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Original Article

Detection of Male DNA in the Vaginal Cavity After Digital Penetration Using Y-Chromosome Short Tandem Repeats

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ABSTRACT

In this study, useful genetic information from male donors was obtained on vaginal swabs taken from female volunteers after male digital vaginal penetration in a time frame relevant to a sexual assault investigation. Vaginal swabs were collected from eight volunteers at intervals of 1, 6, 12, 24, and 72 hours after digital vaginal penetration. DNA was extracted from collected swabs and subsequently genotyped using a commercially available Y-chromosome short tandem repeats (Y-STR) multiplex kit. Fifty-eight vaginal swabs were collected and analyzed in the study. Composite Y-STR profiles from all combined volunteers showed that 85% of all possible alleles were detected at the 1-hour interval, 77% of all possible alleles were detected at the 6-hour interval, 73% of all possible alleles were detected at the 12-hour interval, 66% of all possible alleles were detected at the 24-hour time interval, and 71% of all possible alleles were detected at 72 hours after digital vaginal penetration. Results indicate that a viable possibility exists that probative Y-STR profiles, useful for investigative purposes, can be obtained from vaginal swabs taken from subjects exposed to digital penetration at time intervals up to 72 hours postpenetration.

KEY WORDS:

digital penetration; DNA profiling; sexual assault evidence collection; Y-STRs

The purpose of this study is to determine the viability of obtaining Y-chromosomal DNA profiles consistent with those of a male donor on vaginal swabs taken from female volunteers at various time intervals after male digital vaginal penetration. Successfully obtaining male DNA profiles may encourage jurisdictions to develop the policy of collecting vaginal swabs from victims when this type of assault is alleged.

In 2011, the Federal Bureau of Investigation (FBI) Criminal Justice Advisory Policy Board voted to change the definition of rape in the Uniform Crime Reporting Summary Reporting System because of objections from many women's rights activist groups. The new FBI definition of rape expanded the 82-year-old Uniform Crime Reporting definition of rape from forcible penile–vaginal penetration to "penetration, no matter how slight, of the vagina or anus with any body part or object, or oral penetration by a sex organ of another person without the consent of the victim" (Women's Law Project, 2011). The FBI collects statistics regarding rape annually; in 2011, there were approximately 83,500 rapes of adult women reported to law enforcement agencies. In addition, over 80,000 female children are sexually assaulted in the United States annually (Bonnar-Kidd, 2010). Since the change in the definition of rape, it is important to consider the collection of additional evidence when sexual assaults are reported or when any sexual-assault-related incident occurs. According to the new FBI definition of rape, digital penetration is also considered a form of rape necessitating the need to collect physical evidence from the victim if digital penetration is alleged.

Digital penetration commonly results in genital trauma (Grossin et al., 2003). Although genital trauma is identified through visual inspection, histological techniques, and

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colposcopy (Sommers, 2007), the presence of evidence from the act of digital penetration is not investigated. Digital penetration is most frequently reported in young children who are sexually abused, although the report of these assaults is less frequent than assaults on adult women (Finkel, 1998; Grossin et al., 2003).

It is currently unknown how the vaginal environment affects the detection and presence of male epithelial cells as a function of time after male epithelial cell deposition. No studies have been conducted on the detection of male DNA in the vaginal cavity after digital penetration. A previous study pertaining to digital penetration only investigated the persistence of female epithelial cells under male fingernails up to 72 hours after digital penetration (Flanagan & McAlister, 2011). This indicates that there is transfer and persistence of epithelial cells after digital penetration and suggests that male DNA could also persist and be detected from the vaginal cavity.

Sexual assaults involving penile–vaginal penetration normally result in a DNA mixture of male and female DNA when using autosomal short tandem repeats (STRs; Butler, 2005). However, in some cases of mixed DNA profiles, the quantity of male DNA is too low to obtain a meaningful male profile using autosomal DNA analysis. A previous study successfully utilized Y-chromosome STRs (Y-STR) profiling to analyze vaginal swabs collected from sexually assaulted female victims where spermatozoa were not detected on the swabs and autosomal male DNA typing results were not obtained. The maximum time frame for successful Y-STR DNA typing was found to be 48 hours after the assault and was likely because of low quantities of undetected sperm cell or male epithelial cells (Sibille et al., 2002).

