August 31, 2014

Post-Coital DNA Recovery Study

Final Report

NIJ Grant No. 2009-DN-BX-0023

Performance Period: January 2010 through July 2014

Prepared for

National Institute of Justice
810 Seventh Street, NW
Washington, DC 20001

Prepared by
Principle Investigator
Patricia Speck, DNSc, APN, FNP-BC, DF-IAFN, FAAFS, FAAN
The University of Tennessee Health Science Center College of Nursing
Department of Advanced Practice and Doctoral Studies (Ret.)
University of Alabama Birmingham School of Nursing
Department of Family, Community and Health Systems
NB-302 | 1720 Second Avenue South | Birmingham, AL 35294-1210
P: 205.934-6790 C: 901.488-7723 Email: pmspeck@uab.edu

Co-Principle Investigator
Jack Ballantyne, PhD
University of Central Florida
National Center for Forensic Science

ABSTRACT

Introduction and Background

Deoxyribonucleic acid (DNA) has revolutionized crime scene investigation. Infertility literature benchmarks provide evidence that DNA exists longer in reproductive tracks than general forensic studies historically demonstrate. The result is for most rape victims, timing for evidence collection is limited jurisdictionally to three days, even though recent literature describes novel advancements in Y-STR analysis methods, which is now customary in many forensic laboratories.

Aims

The study aims are: (1) what is timing for DNA recovery in proxy-couples from the vagina and cervix using Y–STR laboratory methods, and (2) what are common physiological conditions influencing recovery of DNA in post-coital proxy-couples?

Design and Methods

The study design is a prospective, mixed-methods design with thematic, descriptive, and inferential statistical analysis, including Generalized Estimating Equations (GEE) methods. Recruitment of volunteer proxy-couples followed a strict protocol of documentation of activities, abstinence, DNA deposit, and timed collections. Subject matter experts' focus group combed literature to identify themes to determine inclusion and exclusion criteria, including the abstinence washout period. Couple and collector recruitment used media, presentations, and flyers. Quality process safeguards included bulk case and kit assembly and study personnel availability to the couples and collectors. Eligible participants provide consents, eligibility data, and agree to complete a diary during four 10-day abstinent periods, and collect post-coital samples at baseline and 4-, 7-, or 9-days. Upon protocol completion, participants mailed samples to the forensic laboratory for Y-STR methods analysis. Secure physical environments and blinding of laboratory personnel and statisticians to all participant information protected personal health information.

Results

The focus group established a timed protocol, which included theme-based eligibility questionnaires, diary cards, collector forms, and kit contents. A full IRB application approval allowed recruitment. Upon completion of the protocol by the first five proxy-couples, Bayesian data analysis validated the complex study design and proposed statistical methods. Sixty-six of 112 consenting monogamous couples completed all phases of the study protocol. Participant couples (N=66) were primarily non-Hispanic white (91%), menstruating (94%), between 18-35 (80%), and college educated (77.3%). Analysis of standard and enhanced Y-STR, data revealed surprising differences between cervix and the posterior fornix recovery respectively. With analysis of standard and enhanced Y-STR methods from cervix or posterior fornix respectively, DNA detection improved significantly with enhanced Y-STR. When combining swabs, the cervix and posterior fornix analysis detected increased alleles in all timed samples, and enhanced Y-STR demonstrated significant DNA detection increases compared to standard Y-STR. Significant variables influencing DNA recovery using standard Y-STR methods include menses and hormonal birth control. The study data revealed odds of DNA recovery using standard Y-STR methods is significantly lower when reporting menstruation ($p=0.0445$), or when using

hormonal birth control (p=0.0004), not replicated with enhanced Y-STR analysis. With GEE modeling, data demonstrated the lowest DNA recovery with the presence of menses and hormonal birth control.

Conclusions and Discussion

The Post-coital DNA Recovery study developed an in vivo data model and study protocol establishing a valid scientific foundation for understanding extended interval post-coital DNA recovery and the influencing variables. Data from this study revealed standard Y-STR methods, when compared to enhanced Y-STR methods, are insufficient in DNA detection from both the cervix and posterior fornix at all timed collections (4, 7, 9, and baseline or 10 days). Data supports a practice change to a single swabbing in the cervix followed by the posterior fornix in forensic medical care. The data also demonstrated an association of two variables, i.e., menses and hormonal birth control, with diminished DNA recovery. This study provides strong pilot data to collect samples in females from the cervix and posterior fornix through their first menses for forensic laboratory analysis.

EXECUTIVE SUMMARY

Brief Synopsis of the Problem

In the 1970s, innovations in the forensic science community combined knowledge derived from the medical community, needing to treat disease and pregnancy exposure, with the forensic scientist community, seeking ABO secretor status and motile sperm in samples collected from victims. Historically, when secretor status was one benchmark for identification, research demonstrated a predictable decrease over time in vaginal semen after a deposit, as measured by acid phosphatase. Using state-of-the-art laboratory methods, forensic science scholars found limited probative value from post-rape vaginal samples after 72 hours. Thus, time limitations of 72 hours for evidence collection remained the recommendation for most jurisdictions responding to victims reporting rape until recently. Responding to the laboratory limitations, law enforcement did not bring victims to health care providers for evaluation and evidence collection after 72 hours, creating a barrier to care for victims traumatized by a rape and unable to report within 72 hours. During the same time over the last 40 years, the medical community researched and disseminated evidence about male and female infertility, finding sperm in the post-coitus deposit from collections with extended time intervals – sometime weeks. The medical community slowly built a body of evidence chronicling sperm viability and they discovered that the female vaginal environment influences sperm viability throughout the lifespan. There is no similar outcome in the forensic science literature to support comparable changes with seminal interaction or sperm persistence in the vaginal environment; therefore, conclusive judgments by medical forensic and forensic scientists about DNA recovery remain conjecture. Without evidence, victims are unable to pursue justice. The impact on the accused is also negative because in many cases DNA recovery and identification determines innocence or guilt.

Forensic evidence containing deoxyribonucleic acid (DNA) obtained from victims of rape contributes to elimination of suspects, conviction of others, and exoneration of those arrested and charged for rape. Today, evolutionary improvements in a variety of DNA technologies expand forensic laboratories' capacity to recover and analyze fresh, degraded, or complex mixtures containing DNA. Recent studies support expanding the timing of DNA sampling and recovery; however, the sample sizes were small and not generalizable. Early forensic research looked at acid phosphatase reduction over time, and today, there is reason to think that sperm cells behave differently than the bolus of semen or the subsequent liquefied semen. Forensic science literature has no data on the recovery of sperm cells, rich in DNA. Recent forensic science literature has little information about motility of sperm from the vagina or cervix because forensic laboratories predominantly receive dried samples or if the evidence is fresh and wet, upon receipt, the laboratory deliberately preserves the samples by drying them. The in vivo literature about DNA recovery in these populations is minimal, so laboratories exercise timing limitations. The scientific silos in medical and forensic laboratories have resulted in a "one size fits all" approach to the timing of collection of all forensic evidence from the vagina and cervix.

Although in vitro trends in expanding protocols related to DNA analysis are building, significant evidence for changing the timing of sampling for evidence is not in the forensic literature. Physical variation, acquired or congenital systemic morphology and changing environments provide three possible explanations for 'why' sperm is scarce in some cases. These explanations aside, sperm viability and therefore, recovery using newer methodologies reflects a complex multi-system challenge to current state of the art methods and processes when combined with human conditions and environments – all restricted by protocols that limit timing

in the forensic science community today. With evolutionary advances in technologies for DNA detection and identification, logically, limiting timing of the collection of evidence from the vagina and cervix to 72 hours begs re-evaluation.

Purpose

Grounded in forensic sciences, this research proposes to apply current and advanced Y-STR DNA technology in forensic laboratories to a large in vivo population of proxy-couples, to provide groundwork for future inquiry about the conditions affecting DNA recovery in the living patient, to determine timing for evidence collection, and to attempt to identify variables influencing DNA recovery. These answers will improve objectivity in the criminal justice system and provide choices for victims seeking care after the 72-hour limit. The objective of this research is to create the evidence base supporting or limiting the expansion of the 72-hour period for evidence collection. Another objective is to identify conditions that might influence the recovery of DNA, and therefore influence policies related to sample collection from the complex post-coital environment. In summary, the purpose of this research is to answer: (1) what is the period for DNA recovery in post-coital samples from the vagina and cervix using Y-STR laboratory methods, and (2) what are the common physiological conditions that may influence recovery of DNA in post-coital samples from volunteer proxy-couples.

Research Design and Methods

The research design is a prospective, mixed methods design with qualitative, descriptive, and inferential statistical analysis. Analysis of the data took place using The Statistical Package for Social Sciences (SPSS-PC) and the Statistical Analysis Software (SAS). This is an exploratory study but with multivariable models which help sort risk factors and their contribution to the outcome. The initial analysis is descriptive and includes means and standard

deviations, calculated from continuous data and frequency counts and proportions for categorical data. Where appropriate, Chi-square, Fishers exact, and *t*-tests compares two variables - younger to older subjects, as well as hymen appearance and other associated variables determined to be important by the Expert Advisor Group members. Multivariable analysis includes Generalized Estimating Equation (GEE), which is an extension of the quasi-likelihood approach, and accounts for repeated measures, the longitudinal design of this study, and the binary outcome of DNA recovery.

A focus group of national subject matter experts (SME) gathered to identify evidence-based themes for inclusion and exclusion criteria for couples, and defined variables, as well as developed the protocol for sample collection reflecting current research and demonstrating clinical rigor in research.

A full Institutional Review Board review and approval preceded nationwide recruitment of collectors, who recruited volunteer proxy couples. Volunteer proxy couples followed a strict protocol of abstinence, defined as 'no unprotected coitus' or 'if coitus occurred, use of barrier methods.' The abstinence period covered no less than 10 days over four different periods. The recruited couples completed demographic information and answered specific questions about their activities, health, and medical conditions. Once determined eligible, couples submitted baseline buccal samples, and the female partner provided separate cervix and posterior fornix samples at or after 10 days of abstinence from all types of unprotected coitus, but before the first DNA deposit. The proxy couples followed the complex protocol to abstain from all types of unprotected digital, oral, or genital-to-genital coitus for a period of 10 or more days, recording their daily activities. Upon sample collection completion, US Post Office delivered all anonymous samples to the laboratory for Y-STR analysis. All proxy couple information was

'blinded' to the forensic laboratory. The core team (e.g., the PI, research nurse, and data entry personnel) accessed all participant information. Statistical team members received all data de-identified for analysis. The analysis included identification of the themes evaluated on the tools for data collection, as well as descriptive and analytical/inferential statistical methods.

No publications existed in the literature that undertook research testing of coherent beliefs to satisfy the rules of probability in DNA recovery from post-coital samples taken from proxy couples utilizing a protocol with four abstinent periods. After a focus group of Subject Matter Experts and analysis by statisticians, a decision supporting a process evaluation of the first group of a minimum of five couples preceded the formal study for the purposes of assessing the validity and ease of all protocol steps. After Bayesian analysis confirmation, nationwide recruitment of registered nurse sample collectors occurred. Those interested recruited volunteer proxy couples and couples recruited collectors in their area. Recruitment and screening questions eliminated those with full hysterectomy (must have a cervix), male vasectomy or infertility (must have sperm DNA) and obesity (which lowers sperm count). If not self-eliminated, interested couples and collectors contacted the Principle Investigator or co-investigator where a telephone discussion about the study and protocol for IRB approved informed consent occurred. Participants received consent paperwork following the telephone-consent. Once the PI received the signed consents from both couple-members, each participant completed the questionnaire and demographic survey. PI determined eligibility and if eligible, sent a protocol kit, protocol diary and collector forms, and study directions to the female participant. Identification of a female nurse collector allowed for provision and individual review of specific instructions for speculum insertion, packaging, and completion of the collector's paperwork. The female participant of the couple was responsible for sending both the

self-addressed kit to the laboratory and the paperwork completed by the female nurse collector to the PI for data processing.

On receipt of the Post-coital DNA Recovery Kit, the couple began coital abstinence according to the protocol. The definition of abstinence for the purposes of this study was 10 or more days of barrier methods for intimate activities, which included digital coitus, cunnilinguis, and penile-vaginal intercourse. Under the protocol and before the first unprotected DNA deposit after the10-day abstinence, the female collector nurse took baseline samples from the buccal surfaces of the male and female couple-participants. At the same time, the collector RN took baseline samples from the cervix and posterior fornix as controls. One unprotected coitus followed by abstinence with sample collection occurred at Days 4, 7, or 9, depending on one of four 10-day periods of abstinence. Once collected, samples packaged according to the study protocol used the assigned unique identifier, and collectors or participants mailed the contents directly to the National Center for Forensic Science DNA laboratory for indexing and analysis using of Y chromosome markers (Y-STR) methods.

Each subject was required to complete a data set that provided their identifying information, which remained confidential and in a secure location, available to the PI, but blinded to the laboratory and evaluation researchers. Labeling all samples in the kit, the unique number with password protection was the common thread for entering data from the laboratory, providing another layer of confidentiality where other systems' protections were in place. Preservation of all raw data sets followed the Data Archiving Plan and the NIJ received the data sets as deliverables.

Consent was twofold. First, the individual was able to opt out without pressure, where continuation with deposits and collection implies assent-type consent to participate in this study.

Second, the collection, packaging and mailing of post-coital samples is self-initiated and implies consent under the protocol. The inclusion criteria for both male and female participants was specific to ensuring fertility, general good health, and determining one consenting and monogamous male – female couple. The exclusion criteria for participants were specific to eliminating infertility with specific health problems or conditions that reduce sperm count or collection post-coitus.

Some bias is inevitable. The design of the self-enrollment and screening process eliminated individuals with known reproductive contraindications. Self-selection bias was a concern. To minimize variability and create consistency among collectors, clear instructions about the sampling technique and packaging enhanced protocol completion. The PI and support personnel packed post-coital DNA collection kits simultaneously on four separate occasions for the purposes of maintaining quality and consistency of the kit contents, which were stored in a secure clinical area. Due to the nature of the sampling of human body fluids, transfer of post-coital proxy-couple samples to the forensic laboratory followed the rules outlined in the USPO Rule 346.326 Exempt Human or Animal Specimens.

