $F = 6.04$ *-DNA yield-*, $p < 0.05$) (Table 1, supporting data in Supp. Table 1). Incubations of the thin-type napkins produced the highest DNA recovery yield (64%). In fact, thin napkins and panty-liner types offered better recaptures than the thick type substrates ($F = 4.82$ *-sperm,* $F = 4.65$ *-DNA yield,* $p < 0.05$; Table 1). The incubation step followed by filtration increased the cellular and DNA quantifications (up to 2.8x) when compared with the simpler protocol of eluting the SAPs-free upper layers [2].

Recapture sperm yields for the four chemical treatments showed generally low values, although incubation with water and TNE presented better results (Table 1, supporting data at Supp. Table 1). It would be expected to observe a dewatering of the hydrogels when the pH is significantly lowered by substitution of $Na^+$ ions with $H^+$ ions, or the sodium concentration is raised by the use of TNE [14,15]. In contrast, our work showed that when incubating hydrogels with a low pH buffer treatment, like the pH4- buffer, recapture results were on average less efficient for all pad types.

**Table 2.** Average ( ± S.D.) recapture percentage of spermatozoa count and male-cell DNA quantification from the semen-embedded pads (1–4) after applying the shredding pressure in PS and PMS *Shredder* tubes. Filtration was followed after the incubation treatments with (a) water, (b) isopropanol –Iso., and (c) TNE.

| Pads type | PS tube | | PMS tube | | Treatment | |
|---|---|---|---|---|---|---|
| | % sperm | % DNA yield | % sperm | % DNA yield | % sperm | |
| (1) Thick sanitary napkin n = 24 | 0.01 | – | 0.08 | – | $H_2O$: 0.05 ± 0.04 | |
| | 0.24 | 1.25 | 0.14 | 0.61 | Iso.: 0.19 ± 0.07 | |
| | 0.01 | – | 0.02 | – | TNE: 0.02 ± 0.01 | |
| (2) Thin sanitary napkin n = 24 | 0.03 | – | 0.20 | – | $H_2O$: 0.12 ± 0.12 | |
| | 0.43 | 11.21 | 0.14 | 2.22 | Iso.: 0.29 ± 0.20 | |
| | 0.08 | – | 0.04 | – | TNE: 0.06 ± 0.03 | |
| (3) Panty-liner n = 24 | 0.02 | – | 0.02 | – | $H_2O$: 0.02 ± 0.00 | |
| | 1.27 | 15.88 | 1.11 | 23.98 | Iso.: 1.19 ± 0.11 | |
| | 0.04 | – | 0.03 | – | TNE: 0.04 ± 0.01 | |
| (4) Diaper n = 24 | 0.38 | – | 0.51 | – | $H_2O$: 0.45 ± 0.09 | |
| | 1.52 | 9.70 | 1.20 | 10.07 | Iso.: 1.36 ± 0.23 | |
| | 0.34 | – | 0.19 | – | TNE: 0.27 ± 0.10 | |
| Treatment | 0.11 ± 0.18 | – | 0.20 ± 0.22 | – | (a) $H_2O$: 0.16 ± 0.19 | (PS + PMS) |
| | 0.87 ± 0.63 | 9.51 ± 6.10 | 0.65 ± 0.59 | 9.22 ± 10.67 | (b) Iso.: 0.42 ± 0.40* | % sperm: $F = 6.91$* |
| | 0.12 ± 0.15 | – | 0.07 ± 0.08 | – | (c) TNE: 0.09 ± 0.11 | |
| | % sperm | % DNA yield | (PS + PMS) | | | |
| Pad type | 0.08 ± 0.09 | 0.93 ± 0.45 | (1) Thick napkin | % sperm: | | |
| | 0.15 ± 0.14 | 6.72 ± 6.36 | (2) Thin napkin | $F = 4.82$* | | |
| | 0.42 ± 0.60* | 19.48 ± 5.09** | (3) Panty-liner | % DNA: | | |
| | 0.36 ± 0.13 | 9.89 ± 0.26 | (4) Diaper | $F = 6.59$** | | |

* $p < 0.05$.
** $0.001 < p < 0.01$.

