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Post-coital vaginal sampling with nylon flocked swabs improves DNA typing

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ABSTRACT

In the examination of sexual assault cases, DNA typing of vaginal samples mostly occurs after differential DNA extraction. Notwithstanding the differential extraction method, the DNA profiles from the seminal fraction often show the male alleles at low-level in combination with female alleles. This unfavorable ratio male to female DNA is due to a limited amount of sperm cells and an overwhelming quantity of female cells. In this study, we compared standard cotton and nylon flocked swabs for post-coital vaginal sampling. Twelve couples donated 88 vaginal swabs – 44 cotton, 44 nylon flocked – which were taken with a time since intercourse (TSI) up to 84 h. These vaginal swabs were sorted into categories on the basis of the TSI and submitted to (1) microscopic examination for the presence of male cells, (2) presumptive tests for the detection of seminal fluid and (3) DNA typing. Cellular elution was found to be 6-fold more efficient from the nylon flocked swabs. This makes microscopic analysis less time consuming as the higher cell yield and better cell morphology simplify detection of male cells. Both swab types reveal similar results regarding presumptive tests and male DNA typing. Positive presumptive tests (RSID-semen and PSA) were obtained up to 60 h TSI and male autosomal profiles up to 72 h TSI. Interestingly, over 50% of the samples negative for both presumptive tests resulted in informative male STR profiles. After differential extraction, less DNA was left on the nylon flocked swabs and more male DNA was isolated. Our results imply that the use of nylon flocked swabs for vaginal sampling will improve microscopic analysis and DNA typing in the medical forensic investigation of sexual assault cases.

1. Introduction

Sexual assault casework often involves the analysis of vaginal samples. The most important aims of this forensic analysis are the detection of sperm cells and the generation of the assailant's DNA profile. Samples are therefore subjected to microscopic analysis, presumptive testing for body fluid identity and DNA isolation followed by DNA profiling. DNA isolation is generally performed by differential extraction (DE) in which non-sperm DNA (mainly from vaginal epithelial) is separated from sperm cells. Clearly, more informative (male) DNA profiles are obtained when more sperm cells are present, which is first of all determined by the number of sperm cells deposited during the assault. Sperm cell preservation is influenced by time between the assault and sampling, victim's behavior during that time and conditions under which samples are collected and stored. When little or no sperm cells are present and/or the male to female cell ratio is unfavorable, low level and incomplete DNA profiles from the male contributor are obtained. In the forensic context, it is often noted that the male contribution is a minor component in a mixed profile, even in a sperm fraction (SF) profile.

Previous studies to improve DNA profiling in sexual assault cases focused on enhancing cell elution from cotton swabs [1–3] or using laser microdissection (LMD) to select sperm cells [4,5]. It has been reported that STR profiles can be generated from as little as 20 sperm cells that were selected using LMD [6]. In cases with no sperm cells present, male diploid cells can be detected by XY fluorescence in situ hybridization (XY FISH) labeling [7–10] and collected with LMD [11,12]. Although LMD provides the ability of individually selecting cells of interest, we have experienced that it requires intact cell nuclei since it is not possible to microscopically distinguish damaged male and female XY FISH-stained nuclei.

The overall effectiveness of obtaining a male DNA profile after DE or LMD is dependent on the efficiency with which the cellular material is collected and subsequently eluted from vaginal swabs [2]. Nylon flocked swabs have been developed to improve sampling due to an open fibre structure, which allows rapid absorption, better sample release and no sample entrapment such

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as occurs for traditional cotton swabs [13]. Nylon flocked swabs are efficient in sampling saliva, blood and skin epithelia from various objects (Loes Schoenmakers, Bas de Jong and Titia Sijen, unpublished results). In this study, we compared standard cotton and nylon flocked swabs for vaginal sampling. The ideal swab for medical forensic examination has the following characteristics: (1) improved elution of intact cells for microscopic examination and LMD, (2) swab is free of extraneous human DNA and (3) swab is suited for all techniques commonly used in sexual assault cases. A total of 140 air-dried cervicovaginal swabs – 70 cotton, 70 nylon flocked – were donated by 14 male–female couples. A selection of 88 vaginal swabs with a time since intercourse (TSI) up to 84 h was used for comparative studies regarding microscopic examination for sperm cells, presumptive tests for seminal fluid and male DNA typing. Nylon flocked swabs were found to improve vaginal sampling and hold promise to advance DNA typing in sexual assault cases.