Support for the female forensic nurse preference for collector role was *a priori*, supported by the forensic nursing education and training; however, additional verbal instructions related to speculum insertion, specimen collection, and labeling developed in an effort to maintain inter-rater reliability during the evaluation procedures. Completion of tools developed to understand the variables present during the cyclic environment of the female participant occurred with each specimen collection. For the female participant, daily activities related to coitus, menses, medication, and physical stresses documented on the diary card provided data for analysis.

Once in the forensic laboratory and following standard procedures for DNA extraction from the single submitted post-coital swab and the reference sample, laboratory personnel used standard techniques for Y-STR evaluation. This included a non-differential extraction procedure to separate the sperm from the non-sperm cells in the post-coital specimens. Quantification of DNA and exposure to male specific DNA amplification and analysis occurred using Y-STRs. Of the samples when the standard approach did not yield results, the forensic laboratory analyst used a number of advanced non-standard approaches using DNA profile enhancements developed by NCFS. The success rate and category assignment (full profile = 17 markers), partial profiles = 1-16 markers) and no profile = 0 markers) recorded the time since unprotected and protected coitus. Samples assigned to one of three categories were dependent on the number of genetic markers up to seventeen, and when analyzed any profile (1-17) counted as DNA recovery. Use of standard descriptive statistical techniques determined whether any of the other metadata associated with the samples yielded information on other factors that affect the DNA typeability of extended interval post-coital samples.

Results

For females, the focus group of SMEs listed common themes from the literature that may affect recovery from the cervix and posterior fornix of the vagina in the female and male. For males, the focus group of SMEs listed common data elements from the literature that may affect DNA deposits. The Bayesian review, which occurred when data was available upon completion of the complex protocol from five proxy-couples, confirmed that when followed, the protocol was sound and would yield the expected data.

Although a nationwide effort, participant proxy-couples (N=66) were primarily non-Hispanic white (91%), menstruating (94%), between 18-35 (80%), and college educated

(77.3%). The majority reported normal menstrual cycles that varied in length, were equal in reports about pregnancy or not, and the women used a variety of birth control methods.

As expected, the DNA recovery from the cohort of proxy-couples dropped with each progressive timed collection regardless of standard or enhanced Y-STR method, where statistical differences using standard and enhanced Y-STR methods in DNA recovery between timings (0-4 days, 4-7 days, and 7-9 days) (Table 7 and Table 10) was expected and explained. Recovery of DNA improved considerably on all timed collections with enhanced Y-STR compared to standard Y-STR in the cervix (Figure 8) and posterior fornix (Figure 9), with enhanced Y-STR methods out-performing standard Y-STR methods on all timed collections respectively. When combined posterior fornix and cervix samples with comparison of standard Y-STR to enhanced Y-STR methods, there is substantial increase in DNA detection (Figure 12), specifically 46.9% to 92.4% on Day 4, 26.6% to 78.8% on Day 7, 26.6% to 78.8% on Day 9, and 25.0% to 67.7% on Day 10 (baseline) respectively. Using standard Y-STR across all times, this research revealed recovered DNA in the cervix slightly more often than in the posterior fornix (Figure 4). Surprisingly, in this study, using results from the enhanced Y-STR, DNA detection occurred more frequently in the posterior fornix on Days 4 and 7 (Figure 7). Supporting this finding is descriptive data (Table 3) revealing that DNA detection using both standard or enhanced Y-STR method from the posterior fornix occurred in 53.7% (N=72) in at least one of four timed collections when it was not present in the cervix. DNA detection from the cervix occurred in 56.9% (N=72) in at least one of four timed collections when it was not present in the posterior fornix where the differences between the posterior fornix and cervix are not significant. Complicating the DNA location, 31.9% of the couples had one of four collections with DNA detection from the posterior fornix but not the cervix, and one of the remaining three collections

from the cervix but not the posterior fornix. These results imply that while present on any given day, DNA location in an extended post-coital environment is unpredictable for location and in an individual. To solve this dilemma, statistical analysis of the Y-STR methods revealed that DNA detection increased by combining samples taken from the posterior fornix and the cervix (Figure 12) rather than from either the posterior fornix (Figure 3, 6) or the cervix (Figures 2, 5) singly. The data supports one swabbing. To prevent iatrogenic injury, the recommendation is collection first from the cervix and then the posterior fornix.

Through literature review, menses and hormonal birth control changes to the genital track prompted a closer look at these two variables. The analysis of the study data revealed that the odds of DNA recovery is significantly lower using the standard Y-STR methods when menses is reported (OR: 0.5412; p=0.0445), and when hormonal birth control is used (OR: 0.2000; p=0.0004) (Table 7). With GEE modeling, data demonstrated statistical significance with the lowest DNA recovery rates, but not absence of DNA recovery, when using the standard Y-STR method. Therefore, DNA recovery reduction when both menses and hormonal birth control are present is statistically significant and occurred only when using standard Y-STR methods (Figure 13). A trend of reduced DNA recovery with menses and hormonal birth control is present using enhanced Y-STR methods, but the reduction is not statistically significant and DNA is not absent in either standard or enhanced Y-STR methods.

Implications for policy, practice, and future research

This research indicates that failure to collect samples from victims with an extended post-coital interval may result in the potential loss of probative evidence that could be crucial to the investigation and prosecution of sexual crimes. Even with menstruating women on hormone birth control, when using enhanced Y-STR, DNA detection is greater than 50% on Day 10,

compared to women without menstruation or hormones at >65%. The data analysis in this study supports evidence collection consideration of 'until a completed menstrual period' in menstruating reproductive females. Additionally, this research supports a change of a current practice from two separate swabbings, e.g., one swabbing of the cervix and one of the posterior fornix, to one swabbing of the cervix followed by swabbing the posterior fornix with the same swab. A simultaneous double-headed swabbing satisfies inevitable legal challenges about forensic laboratory bias from defense attorneys who receive their own sample for a separate laboratory analysis.

The results from this study should enhance the policy debate about evidence collection timing and collection methods among SART members. The policy debates should result in expedited changes to protocols and practices with rapid dissemination in support of evidence collection timing expansion through menses and implementation of the recommended increase in timing a victim can seek evidence collection and forensic medical care following rape in all jurisdictions throughout the U.S. and globally.

The results of this study inform policy, which influences practice and triage protocols that currently exist among SART member organizations. This study also challenges each SART member agency's internal policies and protocols. The results of this study informs policy which influences practice and triage protocols that currently exist among SART member organizations but also challenges each SART member agency's internal policies and protocols.

All SART member organizations should plan policy for the increased economy of scale, specifically, cost reductions in light of increasing demand for services as community awareness increases, by developing and testing new processes, procedures, and methodologies to achieve justice for victims and accused. The debates internal to SART organization administrative policy

maker members should focus on collaborations and cross training on topics such as case triage, evidence collection and medical treatment opportunities, workforce burden, prosecutor, law enforcement and laboratory capacity, as well as economic impact planning, particularly with extended interval complainants. With policy changes associated with the recommendations from this study, these researchers predict there is a corresponding expectation for increased rates of successful sexual assault convictions and exonerations with informed policies and procedures reflecting economy of scale.

The ripple from these research findings will touch all criminal justice, advocacy, and health care systems, which demonstrates the need for further research on many fronts. The volunteer participant population of primarily white college-educated, menstruating females in this study limits generalizability to genetic minorities, long known to have unique medical treatments for disease processes. Vulnerable populations of minority women subjected to the highest rape rates necessitate research that confirms or denies similar post-coital DNA recovery timing. Therefore, future research should concentrate on this vulnerable population of genetic minority women by following the existing complex post-coital DNA recovery protocol validated in this study. Other populations not studied but meriting research under this protocol include the growing number of older women experiencing menopause (with implications for child sexual assault with similar basic vaginal environments), digital penetration of women in all age groups, and DNA recovery from suspects and victims' oral cavities following cunnilingus or fellatio. The population of females with vasectomized male partners, which is a growing popular method of birth control, will yield a comparison group for touch DNA detection and detection of seminal products. Further research is necessary to substantiate the recommended practice change to a single swabbing technique, given the legal challenges that await this evidence-based practice

change recommendation, e.g., defense attorneys wanting to test the sample in a separate laboratory. Future research may include laboratory use of parts of a single swab and determination of differences between the first cut sample and the remaining second sample.

Forensic laboratory research is necessary to delineate a clear standardized process for application of enhanced Y-STR methods, including the threshold for individual identification after DNA recovery, and development of newer novel methods for use on extended post-coital timing samples. With the success of the 9 and 10-day profiles at 25.5-67.7% or greater with standard and enhanced Y-STR methods respectfully, it might even be possible to obtain profiles from samples collected beyond menses, which is the recommended evidence-based timing for collection from this research. The recommendation provides the impetus for extended timing in sample collection rather than the current limitations exercised with today's jurisdictional policies and standard operating procedures. We have concentrated on use of standard and enhanced Y-STR methods in this study, demonstrating with enhanced Y-STR methods substantial DNA detection rates at 9 and 10 days (78.8%, 67.7%). It is likely the final detection limit is unknown. Therefore, research testing targeting samples collected 14 or more days after coitus may provide additional insight about timing and testing of collections. While more challenging and possibly controversial is the recommendation to undertake research to extend the post-coital interval in the future, and possibly obtaining a standard autosomal STR typing of the semen donor.

All systems and agencies responding to victims of sexual assault and rape should implement formative and summative program evaluations to study the impact of the evidence-based recommendations arising from this research on their systems. Systematic economic and program evaluation with summative and formative outcomes and outputs will assist in the

implementation of yet unknown best practices and processes that will affect the efforts to increase 'economies of scale' for all.

Recommendations

This research provides the evidence base for a practice change in timing for evidence collection in cases of rape from SART members who respond to all raped complainants. The recommended timing for evidence collection for menstruating women is through the first post-rape menses. The recommendation is that SART members provide a thorough investigation and medical forensic evaluation using the latest research for detection of injury, pregnancy and illness exposure, as well a single combined sampling from the cervix and posterior fornix.

Standard protocols that currently instruct health care providers to gather multiple swabs samples from each location in the cervix and the vaginal vault should cease as soon as possible, to reflect the evidence from this study, which is to implement swabbing of the cervix first, followed by swabbing the posterior fornix with the same swab to increase DNA recovery.

The research provides the evidence base necessary to extend the timing for sample collection through menses using one swabbing – first from the cervix, followed by the posterior fornix for the purposes of recovering DNA areas using both standard and enhanced Y-STR methods. Policy recommendations include collaborative discussions among SART members about the legal ramifications of the 'one swabbing' recommendation, since defense attorneys typically want a separate swab for testing. Future research should include quantification studies about laboratory analysis of parts of a single swab and determination of differences between the first cut sample and the remaining second sample. Finally, the recommendation for forensic laboratories is to apply the evidence from this study to protocols addressing collected samples from extended interval situations.

This research also supports including menstrual history and type of birth control in the medical forensic history, specifically to understand and anticipate that hormone birth control with menses is positively associated with a statistically significant reduction in (but not absence of) DNA recovery using standard Y-STR methods. The significant reduction in DNA detection did not materialize using the enhanced Y-STR method, although there was a trend toward reduction of DNA recovery. Therefore, since population-research only informs practice, it is necessary for health care providers to advise the individual with both menses and use of hormonal birth control about the study findings of reduced DNA detection with standard Y-STR methods. Importantly, in patient-centered care, advisement includes that the study reflects data from a population of participants, and the study results cannot predict an individual's DNA recovery outcome. Recommended dissemination to forensic laboratories about this study's data, which is significant in DNA recovery from extended post-coital intervals using enhanced Y-STR methods, could spur policy and procedure changes. When samples tested by standard Y-STR methods are negative for DNA detection or if there is a person with an extended post-coital interval, the policy change reflects knowledge of evidence recommending triaging all extended post-coital interval cases directly to enhanced Y-STR methods.

These practice recommendations are evolutionary and based on building the evidence base from a number of small studies cited in this work, and when confirmed by this larger research project, validated expanded post-coital interval sampling and testing through the first menses. However, the ramifications of this recommended practice change is not without impact. Therefore, these authors recommend a common model for systems evaluation with validated tools measuring quality, processes, and economies of scale, which should be available to all SART members in a widely disseminated online toolbox to promote standardization of best

practices among disciplines and between members of the SART. All the recommendations from this study, when implemented, could increase patient exposure to therapeutic interventions, improve DNA detection, increase plea agreements through improved prosecution, and result in conviction or exoneration of the accused, all while creating an 'economies of scale' offsetting the costs of a predicted increase in reporting.

The evidence from this research promotes changes in practice related to timing of collections and improvement in understanding the variables affecting DNA recovery, which are more complex than known previously from published forensic literature. For the SART members and their organizations, resourced organizations make changes quickly, but for others, these practice changes occur slowly, but build over time. In the short run, the practice changes may be disruptive, but in the end, through ingenuity, resource development with an eye on economies of scale, and collaboration, the practice changes supported by this research benefit the victim and the accused by improving criminal justice outcomes.