Y-STR profiles were also obtained in female subjects after extended postcoital intervals (Mayntz-Press & Ballantyne, 2008; Quarino & Kishbaugh, 2012). Usable Y-STR profiles were obtained in vaginal swabs at postcoital intervals as long as 8 days (Quarino & Kishbaugh, 2012).

In this study, vaginal swabs were taken from volunteers at various intervals after digital penetration and subsequently examined for Y-chromosomal DNA using a commercially available Y-STR multiplex to ascertain whether a male-specific DNA profile from the digital penetration can be detected in the vaginal cavity.

Methods

Approval to Work With Human Subjects

The study was reviewed by the institutional review board of Cedar Crest College who approved this project for use with human subjects. Approval for use of human subjects was granted only after information regarding volunteer recruitment procedures, methods of sample collection, and protections of confidentiality were adequately described to the institutional review board of Cedar Crest College. Informed consent procedures were carried out. In addition, the institutional review board ensured that any possible risks associated with the collection of samples in this study were explained to the volunteers and that they had a right to withdraw from the study at any time. The names of human subjects used in this study are known only to the authors.

Sample Collection

Eight couples were asked to participate in this study without regard to age of the subjects. No specific criteria for volunteer recruitment were used. The participants in the study did not have any known chronic medical conditions or acute illnesses. Couples who did not use condoms during sexual activity were asked to abstain from sexual activity for at least 14 days before digital penetration. Those couples who typically used condoms during sexual activity began the study after abstaining from sexual activity for 7 days. After digital penetration was performed for 5 minutes, the vaginal cavity was swabbed at one of the following time intervals: 1, 6, 12, 24, or 72 hours after penetration. Couples also abstained from sexual activity during each of the requested time intervals. Instructions on sample collection were provided by the researchers. Immediately before digital penetration, four control vaginal swabs were collected by the woman. Two swabs were simultaneously inserted into the vaginal cavity and then removed. Two additional swabs were then inserted, creating two sets of two control swabs. After digital penetration, the vaginal cavity was swabbed at one of the time intervals mentioned above by the woman in the same manner as the control swabs. Upon collection of the samples, the two sets of two swabs were randomly designated either A or B. There was no correlation between the letter and the order of collection.

After collection, swabs were reinserted into the original swab packaging, cotton tip facing outward protruding from the open area of the packaging, and allowed to air dry. Once dried, the swabs were placed into manila envelopes to keep each collection separate from the others. Collection of biological samples into a paper medium is recommended to prevent microbial growth from occurring (Butler, 2005). Swab sets A and B for both controls and samples were packaged separately. Four more trials were performed to collect samples at the remaining intervals in the same fashion.

During the tested time intervals, female volunteers engaged in normal activity, but none performed feminine wash. Specific information about bathing and showering during the tested time intervals was not collected.

Buccal swabs were collected from the male and female participants to serve as typing controls for the study. Only five couples finished all of the collection time intervals. Fifty-eight samples were collected and analyzed in the study (two samples each from 29 intervals).

Contamination Prevention Procedures in the Laboratory

Gloves were worn at all times when working with biological samples or handling materials for DNA-related procedures and were changed when handling different samples. All work surfaces and materials (e.g., tubes, tube racks) were cleaned with 10% domestic bleach followed by 70% ethanol before all analytical procedures. Before DNA extraction, quantitative polymerase chain reaction (PCR), conventional PCR, and capillary electrophoresis, all supplies (e.g., molecular grade water, pipettes, microcentrifuge tubes) were irradiated in an Ultraviolet Stratalinker 2400 Crosslinker (Stratagene, La Jolla, CA) for 75 minutes to minimize contamination of samples.

Analytical Methods

All analytical methods to be described are typically used in forensic science laboratories and are consistent with accepted analytical procedures as described by the FBI (2011a). Swab sets A and B were analyzed separately.