2. Materials and methods

2.1. Samples

In order to mimic sexual assault casework samples, 14 voluntary male–female couples donated a total of 140 cervicovaginal swabs with informed consent. Female volunteers were asked to self-sample by inserting the swab approximately 5 cm into the vaginal tract and rotating three times. Couples provided information regarding time between intercourse, use and type of contraceptives and time in female cycle (e.g. menstrual week, 1 week after menstruation, menopause). Possible differences in personal hygiene and activity level were not taken into account. The 14 females sampled each day with two different swabs for five consecutive days. Half of the volunteers sampled first with a cotton swab (Deltalab, Barcelona, Spain), while the other half started sampling with a nylon flocked swab (Copan, Brescia, Italy). All donors provided buccal reference samples to confirm obtained DNA profiles. Nylon flocked swabs were dried in 2 ml vials with a small puncture in the lid, while cotton swabs were dried in a tube punctured four times. The swabs were kept at room temperature until examination.

2.2. Cell elution and presumptive testing

For cell elution, intact nylon flocked swabs and cotton swabs which were cut into small pieces were incubated for 60 min in 500 μl phosphate buffered saline (PBS, Invitrogen, Breda, The Netherlands) with moderate shaking at RT. Cells were separated from the swab remnants by punching a hole in the bottom of the microfuge tube followed by centrifugation (2 min 800 × *g*) on top of a new microfuge tube. Cells were pelleted (centrifugation for 5 min 16,100 × *g*) and 300 μl of the supernatant was transferred to a new tube to be used in PSA tests (200 μl of supernatant per test, SERATEC®, Göttingen, Germany) and RSID-semen tests (20 μl of supernatant per test, Galantos Genetics GmbH, Mainz, Germany). The cell pellet was resuspended in the remaining 200 μl PBS from which 180 μl was subjected to differential extraction. The residual 20 μl of cell suspension represents the microscopic fraction (MF), and was used immediately or frozen (−20 °C) until further examination. Up to 10 μl of this MF was used for cytospin slide preparation and 5 μl for DNA extraction using the QiaAmp mini kit as described by the manufacturer (Qiagen, Venlo, The Netherlands).

2.3. Differential extraction (DE)

Differential extraction was performed to separate sperm and non-sperm DNA. Non-sperm DNA was extracted by incubating the 180 μl cell suspension combined with the vaginal swab in 300 μl PBS with 15 μl proteinase K (10 mg/ml, Invitrogen) at 56 °C for 60 min. Next, the lysate was cleared from the swab remnants as described before and centrifuged for 5 min at 16,100 × *g*. The non-sperm DNA-containing supernatant was transferred to a new tube to which 150 μl 20% chelex (Bio-Rad Laboratories S.A.-N.V., Nazareth Eke, Belgium) was added. The pellet containing sperm cells was washed twice in 750 μl digest buffer (10 mM Tris–HCl, 10 mM EDTA, 50 mM NaCl and 2% (w/v) SDS) and once in 1000 μl dH_2O. Sperm cells were lysed in 184 μl lysis buffer (4.8% chelex, 0.1 mg/ml proteinase K and 0.3 mM DTT, Merck Serono BV, Schiphol, The Netherlands) for 30–60 min at 56 °C. Both the non-sperm DNA (non-sperm fraction, NF) and the sperm DNA (sperm fraction, SF) were boiled for 8 min and centrifuged (5 min 16,100 × *g*). NF and SF DNA extracts were purified and concentrated to a final volume of 40 μl by Microcon® YM-100 spin columns (Millipore B.V., Amsterdam Zuidoost, The Netherlands).

In order to determine the amount of DNA which was left in the swab after DE (retained fraction, RF), cotton and nylon flocked swab remnants were subjected to DNA extraction using the QiaAmp mini kit as described by the manufacturer (Qiagen).

2.4. Interphase XY FISH

In previous optimization experiments, three different membrane slides (Poly-Ethylen-Naphtalate (PEN), Polyester (POL) and Poly-Ethylen-Teraphtalate (PET) all supplied by P.A.L.M. Microlaser Technologies GmbH, Bernried, Germany) were examined, since for microdissection of (diploid) cells a membrane support is needed. PET membrane slides showed less background fluorescence and proved suited for XY FISH, DAPI staining and laser microdissection. Vaginal cell suspensions were distributed onto these PET membrane slides with the aid of a cytospin® 4 Cytocentrifuge (10 min 250 × *g*, Thermo Fisher Scientific, Waltham, USA). XY FISH positive control slides were made using fresh male buccal cells.