TABLE OF CONTENTS

Abstract ..... **iii**

Executive Summary ..... **v**

Table of Contents ..... **xxi**

Introduction **1**

Statement of the Problem 1
DNA Recovery 3
Semen and Sperm 7
Male Contribution to Cervical-Vaginal Sperm Retention 9
Vulva and Vagina 10
Cervix 13
Persistence of DNA on Other Sites 14
Forensic Laboratory 14
Summary 16
Research Aims, Purpose and Objectives 17

Methods **17**

Research Design 17
Methods, Materials and Procedures 18
Focus Group 18
Bayesian Review 19
DNA Recovery Protocol 20
Recruitment and Consent 20
The Protocol Process 21
Protection of Human Subjects and Confidentiality 22
Recruitment Consenting Couples 23
Participant recruitment 23
Inclusion criteria 24
Exclusion criteria 25
Ethical Considerations 25
Confidentiality 25
Bias 26
Kit Distribution Process 27
Quality assurance, packaging, and storage 27
USPO mailing requirement 28
Collection Protocol for Post-coital Interval Samples 28
Collectors 28
Speculum insertion and contamination 28
Sampling 29
Labeling 30
Documentation 30
Daily activities, conditions, condom and coitus history 30
Documentation of clinical appearance 30
Laboratory Method 31

DNA isolation 31
Purification 31
Quantitation 32
Y chromosome-specific nested PCR pre-amplification 32
Y-STR amplifications 32
PCR product detection - capillary electrophoresis 33
Data Processing 33
Summary of Post-Coital DNA Recovery Collection Protocol 34
Statement of Results 36
Qualitative Analysis 36
Focus group 36
Focus group suggested changes in protocol 38
Bayesian review results 38
Descriptive Results 38
Post-coital DNA kits 38
Demographics 40
Inferential Results 41
DNA recovery 41
Hormonal Birth Control and Menstruation 49
Standard Y-STR 49
Enhanced Y-STR 50
Assumptions and Limitations 51
Assumptions 51
Limitations 52
Tables 53
Table 1 53
Table 2 56
Table 3 57
Table 4. 61
Table 5 62
Table 6 63
Table 7 63
Table 8 64
Table 9 64
Table 10 65
Table 11 65
Figures 66
Figure 1 66
Figure 2 67
Figure 3 68
Figure 4 68
Figure 5 69
Figure 6 69
Figure 7 70
Figure 8 70
Figure 9 71

Figure 10 71
Figure 11 72
Figure 12 72
Figure 13 73
Figure 14 73

Conclusions 74

Discussion of Findings 74
Implications for Policy and Practice 77
Implications for Further Research 80
Recommendations 82

References 86

Dissemination of Research Findings 93

Publications 93
Presentations 93

Definition of Terms 95

ACKNOWLEDGEMENTS

National Institute of Justice

Bethany Backes, PhD, NIJ Project Manager
Cathy Girouard, PhD, NIJ Project Manager
Donna J. Davis, PhD, Social Services Scientist

The Interprofessional Team

Erin Hanson, PhD, Co-Investigator
National Center for Forensic Science, University of Central Florida
Elizabeth Lee, PhD, APN, Co-Investigator
University of TN Health Science Center College of Nursing, College of Graduate Health Sciences and University of Arkansas Fayetteville College of Nursing
Simonne S. Nouer, MD, PhD, Co-Investigator
University of Tennessee Health Science Center
College of Medicine, Department of Preventive Medicine
Pamela D. Connor, PhD, Co-Investigator
University of Tennessee Health Science Center
College of Medicine, Department of Preventive Medicine

Subject Matter Experts

Susan Chasson, JD, MSN, FNP-BC
Elise Turner, CNM
Michael Weaver, MD
Wendy Likes, PhD, APN, University of Tennessee Health Science Center
College of Nursing, Department of Advanced Practice and Doctoral Studies
Ann Cashion, PhD, RN, University of Tennessee Health Science Center
College of Nursing, Department of Advanced Practice and Doctoral Studies and
National Institute of Nursing Research

Administrative Support

Marion Donohoe, DNP, APN, Research Nurse Coordinator
Zoran Pavaciek, MPH, Data Entry and Descriptive Analysis
Gail Spake, Editor

INTRODUCTION

Statement of the Problem

Cutting edge technology in the 1970s in the forensic sciences combined knowledge derived from the medical community needing to treat disease and pregnancy exposure; and the forensic scientist community seeking motile sperm and ABO secretor status in samples collected from victims (Archambault, 2000, September). Forensic science research found that after 36 to 72 hours, discharge, disease, and sample degradation limited effective evaluation of collected vaginal evidence. Importantly, research using acid phosphatase testing of post-coital samples determined a predictable decrease in acid phosphatase in the vagina over time (Sensabaugh, 1979). Therefore, the forensic laboratory interpretation of the science about evidence from the genital structures limited collection times (Archambault, 2000, September; A. M. Hall & J. Ballantyne, 2003b; Ledray, 1999). Thus, time limitations of 36 hours (Ledray, 1999) were commonplace and the 48-72 hour limit, confirmed by forensic laboratory study results (A. M. Hall & J. Ballantyne, 2003b), remained the recommendation for most jurisdictions responding to victims reporting sexual assault and rape. Regardless of the probative value of DNA in a particular case, within 72 hours of a rape, the evidenced-based standard of care is to collect evidence from the vaginal and cervical environment. Law enforcement did not bring victims to health care providers for evidence collection after 72 hours; however, many did refer victims to health care providers for pregnancy and sexually transmitted disease testing and follow-up. Evidence from victims shows that reporting within 72 hours remains a challenge for many victims traumatized by a rape (Burgess, Fehder, & Hartman, 1995). A victim's perception of re-victimization in and by the criminal justice and medical systems supports the use of advocates (Maier, 2008; Ranjbar & Speer, 2013; Schonbucher, Maier, Mohler-Kuo, Schnyder, & Landolt, 2014), but for some victims needing help with rape recovery, barriers to follow-up with

counselors and/or health care providers never becomes an option (Boykins & Mynatt, 2007; Dohrenwend, 2011).

Concurrently, during the last 40 years, the medical community researched and disseminated evidence about male and female infertility, as well as men and women's health. The reproductive health challenges chronicled describe congenital, acquired and systemic physical and environmental realities, included sperm quality, pH, as well as vaginal and cervical receptivity in menstruating females (Hess, Austin, Dillon, Chang, & Ness, 2008; Suarez & Pacey, 2006). Females, throughout their life cycle, from birth to death, experience hormonal fluctuations and chronological aging processes reflected in the appearance of the genitalia (Hale, Robertson, & Burger, 2014; Speck & Patton, 2007). Clinical evidence supports the notion that estrogen influences aging and maturation changes, which reflect changing vaginal pH as well as the appearance of the hymen (Hale et al., 2014; Speck & Patton, 2007). Age, illness and environment modify sperm motility, persistence and presence, therefore viability, in the male and recipient female (Fisch, 2009; Suarez & Pacey, 2006), possibly resulting in degradation of DNA. Designed to protect itself from intruders, the female genital structures of the reproductive track, particularly the vagina and cervix, treat sperm and semen as foreign material. Subsequently and throughout the reproductive years, the vagina produces chemicals in secretory glands to eliminate foreign materials by washing out the foreign substances, and maintains an acidic pH (3.0-5) that immobilizes the sperm (Suarez & Pacey, 2006). There is no documentation or studies about cyclic changes in the vaginal environment and the interaction of seminal products with the acidic secretions in the forensic literature, thereby preventing conclusive judgments by medical forensic and forensic scientists about influences in DNA recovery and identification.

The scarcity of forensic DNA recovery research from large in vivo populations, necessary for broad policy and procedures among SART member organizations, and the plethora of infertility research in medical communities exposes gaps in understanding about DNA evidence recovery as it relates to timing of evidence collection in changing genital environments in reproductive females. As a result, the dearth of in vivo DNA research, decisions by Sexual Assault Response Teams (SART) regarding timing of evidence collection from changing genital environments following rape have remained the same. The impact is re-traumatizing for victims unable to seek help within the 72-hour time limit for evidence collection and DNA recovery in most jurisdictions; then without evidence, victims are unable to pursue justice. The impact is just as bad for the accused whose innocence or guilt could be determined by DNA recovery and specific identification. As the evidence supporting cyclic and reproductive track environment changes mounts, concurrently there are advances in DNA recovery and identification in forensic laboratories with smaller and smaller, often degraded, samples. Grounded in forensic sciences, this research proposes to apply current and advanced Y-STR DNA technology in forensic laboratories to a large in vivo population of proxy couples, in hope to provide foundational groundwork for future inquiry about the conditions affecting DNA recovery in the living patient. The research will determine timing limits for evidence collection, DNA recovery, and identification, and to attempt to identify variables, which influence recovery. These answers will improve objectivity of the criminal justice system's response to victims and provide choices for victims seeking care after the 72-hour limit currently set.

DNA Recovery

Forensic evidence containing deoxyribonucleic acid (DNA) obtained from victims of rape contributes to elimination of suspects, conviction of others, and exoneration of those

arrested and charged for rape (Roman, Walsh, Lachman, & Yahner, 2012). When forensic nurses specializing in sexual assault care also collect evidence from victims, evidence collection kits are more accurate and complete (Sievers, Murphy, & Miller, 2003), and prosecution rates increase (Campbell, Patterson, Bybee, & Dworkin, 2009; Crandall & Helitzer, 2003).

Today, evolutionary improvements in a variety of DNA technologies has expanded the forensic laboratory's capacity over time to recover and analyze fresh, degraded, or complex mixtures containing DNA (J. Ballantyne, 2013; Elliott, Hill, Lambert, Burroughes, & Gill, 2003; Garvin, Bottinelli, Gola, Conti, & Soldati, 2009; Giusti, Baird, Pasquale, Balazs, & Glassberg, 1986; A. Hall & J. Ballantyne, 2003; Hatsch, Amory, Keyser, Hienne, & Bertrand, 2007; Mayntz-Press, Sims, Hall, & Ballantyne, 2008; Voskoboinik & Darvasi, 2011). In one recent study, full DNA profiles were found in vaginal/cervical post-coital samples at three and four days, and partial DNA profiles found five to six days post-coitus (Mayntz-Press et al., 2008); however this research is limited by the size of the sample (3 couples) and blind swabbing techniques. Little has appeared in the recent forensic science literature about motility of sperm from the vagina or cervix. This is likely because the forensic laboratory only receives dried samples hours or days after the crime or if the evidence is fresh and wet, the laboratory deliberately preserves the samples by drying them upon receipt and prior to DNA analysis. Conversely, no literature about older or menopausal women and sperm recovery is in the scientific medical or forensic literature; where infertility literature yields many reasons for degradation of DNA in men (Jensen et al., 2014; Meeker et al., 2010; Pacey et al., 2014; Trisini, Singh, Duty, & Hauser, 2004). Children, when studied for DNA recovery from sample collections after rape complaints, yield little promise with poor outcomes outside 24 hours (Christian et al., 2000; Hornor, Thackeray, Scribano, Curran, & Benzinger, 2012).

The scientific silos in medical and forensic laboratories resulted in a “one size fits all” approach to the timing of collection of all forensic evidence from the vagina and cervix. Since the in vivo literature about DNA recovery in these populations is minimal, laboratories exercise timing limitations in the development of samples collected from victims. Consequently, protocols for collection techniques and evaluative measures for sexual assault evidence in the forensic science community have remained the same for all women (and men) of any age and all reproductive capacities. This lack of rigor in forensic scientific inquiry reproach ("'Badly fragmented' forensic science system needs overhaul," 2009; Budowle et al., 2009; Committee on Identifying the Needs of the Forensic Sciences Community National Research Council, 2009; Mnookin, 2009) and the long-standing timing limitations for sexual assault evidence collection, which pre-dates DNA technology, fits the criticism. The evolutionary advances in technologies for DNA detection and identification, logically, limiting timing of the collection of evidence from the vagina and cervix to 72 hours begs re-evaluation.

The speed of elimination of degraded materials (sperm, seminal and cellular products in this case) is dependent on many factors. Understanding is evolving to believe that former studies about acid phosphatase (Sensabaugh, 1979), seminal backflow, sperm motility and viability are unique and distinguishable in today’s laboratory environment. These evidence-based factors include the pre- and post-rape activity (e.g., coitus with partner, condom use, medication application), age of the patient (e.g., young verses old), the time in a female’s cycle (e.g., pre-menarche, reproductive or menopausal), male or female disease states (e.g., infections and cancers) or conditions (e.g., autoimmune, genetic, or medication side effects), post rape activity (e.g., douche, reclining or bathing) and health and viability of the sperm from the assailant (Bouvet, Gresenguet, & Belec, 1997; Christian et al., 2000; Colagar, Marzony, & Chaichi, 2009;

Enos & Byer, 1977; Finkel & Giardino, 2002; Fisch, 2009; Hawkes & Turek, 2001; Hess et al., 2008; Jordan, 1999; Katz, Slade, & Nakajima, 1997; Nicholson, 1965; Sensabaugh, 1978; Speck & Patton, 2007; Suarez & Pacey, 2006; Tucker, Claire, Ledray, Werner, & Claire, 1990; Waugh & Grant, 2006; Young, Jones, Worthington, Simpson, & Casey, 2006). A rape complainant is limited by time in the recovery of cellular and seminal products from the vagina as the body eliminates foreign material rather quickly (Suarez & Pacey, 2006). However, which, if any, variables associated with the elimination or degradation of seminal fluids, sperm, or cellular products is unknown, nor are many of the conditions supporting preservation of sperm or collection timing for DNA recovery from the sperm or cells. However, there is reason to believe that semen and sperm behave differently in a unique person's vaginal environment and compounding the explanation is increasingly sensitive DNA recovery tools. There remains a lack of understanding about individual variability and DNA recovery predictability person to person or couple to couple.

Although in vitro trends in expanding protocols related to DNA analysis are building, significant evidence for changing the timing of evidence collection is not in the forensic literature, which proposes that the absence of sperm does not mean rape did not occur. There are three possible explanations for 'why' sperm is scarce. They include physical, systemic, or environmental explanations (Beckmann et al., 2014). Other factors associated with recovery of sperm from the male include the male did not ejaculate, poorly ejaculated, had a vasectomy or used a condom (Groth & Burgess, 1977). These explanations aside, sperm viability and therefore recovery reflects a complexity of multiple conditions and environments that are not well-understood (McKnight et al., 2014), nor addressed by limited protocols for evidence collection determined by a 1970's 72-hour post-rape timing. Discussions about these intertwining

complexities at professional meetings, including the evidence supporting complex interactions of the post-coital seminal products in the vaginal environment (Suarez & Pacey, 2006), were the stimulus for the Co-PI of this study to query in a pilot study – how long can sperm be found in post-coital samples? In a small sample of three laboratory couples, partial fragments from sperm were found at five and six days post-coitus (Mayntz-Press et al., 2008), more than doubling the time frame for routine testing and DNA recovery! The sample was small (3 couples), too small for protocol changes, but the pilot study set the stage for this collaboration to plan research utilizing interprofessional investigator experts – the healthcare practitioner PI in the clinical arena and the Co-PI in the laboratory. At around the same time, a forensic nurse-laboratory collaboration published study outcomes of a convenience sample of rape cases with DNA recovery and compared the cervix to vagina in the same patient; not unexpected in healthcare practitioner communities treating infertile couples, the results stated that cervical samples produced DNA more often (Morgan, 2008). These two small studies used descriptive statistics to evaluate the phenomena of DNA recovery, thereby setting the stage for a larger, complex inquiry using post-coitus proxy couples to submit samples at timed intervals for forensic laboratory analysis. Without a previous protocol to study post-coital sample collection, researchers combed literature about the heuristic elements necessary for understanding complexities related to DNA recovery and identification.

