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Assessing the presence of female DNA on post-coital penile swabs: Relevance to the investigation of sexual assault

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ABSTRACT

During the investigation of sexual assault, penile swabs from an alleged perpetrator are processed as part of the routine procedure. The results of the forensic DNA analysis may subsequently be presented in court. Documentation on the expectancy of finding female cells on post-coital penile swabs is scarce. Reviews from assault cases show that retrieval of female cells from penile swabs is generally reported for less than 50% of the cases. The perception may therefore be that female cells are not expected to be recovered on penile swabs, even few hours after vaginal penetration. However, these reviews do not provide certainty that penetration of body cavities had taken place. Furthermore, the alleged perpetrator may have washed himself before the examination. In the present study, eleven couples provided fourteen sets of post-coital penile swabs collected between 5 and 24 h after vaginal intercourse. At time intervals between 5 and 12 h, the full female DNA profiles were recovered in 90% of the samples. At the lowest, 67% of the full female profile was typed as an average of two swabs sampled at each time point. Samples collected from three couples at 20, 22 and 24 h post-coital, retrieved 100% (15 AMPFISTR® Identifier markers, in addition to the amelogenin gene) of the female DNA profile from one couple, and 37% and 30% of the full female DNA profiles from the other two couples. For the majority of samples, male DNA was present in slightly greater abundance than female DNA. In this study, female DNA was recovered on all post-coital penile swabs taken at 5–24 h intervals.

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1. Introduction

When investigating alleged sexual assault, penile swabs are examined to establish whether an alleged perpetrator was involved in intercourse with the assaulted. Assessment of success rates for retrieving female cells from penile swabs is in the literature mainly based on records from sexual assault cases retrospectively reviewed.^{1,2} According to these data, the recovery rate of female cells from penile swabs is low. The recovery of female cells on post-coital penile swabs potentially provide key evidence in sexual assault cases demonstrating that penetration is likely to have occurred. However, the dilemma occurs when interpreting the absence of female cells on post-coital penile swabs, and there is scarce documentation of what to expect of recovery.

The aim of our study was to provide greater understanding of the evidential findings in alleged rape cases. In principle, the perseverance of female epithelial cells on the exterior of the penis

may provide useful information on the activity level prepositions when assessing a weight of evidence to scientific findings on penile swabs. The purpose of the project was to investigate whether female DNA can be found on penile swabs sampled at time intervals from 0 to 24 h after vaginal penetration.

2. Materials and methods

Eleven couples participated in the study. Each couple provided one set of penile swabs at a recorded number of hours after vaginal intercourse. In addition, three couples provided control samples collected shortly after intercourse (time 0), and on another occasion samples from 20 to 24 h post-coital, thus covering the outliers of the sampling time-range. The participating males were asked to refrain from washing until after the swabbing. Whether intra-vaginal ejaculation had taken place was not recorded. A Bode Crime Scene Collector (Bode Technology, US) were slightly moistened and used to sample around the base of the penis, underneath the foreskin at the location referred to as the coronal sulcus. The procedure was repeated in duplicate, with the purpose of assessing

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whether sampling had been conducted satisfactorily. The collectors contain a silica clot intended to dry out the sample. Therefore, the swabs were not left out to dry, but placed directly in labelled containers. The participants collected their own samples, guided by detailed written instructions. The participants reference samples were collected on FTA[®] Micro Cards (Whatman Inc., US). All samples were stored at 4 °C and analysed within one week after sampling. DNA was extracted from the swabs using the Quiagen Biorobot EZ1 Investigator programme, according to the manufactures protocol. The procedure involved a proteinase K facilitated sample lysis step of 15–30 min at 56 °C. PCR was performed on fresh eluates on 10 µL of sample (DNA concentrations were not determined) and 28 cycles of PCR for all samples with the AMPF/STR[®]Identifiler kit. Amplified DNA was subsequently analysed with the ABI 3130 Genetic Analyzer and GeneScan and GenoTyper software.

