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Abstract

Despite increased reporting figures for sexual offences, it is believed that this crime type is under-reported. Support information stresses the need to preserve forensic evidence, including the victim not washing themselves/their clothes. The bath scrunchie is a popular choice in the UK as a personal cleaning instrument, and its ability to retain semen was examined in this study. Retention of Acid Phosphatase (AP) and spermatozoa was explored on new and previously used bath scrunchies, in the presence/absence of Dove® body wash. Seventy-seven percent of samples were positive for AP and spermatozoa were recovered from 99%, indicating that the bath scrunchie is suitable for recovering this evidence type. Higher spermatozoa recovery was possible from the rope in comparison to the net, as a consequence of differences in surface area, but recovery in the presence of Dove® body wash requires further examination, as this was variable. The compactness of the bath scrunchie affected spermatozoa recovery from the net, but did not appear to affect recovery from the rope. These preliminary study results show that the bath scrunchie could be of value as an evidential item if the victim has bathed or showered post-sexual offence and prior to reporting to the Police.

Key words

semen, spermatozoa, bath scrunchie, non-invasive evidence collection, sexual offence

Main Text

1.0 Introduction

International figures for total sexual violence, which includes rape, sexual assault and sexual offences against children, show an overall increase in rate from 30.92 per 100 000 population in 2008 to 38.53 per 100 000 population in 2013[1]. Although interpretation of these figures should be cautiously completed, due to the differences in legal definitions and the methods of offence counting and recording found across the world, there is still a worrying upwards trend. In Australia a total of 85.3 (rate per 100 000 population) was reported in 2013 compared with 29.5 in 2008, with the rates for England and Wales being 99.3 in 2013 compared with 74.6 in 2008[1]. The literature on the reporting of rape and sexual assault concludes that these crime types are generally under-reported due to the psychological stresses experienced by the victim[2-5]. These traumas can manifest themselves in a variety of ways, but one which is seen frequently is that of delayed reporting of an offence[6], even more than 72 hours[7]. It is believed that, in addition to better recording methods, the determining factor behind the further increase observed in the UKs rate in 2014-15, is a greater willingness of victims to come forward[8]. This is postulated, by those involved in charitable organisations and the media, to be as a direct result of increased public awareness as a consequence of Operation Yewtree[9] and other high-profile sexual offence cases.

The support that is now available to victims of sexual offences is wide and varied from comprehensive information on the internet (often produced by charitable organisations), handbooks and leaflets (e.g. From Report to Court[10]), directly from Sexual Assault Referral Centres (SARCs) and from the Police and government organisations, such as the NHS in the UK. Advice to victims of recent offences emphasises the need to preserve evidence by, for example, not washing yourself or your clothing. This however, is not the case in all instances as a number of victims will wash themselves after an incident[5,11,12]. The introduction of SARCs was intended to enable the forensic examination of sexual assault victims in a clean and sterile environment, whilst offering suitable support and guidance[13]. As the collection of forensic evidence may cause further distress[2,4], it is important to explore and utilise methods and procedures which aim to limit the potential for this.

In previous work, Page, *et al*[14], showed that it was possible to use an Evidence Recovery System (ERS) to recover spermatozoa from bathwater. It was proposed that the ERS could be used post-sexual assault in a SARC as a non-invasive alternative to a full medical examination or as a means to obtain spermatozoa from the bath/shower of a victim who had washed. Having determined the feasibility of recovering spermatozoa from bath/shower water, possible alternative recovery implements were considered for study. Ultimately, due to its folding, net-like structure a bath scrunchie was chosen (Figure 1). The bath scrunchie is comprised of a hydrophobic mesh sponge usually held together by a nylon band and with a rope handle[15]. The bath scrunchie is designed to be better for lathering and effectiveness during washing, with the additional benefits of reduced water-logging and bacterial growth when compared to other products on the market[15-17]. As a consequence it is a popular choice of personal cleaning instrument in the UK.

This study aimed to determine whether or not semen could be recovered from new or previously used bath scrunchies, to identify a new evidential item not previously examined, or as a new non-invasive mechanism for the recovery of evidence. A new bath scrunchie is very densely packed and becomes more loosely associated as the scrunchie is used. The authors aimed to determine whether the tightness/denseness of the mesh had an effect on the ability to recover spermatozoa and whether this may ultimately limit its use as an evidence type. In addition, and to simulate a victim washing themselves fully, a body wash was also added to ascertain any negative effect on the recovery.