Semen and Sperm

Semen is a mixture of viscous secretions from the prostate, seminal vesicles, and bulbourethral glands that blend with the semen arising from the testis and epididymis (Beckmann et al., 2014) and must meet the normal semen measurements of volume, concentration, motility, rapid progressive motility and morphology (Cooper et al., 2010). Sperm are terminally differentiated cells and do not have the capacity to repair themselves, like many other cell types

(Suarez & Pacey, 2006). Considering the anatomy of the individual male penis and the individual female vagina, for most, sperm in consensual coitus is deposited in the posterior fornix near the cervical os where sperm swim to the cervical canal (Suarez & Pacey, 2006). Numbers of sperm are dependent on the number of ejaculations in a period of time and the volume of ejaculate (Beckmann et al., 2014; WHO/Department of Reproductive Health and Research, 2010). Pheromones and environmental cues in female nervous systems influence sperm motility (McKnight et al., 2014). The human semen coagulates within a minute of coitus but begins to degrade within the hour because of enzymes from the seminal vesicles and prostate (Lilja & Lundwall, 1992; WHO/Department of Reproductive Health and Research, 2010). When a rape victim presents within the first hour after a rape, this mucous gel may be mistaken for Spinnbarkeit, e.g., a German word for the sticky mucous formed in the cervix during ovulation (Waugh & Grant, 2006). In addition, the normal liquefied semen sample's color is influenced by sperm concentration, haemospermia or oral medications, e.g., vitamins (WHO/Department of Reproductive Health and Research, 2010). Flowback is the term used to describe the liquefacation of the gel (e.g., becoming liquid) following semen deposition where high viscosity and sperm agglutination interferes with determining motility, concentration and measurement of biochemical markers (WHO/Department of Reproductive Health and Research, 2010). In 1993, it was found that sperm loss from the vagina occurred within 5 to 120 minutes of deposition, the median of 35% were lost in flowback, but that in 12% of copulations, 100% of sperm were eliminated within 120 minutes (Baker & Bellis, 1993). Reportedly, "this suggests that less than 1% of sperm might be retained in the female reproductive tract and this supports the notion that only a minority of sperm actually enter cervical mucus and ascend..." (Baker & Bellis, 1993), but may be reflective of the state of the science 20 years ago. The variability and rapidity of sperm

loss poses special considerations for the forensic community and answers may be through studying which elements in the female's vaginal environment influence DNA recovery and identification.

Male Contribution to Cervical-Vaginal Sperm Retention

Evolutionary adjustments for propagation of the species results in the increased chance that spermatozoa will neutralize the acidic vaginal secretions in reproductive aged females; thereby, the sperm remain in the vaginal tract by producing a gel of the ejaculate when pooled in the fornix, which effectively holds healthy sperm next to the cervix (Suarez & Pacey, 2006). Sperm next to the cervix enhances ultimate access to any ovum ready for fertilization. Seminal plasma health is critical to the quality and quantity of sperm (and therefore DNA), which diminishes with oxidative stress; and the result is infertility (Agarwal, Durairajanayagam, Halabi, Peng, & Vazquez-Levin, 2014). Conversely, progressive sperm motility improves pregnancy rates (Jouannet, Ducot, Feneux, & Spira, 1988; Larsen et al., 2000; Zinaman, Brown, Selevan, & Clegg, 2000). Seminal plasma interacts with the vaginal environment, possibly influencing the peri- and early placenta implantation and growth (Bromfield, 2014), important to victims of rape worried about pregnancy. Thickened cervical mucus complicates passage of sperm into the cervix and the thinking is that the neutralization process between semen, spermatozoa, and vaginal defenses may also encourage HIV transmission when women are exposed (Bouvet et al., 1997; Katz et al., 1997). With sufficient quantity and quality, a series of complex capacitation processes result in spermatozoa structural and functional changes, which creates hyper-activation necessary to penetrate thick cervical mucus (Ferramosca & Zara, 2014), necessary for pregnancy. However, in spite of these evolutionary environmental and cyclic changes, elimination of most seminal products from the vagina occurs within two hours due to seminal

backflow. Backflow definition is the liquefaction of semen pouring out of the vagina to the perineum leaving less than 1% of ejaculate (e.g., sperm) to migrate into the cervix. External environmental factors notwithstanding, there are a number of reasons why sperm might lack quality and quantity, e.g., malformations, nutrition, age, disease, medications, increasing lipid levels, and obesity (Chavarro, Toth, Wright, Meeker, & Hauser, 2009; Colagar et al., 2009; Eskenazi et al., 2005; Ferramosca & Zara, 2014; Fisch, 2009; Hawkes & Turek, 2001; Schisterman, Mumford, Browne, et al., 2014; Suarez & Pacey, 2006; Trisini et al., 2004). In fact, the obesity epidemic has exposed physical factors affecting the integrity of the spermatozoa DNA including sperm quality that reduces fertility (Chavarro et al., 2009; Schisterman, Mumford, Chen, et al., 2014; Trisini et al., 2004). The quality of seminal fluid as the transporter of sperm, is now thought to regulate the female vaginal and cervical track environment (Bromfield, 2014). Longstanding protocols, tradition, and a lack of resources for recent technology, replication of morphological profiling of spermatozoa among forensic laboratories is non-existent. Infertility researchers have found there are many factors affecting viability of spermatozoa, specifically its presence, the environment, sustained motility, and longevity in the vaginal track; however, these clues to understanding DNA recovery from the vaginal cervical environments have not been thoroughly researched.

Vulva and Vagina

The embryologic development of the female genitourinary system arises from all three embryologic layers – mesoderm, endoderm, and ectoderm. Variations in appearance are due to genetic and nutritional environments (e.g., Hypospadias, or genetic aberrations). After birth, pituitary hormones activate mammary and uterine growth, resulting in foliculaogenesis from follicular stimulating hormonal surges, considered normal for female reproductive development

in early infancy (Kuiri-Hanninen et al., 2013; Kuiri-Hanninen et al., 2011). Throughout the life span, the genitourinary system changes appearance and function as hormonal fluctuations yield to sexual maturity in staged development, e.g., Tanner stages, Hymen Maturation Scale (Speck & Patton, 2007). Stresses (including sexual abuse and rape), irritating environments, conditions or diseases also change the appearance and function of the genitals ("Managing common vulvar skin conditions," 2008; Sargeant & O'Callaghan, 2007). With body-dysmorphic disorder (BDD), "persistent and intrusive preoccupations with an imagined or slight defect in one's appearance," obsessions and anxiety occur (Anxiety and Depression Association of America, 2014). With BDD whether focused on genital or other areas, patients seek surgery to hide or improve their appearance, usually with temporary results (Anxiety and Depression Association of America, 2014).

The normal vulvar area is smooth mucous membrane and contains structures including labia minor, clitoris, vestibular bulbs, and openings to the urinary track (urethra), glands (Skeen's and Bartholin), and vagina through the hymen, (Andrikopoulou, Michala, Creighton, & Liao, 2013; Faugno & Speck, 2011; Puppo, 2013). The physiology of the genitourinary sexual response was first described by Masters and Johnson (Masters & Johnson, 1966). Female sexual arousal occurs in three phases (latent, turgid, and rigid). Congestion occurs in female genital structures (clitoris [glans, body, crura], labia minor, vestibular bulbs and corpus spongiosum), which are homologous to the male, and result in bulbocavernosus muscular orgasmic contractions and lubrication (Marthol & Hilz, 2004; Puppo, 2013). Normal phases of orgasmic female sexual response include capacity for multiple cycles of arousal with continued stimulation (Puppo, 2013), which is particularly concerning for victims who experience orgasm while being raped over long periods of time or by multiple rapists.

Environmental elements effecting the appearance and function of the genitourinary structures include allergies, external application of medication, and chemical contact. Hormonally induced atrophy, from either disease (e.g., cancer), aging (e.g., menopause or childhood) or pharmaceuticals, leads to loss of glycogen content and thinning of vaginal tissue (Beckmann et al., 2014; Puppo, 2013). The lack of glycogen reduces lactic acid and may increase pH altering vaginal secretions, possibly altering sperm and DNA recovery. The increase in glycogen during the luteal phase of the cycle results in copious yeast-like discharge and symptoms, but is distinguished by the pH of 3.5-4.5 (Suresh, Rajesh, Bhat, & Rai, 2009). The pH of the vaginal track is influenced by the age of the woman, the length and period in her unique cycle (usually 28 days during reproductive years), the amount of glycogen (sugar) necessary for lactic acid production and acidic environments in reproductive years, and a host of other influences (Suresh et al., 2009). Conditions affecting the lower genital tract may include normal aging, nutrition, autoimmune or unknown etiologies, e.g., menopausal urinary incontinence, burning, itching, lichen sclerosis or cytolytic vaginitis (Beckmann et al., 2014; "Managing common vulvar skin conditions," 2008; Sargeant & O'Callaghan, 2007; Suresh et al., 2009). Disease states are the most common reason for change in appearance of the genitourinary system of the vulva and vagina, and include STIs e.g., trichomonasis, candidiasis, bacterial vaginitis, Neisseria gonorrhea, and Chlamydia trachoma, and viral etiologies e.g., Human Papilloma Virus (HPV) and Herpes Simplex (HSV).

Other variables are promulgated as reasons for variability in volume of DNA in the female's reproductive track, e.g., activity, personal care, menstruation, and partner factors such as sperm count, volume, and number of ejaculations (J. Ballantyne, 2013). However, there is no generalizable research available to date in forensic literature.

Combining the knowledge about health and disease as well as male spermatozoa and female vulvovaginal environments provides a foundation for subject matter experts to begin to separate common variables that influence post-coital DNA recovery in forensic settings.

Cervix

The cervix arises from the mesoderm (Kurita, 2011), as does the oviduct, uterus and anterior vagina where differentiation among the structures creates unique morphology and function. Exposure to disease, medications, including hormones, or chemical moderators of development change the appearance, function, and ultimate health of the structures (Yin & Ma, 2005), possibly transferring the changes to subsequent generations through epigenetic adaptations (Masse et al., 2009).

In reproductive aged women, the cervix is protection for the upper genital track, providing a thick barrier mucus plug to prevent ascending infections. However, during ovulation, the secretions thin, allowing sperm to penetrate the cervix and travel into the uterine body and fallopian tubes (Beckmann et al., 2014; Harris-Glocker & McLaren, 2013). Estrogen affects the quality and consistency of the cervical mucus and in the past, clinicians qualitatively evaluated post-coital mucus for quality in the sperm-mucus interactions when couples were infertile (Beckmann et al., 2014; Harris-Glocker & McLaren, 2013). The emerging role of estrogen as a regulator and knowledge about the locations of receptors provides an opportunity to evaluate estrogenic contributions to endothelial changes in preparation of reproduction in women (Su, Xin, & Monsivais, 2012). The role of estrogen and the estrogenic environment of the vagina and cervix, as well as other reproductive hormones in the recovery of DNA from the cervical and vaginal environment are absent from forensic literature.

Persistence of DNA on Other Sites

Locard Exchange Principle posits when there is a contact between two surfaces; "exchange of materials" occurs (Maze, Stagnara, & Fischer, 2007; Oien, 2009). The Principle is a mainstay in investigative fieldwork, influencing analytical recreations of crime scenes. When victims have evidence in or on their person, and timing for collection of evidence is limited, other possible locations for evidence deposited or transferred outside the vagina are often unobserved and not retrieved. While not well developed, there are hypotheses about persistence of DNA in other locations with case studies and case series to support associations and the investigative assumptions. The knowledge about flowback posits that drainage will communicate with anal folds, yielding DNA (Enos & Byer, 1977), even after cleaning in living or deceased. When victims are dressed and DNA is deposited on skin, it is suggested that the DNA is better found on clothing or linens (Christian et al., 2000). Fingernails are harbingers of DNA following digital penetration (Flanagan & McAlister, 2011). Use of the Y-STR method recovers DNA when cervicovaginal samples from self-collection are negative for sperm, where the source of DNA is thought to be a result of the presence of leukocytes and epithelial cell nuclei (Johnson, Giles, Warren, Floyd, & Staub, 2005; Sibille et al., 2002). Finally, there is a subset of rapists who use condoms, e.g., younger rapists and those using a weapon (O'Neal, Decker, Spohn, & Tellis, 2013); conversely, alcohol use was negatively associated with condom use (Davis et al., 2012) yielding implications not only for evidence collection, but for prophylactic medical treatment when alcohol use is reported by the victim about the rapist.