DNA Extraction

DNA from each swab set was extracted in an AirClean600 PCR Workstation (AirCleanR Systems, Raleigh, NC) using the DNA IQ System (Promega, Madison, WI). The procedure for DNA IQ is described by the manufacturer (Promega Corporation, 2013). The final sample was eluted in 100 mL of DNA IQ Elution Buffer.

DNA Quantitation

DNA quantitation of exemplar male buccal swabs was performed by real-time PCR with a Rotor-Gene 6000 (Qiagen, Germantown, MD) using the Alu-based method employing SYBR Green for detection as described (Nicklas & Buel, 2003). Vaginal DNA samples were quantified for male-specific DNA using the Quantifiler Trio DNA Quantification Kit (Life Technologies, Carlsbad, CA) and a 7500 Real-Time PCR System (Life Technologies, Carlsbad, CA) according to the manufacturer's guidelines (Life Technologies, 2014).

DNA Amplification and Genotyping

DNA samples were amplified and subsequently genotyped using the PowerPlex Y23 kit (Promega, Madison, WI). The protocol was followed according to the manufacturer's guidelines (Promega Corporation, 2014), and the specific parameters for genotyping were obtained from those reported (Pasino, Caratti, & Robino, 2012). Total reaction volume of 25 ml was used for all amplification reactions. The samples were amplified with a Life Technologies Veriti 96 Well Thermal Cycler (Life Technologies, Carlsbad, CA) and analyzed by capillary electrophoresis using an Applied Biosystems Prism 310 Genetic Analyzer (Life Technologies, Carlsbad, CA) with an injection time of 5 seconds. DNA amplicons were sized using CC5 ILS 500 (Promega, Madison, WI; standard size) and genotyped using Genemapper ID-X software version 1.0 (Life Technologies, Carlsbad, CA). An analytical threshold of 50 relative fluorescence units (RFU) was set for allele identification. An analytical threshold of 50 RFU is typically used in forensic biology laboratories. Below the 50 RFU threshold, users run the risk that artifactual peaks may be misidentified as true DNA alleles and allelic drop-in and dropout because of stochastic effects (only one allele detected in a heterozygote genotype because of preferential amplification of the detected allele) at low DNA concentrations (typically below the 50 RFU threshold) may become problematic (Bregu et al., 2012). The analysis method was created according to the manufacturer's guidelines (Promega Corporation, 2014).

DNA Concentration and Purification

Swabs from each couple yielding incomplete profiles were subsequently concentrated using Amicon Ultra 0.5-mL 100K filters (Millipore, Billerica, MA). Concentration of DNA samples is routine in forensic science laboratories when the amount of human DNA is very low (below the recommended amount of DNA needed for amplification in commercial forensic DNA genotyping kits) or when initial testing results in incomplete profiles. The remaining extract (approximately 80 µL) was applied to the filter, and manufacturer's specifications were followed (Millipore Corporation, 2009). Concentrated samples were reamplified and genotyped using the described procedures. The remaining amplified product was then purified using a MinElute PCR Purification Kit (Qiagen, Germantown, MD) according to manufacturer's guidelines (Qiagen Sample Assays and Technologies, 2008) and reinjected on the genetic analyzer with no change in electrophoretic parameters. Extracts that yielded full profiles did not undergo concentration and purification.

Results

Data Interpretation

Interpretation of data in this study is consistent with typical guidelines employed in forensic science laboratories and does not deviate from accepted practice as described by the FBI (2011b).

Male-specific DNA Quantitation

With the exception of two test swab extracts, all tested extracts produced DNA quantities below 1 ng of male DNA at the amplification input volume of 17.5 mL. Two 1-hour post-digital-penetration swabs yielded male-specific DNA quantities of 0.0608 and 0.0528 ng/mL, respectively. Because all samples had input volumes of 17.5 mL regardless of quantitation value, 1.18 ng of male-specific DNA was added from the former sample, and 1.02 ng of male-specific DNA was added from the latter sample to their respective amplification reactions. This only slightly exceeds the manufacturer recommendation (Promega Corporation,

2014) of adding up to 1 ng of male DNA. These 2 samples, however, produced full Y-STR profiles and thus were unaffected by the slight increase in input male DNA.