AP testing is the standard protocol used for presumptive testing of semen[18]. Detection of AP after washing has occurred is reported to be difficult, due to its water-soluble nature[19-21], but some success has also been observed[14]. It is possible to recover spermatozoa from a variety of washed sample types and from bathwater[14, 19-21], even those testing negative for AP[13], so staining for the identification of spermatozoa was completed for all samples irrespective of their AP result.

2.0 Method

2.1 Seeding and washing unused scrunchies

Before each sample, the bath area and shower equipment were cleaned using Virkon®.

A single scrunchie was seeded with the specified quantities of spermic semen in an equal distribution over the scrunchie according to Table 1. The scrunchie was then agitated, to simulate a washing motion, underneath running water from a shower head for 3 minutes. The temperature of the water was maintained at approximately 37°C. The scrunchie was then hung and left to air dry. This process was repeated, with the addition of Dove® Original body wash at the same time as the semen, according to the quantities detailed in Table 1. Each experiment was replicated ten times.

2.2 AP testing and dissecting

The bath scrunchies were fully unravelled and a large piece of dampened filter paper was placed on top of the scrunchie. Pressure was applied to the filter paper to ensure even contact with the scrunchie before the filter paper was sprayed with AP reagent and positive areas noted.

From each unravelled scrunchie two samples types were taken: (1) five randomly selected dissections of the net, each approximately 5 x 5 mm^2 in size, and (2) a dissection of the rope used to bind the scrunchie together. The net material or rope was placed into a spineroo (a 0.5 ml microtube with a small hole in the bottom, placed inside a 1.5 ml microtube) with 2-3 drops of sterile water before being mashed with a cocktail stick to release any cells. The spineroo was then centrifuged at 9 000 g for 3 minutes and the microtube containing the net or rope was removed. The eluate was then lightly mixed using a pipette to resuspend the contents. The five net samples were individually processed before being combined at the eluate stage.

2.3 Haemotoxylin and Eosin (H&E) staining for the detection of spermatozoa

A small quantity of the eluate was placed on a glass slide and the stain fixed by slow drying on a hotplate. A visible film of sample was built up on the slide using the complete eluate.

The slide was then stained using the H&E method employed by Page, *et al*[14].

Once dry, the slides were examined at 400 X magnification, using the standard scale to grade the number of spermatozoa observed as “Trace – less than five” up to “++++ - very abundant”[22].

2.4 Creating the used scrunchies

To simulate the recovery of semen from a previously used scrunchie, sections 2.1 to 2.3 were repeated on bath scrunchies that had previously been used once a day for a period of seven days. To avoid accidental contamination, the scrunchies were used by females only who abstained from sexual contact for the seven day period. Between each use, the bath scrunchies were hung to air dry.

2.5 Statistical analysis of results

Statistical analysis of the results was performed using the Mann-Whitney-*U* test. p values are quoted.

3.0 Results

Of the 140 samples tested using the AP test, 108 of them were found to be positive, or weakly positive, and 32 were negative. The positive AP results were not dependent upon the quantity of semen, the presence or absence of Dove® Original body wash, nor whether the scrunchies were new or had been used previously.

The results from the H&E staining can be found in Table 2, for the new scrunchies, and Table 3 for the used scrunchies. One can see that recovery and visualisation of spermatozoa is possible from bath scrunchies that have been subjected to a simulated wash for 3 minutes at approximately 37°C. Of the 140 slides produced, spermatozoa were visualised on 139: a recovery rate of 99%. The single sample for which no spermatozoa were observed was with the addition of 2 ml semen and 3 ml Dove® Original on to a new scrunchie.

Generally, the trend was to observe a higher number of spermatozoa from the rope in comparison to the net material of the scrunchie, with average results from all 140 samples of "+++ - many or some in most fields" for rope and "++ - some in some fields, easy to find" for the net material ($p = 0.0003$). In addition, an aggregating phenomenon was noted on slides prepared from the rope as shown in Figure 2.

With new bath scrunchies, there was no significant difference in the recovery and visualisation of spermatozoa from the net material when only 5 ml, 2 ml or 1 ml of semen was seeded. However, there was a significant difference in recovery from the rope when only 1 ml was added (5ml semen $p = 0.002$ and 2 ml semen $p = 0.002$). The average recovery from the rope when 1 ml was added was still "+++ - many or some in most fields".