Forensic Laboratory

Autosomal short tandem repeats (STR) testing is in use since the middle 1990s specifically to amplify samples from rape victims in forensic laboratories (Butler, 2006; Ludes &

Clisson, 2000; Tamaki & Jeffreys, 2005). Forensic samples contain both female and male DNA – whether cellular or seminal. However, the ability to obtain an autosomal STR profile of the semen donor diminishes as the post-coital interval is extended (Daniels, Hall, & Ballantyne, 2004; A. M. Hall & J. Ballantyne, 2003b; Ludes & Clisson, 2000; Mayntz-Press & Ballantyne, 2007; Mayntz-Press et al., 2008). While obtaining an autosomal STR profile can occur from samples taken 0 to 48 hours after intercourse in most cases, it becomes increasingly difficult to obtain such a full profile from samples taken more than 48 hours after intercourse. Moreover, the mixed sample (that is unavoidable from the female sample with female and male cells) poses challenges to interpretation with STR methods. The Y-chromosome-STR (Y-STR) testing, however, is specific for the male cell because the female cells essentially are ignored and undetected during the testing. Evidence supports Y-STR as the most suitable STR method for extended interval post-coital samples when male cells gradually diminish in number (A. M. Hall & J. Ballantyne, 2003b, 2003c). When amplified with this method, mixed samples of multiple rapists can be distinguished in forensic laboratories and identified, even with small quantities of DNA, making it the ideal test for this study (J. Ballantyne, 2013; J Ballantyne, van Daal, & Lubenow, 2013; Berger, Niederstatter, Kochl, Steinlechner, & Parson, 2003; Cerri, Ricci, Sani, Verzeletti, & De, 2003; Daniels et al., 2004; A. M. Hall & J. Ballantyne, 2003a, 2003b, 2003c; Hanson & Ballantyne, 2004; Hanson, Berdos, & Ballantyne, 2006; Ludes & Clisson, 2000; Mayntz-Press & Ballantyne, 2007; Parson, Niederstatter, Brandstatter, & Berger, 2003; Prinz, Boll, Baum, & Shaler, 1997). However, even Y-STR has limitations resulting in continued studies from forensic laboratories seeking to find the elusive extended interval DNA, which is readily available for decades in medical settings in the female genital track (Ahlgren, 1975; Davies & Wilson, 1974).

As forensic laboratories investigate and improve methods to detect male DNA from large amounts of female epithelial cells using Y-STR methods, when delays in reporting result in degradation of the sample, newer methods of analysis are needed to overcome laboratory barriers in the recovery of DNA and identification (J Ballantyne et al., 2013). A method for selective amplification prior to using Y-STR methods is effective in clearly identifying male profiles from touched items (e.g., "Probative partial profiles were obtained using as little as a single buccal epithelial cell after pre-amplification" p. 7) even when exposed to heat, light, and humidity. Importantly, with a limitation of four couples and blind swabs in the study, the newer method was successful in DNA recovery at nine days (J. Ballantyne, 2013). For laboratories, the expectation is that newer research will define conditions under which standard versus more advanced non-standard methods would be required to obtain the male donor DNA profile in extended interval post-coital swabs.

Summary

Understanding the female and male reproductive environments and in vivo interaction, as well as the structures and function with unique couple, variations provide the basis for understanding DNA recovery. Additionally, advances in forensic laboratory DNA detection methods over the last few years are serendipitous in this study's quest to expand timing of evidence collection and to determine human factors most likely to influence DNA recovery and identification with the newer methods. While the small studies provide a foundation for this research, the attempt to complete a large-scale project using a population of proxy couples who follow a strict protocol of abstinence and timed DNA deposits is absent from the forensic science literature to date.

Research Aims, Purpose and Objectives

The research aims are two and include (1) what is the period for DNA recovery in proxy couples from the vagina and cervix using Y–STR laboratory methods; and (2) what are the common physiological conditions that may influence recovery of DNA in proxy post-coital couples.

The purpose of this research is to answer: (1) the period for DNA recovery in post-coitus samples from the vagina and cervix using Y-STR laboratory methods, and (2) identify the common physiological conditions that may influence recovery of DNA in post-coital samples from proxy volunteer couples.

The objective of this research is to address potential factors that could enhance evidence supporting or limiting the expansion of the 72-hour period for evidence collection from the vagina and cervix after a rape event in adult females. Another objective is to begin to identify and support heretofore suggested conditions, not yet supported by generalizable research, which might influence the recovery of DNA, and therefore policies and procedures about sample collection from the complex post-coital environment.

METHODS

Research Design

The research design is a prospective, mixed methods design with qualitative, descriptive, and inferential statistical analysis. This is also an exploratory study but with multivariable models which help sort risk factors and their contribution to the outcome. Analysis of the data occurred using the Statistical Package for Social Sciences (SPSS-PC) and the Statistical Analysis Software (SAS). The initial analysis was descriptive and included means and standard deviations, calculated from continuous data and frequency counts and proportions for categorical data. Where appropriate, Chi-square, Fisher's exact, and *t*-tests use compared the variables of

younger to older subjects, as well as hymen appearance and other associated variables determined to be important by the Expert Advisor Group members. An exploratory study with multivariable models for analysis resulted in the use of Generalized Estimating Equation (GEE), which is an extension of the quasi-likelihood approach for multivariable models, accounting for repeated measures, the longitudinal design of this study, and the binary outcome of DNA recovery.

Methods, Materials and Procedures

Focus Group

A focus group of national experts in emergency and women's health care delivery gathered to identify themes for inclusion and exclusion criteria for couples, and define variables to study, as well as develop the protocol for sample collection reflecting current research and demonstrating clinical research rigor.

After a full Institutional Review Board review and approval, nationwide recruitment of registered nurse collectors (preferred forensic nurses) occurred, and they recruited volunteer proxy couples. Volunteer proxy couples followed a strict protocol of abstinence, defined as 'no unprotected coitus' or 'if coitus occurred, use of barrier methods' (specifically including condoms). The abstinence period covered no less than 10 days over four different periods. The choice of ten days in 2009 reflected the state of the science, as 10-days doubled the time of five days for DNA recovery and identification. The couples' instruction was that only females could handle the condom and there could be no male contact of any kind with her genitalia. The recruited couples completed demographic information and answered specific questions about their activities, health, and medical conditions; once determined eligible, the couples submitted baseline buccal samples, and the female partner provided cervix and posterior fornix samples at or after 10 days of abstinence from all types of unprotected coitus, but before the first DNA

deposit. The proxy couple followed the complex protocol to abstain from all types of unprotected coitus for a period of 10 or more days, recording daily activities identified by the focus group on a diary card, and then the male deposits DNA in the vagina. The second 10-day period of abstinence from all types of unprotected coitus begins after the first DNA deposit, and the proxy female submits to collection of one sample on either Day 4, 7, or 9. The 10-day or more days of abstinence from all types of unprotected coitus, and repetition of the DNA deposits and scheduled collections occurs over four 10-dayperiods total. Upon sample collection completion, US Post Office delivered all samples to the laboratory for Y-STR and enhanced Y-STR analysis. All proxy couple information is 'blinded' to the forensic laboratory. The samples received in the lab had labels with unique identifiers necessary for distinguished common reference, as well as the dates and times of coitus, and the dates and numbers of protected coitus during each of the 10-day abstinence periods. The core team (e.g., the PI, research nurse, and data entry personnel) had access to all participant information. Statistical team members received all de-identified data for analysis. The participants mailed the demographic data, daily activity and symptom diary, and observational information to the PI for entry into the data system. The analysis included identification of the themes evaluated on the tools for data collection, as well as descriptive and analytical/inferential statistical methods.

Bayesian Review

No publications existed in the literature that undertook research testing of coherent beliefs to satisfy the rules of probability in DNA recovery from post-coital samples taken from proxy couples utilizing a protocol with four abstinent periods. After a focus group of Subject Matter Experts and analysis by statisticians, a decision supporting a process evaluation of the first group of a minimum of five couples would precede the formal study to assess the validity and ease of all protocol steps.

DNA Recovery Protocol

Recruitment and Consent

A nationwide recruitment of registered nurse specimen collectors occurred through organization media and meetings in Years 1-3. Those interested recruited volunteer proxy couples in their area; for forensic nurses or nurses interested in participating as subjects, they recruited collectors. Recruitment and screening questions by unknown supporters of the study provided information to interested parties and eliminated those with full hysterectomy (must have a cervix), male vasectomy or infertility (must have DNA) and obesity (which lowers sperm count). If not self-eliminated, interested couples and collectors contacted the Principle Investigator or co-investigator where a discussion for IRB approved informed consent and about the study and protocol occurred; participants were encouraged to ask questions, clarify understanding using a "teach-back" method (specifically, verbally demonstrating understanding about the study's consent and the complex protocol and paperwork). Study principle and co-investigators' cell numbers were provided to the inquiring potential participants. Participants received consent paperwork through the U S Post Office or email following the telephone-consent from study participants to receive the materials according to their preference, which included IRB-approved consent forms with tagged signature and initials locations for individual male and female participants. In addition, a return self-addressed envelope was included. Once the PI received the signed consents from both members of the couple, each participant completed the questionnaire and demographic survey either by telephone or by email. Upon receipt of the questionnaires and demographic survey from the couple, the PI determined eligibility and if eligible sent a protocol kit, protocol diary and collector forms, and study directions to the female participant in the couple. A female nurse collector was identified (as a male collector could influence results through "touch" contamination), and instructions reviewed for speculum

insertion and completion of the collector's paperwork. The female participant of the couple was responsible for sending both the self-addressed kit to the laboratory and the paperwork completed by the female nurse collector to the PI for data processing.

The Protocol Process

On receipt of the Post-coital DNA Recovery Kit, the couple began coital abstinence according to the protocol, which is for 10 or more days using barrier methods placed on the male by the female for digital coitus, cunnilingus, and penile-vaginal intercourse. Under the protocol and before the first unprotected DNA deposit after the10-day abstinence, the collector nurse takes samples from the buccal surfaces of the male and female couple-participants. At the same time, the collector RN takes baseline samples from the cervix and posterior fornix as controls. After the period of abstinence, followed by one unprotected coitus, followed by abstinence, sample collection occurred at Days 4, 7, or 9, depending on one of four 10-day periods of abstinence. Cervical and posterior fornix collection strategies did not include serial collections, as removal of DNA from either surface more than one time may reduce cellular materials, including DNA, which may affect detection outcomes by the laboratory. Once collected, samples packaged according to the study protocol, using the assigned unique identifier, were mailed directly to the National Center for Forensic Science DNA laboratory for indexing and analysis using of Y chromosome markers (Y-STR) methods. Once the samples arrived in the forensic laboratory, DNA extracted from the post-coital swabs and reference samples used standard techniques for evaluation. This included a non-differential extraction procedure to separate the sperm from the non-sperm cells in the post-coital specimens, useful with large amounts of female DNA. DNA was then quantified and subjected to male specific DNA amplification and analysis using Y-STR methods. The expectation is that sample sets from the

cervix or posterior fornix had no quantifiable male DNA at baseline. However, all sample sets that demonstrated no quantifiable male DNA in the associated pre-coital samples was subjected to DNA analysis. The expectation was that standard amplification methods for a sub-set of the samples would be amenable to this approach, which was likely to include three-day samples and some four- and five-day samples. For those samples in which the standard approach did not yield results, a number of advanced non-standard DNA profile enhancement approaches developed by NCFS were used. To explain the reporting from the laboratory, for categorization, each sample's assignment depended on the number of genetic markers obtained out of a total of seventeen. The success rate (full profile = 17 markers), partial profiles = 1-16 markers) and no profile = 0 markers) was correlated with the time since intercourse. Standard descriptive statistical techniques was used to determine whether any of the other data associated with the samples yielded information on other factors impacting the DNA typeability of extended interval post-coital samples.

Protection of Human Subjects and Confidentiality

Each subject was required to complete a data set that provides their identifying information, e.g., name, address, contact phone, email, and specific questions about eliminating factors, e.g., no cervix, no male partner, known disease states, etc. This data set remains confidential and in a secure location, available to the PI who had information about those applying to be study subjects and those who did not meet the criteria for participation. Every effort was made to protect the identity of the study subject, including blinding the survey with a unique subject identifier and blinding the laboratory personnel to the personal health and survey information. The core Expert Advisor Group reviewed data on the participation application data without identifying demographic data. Assignment of a unique identifying number, with

password protection, provided entry into the second data collection system with information about qualifying participant subjects. The unique number was the common thread, labeling all samples in the kit and for entering data from the laboratory, providing another layer that preserved confidentiality about subject information. Combination of the laboratory data set with the raw data from the eligibility, diary, and collector forms occurred at the completion of the laboratory tests, and analysis of the complete data set began. The PI, co-PI researchers, and administrative personnel had access to all de-identified information; however, protections were in place to deny the Evaluator, statistician and the forensic laboratory personnel the subjects' demographic and personal health information.

Preserved according to the Data Archiving Plan, the NIJ received all raw data sets as deliverables.

Consent was twofold. First, the individual was able to opt out without pressure, where continuation implies assent-type consent to participate in this study. Second, the collection, packaging and mailing of post-coital samples is self-initiated and implies consent under the protocol. To assist the subject with knowledge about the study, benefits and risks, as well as the study outcomes, general information about the study in a consent form was included in the initial documentation and a copy provided to the consenting subjects. Table 1 provides a summary of information related to consent for subjects participating in the study, *Post-Coital DNA Recovery*.

Recruitment Consenting Couples

Participant recruitment

Forensic Nurse (for sample collection) and participant couple recruitment occurred via email notification by IAFN (International Association of Forensic Nurses) on 3 occasions. Additionally, word-of-mouth spread among other health care professionals associated with

forensic nursing programs. Expanded recruitment included distribution of electronic or hard copy flyers to requesting communities that had health care providers with forensic training (i.e. colleges, universities, churches, and adult community service organizations, etc.). Inquiring prospective participants received informed consent forms through the USPO via mail or internet email. Then, participants called the PI or co-PI to discuss elements in the study. If participants agreed to volunteer to participate in the study, the male and female participants returned the forms with signatures and initials where directed. When received, and the signed and initialed form was in PI or co-PI possession, a phone call to each participant for the purposes of obtaining data for the eligibility criteria and questionnaires from both the male and female participant followed. Subject validation of desire to participate during the phone call was a confirmation assurance, which was an additional declaration from the PI to determine the subject understands the risks and benefits for participation in the study, required by IRB. The questionnaires review for eligibility occurred with the individual participant while on the phone, and the information about eligibility or lack thereof shared. If eligible, the female participant received a collection kit with instructions through the USPO. If ineligible, the PI expressed gratitude to both the male and female inquirer and closed the file.

Inclusion criteria

The inclusion criteria for both male and female participants was specific to ensuring fertility, general good health, and determining one consenting male – female couple. Participant inclusion required they have only one partner for the study period and participation in consensual coitus with their consenting partner. The partners would be able to and agree to abstain from coitus or use barrier methods placed by the female with vaginal, oral or digital intercourse (to avoid the possibility of "touch" DNA transfer) for four 10-day periods up to 39 days as required

by calendar of the study protocol. The couple would need to agree to abstain from vaginal lubricants of any kind. Specifically for consenting male participants, the ability to ejaculate directly in the posterior fornix with viable sperm (by self-report during eligibility criteria questionnaire) was necessary. Specifically for females, they must have a cervix and understand their results will be separated into one of two cohorts – menstruating or peri- or post-menopausal, determined by their self-report.