3. Results

The aim of our project was to investigate whether female cells can be found on penile swabs after vaginal intercourse. 11 couples provided 14 sets of penile swabs at time intervals between 5 and 24 h after intercourse. In addition, three of the couples also provided samples collected immediately after intercourse as references (time 0). A DNA profile, male or female, was considered a full profile only when all 30 alleles from the 15 STR-markers and the amelogenin gene sex marker were typed. Thus, a profile reported as 98.34% of its full profile would mean that a full female DNA profile was recovered on the one swab and all but one allele of 30 total was missing from the profile of the duplicate sample. The recovery of female epithelial cells from the penile swabs in relation to time elapsed since sexual intercourse is listed in Table 1. The results show that female DNA was recovered with an average of 90% of its full DNA profile from a male/female mixture in samples collected between 5 and 12 h after vaginal intercourse. The sample with the lowest female DNA recovery averaged 67% of the full DNA profile, which were collected 10 h post-coital. However, another couple provided swabs taken 12 h post-coital, with 100% of the full female DNA profile. Yet another couple recovered 98% of the full female DNA profile after 11 h, another 97% after 10 h. From the electropherogrammes, the male DNA component was slightly

larger in all swabs expect for the time 0 samples which had predominance of female DNA. The female contribution was at its highest at time 0 post-coital, and lowest in samples collected between 20 and 24 h after intercourse, however still present at 30% of the expected AMPF/Identifiler STR profile in the sample with the lowest female DNA content. The result from all time intervals is illustrated in Fig. 1.

4. Discussion

In our study, female DNA was found on all penile swabs collected between 0 and 24 h after consenting sexual intercourse. The success rate of recovery of female DNA was very high. In the time intervals between 5 and 12 h, full female DNA profiles were recovered in 90% of the samples. The lowest partial profile typed had an average from two swabs of 67% of the full female DNA profile.

Although close to full female DNA profiles were typed from the samples, there is some variation in the recovery rate for the full female DNA profile within the 5–12 h time range. The highest recoveries were at 100%, the lowest 67% of the expected full DNA profile. In a study on shedder ability, we found that individuals can be divided into high, medium or low ability to shed cells onto inert surfaces.³ Differences in the propensity of individuals to deposit DNA has also been reported by others.⁴ Thus, variations in persons shedder abilities may be reflected in the recovery rate of female cells on the post-coital penile swabs. Other factors, such as the ovarian cycle, may be of consequence. In our study, three couples provided additional samples on separate occasions at time 0 and between 20 and 24 h post-coital. The purpose was to assess the expectancy of detecting female cells at the out-liners of the sampling time-range. We allowed for the repetitious use of the individuals since the outcomes at between 5 and 12 h did not differ considerably between all of the participating couples.

The high recovery rate observed deviate from the records from real rape cases. A Danish report reviews rape cases where penile swabs or imprints had been collected and examined. In 227 cases, 57% of the cases held no samples with suitable material for DNA extraction. The mean time interval from assault to examination was 18 h (range 1–154 h).¹ Of 97 cases, 26 provided a DNA profile from the female victim, giving a low success rate. However, no insight is given into whether vaginal, oral or anal penetration had taken place, whereby giving the conditions for deposition of female cells, or if the suspect had washed himself after the alleged assault. Furthermore, sampling may not have been performed correctly, as some physicians for instance collect samples from the urine tract. In our study, the participants themselves provided the duplicate swabs. Since none of the participants work within the field of forensic medicine, the sampling procedures held no advantage over personnel trained in sampling evidence, thus not providing an explanation for the high recovery of female cells in our study.

Although the study was conducted under controlled conditions, technically we are detecting female epithelial cells on a mans penis under the comprehension that consented intercourse will resemble un-consented sexual intercourse for the purpose of this study. Biological materials may deteriorate during storage, implicate that time between sampling and the DNA analysis may be of consequence for successful DNA typing. Furthermore, if the sample is not dried properly before being contained, and then stored at room temperature for lengths of time, bacteria and fungi may further damage human cells in the sample. Therefore, both the sampling method and rapid DNA extraction may have contributed to the high recovery of female cells, and such conditions may not always be as favourable in real case work.