Individual Swab Sets

Five of the 8 couples in the study produced at least 1 full profile in an individual swab set. Not surprisingly, the trend in the amount of alleles obtained in individual swab sets generally decreased with increasing time interval although there is a slight increase from 24 to 72 hours (see Table 1). Of the 58 swabs collected in the study, 55 underwent concentration and purification. The 3 swabs that did not yielded complete profiles with the initial extracted sample.

At 1 hour after digital penetration, 2 swab sets were collected from seven couples. Before concentration and purification (listed as "Original" in Table 1), at least 1 set of swabs from 3 of the couples produced full Y-STR profiles (23 alleles). Test swabs from 2 other couples yielded profiles containing 19 alleles, one couple yielded 11 alleles, and one couple yielded eight alleles. With concentration, 2 additional couples (the 2 couples who produced 19 alleles with the original extract) produced full Y-STR profiles. Results from swabs from 2 of the couples who underwent both concentration and purification were relatively unchanged. Five of the 7 couples (71%) were therefore able to produce full profiles or nearly full profiles (defined as profiles with 20–22 alleles) 1 hour after digital penetration.

At 6 hours after digital penetration, two swab sets were collected from 6 couples. Before concentration and purification, swab sets from 2 couples yielded full profiles. No more than 6 alleles were detected in any of the tested swabs from the other 4 couples. With concentration and purification, the 4 couples did see an increase in the number of alleles with one couple producing a maximum of 20 alleles. Three of the 6 couples (50%) were therefore able to produce full profiles or nearly full profiles 6 hours after digital penetration.

At 12 hours after digital penetration, two swab sets were collected from 6 couples for 12 swab sets. Before concentration and purification, no swab set yielded a full or nearly full profile with the maximal number of alleles for all couples combined ranging from 4 to 18. Five of the 6 couples did increase the number of alleles detected after concentration and purification ranging from 10 to 20 (two couples were able to produce 20 alleles). Two of the 6 couples (33%) were therefore able to produce full profiles or nearly full profiles 12 hours after digital penetration.

At 24 hours after digital penetration, two swab sets were collected from five couples for 10 swab sets. Before concentration and purification, 1 couple was able to produce a nearly full profile (22 alleles), whereas the maximum number of alleles from the other couples ranged from 0 to 10. The couple who yielded 22 alleles was able to produce a full 23-allele profile after concentration. Concentration and purification did increase the number of alleles in three of the 4 other couples resulting in profiles ranging from 8 to 12 alleles. One of the five couples (20%) was therefore able to produce full profiles or nearly full profiles 24 hours after digital penetration.

At 72 hours after digital penetration, 2 swab sets were collected from 5 couples for 10 swab sets. Before concentration and purification, no swab set yielded a full or nearly full profile with the maximal number of alleles for all couples combined ranging from 0 to 19. Concentration and purification did increase the number of alleles in 4 of the 5 couples, whereas 1 couple showed a significant decrease in the number of alleles (19 alleles before concentration, 5 alleles after concentration, and 8 alleles after purification). Two of the 4 couples who showed an allele increase produced profiles of 20 and 21 alleles, respectively. The electropherogram of the Y-STR DNA profile from the couple who yielded 21 alleles after concentration and purification is found in Figure 1. The other 2 couples yielded only a maximum of 7 alleles after concentration and purification. Two of the 5 couples (40%) were therefore able to produce full profiles or nearly full profiles 72 hours after digital penetration.

None of the control vaginal swabs in the study yielded male DNA.

Composite DNA Profiles

Composite profiles for each couple at each time interval were generated based on the presence of an allele obtained with the original extract, concentrated extract, or concentrated and purified amplification product in either set of swabs (see Table 1).

At 1 hour after digital penetration, five of the 7 couples had full composite profiles (23 alleles), whereas 2 couples had respective partial composite profiles of 11 and 13 alleles. Of all possible alleles, 85% were detected for all combined couples at the 1-hour interval.

