Can. Soc. Forens. Sci. J. Vol. 29. No 1 (1996) pp. 7–11

THE RETENTION AND TRANSFER OF SPERMATOZOA IN CLOTHING BY MACHINE WASHING[1]

E. KAFAROWSKI[2,3], A.M. LYON[2] AND M.M. SLOAN[2]

ABSTRACT

The interpretation of trace findings on spermatozoa on clothing is often problematic, as the manner of deposition may not be readily determined. Particularly troublesome are cases involving complainants who are unable to relate a complete history. Small numbers of spermatozoa may be a result of some type of sexual activity or may be due to an unrelated, innocuous incident. Transfer of spermatozoa between items during machine washing has been theorized as one possible method of indirect deposition. This research was undertaken to determine the likelihood of such transfer. A normal machine wash was simulated in three independent experiments. Pristine items of clothing were washed together with one pair of semen-stained panties. After washing, random samples (n=162) from nine unstained items were examined microscopically. Some spermatozoa were detected on all nine previously pristine items included in the wash loads. Three to eight spermatoza were identified in 16% of the samples. One or two spermatozoa were identified in a further 38% of the samples. The original semen-stained panties were also examined following washing. Although there was no visible staining or acid phosphatase activity, significant numbers of spermatozoa were retained in the original stain areas. The analysis and interpretation of these findings is discussed with reference to current DNA methods.

RÉSUMÉ

Il est souvent problématique d'expliquer une faible présence de spermatozoïdes sur des vêtements puisque la manière dont ils ont été déposés n'est pas toujours explicite. Ceci s'applique particulièrement dans des enquêtes où les plaignant(e)s se trouvent incapables de décrire les évênements. Un petit nombre de spermatozoïdes peut-être le résultat d'une activité sexuelle quelconque ou bien la conséquence d'un incident inoffensif et sans rapport. Le transfert de spermatozoïdes entre plusieurs articles lors d'une lessive représente un des mécanismes possibles pour ce genre de dépôt indirect. Cette étude fût entreprise dans le but de déterminer la probabilité d'un tel transfert. Un cycle de lessive régulier fut simulé dans trois expériences indépendantes. Des vêtements propres furent lavés en présence d'un article taché de sperme. La lessive terminée, des échantillons choisis au hazard (n=162) parmi 9 des vêtements propres furent examinés microscopiquement. Des spermatozoïdes furent détectés sur tous les 9 vêtements préablement propres inclus dans le lavage. De 3 à 8 spermatozoïdes furent identifiés dans 16% des échantillons. De 1 à 2 spermatozoïdes furent identifiés dans 38% des échantillons additionels. L'article taché fut également examiné après le

1. Poster presented at the 42nd Annual Meeting, Canadian Society of Forensic Science, Toronto, Ontario, September 1995.
2. Forensic Biologist; Biology Section, Centre of Forensic Sciences, 25 Grosvenor Street, Toronto, Ontario, M7A 2G8, Canada.
3. To whom correspondence should be addressed.

lavage. Malgré l'absence de tache visible et d'activité enzymatique (phosphatase acide), il restait un nombre significatif de spermatozoïdes au site de la tache originale. L'analyse et l'interprétation de ces résultats sont présentés dans le contexte des méthodologies de typage d'ADN disponibles aujourd'hui.

INTRODUCTION

Sexual assault cases submitted to the Centre of Forensic Sciences (CFS) commonly involve sexually experienced complainants and include a comprehensive case history and biological samples which are collected as part of a sexual assault evidence kit. However, a notable number of submitted cases involve children, the elderly or mentally challenged individuals who are unable to provide a detailed account of the occurrence. In some cases, only underwear or other garments are submitted for examination.

Semen identification on clothing begins with an examination for visible staining and acid phosphatase (AP) activity. Positive areas are excised for extraction and further testing. Samples may be taken from relevant locations even in the absence of detectable AP activity to avoid false-negative situations where AP may be absent or lost due to degradation. The presence of semen is confirmed by the detection of spermatozoa.

When semen is identified on internal samples, sexual activity involving the complainant is indicated. In contrast, interpretation of semen on clothing is more problematic as the time and manner of deposition may not be readily evident. Expert testimony is frequently necessary to explain the significance of semen on clothing, particularly when only trace quantities of spermatozoa are identified. In addition to situations involving sexual contact or activity, other innocuous sources of spermatozoa exist such as secondary transfer, or theoretically, transfer during laundering.