Exclusion criteria

The exclusion criteria for both male and female participants was specific to eliminating infertile males with specific health problems that reduce sperm count or introduce additional male DNA, specifically more than one partner.

Exclusion criteria for females included women with surgical menopause, without a cervix, multiple male partners during study, current use of Depo-Lupron in the previous 6 months, avaginosis, vaginal or surgical construction or reconstruction, abnormal PAP specimen within the last 12 months, total hysterectomy, douching during study protocol period, and pregnancy.

Male exclusion criteria included known sterility or physical anomalies (e.g., hypospadias), hot tub use in the preceding three months and during the protocol study time, morbid obesity (e.g., >35 BMI evaluated by self-reported height and weight), erectile dysfunction with or without medication use, illicit drug use, or vasectomy.

Ethical Considerations

Confidentiality

Advisement and discussions about potential consequences for participation in this study happened at the first and second subsequent contact with study subjects. After subjects

consented, each subject was required to complete a data set that provided their identifying information, e.g., name, address, contact phone, email, and specific questions about eliminating factors, e.g., no cervix, no male partner, known disease states, to name a few. While confidential, the data set remained secure, available to the PI and the research staff and had information about those who applied to be study subjects and those who met the exclusion criteria for participation. Using the criteria recommended by the Expert Advisor Group, the research staff reviewed data on the application for inclusion criteria and subsequent invitation for participation. If the subject qualified, a unique identifying number creation linked their personal health information to a case number on a secure Excel file for the purpose of linking the couple to their information, known only to the PI, co-PI, data entry personnel and finance officer for incentive payments. The unique number labeled all samples in the kit and for data entry in the laboratory, preserving the confidentiality of the participant, both in the laboratory and with the evaluators. At the completion of the laboratory tests, the laboratory data set added laboratory variables to the data dictionary for analysis. Preservation of all raw data sets according to the Data Archiving Plan was a NIJ deliverable, transferred at the end of the study.

Bias

In questionnaires, some bias is inevitable because of non-response, self-selection, recall bias and the quality of the questions in the survey. The design of the self-enrollment and screening process eliminated individuals with known reproductive contraindications, e.g., no cervix or a vasectomy, specifically to create a homogenous cohort of reproductive aged, peri-menopausal, and menopausal subjects. Self-selection bias was a concern as many voiced desires to help rape victims. To minimize variability and create consistency among collectors, clear instructions about the sampling technique, e.g., speculum insertion, with identification of the

target structures as well as location placement and handling of the cotton-tipped applicator for sampling, minimized subject repetition of the specific timed collection, which enhanced protocol completion and minimized recollection.

Kit Distribution Process

Quality assurance, packaging, and storage

The PI and support personnel packed post-coital DNA collection kits simultaneously on four separate occasions for the purposes of maintaining quality and consistency of the kit contents.

Kit materials included:

1. Labels that assigned a case number specific to the consenting and participating couple, which was applied to all documentation;
2. Diary cards and description tools, including one for each 10-day abstinence period and each timed collection;
3. Six packets of sterile cotton-tipped, wooden stick applicators with plastic drying covers for collection and packaging following collection;
4. Envelopes labeled with collection identification information and case number, for the purpose of keeping collections separate and drying after collection; and
5. Stamped envelopes to mail diary and description tools to the PI when the couple completed the materials.

For efficiency, packaging of approximately 30 kits at one time created a rapid response to the eligible and consented participating female partners, and a supply of kits stored in a secure clinical area.

USPO mailing requirement

Due to the nature of the sampling of human body fluids, transfer of post-coital proxy couple samples to the forensic laboratory followed the rules outlined in the USPO Rule 346.326 Exempt Human or Animal Specimens (Found at http://pe.usps.com/text/pub52/pub52c3_023.htm#ep925305).

Collection Protocol for Post-coital Interval Samples

Collectors

Due to this study's use of standard Y-STR and enhanced Y-STR methods used in the forensic laboratory, the desired collector gender was female. During the washing process for identification of the Y-chromosome, ignoring the female DNA minimizes the possibility for identification of another male's DNA. Support for the female forensic nurse preference for collector role was *a priori*, supported by the forensic nursing education and training; however, additional verbal instructions related to specimen collection developed after knowledge of final Bayesian results.

Instructions to collectors included the use of gloves throughout the collection process for contamination avoidance from inadvertent touched objects, i.e., lighting equipment or lighting cords, or other inconsistent speculum psychomotor techniques. Instructions to the male partners included denied access to the vulva and vagina via digits, oral contact (cunnilinguis), or condom application to minimize the transfer of "touch" DNA.

Speculum insertion and contamination

After Bayesian results, speculum insertion instructions were reviewed and provided to collectors via phone conversation. The instructions specifically included "how to" methods of speculum insertion method for the study to avoid contamination, which is, insert speculum

blades at an angle to no more than 2 cm before opening the blades. Then open the blades and look for the cervix as insertion continues in a caudal (toward the spinal cord) direction following the slope of the vagina. Once the cervix is sighted, stop the insertion and stabilize the speculum instrument for visualization of the target locations for collection. The *a priori* justification is that the former and traditional method of blind and full insertion followed by opening the speculum at the cervix was potentially dragging contaminants from the vulva and hands of the collector to the cervix and posterior fornix, possibly influencing outcomes. Conversations that included warnings about the traditional speculum contributing to the difficulty in seeing the study collection sites, specifically the cervix and posterior fornix, ensued. Also, warnings that the speculum use may also become areas of potential contamination due to excessive vaginal wall encroachment between the blades of the speculum and unintended touching (i.e., contamination) as the collector guides the cotton-tipped applicator toward the cervix or posterior fornix.

Sampling

Using the new technique for speculum insertion, but unable to control for vaginal wall encroachment, the instructions to collectors included to collect from the cervix first and the posterior fornix second, packaging each swab after the collection, according to instructions for use (including labeling and packaging in the drying cap) before the second swab was collected. The sampling technique included avoiding "twirling" the swab, as iatrogenic injury is a risk; instead, to allow the swab to absorb the fluid for 5-10 seconds, then placing the second swab in the fornix and sweep the fluids. This technique *a priori* loads both swabs with fluid by wicking fluid. The specimen packaging for each swab included a plastic self-container dryer chosen by the forensic laboratory. Additional instruction to the forensic laboratory included information about potential points of contamination with the drying cap. The points included threading the

wooden stick handled by the collector through the opening, the swab touching sides of the drying cap, and possible contamination of the inner surface of the cap with sealing, wasting precious sample.

Labeling

All printed labels were available and included in the kit at the time of assembly with case numbers, a place for date and time, and the collector's signature. At the point of collection, the label sealed the specimen and the specimen rested in the kit for dry storage in a temperature-controlled environment. The kit stayed with the female subject and she had instructions to keep it in a temperature controlled storage area until all collected samples assembled for mailing to the forensic laboratory.

Documentation

Daily activities, conditions, condom and coitus history

The IRB approved Diary Card assists the female in the collection of information about activities (particularly condom use and other sexual activity), illnesses, stress, and medication use (including hormones, medications prescribed, or over-the-counter and other herbals) during the abstinent and unprotected coital experiences.

Documentation of clinical appearance

The IRB approved Hymen Estrogen Response Scale (HERS©), cervical appearance, and photographic discharge description form provided an opportunity to determine cyclic changes that are predictable in menstruating and reproductive aged females.

Laboratory Method

DNA isolation

DNA extracted from the samples, used a standard non-differential organic extraction as previously described with a minor modification involving re-solubilization in 75 µl of TE^{-4} rather than 100 µl. The entire extract (75 µl) was subsequently purified and concentrated using the MinElute PCR Purification kit on the QIACube (QIAGEN, Germantown, MD). The samples were eluted in 12 µl of nuclease-free water. DNA from purified post-coital samples (2 µl) was quantitated using the Quantifiler® Y Male DNA Quantification kit (Applied Biosystems (AB) by Life Technologies, Foster City, CA) in accordance with the manufacturer's instructions. Buccal swab (reference) extracts were quantitated using the Quantifiler® Human DNA Quantification kit (AB). Included was an extraction blank with each extraction as a negative control. This extraction blank use was also in subsequent Y chromosome targeted pre-amplification and Y-STR amplification reactions. There was no observation of contamination in extraction blanks at any point during the course of this study. Discussion of any modifications to the extraction protocol is in the results section.

Purification

The MinElute PCR Purification kit (QIAGEN) was utilized for DNA extract purification and concentration, as well as post-PCR purification of the Y chromosome-specific nested PCR pre-amplification samples. Use of the semi-automated QIACube (QIAGEN) protocol was for all MinElute reactions in accordance with the manufacturer's instructions. All samples were eluted using nuclease free water (12 – 25 µl elution volumes).

Quantitation

Quantification of DNA samples (2 □l) used the following real time PCR quantification kits in accordance with the manufacturer's instructions: Quantifiler® Y Male DNA Quantification kit and Quantifiler® Trio DNA Quantification kit (Life Technologies). Quantitation analyses were performed on an ABI 7000 or 7500 real-time PCR instrument.

Y chromosome-specific nested PCR pre-amplification

Amplification took place in a 25 □l reaction mix which utilized the Type-It Microsatellite kit (QIAGEN) and consisted of the following: 1X Type-It Multiplex PCR master mix, 0.5X Q-solution, and 2.5 □l of a proprietary primer mix (15 primer sets to amplify 17 Y-STR loci: DYS19, DYS385 a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, Y-GATA-H4). The cycling conditions for the pre-amplification were 95ºC 15 min; 15 cycles 95ºC 30 sec, 60ºC 90 sec, 72ºC 60 sec; 68ºC 10 min (final extension). Positive and negative controls were included with each amplification (positive controls consisted of a male DNA standard; negative controls consisted of sterile water). All controls were verified during analysis and only data from amplifications with proper control results were accepted.

Y-STR amplifications

Y-chromosome STR analysis was performed using the AmpFlSTR® Yfiler® PCR Amplification kit (AB by Life Technologies), the PowerPlex® Y23 Amplification kit (Promega), and the AmpFlSTR® Yfiler® Plus PCR Amplification kit (AB by Life Technologies). All amplifications were performed in accordance with the manufacturer's instructions using ABI 9700 thermal cyclers (AB by Life Technologies). Positive and negative controls were included with each amplification (positive controls consisted of male DNA provided with the kit; negative

controls consisted of sterile water). All controls were verified during analysis and only data from amplifications with proper control results were accepted. Contamination in the amplification blanks was not observed in any extraction blank or amplification was not observed at any point during the course of this study. The accuracy of any obtained profile was verified by comparison to reference profiles (donor buccal swabs).

PCR product detection - capillary electrophoresis

All amplified fragments were detected with the ABI Prism 3130 Genetic Analyzer capillary electrophoresis system (AB by Life Technologies). A 1.0 µL aliquot of the amplified product was added to 9.7 µL of Hi-Di™ formamide (AB by Life Technologies) and 0.3 µL of GeneScan™ 500 LIZ® (G5 dye set) (AB by Life Technologies), GeneScan™ 600 LIZ® (J6 dye set) (AB by Life Technologies) or CC5 ILS-500 (Any5Dye dye set) (Promega). The electrophoretic conditions used were as follows: 16 sec injection time, 1.2 kV injection voltage, 15 kV run voltage, 60°C, 20 min run time, dye set G5 (Yfiler®); 16 sec injection time, 1.2 kV injection voltage, 15 kV run voltage, 60°C, 25 min run time, dye set J6 (Yfiler Plus®); 5 sec injection time, 3 kV injection voltage, 15 kV run voltage, 60°C, 25 min run time, or Any5Dye (PowerPlex® Y23). All samples were analyzed with GeneMapper® Software v4.0 or GeneMapper® ID-X v.1.4 (peak detection thresholds of 50 RFUs).

The data shared with the PI included case number, alleles and timing of samples (e.g., date and time) recorded on the samples. The charting of this data is reflected in the results.

Data Processing

Data collection begins with the PI or co-PI's first contact with a monogamous heterosexual fertile couple responding to call for study participants, interested in participating in the study. The location of identifying data provided by the couple is on a password-protected

computer with PI and Nurse Co-Investigator access, which was stored in a locked desk or cabinet, in a locked clinical area on campus. Once an interested person contacts the PI to inquire about the study, the participant receives an explanation of the research; the participant subject either wants more information or not. If they are interested, they answer eligibility-screening questions, such as but not limited to, "Have you had a hysterectomy?" or "Has your partner had a vasectomy?" If the individuals in a couple continue to express interest, mail or email delivers the consent forms to each subject. If the couple returns the consent forms and completes the eligibility form with protected personal health information (PHI), the couple a unique identifier, which is a case number used to separate them from their PHI data, assigned. All data collected is entered into the excel files as raw data and clustered to *posteriori* groupings for the purposes of analysis. The forensic laboratory receives case number data only, collects data, and returns data on charts with the Y-STR methods results, linking the couple to other gathered data by the study's case number. The evaluators who have access only to the case number receive all de-identified data entered on spreadsheets. When the lab and evaluator notify the PI the case is complete, a match of the case number with the individual's name and address, the Assistant Dean of Finance in the College of Nursing processes all required paperwork for university payment. In summary, Figure 1 represents the Post-coital DNA Recovery protocol and process, emphasizing data protection.