At 6 hours after digital penetration, 1 couple had a full composite profile, 2 couples had nearly full composite profiles (20–22 alleles), and 3 couples had respective partial composite profiles of 11, 12, and 16 alleles. Of all possible alleles, 77% were detected for all combined couples at the 6-hour interval.

At 12 hours after digital penetration, 3 of the 6 couples had nearly full composite profiles, and 3 couples had respective partial composite profiles of 12, 12, and 16 alleles. Of all possible alleles, 73% were detected for all combined couples at the 12-hour interval.

At 24 hours after digital penetration, one couple yielded a full profile, whereas 4 couples produced respective partial composite profiles of 9, 14, 16, and 16 alleles. Of all possible alleles, 66% were detected for all combined couples at the 24-hour interval.

Finally, at the 72-hour postpenetration interval, 1 couple had a full composite profile, 2 couples produced a nearly

TABLE 1. Number of Alleles Obtained From the Original, Concentrated, and Purified Samples for Swab Sets A and B From Each Couple at All Time Intervals

		1 hour		6 hours		12 hours		24 hours		72 hours	
		A	B	A	B	A	B	A	B	A	B
Couple 1	Original	19	19	6	3	0	4	0	10	19	5
	Concentrated	23	22	4	2	6	0	9	0	5	1
	Purified	23	23	9	7	6	10	11	5	4	8
	Composite	23		11		12		14		21	
Couple 2	Original	19	10	3	23	8	6	0	5	16	12
	Concentrated	23	13	12	23	8	12	5	4	18	10
	Purified	23	17	14	0	13	20	0	12	21	20
	Composite	23		23		20		16		23	
Couple 3	Original	8	0	0	1	6	0	0	0	0	7
	Concentrated	9	0	11	2	1	0	8	0	4	5
	Purified	9	0	14	11	11	0	8	0	7	7
	Composite	11		16		12		9		10	
Couple 4	Original	11	2	6	0	15	6	10	3	3	1
	Concentrated	11	0	12	1	0	2	7	2	12	2
	Purified	6	2	12	6	0	5	9	9	20	9
	Composite	13		12		16		16		21	
Couple 5	Original	23	23	3	5	NC	NC	NC	NC	NC	NC
	Concentrated	NT	22	4	11	NC	NC	NC	NC	NC	NC
	Purified	NT	22	6	20	NC	NC	NC	NC	NC	NC
	Composite	23		21		NA		NA		NA	
Couple 6	Original	NC	NC	NC	NC	7	2	NC	NC	NC	NC
	Concentrated	NC	NC	NC	NC	19	0	NC	NC	NC	NC
	Purified	NC	NC	NC	NC	20	1	NC	NC	NC	NC
	Composite	NA		NA		20		NA		NA	
Couple 7	Original	23	17	10	23	18	0	22	19	0	0
	Concentrated	NT	21	0	23	7	0	23	16	2	0
	Purified	NT	22	0	23	12	0	23	20	7	0
	Composite	23		23		21		21		7	
Couple 8	Original	23	23	NC	NC	NC	NC	NC	NC	NC	NC
	Concentrated	NT	23	NC	NC	NC	NC	NC	NC	NC	NC
	Purified	NT	23	NC	NC	NC	NC	NC	NC	NC	NC
	Composite	23		NA		NA		NA		NA	

NC = not collected; NT = not tested; NA = not applicable. The number of unique alleles obtained from composite profiles at each time interval is also listed.