No reports were found in the literature on the likelihood of spermatozoal transfer among items during laundering. Of the few studies published on the subject of semen and washing, only the retention of semen in fabric has been addressed (1–3). These reports have shown that while AP activity is generally lost, spermatozoa are still detectable.

This study was undertaken to test the likelihood of transfer of spermatozoa during machine washing. The extent to which spermatozoa are retained in fabric after laundering is also reviewed.

MATERIALS AND METHODS

A single semen stain was deposited in a clean pair of cotton panties by natural drainage following vaginal intercourse. The stain was air dried and outlined with thread to assist in later re-location. The AP activity of the stain was tested directly, using the Brentamine fast blue technique (4). A sample (0.4 cm^2) was excised from the centre of the stain and retained for further analysis. The preparation of such a stain was repeated by two additional couples.

In each of three independent washes, one pair of semen-stained panties was machine washed with three pristine pairs of cotton or cotton blend panties. Other clean items such as cotton and cotton-blend tea towels, bath towels, pillowcases, T-shirts and socks were added to simulate a normal load. A 10 minute warm wash, cold rinse setting and phosphate-free detergent were used. The items were machine dried.

After laundering, the original semen-stained panties were examined using visible and ultraviolet light (366 nm). A sample (0.4 cm^2) was taken from the periphery of the

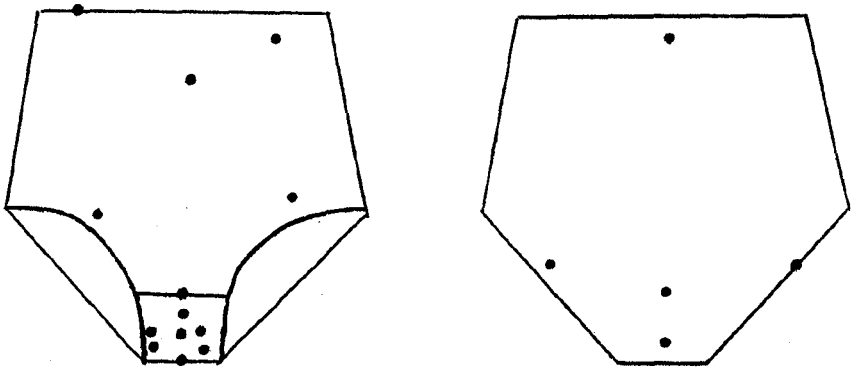

Figure 1. Location of samples excised for sperm transfer analysis.

pre-washed sample site and assessed for AP activity. From the three pristine pairs of panties, eighteen random samples (1.0 m^2) were excised (Figure 1)

All samples were passively extracted in 400 µL of distilled water for twenty minutes and then actively extracted with a Moto-Tool™ for twenty seconds. Each substrate was transferred to a 1000 µL eppendorf tip. The tip was then placed into the original extraction tube and spun at maximum speed for ten minutes in a clinical centrifuge. The resultant pellet was resuspended in approximately 50 µL of distilled water and placed on a microscope slide. The slide was heat fixed, stained with Christmas Tree stain (5) and examined for spermatozoa using oil immersion and 1000X magnification.

Spermatozoa were identified based on morphological and staining characteristics. Clear differential staining of each sperm was essential while some variability in colour or size was acceptable. Sperm density was graded using a non-linear scale ranging from "few" to "4+". The ratings refer to the number of spermatozoa identified per microscopic field of view as follows: "few", few per slide, "1+", 1 in some fields; "2+", 1–5 in most fields; "3+", 5–10 in most fields; "4+", more than 10 per field. For samples with few spermatozoa, the entire slide was examined and the total number was recorded.

RESULTS AND DISCUSSIONS

Sperm Transfer

In all three independent trials, trace quantities of spermatozoa on clothing resulted from transfer during machine washing. A high likelihood of occurrence is suggested as spermatozoa were identified on each of the nine original pristine items examined after washing (Table 1). In over 50% of the 162 samples excised, at least one sperm head was observed. One or two sperm heads were presented in 38% of the samples (62/162). Three to a maximum of eight sperm heads were observed in a further 16% of the samples (25/162). Within each trial the total number of spermatozoa varied on each item, possibly due to random movement in the wash. Differences between trials may also be attributed to random movement and to variation in the initial semen concentrations.

The fact that spermatozoa can be present on a garment that has in no way been involved in any sexual event can have strong implications in relation to opinion testimony. This is particularly important in cases involving complainants who are not sexually active and who are unable to provide a detailed account of the occurrence.