Cells were fixed using 10% acetic acid–90% ethanol (both Merck) for 15 min and cell membranes were cleaved using 0.01% pepsin (VWR International B.V., Amsterdam, The Netherlands) in 10 mM HCL (Merck) at 37 °C for 2 min. For fixation, slides were treated with 1.1% formaldehyde (Merck)/0.05 M $MgCl_2$ (Merck)/PBS and dehydrated in a series of alcohol solutions (70%, 85% and absolute ethanol, 3 min each). Ten μl DNA probe solution (CEP X alpha satellite SpectrumGreen™ in combination with CEP Y satellite III SpectrumOrange™, Abbott B.V., Hoofddorp, The Netherlands) was added to each slide, which was sealed using a coverslip and rubber cement. The DNA was denatured for 5 min at 80 °C and hybridization was performed at 37 °C for at least 6 h. Three 5 min washes were performed using twice 0.4× SSC (Abbott)/0.05% Tween20 (Dako Netherlands B.V., Heverlee, Belgium) and once 2× SSC. After washing, slides were dehydrated as described above and incubated for 5 min with 200 μl 100 times in PBS diluted Vectashield mounting medium with DAPI (Vector, Burlingame, USA). Excess of DAPI was removed by washing in PBS for 5 min. Finally, slides were dehydrated as described above and air-dried. In microscopic examination, X (green) and Y (orange) chromosomes in DAPI-stained nuclei were detected with FITC, Rhodamine and DAPI filter sets and Axiovision 4.6.3 software.

2.5. Laser microdissection (LMD) and DNA isolation

The XY FISH-stained slides were microscopically analyzed for male cells. If available, 30 sperm heads were collected using the PALM® MicroBeam C HT robomover. If not available, less sperm heads were taken. Also for male diploid cell analysis 30 nuclei were selected (if available). The cells were catapulted into the cap of a 0.5 ml microfuge tube filled with 35 μl isolation buffer (4.3%

chelex, 0.3 mg/ml proteinase K and 0.9 mM DTT). As a negative control, empty parts of the membrane were catapulted and treated similarly. DNA was isolated by incubation at 56 °C for 60 min followed by 8 min boiling and centrifugation. DNA was precipitated from the supernatant by O/N incubation at −20 °C in 0.09 M NaAc pH 5.6 (Merck), 64% ethanol, 0.09 μg/ml glycogen (GlycoBlue, Ambion, Nieuwerkerk a/d IJssel, The Netherlands), in a volume of 332 μl. DNA was collected by centrifugation for 15 min at 16,000 × *g* and the DNA pellet was washed using 250 μl 70% ethanol. The remaining DNA pellet was air-dried and dissolved in 10 μl dH_2O.

2.6. DNA quantification and STR profiling

DNA concentrations were determined by real-time PCR (Quantifiler human, Applied Biosystems (AB), Nieuwerkerk aan de IJssel, The Netherlands). One nanogram – or less when DNA concentrations were below 0.1 ng/μl – was amplified using the AMPF*l*STR® SGM Plus™ (SGM+) kit (AB). Sperm fraction DNA extracts that resulted in no male autosomal profiles were also amplified using the AMPF*l*STR® Yfiler™ kit (AB). For DNA amplification of LMD samples, the full DNA isolate (10 μl) was used as a template in the SGM+ PCR. Amplifications were carried out in a 25 μl volume for 28 cycles (SGM+) or 30 cycles (Y-filer). DNA fragments were separated and detected by capillary electrophoresis on the 3130xl ABI Prism® Genetic Analyzer™ (AB). DNA profiles were analyzed using Genemapper®ID v3.2.1 software (AB).

When partial DNA profiles were obtained for the LMD samples, the sensitivity of STR typing was increased by performing a capillary electrophoresis rerun with 9 kV injection settings preceded by removal of residual dye molecules using Performa DTR gel filtration cartridges (Edge BioSystems, Gaithersburg, USA) as described in Westen et al. [14].