Summary of Post-Coital DNA Recovery Collection Protocol

When determined eligible, the proxy couple planned their schedule with the collector and the female participant recorded on her diary daily. Once timing for DNA deposit and collection is scheduled, the collector notice to meet the female participant on the scheduled days and time for DNA sampling and to documents the appearance and characteristics of the genitalia

transpires. The first collection occurs after 10 days of abstinence and/or protected coitus as described above. The collector nurse opened the sterile container with two cotton-tipped applicators and removed one by holding the wooden stick with gloved hands, taking care to avoid touch of the cotton tip. After speculum insertion according to protocol instructions, the nurse collects one swab from the cervix, slips that sample in the drying cap, and proceeds to collect the second swab from the posterior fornix of the vagina, slipping that sample in the drying cap. When removing the speculum, the nurse avoids injury to the participant. The sample collections occurred on the scheduled days i.e., 4, 7, or 9 days but *before* the next unprotected coitus, which occurs *after* 10 days of protected abstinence. The next unprotected sexual intercourse deposited DNA in the vagina at the posterior fornix. The female partner recorded the daily activity on the diary calendar. After the unprotected sex, the couple abstains again from unprotected sexual intercourse or use a condom for the prescribed 10-day period. Collection of the 2nd, 3rd and 4th sets of swabs occur on the assigned days, which included either 4, 7 or 9 days according to the woman's diary calendar. The collector nurse used the same procedure for removal of the sterile cotton tipped applicators with gloved hands from the packaging, and collecting first from the cervix, followed by a separate swab for collection from the posterior fornix.

Upon completion of the entire protocol, which included completion of the diary card over at least 39 days, collection of baseline specimens (Day 10 of abstinent period), and 4, 7 and 9-day post-coital samples, the specimens packaging occurs according to protocol at the time of collection. Complete diary cards and calendars along with the collector's documentation of the appearance of the genitalia on each collection by the nurse collector, was packed in the self-stamped and addressed envelope, sealed, and mailed to the PI. The packaging of swabs collected

and labeled with date and time of collection, e.g., baseline, 4, 7 or 9-day post DNA deposit, occurred according to the protocol, packaged separately in the self-stamped box, labeled according to USPO regulations, and put in the mail to the forensic laboratory. Once developed, the forensic laboratory confirmed that the sample was complete using case number, date and time of deposit and collection, and results from the Y-STR and enhanced Y-STR methods, reported in alleles. After receiving confirmation of completion of the DNA analysis for the case, case forms evaluation, and if the couple completed all portions of the protocol, the finance officer in the College of Nursing received their incentive payment forms for payment.

Statement of Results

Qualitative Analysis

Focus group

A focus group of subject matter experts convened to identify themes from their practice, learned from published literature to establish variables of interest in post-coital DNA recovery efficacy. The focus group consisted of persons with extensive experience in care of rape victims, including an emergency room physician specializing in medical forensic response to patients, a family nurse practitioner/midwife/attorney, a nurse midwife, an expert nurse in genetics, a women's health expert in vulvar diseases, and a family nurse practitioner. Pre-meeting assignments included a topic outline with instructions to read the grant application to become familiar with the grant's purpose and objectives. Instructions included reviewing and bringing literature on search terms: infertility – male and female, fertility, semen, sperm, vagina and vaginal secretions, menstruation, menopause, medication side effects related to fertility, sexual assault, rape, rape evidence, STI's, STR, Y-STR, PCR, DNA identification, and DNA. The PI conducted a literature search using the same search terms. An initial grouping of published evidence assembled demographic and common data themes that may influence DNA recovery in

the male and female. The list included age, race, basil metabolic index, medication (particularly prescription and over the counter), herbal use, illicit drug use, genital lubricants and supplements. Additionally, the social history provides insight about the overall demographics of the participants. For females, the focus group of subject matter experts listed common data elements from the literature that may affect recovery from the cervix and posterior fornix of the vagina. These included last menstrual period; hymen estrogenic effect; gravida and para; surgical history and hormonal replacement (hysterectomy and oophorectomy); other uterine or cervical surgical procedure; cancer that is estrogen dependent; medications; medical history (e.g., diseases of the integument, reproductive systems, genitourinary, pulmonary, gastrointestinal, musculoskeletal, neurological); and history of sexually transmitted diseases, which diseases, and types of birth control used, if any. For males, the focus group of subject matter experts listed common data elements from the literature that may affect DNA deposits such as partner fertility (for instance, does he have biological children?), testicular trauma, infections that affect fertility, hypospadias, circumcision, testicular torsion, epididymitis, prostatitis, or prostate cancer. In addition, the researchers identified use of birth control and types used.

The subject matter experts identified elements to include in the participant sample kits, which included cotton tipped applicators, wax paper in bindle fold, instructions for collection from the cervix, and vaginal fornix, speculum use, and possible Dacron tipped applicators.

Protocols for post-coital recovery of DNA defined data elements and timing instructions, information sheets related to pre- and post-coital deposit activities, pre-and post-coital collection, shipping and transport instructions, the collection protocol and random assignment of collection days, with preparation of unprotected coitus on assigned day. Instructions also included

activities to consider when ill, approved condom or lubricant use, avoidance of male touching condom or digitally penetrating or touching vulva to avoid contamination.

Focus group suggested changes in protocol

After the award of the grant, the focus group of subject matter experts modified the design outlined in the Methods section above and the results follow in Table 2. The new design would make it easier for eligible couples to participate and would facilitate data collection to answer the aims of the study – timing for DNA recovery and influences in the recovery of DNA.

Bayesian review results

The Bayesian review, which occurred when data was available from five proxy couples upon completion of the complex protocol, confirmed that when followed, the protocol was sound and would yield the expected data. Additionally minor weaknesses, when identified, resulted in slight modifications, not requiring IRB adjustments. Finally, the Methods implemented yielded a set of results that satisfied the rules of probability, were measurable, and supported the aims of the study.

Descriptive Results

Post-coital DNA kits

Post-Coital DNA Recovery Study, Award Number NIJ Grant No. 2009-DN-BX-0023 assembled 125 proxy couples kits in partnership with the University of Central Florida, National Center for Forensic Science for development using Y-STR methods. Under a separate grant, Dr. Ballantyne reported laboratory findings to the NIJ in 2013 under Award Number: 2009-DN-BX-K007 and disseminated findings at various meetings about Y-STR methods and Y-STR enhancement using the samples collected under this study. The minor differences between the two study reports can be explained by the analytical methods necessary to answer Aim 2 of this

study – what are the variables affecting recovery of DNA in post-coital samples at timed intervals following abstinence periods of 10 days? All documentation is necessary to answer this question, which reduced the number of couples with useful data to 66, down from the 75 couples in the forensic laboratory study. The results are similar. Both studies were able to use the Post-Coital DNA Recovery Study kits collected under this study's protocol, which contained buccal reference samples for the male and female participant, a baseline posterior fornix and cervical swab, and swabs collected from the same locations following DNA deposits at 4, 7 and 9 days. Condom use history completed by the proxy couples recording on diary cards provided the cross-reference case number. To review the detailed laboratory methods, see the study details under Award Number: 2009-DN-BX-K007.

At the time of the collaborative effort to design this study, the thinking was one baseline sample followed a 10-day abstinence period would double the current DNA recovery time of 5 days using Y-STR methods in 2009. While collection of a baseline before each deposit is ideal, the limitations in the study, e.g., cost of collection and development, and scheduling collection before and after DNA deposit, created constraints to subsequent baseline collections, particularly during the three abstinent periods, and after baseline collection at 10 days. When there were problems reported by the laboratory, e.g., timing of collections, recollection of the baseline for the time as well as the post-coital DNA deposit time i.e., 4, 7 or 9 days was encouraged. Given the complexity of the protocol, coordination between partners needing to reschedule their lives and abstain 10 days and then deposit DNA with an unprotected coitus, to collection scheduling with re-collection proved to be a barrier for three couples. Therefore, for the purposes of this study, sixty-six couples completed all elements of the Post-Coital DNA Recovery Study (i.e.,

consents, eligibility questionnaires, diary cards, collector observations, laboratory results, and recollections when asked) revealed in this report.

One hundred-twelve Post-Coital DNA Recovery study kits (N=112; 100%) were sent to collectors and consenting couples. Seventy-five couples (67%) completed the post-coital recovery study kits. Seventy-four couples (66%) completed all demographic data, including diary cards, and collector observations. Sixty-six proxy couples (59%) completed all components, i.e., kits, data questionnaires, diary, and collector observations for this study. Forty-six cases (47%) closed or were not included for a variety of reasons. They include physical issues, e.g., illness or pregnancy (n=8), partner issues, e.g., break up or deployment (n=4), or unresponsive to study personnel requests for completion of data collection or recollection of samples, e.g., "changed my mind," moved or declined recollection (n=19), no collector available (n=10), and other, e.g., kit mix-up in lab (n=2). While there were 34 RN collectors representing over 20 states and DC, some volunteer couples could not find collectors, and some collectors could not find eligible volunteer couples in their regions. Phone calls and emails (N=725; Average 6.5 per couple; Range 1 to 17) helped some couples (N=66) struggling with the protocol and collectors (N=43) needing support for the collection process and documentation. Word-of-mouth, meeting presentations, and media recruitment strategies generated considerable interest, but many persons subjectively reported barriers to participation due to eligibility criteria, e.g., obesity, vasectomy, hysterectomy, and other criterion.

Demographics

The demographic data about proxy couples who completed the complex Post-coital DNA Recovery research protocol and all accompanying data collection forms (N=66) and DNA recovery information submitted by the forensic laboratory that performed Y-STR and enhanced

Y-STR on the submitted samples, reported in alleles is presented. Participant proxy couples (N=66) were primarily non-Hispanic white (91%), menstruating (94%), between 18-35 (80%), and college educated (77.3%). One in five reported trauma in their lives and the majority (64%) had between 5 and 20 lifetime sexual partners. The majority reported normal menstrual cycles that varied in length, were equal in reports about pregnancy or not, and the women used a variety of birth control methods.

Inferential Results

DNA recovery

To answer the first aim of the study, all sample testing employed the Y-STR methods; however, we obtained undetectable quantities of male DNA by using a nested method named enhanced Y-STR method, which included a nested PCR pre- and post-amplification process. The nested process amplifies non-specific products in the first amplification, corrected in the second amplification, yielding a highly sensitive process that likely identifies residual DNA in couples with extended post-coital intervals, even in the absence of intervening sexual activities.

The UCF forensic laboratory subjected the received specimens from couples to evaluate for detectable amounts of DNA. Table 3 shows the raw data for allele recovery using a Standard Y-STR method and an Enhanced Y-STR method across all dates (N=66), including at baseline (which was 10 days), 4, 7 and 9 days post-coitus. After completion of evaluation for DNA presence, the forensic laboratory transferred de-identified data to the core evaluation team, and when matched to the couple, the de-identified data entered in Excel files was for the purpose of analysis using SPSS and SAS. Table 3 also reveals interesting data. Supporting this finding is descriptive data (Table 3) (N=72) revealing that DNA detection using either standard or enhanced Y-STR method from the posterior fornix occurred in 53.7% in at least one of four

timed collections when it was not present in the cervix. DNA detection from the cervix occurred in 56.9% in at least one of four timed collections when it was not present in the posterior fornix. Of the proxy couples, 31.9% had one collection with DNA detection from the posterior fornix but not the cervix and one collection from the cervix but not the posterior fornix. Recognizing that profiles of less than five alleles in Y-STR profiles are not demonstrative for laboratory professionals, Table 4 Counts of DNA according to numbers of allele recovery by technique and timeline (n=66). Table 5 provides a breakdown of the mean and standard deviation for allele recovery by collection day, site and method.

All except one proxy couple had DNA detected in at least one of the four collection days during one of four 10-day abstinence periods from all types of unprotected coitus; when asked to recollect the absent data, the couple chose not to recollect when asked; therefore, no data from their collections were included in the analysis. There was elimination of two more cases following laboratory error when case numbers were transposed, which resulted in elimination of an additional two couples' results, leaving 66 proxy couple's data to analyze. The comparison of couples for condom use and no condom use during abstinent periods, and Bayesian couples compared to participant couples after implementation of strict instructions to minimize DNA transfer resulted in no differences in DNA recovery detection between the couple-groups.

While not complex, to reduce confusion about standard or enhanced Y-STR methods results, reporting of the analysis results that answer the first study aim follows a pattern of first reporting standard Y-STR findings, then enhanced Y-STR findings, then reporting combined comparisons of standard and enhanced Y-STR data across locations and days. Incidental findings related to Aim 1 will follow. The second aim results follow reporting of the first aim and incidental analysis' results.

The percentage of allele recovery from the cervix using standard Y-STR was 37.5% on day 4, 23.4% on day 7, and 17.5% on day 9 and baseline (day-10) and is reflected in Figure 2. Figure 3 reflects the percentage of allele recovery from the posterior fornix using standard Y-STR, which was 26.6% on day 4, 15,6% on day 7, and 17.2% on day 9 and 11.1% at baseline (day-10). Figure 4 reflects the percentage of allele recovery comparing standard Y-STR from the cervix and posterior fornix respectively, which was 37.5 v 26.6% on day 4, 23.4 v 15.6% on day 7, 17.2 v 17.2% on day 9, and 17.5 v 11.0% at baseline (day-10).

The percentage of allele recovery from the cervix using enhanced Y-STR was 81.8% on day 4, 60.6% on day 7, and 60.6% on day 9 and 60.0% at baseline (day-10) and is reflected in Figure 5. Figure 6 reflects the percentage of allele recovery from the posterior fornix using enhanced Y-STR, which was 87.9% on day 4, 68.2% on day 7, and 59.1% on day 9 and 54.7% at baseline (day-10). Figure 7 reflects the percentage of allele recovery comparing enhanced Y-STR from the cervix and posterior fornix respectively, which was 81.8 v 87.9% on day 4, 60.6 v 68.2% on day 7, 60.6 v 59.1% on day 9, and 60.0 v 54.7% at baseline (day-10).

Figure 8 reflects the percentages across days from the cervix comparing enhanced to standard Y-STR methods respectively, reflecting 81.8 v 37.5% on day 4, 60.6 v 23.4% on day 7, 60.6 v 17.2% on day 9, and 60.0 v 17.5% at baseline (day-10). Figure 9 reflects the percentages across days from the posterior fornix comparing enhanced to standard Y-STR methods respectively, reflecting 87.9 v 26.6% on day 4, 68.2 v 15.6% on day 7, 59.1 v 17.2% on day 9, and 54.7 v 11.1% at baseline (day-10).