The time elapsed between assault and sampling is of significance. Kaarstad et al. reported that the time interval from assault to

Table 1

The recovery of female epithelial cells transferred onto the penis in relation to time elapsed since sexual intercourse. Profile outcome presents the average number of male and female allele recovery from duplicate samples. The couples 3,4 and 11 provided three sets of samples each, sampled at different occasions at different time points.

Sampling time	Samples	Profile outcome male DNA (%)	Profile outcome female DNA (%)
Time 0	Couple 3	100	100
Time 0	Couple 4	93.33	100
Time 0	Couple 11	40	100
5 h	Couple 10	96.67	85
7 h	Couple 8	100	98.34
7 h 30 min	Couple 2	100	73.34
8 h	Couple 6	100	100
8 h	Couple 11	100	93.33
8 h 30 min	Couple 9	100	96.67
9 h 15 min	Couple 1	100	81.67
10 h	Couple 3	100	96.67
10 h	Couple 7	100	66.67
11 h	Couple 5	65	98.34
12 h	Couple 4	98.34	100
20 h	Couple 3	100	30
22 h 15 min	Couple 4	100	100
24 h	Couple 11	100	36.67

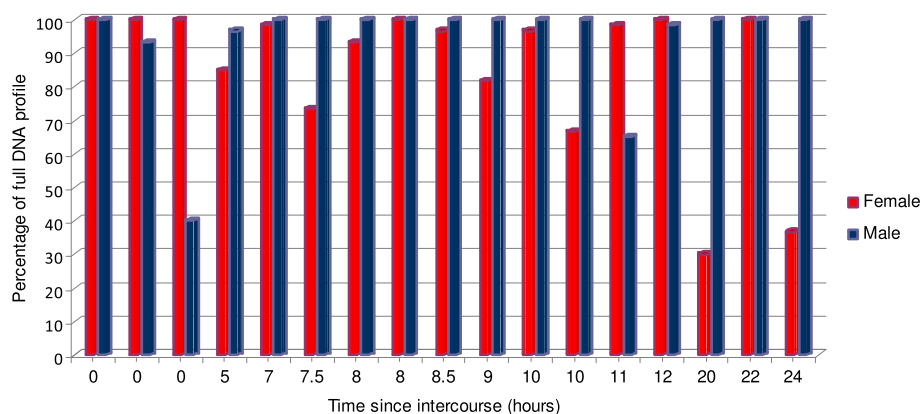


Fig. 1. Comparison of the female vs. male percentage recovery of respective DNA profiles at successive time points between intercourse and sampling. Eleven couples participated providing a total 17 duplicate sets of post-coital penile swabs. Each bar represent the average DNA results from two swabs.

examination was shorter in the cases with successful DNA profiling (mean 7 h, range 1–15 h) than in the remaining cases (mean 19 h, range 2–154 h). This difference was highly significant (t -test $p < 0.003$). In the UK the evidential value of tests carried out on 660 casework penile swabs was reviewed.² The purpose of the examined swabs reported in the survey from 1989 was to see if the donor had had recent vaginal, anal or oral intercourse. An ABO group different from the donor was obtained from a fifth of the swabs typed. Out of the 660 swabs, 364 of the swabs were from a total of 181 female rape cases. The remaining penile swabs were collected in connection with other types of crime cases. The time interval between the offence and collecting the swab was recorded for 335 swabs, whereof over 50% were taken within 12 h, and many of these were taken within 7 h. Information is provided that mostly the penile swabs had been air dried and stored refrigerated prior to scientific examination. Only 63 samples were ABO grouped, whereof 14 samples gave a positive result. The authors concluded that vaginal secretions were sometimes detected, although the routine tests at the time were not ideal. The shortcoming of these surveys is that there is no knowing of whether body cavity penetration had occurred. Furthermore, the way the samples are analysed are not directly comparable, as in the case of the UK study, tests were applied prior to the onset of DNA profiling.