3. Results and discussion

3.1. Cervicovaginal sampling and sample selection

A total of 140 vaginal swabs was obtained of which 88 swabs – 44 cotton, 44 nylon flocked – were selected for further analysis using the following criteria: (1) maximum of 84 h TSI, (2) no condom use and (3) swab not visually overgrown with microbes since microbial growth could negatively affect DNA typing. We observed microbial contamination on three nylon flocked swabs. The 88 selected swabs (Table 1) were obtained from 12 couples (couple A–L) with the majority (57%) of a TSI up to 24 h which is the time period in which 77% of the casework samples are taken (determined for 44 cases).

Table 1
Number of selected swab sets per category and couple (one set consists of one cotton swab and one nylon flocked swab taken consecutively at the same day).

Couple	Time since intercourse (TSI, h)							
	0–6	6–12	12–24	24–36	36–48	48–60	60–72	72–84
A			1		1			
B	2		1		1		1	
C		1		1				
D	1							
E	1	1	1	1		1		
F				1				
G	1		1	1		1		1
H	1	1	1	1				
I	2		1	1	1			
J	1	2		1				
K		1	2	1		1		
L		2		2		1		
# swab sets[a]	9	8	8	10	3	4	1	1

[a] The number of swab sets for each category is presented.

3.2. Microscopic examination and laser microdissection

To examine cell morphology and quantity, two types of slides were made: (1) standard smear slides and (2) cytospin slides using eluted cells. Approximately 5–10 times higher cell yields were observed for the nylon flocked swabs, and the cytospin slides were prepared from 10 μl cell suspension for cotton swabs and 2 μl cell suspension for nylon flocked swabs. Most cells derived from cotton swabs were damaged and few intact nuclei were seen, while most cells eluted from nylon flocked swabs had an intact nucleus. Cytospin slides were prepared for all 50 swabs (25 cotton and 25 nylon flocked) taken 0–24 h post-coitus (Table 1). On 6 slides (all corresponding to cotton swabs) almost no (intact) cell material was present, leaving 44 slides for microscopic analysis. Sperm cells were detected on 27 slides (corresponding to 11 cotton and 16 nylon flocked swabs). Generally the number of sperm cells was higher for the nylon flocked swab preparations, even though five times less cell suspension was used. The 17 slides which were sperm cell negative and 12 of the slides that were sperm cell positive, were subjected to XY FISH and DAPI staining. Male diploid cells were convincingly seen on only 1 slide that was also positive for sperm cells. This slide was derived from a nylon flocked swab.

XY FISH-stained slides from 7 swab sets were selected for male cell collection by LMD. The 7 slides prepared from the nylon flocked swabs all contained sperm cells (and 1 slide was the slide containing XY FISH positive cells), while sperm cells were only detected on 5 of the cotton swab-derived slides (Table 2). For each positive slide, a maximum of 30 sperm or male diploid cells were

Table 2
Results of DNA profiling of male cells, isolated by LMD, after elution from vaginal cotton or nylon flocked swabs. If available, 30 sperm cells or 30 male diploid cells were selected. DNA was isolated, and after DNA profiling the percentage of detected alleles was determined. All typing results were concordant to the reference profiles obtained from buccal samples.

Sample #	Couple	TSI (h)	Cotton[a]		Nylon flocked[b]		Cotton		Nylon flocked	
			% alleles	# sperm[c]	% alleles	# sperm	% alleles	# XY+[c]	% alleles	# XY+
1	B	0–6	91%	30	59%	30	–	0	–	0
2	B	0–6	–	0	95%	30	–	0	100%	30
3	G	0–6	–	0	100%	30	–	0	–	0
4	I	0–6	100%	30	100%	30	–	0	–	0
5	I	0–6	95%	30	100%	30	–	0	–	0
6	I	12–24	91%	20	100%	30	–	0	–	0
7	B	12–24	82%	6	82%	18	–	0	–	0

[a] 10 μl cell suspension on cytospin slide.
[b] 2 μl cell suspension on cytospin slide.
[c] A maximum of 30 sperm or male diploid (XY+) cells were used.

Table 3
Results of presumptive tests (RSID-semen and PSA) and sperm fraction DNA typing for vaginal cotton ($n = 44$) and nylon flocked swabs ($n = 44$).