### *CHEMICAL AND SHREDDING TREATMENT*

When a physical treatment was also applied, the manual pressure device promoted a rapid, progressive and reproducible breakdown of the supporting material. After shredding with the two types of available vials, PS and PMS shredder tubes, the released solution was then centrifuged and filtered in the NAO®Baskets with the manually inserted nylon membranes. There was no significant difference in results be- tween the PS and PMS tubes for sperm recapture ($F = 0.31$, $df = 22$, $p > 0.1$) and DNA yields ($F = 0.17$, $df = 6$, $p > 0.1$). The combination of chemical and physical treatments was more efficient when using isopropanol, rather than water or TNE ($F = 3.13$, $d.f. = 69$, $p < 0.001$). Thus, only isopropanol incubation with pressure shredding improved the results for sperm cell and male

cell DNA recapture (Table 2). In fact, it was not possible to perform the DNA extraction after incubation with either water or TNE after the shredding process, due to the persistence of the hydrogel structure, which hampered the recovery of the liquid volume. Therefore, the aliquots for DNA quantification could not be tested with these treatments, as it was not possible to pipette the resulted elution due to the presence of the hydrogel. With regard to the incubation with isopropanol, one previous study, aside from the present work, has similarly reported the successful ex- traction of a bodily fluid (urine) from a hydrogel substrate, yielding positive results [3].

The combination of shredding plus isopropanol proved more efficient than isopropanol elution alone. This appears to indicate that physical treatments may assist the dewaterisation of SAP hydrogels [1,3]. Other equipment, such as rheometers or pressure cycling technology, applied prior to filtration, may have a stronger effect in changing the physical conditions of hydrogels [1,3,18]. Other previous studies have also showed that raising the temperature while applying pressure has a great effect on the kinetics of the hydrogels [16]. However, in our experiment, temperature variance was not tested due to the risk of lysing the sperm cell membranes.

The routinely used method in forensic laboratories for centrifuging a previously incubated sample, swab, cloth remains, or other substrates, such as pad tissue, is the piggyback method. This consists of inserting a previously perforated 1.5-ml eppendorf inside another 1.5-ml tube to be centrifuged; the evidence is introduced into the perforated tube, once the samples have been incubated in elution buffers or deionized water. During the centrifugation, the eluted solution will pass into the second tube and form a pellet. The advantage provided by *NAO®Baskets* over the standard piggyback method is the possibility of installing filter membranes horizontally in the basket vial, prior to incubation, to allow the subsequent filtration of a SAP-free bodily fluid. When applying the filters, it was necessary to form a single perforation in the bottom of the basket in sterile conditions to help the filtration process in the *NAO®Baskets* during centrifugation. It should be noted that pad sub- strate evidence presents polyacrylate salts (in the lower core) which form the hydrogel in combination with liquid, and hence the common incubation procedure for this type of substrate by the piggyback

method, at the forensic laboratory, avoids the lower core and only uses the upper layer which is SAP-free [2]. Therefore, the filter membranes in the basket vials presented an important barrier, which enhanced the separation of fluids from the SAP hydrogels that were formed when liquid volumes were absorbed by the pad substrates in our experiments.

When considering all the types of treatment and pads presented in our work, the most efficient was water elution in thin napkins (up to 64% DNA recapture); therefore, increasing the water volume ratio be- fore filtration could improve the recovery of sperm cells, as has been demonstrated for other bodily fluids [20]. For practical reasons at the forensic laboratory, simple water incubation seems to be efficient for the extraction of semen from sanitary pads, providing a simpler methodology than the combination of a shredding procedure with isopropanol. Also, some SAPs substrates have been reported to be highly pH-sensitive and to exhibit reversible swelling/deswelling be- haviour when the gel is alternatively exposed to deionized water as well as to sodium chloride [20]. However, drastic pH variations at the in- cubation step did not significantly increase semen extraction in our study.