Relevant for comparison of data from our prospective study is the work of Cina et al.⁵ Their project was also designed to determine whether female DNA could be isolated from post-coital penile swabs taken at varying time intervals (1–24 h). A total of four swabs were taken at each time point at 1, 3, 5, 7, 9, 14 and 24 h post-coital. One volunteer couple provided all the samples in the study. DNA yield was determined, and eight STR loci were amplified using the Promega PowerPlex kit (Promega). Female DNA was extracted from all samples. Interestingly, in Cina's report, only female DNA was detected from the samples during the entire 1–24 h post-coital interval. Intravaginal ejaculation was reported for all cases, and still the male DNA profile was not apparent on any of the penile swabs. This observation contradicts with ours where the male DNA profiles were typed at near 100% at 15 of 17 time points, and in slightly larger abundance than female DNA. The discrepancy between these two observations may be explained by the differences in DNA extraction methods used. In Cina et al.,⁵ DNA was extracted from each swab by standard organic extraction; spermatozoa were not lysed because of an expectation of a preponderance of male DNA in the samples. We used an automated DNA extraction method based on magnetic silica beads with affinity for DNA, involving a cell lysis step with proteinase K. Moreover, Cina

et al.⁵ speculates that vaginal abrasion during intercourse caused shedding of a relatively DNA-rich intermediate to the sampled secretions. Another explanation suggested was that the only male epithelial cells collected from the penis were anucleate superficial squamous cells.

Cina et al.⁵ reports that the total DNA yield decreased significantly during the 1- to 24 h post-coital interval, however still in quantities sufficient for DNA profiling. The authors suggest that based on the significant decrease in the amount of female DNA recovered at 24 h compared to 14 h that the majority of female DNA would be degraded or lost within 2–3 days after intercourse.

In our study, all samples were kept refrigerated in a humidity absorbing container and analysed within one week of sampling. This may have provided favourable conditions for a high recovery rate. However, the literature here cited do not provide information about the storage time and conditions of the samples included in their reviews. Particularly when evaluating the absence of female cells on a penile sample after alleged vaginal penetration, therefore, it is essential that background data on the tests are provided.

The presence of semen in forensically significant specimens such as cervicovaginal samples or stains on the victim's clothing traditionally presents positive evidence of sexual contact. However, semen stains and spermatozoa is not always found on the victim of sexual assault. One such case was presented by K. Drobnic,⁶ where female cells were instead recovered from a penile swab from a suspect matching the victim. It is evident that female cells will deposit on the penis of an assailant during vaginal penetration. And female cells will deposit irrespective of whether the perpetrator has left sperm on the victim's body, and thus provide evidence indicating bodily contact. However, female secretions may deposit through secondary transfer, for instance when the suspected offender has been in touch with body fluids from the assaulted, that is saliva, blood or vaginal secretions, and subsequently transferred it via his hands to his penis. A study of the prevalence of foreign DNA on couples who co-habit reported 17% of mixed DNA profiles from under the fingernails of twelve couples sampled on three separate days.⁷ The report found that variable amounts of foreign DNA can be found in fingernail samples from co-habiting couples without any sexual contact having occurred. Predictably, a variable of statistical significance was the amount of time spent together. Numerous reports have investigated into the transfer of epithelial cells (touch DNA) showing that cells can indeed wander.^{3,4} Obviously, body secretions transfer more easily than epithelial cells deposited on an item through touch. The presence of non-self cells may thus indicate physical contact, but is not proving that actual penetration of

body orifices must have occurred. Such assumptions must be evaluated in connection with other evidential considerations.

We recovered female DNA on all penile swabs collected within 24 h of vaginal intercourse. Although the success of recovering female cells on post-coital penile swabs will depend on the time interval from sexual assault until the forensic examination, we observed greater inter-individual variation at similar time points than throughout the time range from 5 to 12 h after intercourse. This study demonstrates that vaginal intercourse leave female cells on the male penis, and in sufficient amount for successful DNA typing up to 24 h post-coital.

Conflict of interest

No conflicts of interest.

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Ethical approval

None declared.

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