		Time since intercourse (TSI, h)														Male SGM+ profile in SF after DE			Total number[a]
		0–6		6–12		12–24		24–36		36–48		48–60		60–72		72–84		Full	Partial	None	
		C[b]	N[c]	C	N	C	N	C	N	C	N	C	N	C	N	C	N				
Presumptive tests																					
RSID+	PSA+	9	9	6	8	5	4	5	3	1	–	2	2	–	–	–	–	47	2	5	54
RSID+	PSA–	–	–	1	–	1	1	3	3	–	1	1	1	–	–	–	–	4	7	1	12
RSID–	PSA+	–	–	1	–	1	1	–	2	–	–	–	–	–	–	–	–	3	1	1	5
RSID–	PSA–	–	–	–	–	1	2	2	2	2	2	1	1	1	1	1	1	5	4	8	17
Male SGM+ profile in SF after DE																		59	14	15	88
Full		8	8	5	7	5	5	6	5	2	2	3	2	–	1	–	–				
Partial		–	–	3	–	1	1	2	3	–	–	1	2	1	–	–	–				
None		1	1	–	1	1+1[d]	2	1+1[d]	1+1[d]	1	1	–	–	–	–	1[d]	1[d]				
Total number[a]		9	9	8	8	8	8	10	10	3	3	4	4	1	1	1	1				

[a] Number of swabs for each category is presented.
[b] Cotton swab.
[c] Nylon flocked swab.
[d] Y-STR profile obtained.

collected by LMD (Table 2). DNA profiling showed that the male cell identification was correct, as for all LMD collections male DNA profiles concordant with the reference profile of each donor were obtained. Full DNA profiles were generated for five of the sperm cell collections and the diploid cell collection (Table 2).

3.3. Presumptive testing and DNA typing

The cell suspensions obtained by elution of the vaginal swabs were separated into a cell pellet and a supernatant. The supernatants were used in RSID-semen and PSA tests to assay for the presence of seminal fluid. Table 3 shows the results of the presumptive testing of the 88 post-coital vaginal swabs described in Table 1. Positive RSID-semen tests were obtained for 66 of the 88 supernatants, while 59 of the 88 showed positive PSA test results. The positive results are evenly distributed over both swab types (36 cotton and 35 nylon flocked swabs) (Table 3). No variation was found for sampling order; first cotton swab or first nylon flocked swab (data not shown). Both presumptive tests are based on an immunochromatographic assay, but detect a different antigen (semenogelin in the RSID-semen test and prostate specific antigen in the PSA test). The increased sensitivity for the RSID-semen test is not seen when using diluted sperm: for one specific donor the PSA test can detect 0.2 nl sperm fluid, while the RSID-semen test can detect 1.3 nl sperm fluid (Bas de Jong en Titia Sijen, unpublished results), which is partly due to the fact that 10 times more supernatant can be analyzed in the PSA test. Possibly, the stability of PSA is relatively low in a vaginal environment, while semenogelin stability is less affected. The levels of PSA and semenogelin also seem to be donor-dependent, since some couples rather show PSA positive results and other couples RSID-semen positive tests (results not shown).

Positive presumptive tests have been reported up to 4 days TSI [10]. Sperm cells persist longer than seminal fluid components [3,15,16] and are seen through to 6–12 days post-coitus [10,17–24]. Male autosomal STR profiles are generally only obtained from post-coital vaginal samples with a TSI below 24 h (when TSI exceeds 48 h male autosomal profiling is regarded impossible) [18,20]. In this study, positive presumptive tests were obtained only up to 60 h after intercourse, with the note that we only tested 4 swabs in the 60–84 h TSI category (Table 1). On the other hand, male autosomal STR profiles were obtained up to the 60–72 h TSI category (Table 3). Interestingly, over 50% of the samples negative in both RSID-semen and PSA testing resulted in