With regard to the comparison of pad types, the thin panty-liner type provided better outcomes for the male-cell quantification (19.5 ± 5% DNA yield) with the combination of isopropanol/shred- ding procedures (Table 2). These values were among the highest, and for the four pad types, isopropanol produced a 9.4% mean recapture of the total inoculated semen. In fact, when the shredding treatment was applied, the DNA extraction was only possible after the isopropanol incubation. Thus, both sperm and male cell DNA yields varied ac- cording to the type and consequent composition of sanitary pads. Some pads may have a lower core filled with a mix of cellulose fluff and SAPs, others may have thinner lower cores filled mostly with SAPs [5]. Ge- netic profiles could be obtained for the different treatments without noticing interference from the type of incubation reagent (water, iso- propanol and TNE) or SAP-containing substrate (thick/thin pads; Supp. Fig. 1), the latter having also been assessed by Camarena et al. [6]. The global application of a validated method for treating this type of evi- dence is important in forensic genetic analysis to be able to genetically process the biological evidence without the

presence of SAPs and thus avoid the formation of hydrogels.

With the present methodology, it is possible to obtain genetic sperm yields from the SAP-containing material in sanitary pads, after in- cubation and the described centrifugal filtering method, which im- proves the extraction of the bodily fluid by elution from the upper layers that are free of SAPs. Future research into semen extraction from different SAP compositions should consider saturation of the gelled solution in water to facilitate the release of biological fluids, as achieved recently by Liang et al. (2009) [20]. It is likely that to con- tribute to the extraction of absorbed body fluids, the free water com- ponent must be greater than the water retained in the polymer [3]. Our findings showed that water and TNE elutions and the isopropanol- eluted shredding of the whole core (upper and lower layers), prior to filtration, yielded better results than the routinely used method that is only applied to the upper layers [2]. Although there are innumerable publications describing methods for dewaterisation of SAPs, forensic practitioners must take into account the possible effects of those methods on the viability of sperm cells and the quality of their DNA for further genetic profiling.

## CONCLUSION

Treatments applied to the lower core of pads may maximize semen extraction from sanitary pads, panty-liners and diapers. Methods that interact with the chemical and physical properties of SAP hydrogels and promote their dewaterisation must be pursued at the forensic laboratory.

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## CONFLICT OF INTEREST

The authors declare they have no competing interests.

## APPENDIX A. SUPPLEMENTARY DATA

Supplementary material related to this article can be found, in the online version, at doi: https://doi.org/10.1016/j.fsigen.2019.07.014.

## REFERENCES

[1] D.V. Nori, A Novel Method for Rapid and Selective Extraction of Male DNA From Rape Kits Using Alkaline Lysis and Pressure Cycling Technology (PCT), Florida international University, FIU Electronic Theses and Dissertations, (2014) (Accessed 05 February 2019), https://digitalcommons.fiu.edu/cgi/viewcontent.cgi?referer= https://www.google.com/&httpsredir=1&article=2709&context=etd.

[2] I. Gregório, P. Martínez, Characterising sanitary napkins, panty-liners and diaper types: intimate evidence for semen identification, Forensic Sci. Int. Genetics Sup. Ser. 6 (2017) e582–e584.

[3] M.J. Zohuriaan-Mehr, K. Kabiri, Superabsorbent polymer materials: a review, Ir. Polymer J. 17 (2008) 451–477.

[4] M. Elliot, Superabsorbent polymers, Product Development Scientist for SAP BASF Aktiengesellschaft, BASF, Ludwigshafen, Germany, 2008, pp. 1–14.

[5] F.L. Buchholz, Superabsorbent polymers: an idea whose time has come, J. Chem. Educ. 73 (1996) 512–515.