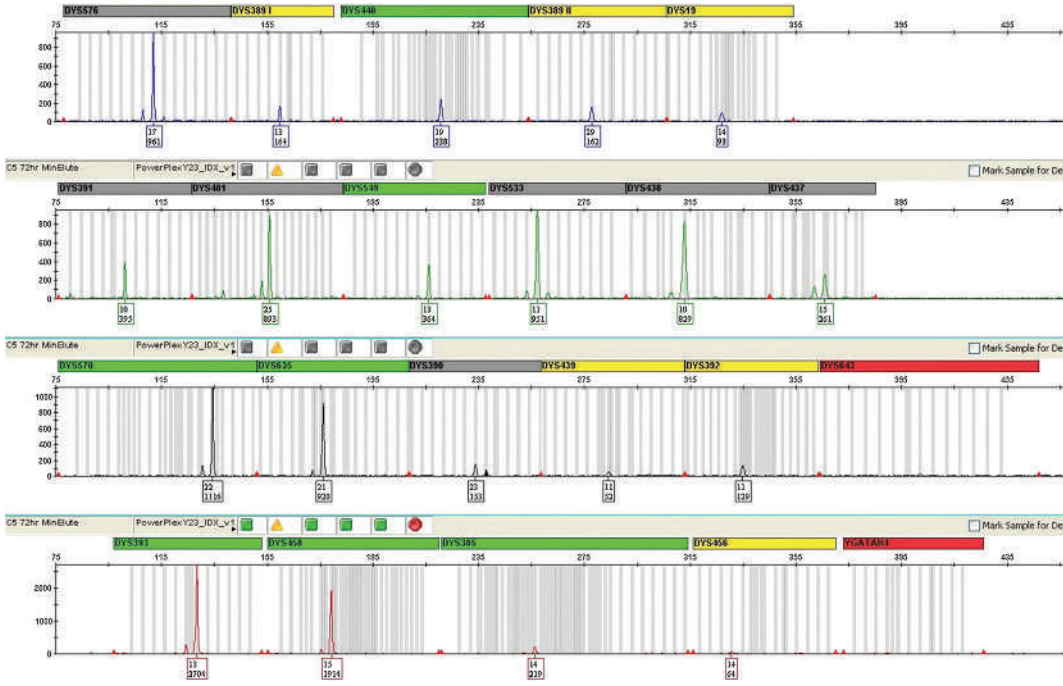

FIGURE 1. Electropherogram of 21-allele Y-SYR DNA profile from a vaginal swab taken 72 hours after digital penetration. Only two loci (DYS643 and Y-GATA-H4) did not yield a result. The 14 alleles detected at DYS385 is a duplicate allele because two copies of this locus are commonly found on the Y-chromosome.

full composite profile, and 2 couples yielded respective partial composite profiles of 7 and 10 alleles. Of all possible alleles, 71% were detected for all combined couples at the 72-hour interval.

Discussion

The results indicate that considerable variation exists between different couples. The variability between couples may be attributed to multiple factors. As reported from the survival of sperm in the vaginal cavity, factors may include oral contraceptives (Davies & Wilson, 1974), self-sampling (Quarino & Kishbaugh, 2012), and changes in the vaginal cavity at various points in the menstrual cycle (Morrison, 1972; Stein & Cohen, 1950). Although it is not entirely known to what extent these factors played in the variability from couple to couple in this study, these factors could possibly play an even bigger role in the survival of foreign epithelial cells in the vaginal cavity given that epithelial cells are far more susceptible to degradation. Sperm cell membranes include resistant disulfide bonds in the outer cell membrane of sperm cells, which are not present in epithelial cells (Butler, 2005).

Variability in results between couples may also be influenced by the men's ability to shed epithelial cells. Past studies have shown that individuals vary in their ability to shed epithelial cells (Lowe, Murray, Whitaker, Tully, & Gill, 2002) although the reasons for this may be complex (Phipps & Petricevic, 2007). Varying ability to shed epithelial cells may influence the number of deposited male epithelial cells in the vaginal cavity affecting male-specific DNA profiling.

It is also not known if the pattern of data was affected to any significant extent by missing data points because of attrition among certain couples in the study.

Despite variation seen in different couples, Y-STR alleles were obtained from every swab collection obtained from the eight couples in the study regardless of the time interval (29 of the 29 collections). As hypothesized, the number of alleles obtained decreased as the post-digital-penetration time interval increased. At 1-hour postpenetration, 71% of couples had profiles with greater than 20 alleles (full or nearly full profiles). This percentage from the 6-, 12-, and 24-hour intervals predictably decreased to 50%, 33%, and 20%, respectively. However, the percentage of couples with greater than 20 alleles increased to 40% at 72 hours after penetration, although this result is only based on two of the five couples yielding the high number of alleles. One of these couples (Couple 4) obtained their highest number of alleles at 72 hours (no other full or nearly full profiles were obtained at all other time intervals), whereas the other couple (Couple 2) yielded full or nearly full profiles at 1-, 6-, and 12-hour intervals.