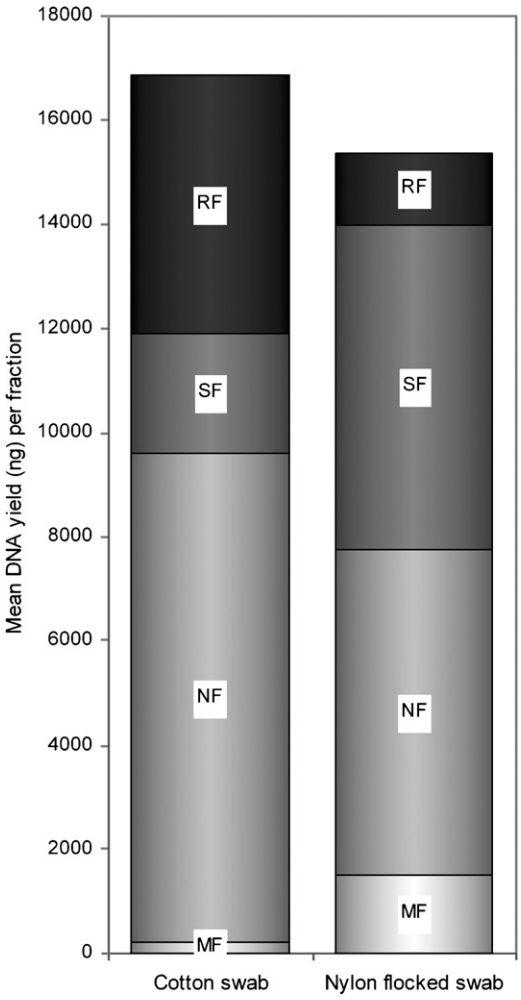

Fig. 1. Mean total DNA yield (ng) in MF (microscopic fraction obtained from eluted cells), NF (non-sperm fraction obtained by differential extraction, DE), SF (sperm fraction obtained by DE) and RF (retained fraction from DNA left in swab remnants after DE) for 8 cotton and 8 nylon flocked swabs sampled 0–6 h post-coitus. The values are the following: MF: cotton 228 ± 116 ng, nylon 1499 ± 808 ng, NF: cotton 9379 ± 4110 ng, nylon 6259 ± 2726 ng, SF: cotton 2302 ± 982 ng, nylon 6222 ± 2750 ng and RF: cotton 4951 ± 2281 ng, nylon 1377 ± 815 ng.

autosomal male profiles (Table 3; some profiles are partial). For the two swab types similar genotyping efficiencies were observed: 37 cotton and 36 nylon flocked swabs resulted in autosomal male profiles.

In total 15 SF samples resulted in no male autosomal profiles (Table 3) and were subjected to Y-STR profiling which is more sensitive in detecting male DNA [10,18,20,25]. Informative (full or nearly full) Y-STR profiles were obtained from five of these 15 SF samples of which two correspond to swabs taken at long TSI; 72–84 h (Table 3). With that, only 10 of the 88 swabs (corresponding to 2 couples) yielded no male genotyping data.

The samples of one couple (couple H) showed noteworthy results; four samples in the 0–24 h TSI category gave positive PSA and RSID-semen results but hardly male genotyping data. This suggests that the male donor is oligospermic or azoospermic. Accordingly, microscopic analysis of swabs from this couple did not reveal any sperm cells. XY FISH positive cells were seen neither. We detected XY FISH positive cells in only 1 of our 12 sperm positive slides (see section '*microscopic examination and laser microdissection*'), indicating that the occurrence of male diploid cells is not frequent even at low TSIs (0–24 h). Only 4 of the 17 negative results in the sperm and male diploid cell examination are explained by the oligo-/azoospermic nature of couple H.

3.4. DNA yield of cotton and nylon flocked internal swabs

In our experimental setup four different DNA-containing fractions were obtained from each swab: (1) the portion of the eluted cells saved for microscopic analysis (microscopic fraction, MF); (2) the non-sperm fraction (NF); (3) the sperm fraction (SF) and (4) the remnants of the swab possibly retaining cell material (retained fraction, RF). Due to the open fibre structure, the nylon flocked swabs may result in a different total DNA yield that is distributed differently over the fractions. To study this aspect, DNA was isolated from one-fourth of the MF and from swab remnants for 16 swabs taken 0–6 h post-coitus (Table 1, swabs couple H excluded). Total DNA yields for each fraction were calculated using the concentration determined by real-time PCR (Fig. 1). The standard deviation is high, as expected, since samples from various couples were used. Importantly, the same set of couples for cotton and nylon flocked swabs was used, which enables comparison in trend-wise manner. The total amount of human DNA was slightly higher for cotton than for nylon flocked swabs. However, during casework the RF is discarded. This RF consists of approximately 30% of the total DNA yield for the cotton swabs but only 9% for nylon flocked swabs. Therefore, the realistic yield (combined yields of MF, NF and SF) appears higher for nylon flocked than for cotton swabs (Fig. 1). When comparing the two swab types for MF, NF and SF, nylon flocked swabs show higher yields for MF and SF and a lower yield for NF. The approximately 6-fold higher yield for the MF is consistent with the earlier described higher number of cells seen during microscopic analysis. The at least 2-fold higher yield in the SF may be important, especially if also the yield of male DNA is increased.