When comparing the recovery of spermatozoa from new scrunchies washed with or without the presence of Dove® Original, a significant difference was observed when recovering from 2 ml semen for both the net material and the rope. Lower recovery was noted from "+++ - many of some in most fields" to "+ - hard to find" for the net material and "++++ - many in every field" to "+++ = many or some in most fields" for the rope (net material $p = 0.0018$ and rope $p = 0.002$). However this was not consistent when only 1 ml of semen was seeded, where there was no significant difference in recovery.

No difference was noted in the recovery of spermatozoa when 2 ml semen and 3 ml Dove® Original was seeded on to new and previously used scrunchies. However, the p value for the recovery of spermatozoa from the net material ($p = 0.1315$) was lower than that from the rope material ($p = 0.2923$) suggesting that some effect had been observed. A significant difference was observed between the new and previously used scrunchies when only 1 ml of semen was added with 3 ml Dove® Original. As can be seen in Table 2 and Table 3, the recovery of spermatozoa was lower for the used scrunchies from both the net material (new "+++ - many or some

in most fields", used "trace") and the rope ("+++ - many or some in most fields", used "+ - hard to find) at p = 0.003 for both net and rope.

4.0 Discussion and Conclusion

Obtaining 77% positive AP results from the net and rope components of the bath scrunchies appears to be a much higher detection rate to that previously observed in the literature[14, 19-21]. Davidson & Jalowiecki[23] observed diffusion of seminal stains when fabric was wetted prior to AP testing. This supports the understanding that, due to its water-soluble nature, acid phosphatase can, and is, washed away. The higher detection rate observed in this study may well be a consequence of the limited (three minute) time period used for the washing stage. Extending this time period may well see the detection of AP reduce as a consequence. Irrespective of the AP results, the recovery of spermatozoa from 99% of the samples aligns with previous work showing that spermatozoa recovery is possible even with negative AP results[14, 19-21]. When positive, the recovery of spermatozoa did vary across all four scoring categories with a reduction in recovery rate as the quantity of sample decreased when analysing the rope. Results are comparable with those seen in the literature when evidential items seeded with semen are washed and spermatozoa are recovered at "++ - some in some fields, easy to find"[20] and 2.9+ and 1.3+ densities[21] on cotton and nylon.

Despite being able to recover spermatozoa from only 1 ml of original seeded sample, it would be beneficial to determine the lowest recovery limit for which DNA profiles could consistently be obtained (where recommended quantities of DNA for PCR are between 0.5-1 ng, and with approximately 3 pg of genomic DNA per haploid spermatozoa, this is estimated at between 165-335 spermatozoa). For some of the current conditions, production of full DNA profiles may not be possible, for example 2 ml semen and 3 ml Dove® where the result was "+ - hard to find". It is worth noting that DNA profiles have been obtained from only 10 haploid cells[24], although the quality of these profiles is not specified. It would also be possible to improve success with additional DNA concentration methods.

The trend of recovering higher spermatozoa counts from the rope material, rather than the net, and the clumping phenomenon observed may be a consequence of two factors. The coating on the rope may be acting as an attractant or an adhesive, causing the spermatozoa to be retained, and in a number of instances clumped together increasing recovery. Or, it may simply be a case of the semen being more dispersed over the larger surface area of the net in comparison to the rope, making the recovery of spermatozoa lower. To maximise recovery from the net, it would be possible to extract spermatozoa from all of the net (rather than portions of it). Due to size limitations of the spineroos, however, this would be time intensive unless an alternative, larger spineroo system was employed.

The addition of Dove® body wash affected the recovery variably, with no effect when 1 ml semen was added, but a reduction in recovery when 2 ml semen was added. Because this is not a consistent variation this requires further examination to determine the effects of the body wash on recovery including different types and ranges of body wash/shower gel. The quantity of body wash/shower gel should also be explored to determine whether there is a quantity at which recovery is no longer

possible. It may be possible to determine a specific body wash/shower gel brand or type which had very limited effect on the recovery of spermatozoa and so could be recommended for use if the bath scrunchie was to be introduced as a non-invasion means of evidence collection. This analysis would need to explore the effect of recovery, not just on spermatozoa, but also on other trace evidence types, such as hairs and fibres, to ensure that there wasn't a subsequent negative effect on recovery of other evidence.