TABLE 1

TRANSFER OF SPERMATOZOA BY MACHINE WASHING

Trial/Item	Total Sperm*	Maximum** Observed	No. of Samples Observed		
			neg	1-2 sp	≥ 3 sp
1–1	50	8	5	6	7
1–2	27	6	7	7	4
1–3	20	3	5	12	1
2–1	10	4	12	5	1
2–2	16	3	9	8	1
2–3	30	7	10	2	6
3–1	14	5	11	5	2
3–2	22	5	7	9	2
3–3	11	3	9	8	1
TOTAL			75	62	25

sp = sperm, neg = negative

* on 18 samples, from each item

** maximum number of sperm observed in any single sample

The potential for further characterization of trace amounts of spermatozoa is limited. In this study, up to eight spermatozoa were observed in any single sample, as a result of transfer during washing. Even sensitive DNA techniques such as the polymerase chain reaction (PCR) require a minimum of 50–100 sperm heads (or at least 0.1 ng amplifiable DNA) to produce a profile. The initial quantity of spermatozoa, fabric type and washing conditions will likely influence the degree of spermatozoal transfer. Although it is unlikely, the total number transferred to a single location could, in theory, meet or exceed the minimum number required to produce a profile.

If a DNA result is obtained, the circumstances of the case must be considered. The analysis of comparison samples from relevant members of the household may be necessary. In the absence of DNA results and other indicators such as AP activity, transfer during machine washing warrants equal consideration with direct and secondary transfer as a possible explanation for the presence of small numbers of spermatozoa.

Sperm Retention

The retention of spermatozoa on clothing following laundering has been previously addressed. Spector and Von Gemmingen (1) examined the effects of various washing conditions on two fabric types. While their results indicated that spermatozoa were retained after washing, no actual reference to quantity was stated. In the present study, the original semen-stained panties, prior to washing, showed visible staining, strong AP activity (within 5 seconds) and a sperm density of 3+ to 4+ (Table 2). Following machine washing, no staining or AP activity was observed, however, a sperm density of 2+ was still present. Based on experience, samples with such gradings contain sufficient DNA to conduct PCR and in some cases, restriction fragment length polymorphism (RFLP) analysis. Studies at the CFS have confirmed that washed semen stains can be successfully profiled by both of these methods[4].

Interpretational problems potentially exist at the level of both semen identification and subsequent individualization. For example, panties worn by a sexually active female may

4. P. Newall. Biology Section, Centre of Forensic Sciences. Personal communication. 1995.

TABLE 2
RETENTION OF SEMEN AFTER MACHINE WASHING

Examination	Original Stains (n=3)	Washed Stains (n=3)
Visible light	+	-
Ultraviolet light	not done	-
AP activity	strong +	-
Spermatozoa	3+ to 4+	2+

Sperm density grading refers to number of spermatozoa per high power field: 4+ = greater than 10 per field; 3+ = 5–10 in most fields; 2+ = 1–5 in most fields; 1+ = 1 in some fields; few = few per slide

+ = positive; - = negative

contain a visible stain and AP activity due to normal vaginal secretions coincident with spermatozoa retained after washing from a prior deposition. Such panties examined as part of a sexual assault case could lead to the incorrect conclusion that the semen was deposited sometime since the last washing. If this stain was further tested by DNA analysis, the results could erroneously exclude a perpetrator who may not have ejaculated during the assault. To assist with interpretation, comparison samples from consensual partners should also be profiled.

ACKNOWLEDGEMENTS

The authors wish to thank P. Newall, C. Hageman and K. Johnston for their helpful suggestions in the preparation of this manuscript.

REFERENCES

1. Spector J. and Von Gemmingen D. The effects of washing on the detection of blood and seminal stains. Can. Soc. Forensic Sci. J. 1971; 4: 3–9.

2. Joshi U.N., Subhedar S.K. and Saraf D.K. Effect of water immersion on seminal stains on cotton cloth. Forensic Sci. Int. 1981; 17: 9–11.

3. Nata M., Toyama H., Nagae M., Aoki Y. and Sagisaka K. Stability of acid phosphatase activity and spermatozoa in semen stains washed with water. Act. Crim. Japon. 1993; 59: 247–253.

4. Seligman A.M. and Mannheimer L.H. A new method for the histochemical demonstration of acid phosphatase. J. Nat. Cancer Inst. 1949; 9: 427–434.

5. Oppitz E. Eine neue Fäbemethode zum Nachweis der Spermien bei Sittlichkeitsdelikten. Arch. Kriminol. 1969; 144: 145–148.