To estimate the male contribution in each fraction, the peak height ratio at the amelogenin (AMEL) locus was used. The percentage of male DNA is calculated as $[(2 \times \text{Y rfu})/(\text{X rfu} + \text{Y rfu})] \times 100\%$ and the percentage of female DNA is calculated as $[(\text{X rfu} - \text{Y rfu})/(\text{X rfu} + \text{Y rfu})] \times 100\%$. Fig. 2 shows the percentages of male and female DNA in the various fractions (MF, NF, SF and RF) for the samples in the TSI 0–6 h category and Fig. 3 shows these percentages for the SF in all TSI categories. The nylon flocked swabs show higher or similar percentages of male DNA in all fractions. This trend is confirmed for individual swab sets (TSI 0–6 h; data not shown) and is not dependent on order of sampling (first cotton or first nylon flocked swab). Thus, both the total (Fig. 1) and male (Figs. 2 and 3) DNA yield in the SF is higher for the nylon flocked swabs. Concomitantly, less DNA extract is needed for amplifications when sampling with nylon flocked swabs and more DNA extract is left for additional DNA research (like Y-STR typing or low-template analyses). In the NF, more male alleles are seen in DNA profiles derived from nylon flocked swabs, but the female contributor is still clearly the major component in the NF.

When the time since intercourse increases, the percentage of male DNA in SF profiles decreases from 96% for swabs sampled at TSI 0–6 h down to 22% for swabs sampled 36–84 h post-coitus (Fig. 3). For both swab types male DNA profiles become more incomplete (Table 3), with lower peak heights and more contaminating female alleles (results not shown). Consequently, the male donor moves from major to minor contributor in the SF profiles with increasing TSI.

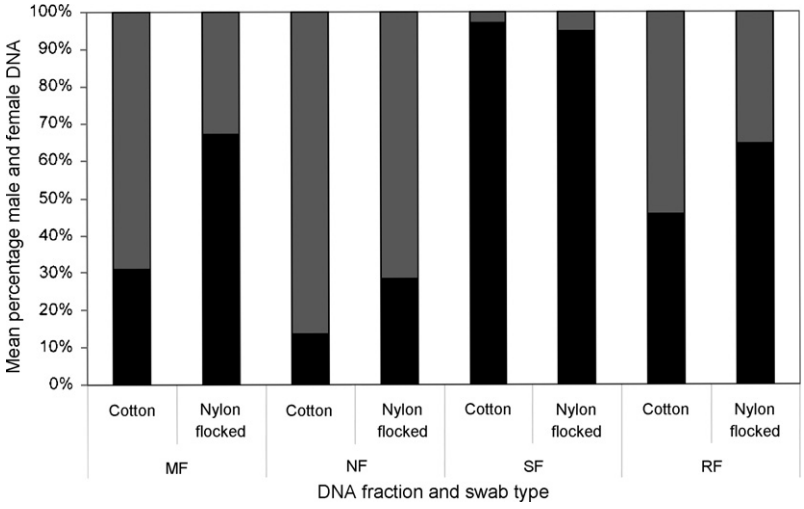

Fig. 2. Percentage of male DNA (black bars) and female DNA (gray bars) based on peak heights at locus AMEL in the various fractions (MF, microscopic fraction obtained from eluted cells; NF, non-sperm fraction obtained by differential extraction, DE; SF, sperm fraction obtained by DE; RF, DNA fraction which retained in swab remnants after DE) for cotton and nylon flocked swabs. For each type 8 swabs with a TSI of 0–6 h were analyzed.