[6] L.R. Camarena, B.K. Glasscock, D. Daniels, N. Ackley, M. Sciarretta, S.J. Seashols-Williams, An optimized centrifugal method for separation of semen from super-absorbent polymers for forensic analysis, J. Forensic Sci. 62 (2017) 411–416.

[7] I. Gregorio, F. Zapata, C. Garcia-Ruiz, Analysis of human bodily fluids on super-absorbent pads by ATR-FTIR, Talanta 162 (2017) 634–640.

[8] A. Sannino, C. Demitri, M. Madaghiele, Biodegradable cellulose-based hydrogels: design and applications, Materials 2 (2009) 353–373.

[9] S. Bialasiewicz, D.M. Whiley, M. Buhrer-Skinner, C. Bautista, K. Barker, S. Aitken, R. Gordon, R. Muller, S.B. Lambert, J. Debattista, M.D. Nissen, T.P. Sloots, A novel gel-based method for self-collection and ambient temperature postal transport of urine for PCR detection of Chlamydia trachomatis, Sex. Transm. Infect. 85 (2009) 102–105.

[10] P. Gill, R.M. Brown, M. Fairley, L. Lee, M. Smyth, N. Simpson, B. Irwin, J. Dunlop, M. Greenhalgh, K. Way, E.J. Westacott, S.J. Ferguson, L.V. Ford, T. Clayton, J. Guiness, Technical UK DNA working group, National recommendations of the Technical UK DNA working group on mixture interpretation for the NDNAD and for court going purposes, Forensic Sci. Int. Genetics 2 (2008) 76–82.

[11] N. Hu, B. Cong, S. Li, C. Ma, L. Fu, X. Zhang, Current developments in forensic interpretation of mixed DNA samples (Review), Biomed. Rep. 2 (2014) 309–316.

[12] P. Hulme, J. Lewis, G. Davidson, Sperm elution: an improved two-phased recovery method for sexual assault samples, Sci. Justice 53 (2013) 28–33.

[13] A. Giusti, M. Baird, S. Pasquale, I. Balazs, J. Glassberg, Application of deoxyribonucleic acid (DNA) polymorphisms to the analysis of DNA recovered from sperm, J. Forensic Sci. 31 (1986) 409–417.

[14] G.K. Elyashevich, N.G. Bel'nikevich, S.A. Vesnebolotskaya, Swelling-contraction of sodium polyacrylate hydrogels in media with various pH values, Polym. Sci. 51 (2009) 550–553.

[15] J.E. Elliott, M. Macdonal, J. Nie, C.N. Bowman, Structure and swelling of poly (acrylic acid) hydrogels: effect of pH ionic strength, and dilution on the crosslinked polymer structure, Polymer 45 (2004) 1503–1510.

[16] M. Bakass, A. Morhlisse, M. Lallemant, Absorption and desorption of liquid water by a superabsorbent polymer, J. Appl. Polym. Sci. Symp. 83 (2002) 234–243.

[17] C. Carson, A. Garvin, K. Gorman, Final Technical Report: Automated Processing of Sexual Assault Cases Using Selective Degradation, National Criminal Justice Reference Service, 2013.

[18] D.V. Nori, B.R. McCord, The application of alkaline lysis and pressure cycling technology in the differential extraction of DNA from sperm and epithelial cells recovered from cotton swabs, Anal. Bioanal. Chem. 407 (2015) 6975–6984.

[19] L.R. Camarena, S.J. Seashols-Williams, Optimized Centrifugal Methods for Separation of Semen From Superabsorbent Polymers for Forensic Analysis, American Academy of Forensic Sciences, Washington DC, 2013.

[20] R. Liang, H. Yuan, G. Xi, Q. Zhou, Synthesis of wheat straw-g-poly (acrylic acid) superabsorbent composites and release of urea from it, Carbohydr. Polym. 77 (2009) 181–187.