When comparing new versus previously used bath scrunchies there was a decrease in spermatozoa recovery at lower seeded semen levels from the net material (1 ml). It is likely that the recovery from the net was negatively affected by how tightly packed the new bath scrunchies are in comparison to new ones. In comparison, the results from the rope for the new versus previously used scrunchies were less affected by this change in overall structure of the bath scrunchie. It appears that the more tightly packed the overall structure of the bath scrunchies is, the better able it is to retain spermatozoa, probably because the spermatozoa are entangled and lodged within the net and are less likely to be washed away. If this prediction is true, it means that ascertaining the most tightly packed bath scrunchie on the market would be beneficial for any recommendation for a non-invasive collection method. In addition, when considering a used bath scrunchie as a possible evidence type, the compactness of it should be assessed and a greater area of net should be selected for extraction of spermatozoa in order to maximise recovery. Furthermore, as the recovery from rope was greater than that from the net, if the used scrunchie had a very open and loose overall structure, the rope may have a greater potential for evidence recovery.

In conclusion it is possible to recover AP and spermatozoa from both new and previously used bath scrunchies in the presence and absence of Dove® body wash, particularly from the rope material. If a victim of an alleged sexual offence has bathed or showered before reporting the incident therefore reducing or removing biological evidence from themselves, it is possible that semen has been deposited onto the bath scrunchie via natural drainage and washing. In this instance, given the ability to obtain spermatozoa from previously used bath scrunchies, the collection of the victims own personal cleaning instruments, including bath scrunchies and flannels, is a viable option and could lead to the recovery of useful biological and other trace evidence. One would need to consider the impact of extraneous contamination if the personal cleaning instrument has been located near to where others have bathed, for example hung in a shower cubicle in a shared house, and whether it has been used again since the bathing. As this is a preliminary study, further work reflecting a more realistic application of semen onto the bath scrunchie is required. Determination of spermatozoa recovery when a bath scrunchie is used to wash semen directly from skin, rather than being seeded directly on to it, would be beneficial before this could be utilised as a non-invasive means of evidence collection. Being able to offer an alternative to the full forensic medical examination may encourage higher reporting for those who may fail to come forward due to the psychological traumas associated with the forensic medical examination[25-26].

Further work is currently being undertaken to examine the retention of spermatozoa on bath scrunchies after repeated washes/uses. Preliminary work indicates spermatozoa are recoverable from bath scrunchies after three washes, similar to

outcomes noted by Brayley-Morris, *et al*[27]. What this means is that, even with delayed reporting of more than 72 hours[7], it may still be possible to obtain results of evidential value from bath scrunchies which have continued to be used post-assault. Obtaining a useable DNA profile from bath scrunchies left for this extended period of time may be hampered by accelerated degradation due to the humid conditions of the bathroom.

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Table caption/s

Table 1: Quantities of semen and Dove® Original used to seed the scrunchies

Table 2: Average results, from ten repeats, of microscope examinations for spermatozoa on the net and rope of new scrunchies. For the H&E results, the standard scoring scale is used[22].

Table 3: Average results, from ten repeats, of microscope examinations for spermatozoa on the net and rope of used scrunchies. For the H&E results, the standard scoring scale is used[22].

Figure caption/s

Figure 1: Folded and un-folded bath scrunchie.

Figure 2: Slides showing spermatozoa aggregation from rope extracts (400X magnification).

Title: The recovery of semen from bath scrunchies

Tables

Table 1: Quantities of semen and Dove® Original used to seed the scrunchies

Semen quantity (ml)	Dove® Original quantity (ml)
5	0
2	0
1	0
2	3
1	3

Table 2: Average results, from ten repeats, of microscope examinations for spermatozoa on the net and rope of new scrunchies. For the H&E results, the standard scoring scale is used[22].

	5 ml semen	2 ml semen	1 ml semen	2 ml semen + 3 ml Dove®	1 ml semen + 3 ml Dove®
Net	+++	+++	++	+	+++
Rope	++++	++++	+++	+++	+++

Table 3: Average results, from ten repeats, of microscope examinations for spermatozoa on the net and rope of used scrunchies. For the H&E results, the standard scoring scale is used[22].

	2 ml semen + 3 ml Dove®	1 ml semen + 3 ml Dove®
Net	+	Trace
Rope	++	+

Title: The recovery of semen from bath scrunchies

Figures

(Black and white image for print edition)

Figure 1: Folded and un-folded bath scrunchie.

Figure 2: Slides showing spermatozoa aggregation from rope extracts (400X magnification).