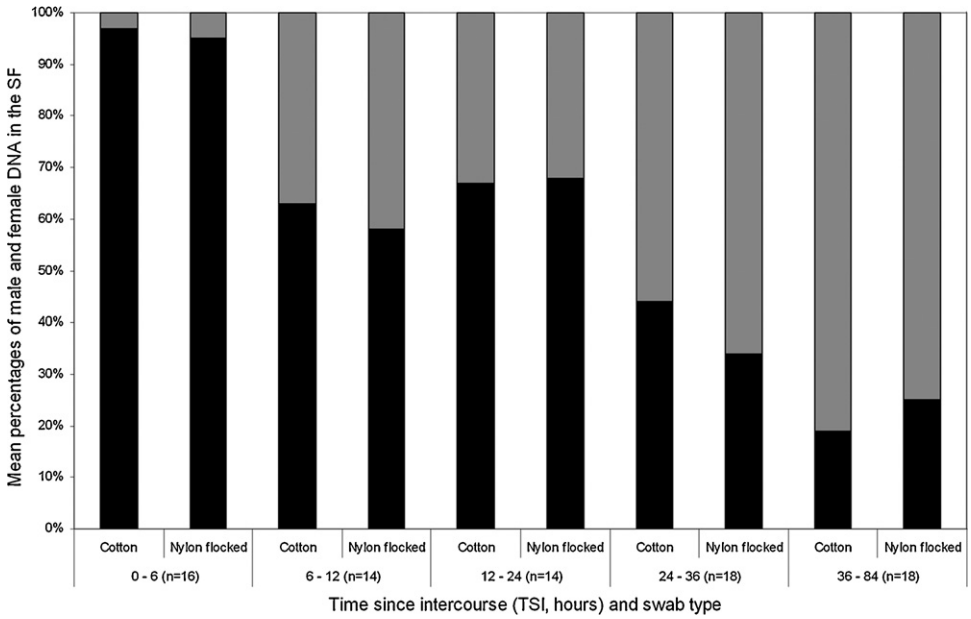

Fig. 3. Percentage of male DNA (black bars) and female DNA (gray bars) based on peak heights at locus AMEL in sperm fraction (SF) STR profiles obtained from cotton and nylon flocked swabs with a TSI up to 84 h. Swabs of the couple with the oligo- or azoospermic male were not included.

4. Concluding remarks

In this study, standard cotton and nylon flocked swabs were compared for vaginal sampling and subsequent analysis. Improvements for the nylon flocked swabs were found regarding two aspects: (1) cell release during elution and DE and (2) total yield of male DNA. The cell elutions from the nylon flocked swabs contained a higher number and more intact cells, which facilitates microscopic examination and made LMD more successful. In cases with an oligospermic or azoospermic assailant, male diploid cells may be detected, although the XY FISH analyses of 4 swabs of the oligo-/azoospermic couple in this study (couple H) did not reveal these cells. Also for the fertile donors, male diploid cells were not readily detected as XY FISH positive cells were only seen on 1 of our 12 sperm positive slides.

Considerably less cell material is retained in the nylon flocked swabs after the cell and DNA extractions and the yield of extracted DNA is higher, which may be due to the more open fibre structure of the nylon flocked swabs [13]. Interestingly, also the male DNA yield is higher for nylon flocked swabs. Possibly, sperm cells retain more efficiently (or vaginal epithelial cells less efficiently) on nylon flocked swabs. Alternatively, the slower adsorption seen for nylon flocked swabs leads to the collection of relatively more sperm cells for instance due to the (more superficial) sampling of a larger area.

Nylon flocked swabs were dried in 2 ml vials with a puncture in the lid while cotton swabs were dried in a tube pierced four times with a hot needle. Consequently, nylon flocked swabs dried significantly slower than cotton swabs (results not shown). This may allow for microbial growth, which was seen on 3 of the 70 nylon flocked swabs received from the volunteers. To stimulate drying of the nylon flocked swabs, we have tested other storage tubes and obtained very promising results using forensiX tubes provided by Prionics AG [26]. Importantly, nylon flocked swabs are gamma irradiated, but provided in forensiX tubes they will be ethylene oxide sterilized as well, which will further reduce contamination from extraneous DNA [27].

Cotton and nylon flocked swabs showed similar results in presumptive tests for the presence of seminal fluid; less positive tests were seen with increasing TSI. Differences were seen between donor couples, which could be due to e.g. variable levels of PSA and/or semenogelin for the male donor or different behavior of the females between intercourse and sampling. Remarkably, more than half (9 of 17) of the samples negative in both RSID-semen and PSA testing resulted in partial or full autosomal male profiles, and two-third (12 of 17) resulted in informative male profiles when additional Y-STR typing was applied. Taken together, our results imply that the use of nylon flocked swabs for vaginal sampling will improve forensic analysis in sexual assault cases.

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