It appears that results for each couple in the study were consistent over the five collections. Three of the five couples who completed the study had very similar results at 1- and 72-hour intervals. Couples who obtained full or nearly full Y-STR profiles at the shorter time intervals also obtained full or nearly full profiles as the intervals increased. This is

true for Couple 2 with the exception of 24 hours. One possible reason is that the collection at 24 hours after digital penetration occurred at a specific time during pregnancy where thick cervical mucus was excreted. This thick mucus may explain the decreased results compared with the other four profiles that were full or nearly full. In Couple 7, all profiles were full or nearly full except for the 72-hour post-digital-penetration interval. This couple reported having a cervical biopsy the week of that particular collection. This disruption may have decreased the DNA profile obtained and, therefore, may not be a true representation at this interval.

Couple 1 had full or nearly full profiles at the 1- and 72-hour time intervals and only partial profiles for the 6-, 12-, and 24-hour intervals. The samples collected at the 6- and 12-hour intervals had the lowest results. These samples were packaged incorrectly after collection, which may have contributed to the decreased number of alleles.

The increase in the number of alleles obtained from 24 to 72 hours for the three couples could be a consequence of self-sampling. Participants in this study were responsible for the collection of their samples. This introduces variation because of participants blindly inserting swabs into the vaginal cavity. Depending on the area the swab enters (e.g., the cervix), the amount of material collected may vary. Sperm are known to survive longer in the cervix, and foreign epithelial cells may also follow the same trend (Nicholson, 1965). During each collection, the women may have collected from a different area of the vaginal cavity.

For the five couples who participated in all sample collections, the average number of alleles detected was determined. The couple who averaged the least number of alleles was Couple 3 with an average of 11 alleles for the five time interval collections. Observations during analysis indicated that swabs from Couple 3 were discolored in appearance compared with swabs from other women. This color variation may be because of a deviation in pH or thicker cervical mucus as a possible result of a clinical manifestation (Majeroni, 1998) and may have contributed to lesser profiles obtained.

The highest allele average (21 alleles) was obtained from Couple 2. Because the female in Couple 2 was pregnant for most of the study, the collections were not susceptible to normal menstrual cycle changes in the vaginal environment and, therefore, may have aided in the survival of epithelial cells. An optimal vaginal environment for cell survival may be a factor in fertility (Morrison, 1972).

In recent years, the trend in the United States has been to increase the time interval that postcoital samples are traditionally collected in sexual assault cases because of empirical evidence. In some jurisdictions, the window for collection has been increased to 72 hours although this shift in policy is far from universal (United States Department of Justice Office on Violence Against Women, 2013). In cases of sexual assault where only digital penetration is reported, historically, samples have not been collected for DNA analysis. On the basis of the findings of this study, a similar change in public policy is warranted.

Although not every jurisdiction offers Y-STR testing, the National DNA Index reports that over 60% of public sector forensic science laboratories in the United States have Y-STR capability (D. Hares, personnel communication, November 24, 2014) as well as many private laboratories. The number of laboratories having Y-STRs online has greatly increased in the last decade offering the viability that this type of testing can have widespread use in investigations of this type. Although, at present, Y-STR DNA profiles are less discriminating than autosomal profiles, they can still provide investigative information and can be the basis for exclusion particularly in cases of adolescent sexual abuse where digital penetration is very common (Grossin et al., 2003). A recent study, however, offers the potential of using Y-STR to differentiate close paternal relatives by showing the high mutation rates of some Y-STRs (Ballantyne et al., 2010). If Y-STRs can be used to distinguish individuals along the same paternal line, their importance in sexual assault cases will become magnified. This work may also provide the foundation for examination of sexual assault cases involving female victims whose assailant are vasectomized men and in cases where ejaculation was not alleged. Male DNA may be detected and subsequently genotyped from epithelial cells foreign to the victim even if sperm is not present.

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