When combining the samples from the posterior fornix and cervix, Figure 10 reports allele recovery using standard Y-STR in percentages of 46.9% on day 4, 26.6% on day 7, and 26.6% on day 9 and 25.0% at baseline (day-10). Figure 11 represents percentages of 92.4% on

day 4, 78.8% on day 7, and 78.8% on day 9 and 67.7% at baseline (day-10) after combining the cervix and posterior fornix samples and using enhanced Y-STR methods. When results were compared of combined cervix and posterior fornix samples using standard vs enhanced Y-STR methods, the results reflected 46.9% v 92.4% on day 4, 26.6% v 78.8% on day 7, 26.6% v 78.8% on day 9, and 25.0% v 67.7% at baseline (day-10) respectively (Figure 12).

When using Y-STR method, not accounting for repeated collections and adjusting for OC use, although not significant, the odds of recovering DNA is lower when menses is reported on every collection day (Table 5). Likewise, when adjusting for menstruation period, the odds of detecting DNA when OC use was reported is lower and not statistically significant on day 4, but the odds is significantly lower on days 7 and 9 (Table 6).

When found at baseline using the standard Y-STR method, actual DNA survival occurred more often in the cervix than the posterior fornix, seen in Figure 4, which supports former smaller studies defining the cervix as the location of choice for sampling. However, Figure 7 demonstrates that using the enhanced Y-STR method, the differences in recovery are less prominent and actual DNA survival occurred more often in the posterior fornix at Day 4 and 7, increasing slightly in the cervix at Day 9 and 10 (baseline). Of note, Table 3 reveals that in some cases (N=72), whether using standard or enhanced Y-STR methods, when DNA was found in the posterior fornix (53.7%), there was no cervical DNA detected. Conversely, when DNA found in the cervix (56.9%), there was no corresponding DNA found in the posterior fornix. In all timed collections documented by the forensic laboratory, 31.9% of couples had both cervix without posterior fornix DNA detection and on another timed collection, DNA detection in the posterior fornix without cervix DNA detection.

The quality of the DNA report from Dr. Ballantine using samples from this study is as follows:

1. The ability to obtain an autosomal STR profile of the semen donor from a living victim of rape rapidly diminishes as the post-coital interval is extended. This is of particular concern in those instances where victims of sexual assault provide vaginal samples several days after the incident. In an attempt to overcome the technological impediments of typing success with these samples, we previously employed the use of Y-chromosome STR profiling. By specifically targeting only the male DNA in the sample, there is reduction or even elimination of the possibility of male profiling masking or critical PCR reagent titration due to the presence of an overwhelming amount of female DNA. In our early work, using Y-STR profiling and additional strategies such as cervical sampling and post-PCR purification, we were able to obtain Y-STR profiles from samples collected 5-6 days after intercourse. However, the reproductive biology literature indicates that spermatozoa are detectable in the human cervix up to 7-10 days post-coitus. The question thus arises as to why, even with improved extraction and profiling techniques, we still fail to routinely recover DNA profiles from samples collected $\geq$6 days after intercourse.
2. In more recent work (manuscript submitted, Medicine Science and the Law), a novel Y-chromosome specific nested PCR pre-amplification multiplex was designed to produce sufficient quantities of template male DNA prior to subsequent Y-STR amplifications. Validation of this pre-amplification multiplex demonstrated that obtaining full Y-STR profiles could occur with as little as 5 – 10 pg of input male DNA. The use of the pre-amplification multiplex, in combination with extract

purification/concentration, resulted in a significant improvement in the period in which male DNA profiles recovery was possible. Thus, full and (still-probative) partial Y-STR profiles were obtained from samples collected up to 9 days after intercourse.

3. The previous studies referred to above involve the testing of samples from a small number of donor couples (4 couples). The current collaborative study with the University of Tennessee involved the testing of a significantly larger number of 4-, 7- and 9-day post-coital samples (69 donor couples) using our developed Y-chromosome specific nested PCR pre-amplification method ("enhanced Y-STR profiling method"). We also performed a comparison to standard Y-STR testing using a commercial kit (AmpFlSTR Yfiler® PCR amplification kit).
4. The results of this study demonstrate the ability to obtain probative genetic information from extended interval post-coital samples (4, 7 and 9 day samples) and suggest that the time frame in which sexual assault evidence is routinely collected might be extended. Since probative information was obtained from ~33% of 9 day samples, it is possible that the limit of detection has not been reached and further research should include an evaluation of samples collected 10 or more days after intercourse.
5. We evaluated the use of next generation Y-STR quantitation and amplification kits as alternative 'enhanced' strategies that would not require the use of additional pre-amplification steps. Using both the Promega PowerPlex® Y23 and Life Technologies Yfiler® Plus amplification kits, probative profiles were obtained using standard manufacturer's conditions from 4, 7 and even 9 day samples using

'standard' DN_A extracts. These kits include additional Y-STR loci that also provide additional discriminatory capacity, particularly when only partial profiles are obtained.

6. In addition to DNA analysis, this study also included an evaluation of a number of variables pertaining to the reproductive stages and health of the donor couples (mainly the female donor) and how such variables correlate with DNA profile recovery. However, the protocol blinded the laboratory to the details of this latter statistical analysis and any subsequent findings. Therefore, the results and conclusions in this DNA report pertain strictly to DNA profile recoverability from extended interval post-coital samples using standard and enhanced Y-STR typing strategies and we did not attempt to correlate them with any sample- or donor-specific meta-data.

Adding to Dr. Ballantyne's report, there is no difference in the population of couples that had negative baselines and those who had recoverable DNA at baseline. Of note, however, is that in both analyses there is continual drop in DNA recovery from DNA deposit, through Day 4, 7, and 9; when one considers that the Baseline *is* Day 10, the continual drop is persistent through all data fields revealed by analyses.

Figures 4 demonstrate the differences between DNA recovery from the cervix and the posterior fornix using standard Y-STR (baseline – 17.5/11.1%; 4 days – 37.5/26.6%; 7 days – 23.4/15.6%; and 9 days – 17.2/17.2%). With the enhanced Y-STR methods, the percentages for DNA recovery improve significantly for the cervix and posterior fornix respectively (baseline – 60/54.7%; 4 days – 81.8/87.9%; 7 days – 60.6/68.2%; and 9 days – 60.6/59.1%) as demonstrated in Figure 7. When data comparing posterior fornix using standard Y-STR with enhanced Y-STR

recovery, DNA with enhanced Y-STR demonstrates in Figure 9 a large increase in DNA recovery, reported in alleles (baseline – 11.1 vs 54.7%; 4 days – 26.6 vs 87.9%; 7 days – 15.6 vs 68.2%; and 9 days – 17.2 vs 59.1%). When the data is combined to compare recovery of alleles, standard vs enhanced Y-STR from the cervix seen in Figure 8, the percentage change is also large (baseline – 17.5 vs 60%; 4 days – 37.5 vs 81.8%; 7 days – 23.4 vs 60.6%; and 9 days – 17.2 vs 60%).

To answer the second aim of which variables influence the recovery of DNA, the first incidental finding was that combining swabs increases the percent of DNA recovery using the standard and the enhanced Y-STR methods in the laboratory. When swabs are combined and recovery of alleles is evaluated, standard Y-STR vs enhanced Y-STR from the posterior fornix and the cervix, the percentage change in DNA recovery is evident when the two swabs are combined for evaluation and Y-STR compared to enhanced Y-STR (baseline – 25% vs 67.7%; 4 days – 46.9% vs 92.4%; 7 days – 26.6% vs 78.8%; and 9 days – 26.6% vs 76.8%). Figures 17 and 18 demonstrate increasing differences between swabs collected singly from different locations, and swabs that combine sample locations. An incidental finding of increased DNA volume using enhanced Y-STR methods resulted from combining both swabs in the lab reflected in Figure 7 and 12 (see enhanced only). The comparison of enhanced methods from individual locations (reflected in two data percentages) compared to combined results reveals an increase in DNA detection using combined swabs (reflected in one data percentage) across all timed collections (baseline – 60.0/54.7 vs 67.7%; 4 days – 81.8/87.9 vs 92.4%; 7 days – 60.6/68.2 vs 78.8%; and 9 days – 60.6/59.1 vs 76.8%). The combined cervix and posterior fornix comparison of standard to enhanced Y-STR revealed double the odds of DNA detection at Day 4, slightly

over triple the odds of recovery at Day 7 and 9, and slightly under triple the odds at baseline (Day 10) as seen in Figure 12.

Hormonal Birth Control and Menstruation

Standard Y-STR

Fifty-nine percent of the females (N=66) in this study took oral hormones as a method for birth control. As noted in Table 5, when adjusting for menstruation, the odds of detecting DNA with the standard Y-STR methods, when oral hormones use was reported, is statically lower on the baseline (p=0.0402), 95% CIs [0.179147, 0.9259], Day 7 (p=0.0098), 95% CIs [0.061774, 0.5114], and Day 9 (p=0.0579), 95% CIs [0.057969, 0.4877], but predictably, the loss of DNA over time was not statistically significant on day 4 (p=0.7158), 95% CIs [0.2155, 2.8692], when Y-STR methods identified DNA from the posterior fornix and cervix, similar to women in this study where Y-STR found DNA in 26.6 to 36.5% of women respectively (see Figure 11 and 12) on Day 4 in this study.

In Table 6, when using the standard Y-STR method, not accounting for repeated collections and adjusting for hormonal birth control use, although not significant, the data suggests that the odds of recovering DNA is lower when menses is reported on every collection day, including baseline (p=0.3574), OR=0.4947 95% CIs [0.1105. 2.2146], Day 4 (p=0.7158), OR=0.7863 95% CIs [0.2155. 2.8692], Day 7 (p=0.6951), OR=0,7640 95% CIs [0.1989. 2.9352], and Day 9 (p=0.1600), OR-0.3393 95% CIs [0.-851. 1/5326].

The adjusted odds of DNA recovery from the cervix or posterior fornix when using the standard Y-STR method, while accounting for repeated measures, adjusting for the occurrence of a menstrual period and use of hormonal birth control is in Table 7. The odds of DNA recovery is significantly lower when menstrual period is reported (p=0.0445), OR= 0.5412 95% CIs [0.2974.

0.9849], and when hormonal birth control is used (p=0.0004, OR=0.20) 95% CIs [0.0959. 0.504f7]. As expected the odds of recovering DNA is higher when comparing Day 4 to baseline (p=0.0046), OR=3.0906 95% CIs [1.4155. 6.7482], while the odds of recovering DNA is significantly lower when comparing Day 7 to Day 4 (p=0.0114), OR=0.4391 95% CIs [0.2321. 0.8306]. The association is, when comparing Day 9 to Day 7 (p=0.8994) 95% CIs [0.43841. 2.0636] implies a confidence that DNA is at or close to the same recovery amount.

Enhanced Y-STR

Fifty-nine percent of the females (N=66) in this study took oral hormones as a method for birth control. As noted in Table 8, when adjusting for menstruation, the odds of detecting DNA with the enhanced Y-STR methods, when oral hormones use was reported, is lower but not statically significant on the baseline (p=0.8011), OR= 0.8593, 95% CIs [0.2641, 2.7962], and Day 4 (p=0.69), OR=0.6402, 95% CIs [0.0715, 5.7291], but predictably when adjusted for menses, the loss of DNA over time was statistically significant on day 9 (p=0.0234), OR=0.2126, 95% CIs [0.0557, 0.8107], when enhanced Y-STR methods are likely to identify minute amounts of DNA from the posterior fornix and cervix.

The women in this study (92%) were in the reproductive development of life and therefore, menstruation is a common experience. When using the enhanced Y-STR method, not accounting for repeated collections and adjusting for hormonal birth control use, although not significant, the data predicts that the odds of recovering DNA is significantly lower when menses is reported on Day 4 (p=0.0232) 95% CIs [0.0059. 0.6870], but not significant at baseline (p=0.1804) 95% CIs [0.1290. 1.4698], Day 7 (p=0.9312) 95% CIs [0.13710. 5.2075], and Day 9 (p=0.1380) 95% CIs [0.1380. 1.9585], meaning the odds of recovering DNA when hormonal birth control is reported is significantly lower on Day 4, after adjusting for menses (Table 9).

The adjusted odds of DNA recovery from the cervix or posterior fornix when using the enhanced Y-STR method, while accounting for repeated measures, adjusting for the occurrence of a menstrual period and use of hormonal birth control in Table 10 results in non-significant findings with a report of a menstrual period (p=0.06028) 95% CIs [0.2664. 1.0350], and hormonal birth control (p=0.2152) 95% CIs [0.2977. 1.3138], but significant findings on Day 4 to baseline (p=0.0037) 95% CIs [1.7522. 18.1545], and Day 7 to Day 4 (p=0.0126) 95% CIs [0.1016. 0.7601]; but not significant and not expected to be significant findings on, Day 9 to Day 7 (p=0.9854) 95% CIs [0.3890. 2.4802].

Generalized Estimating Equations method, which is an extension of the quasi-likelihood approach above, is used to analyze longitudinal data with repeated measures when the outcome is binary (specifically presence of menses and hormone birth control). Using this data, the estimated probabilities of DNA detection using the standard Y-STR is predictable for each of four possible circumstances with and without menses and hormone birth control across all four periods. Figure 13 reflects GEE modeling using standard Y-STR methods. Figure 14 reflects GEE modeling using enhanced Y-STR methods.

Assumptions and Limitations

Assumptions

Assumptions include the following:

1. The thorough literature search revealed all possible variables necessary to develop strong inclusion and exclusion criteria for the inquiring sample of proxy couple participants;
2. The protocol was comprehensive, addressing all possible pitfalls;

3. The abstinence and collection instructions were understood and followed by the volunteer proxy couple participants and collectors; and
4. Participation derived from altruistic motivation.

Limitations

Limitations include a small sample size of predominantly white, college-educated reproductive-aged females and their fertile partners, which may be insufficient to inform practice globally, particularly in the non-white minority female populations.

Additionally, participant proxy couples' coitus in vivo represents unique consensual sexual activities controlled only by a strict and complex study protocol, and does not represent the diverse experiences of sexual assault or rape victim populations.

Tables

Table 1. Consent information for subjects participating in the study, *Post-coital DNA Recovery*.

CONSENT TOPIC	DESCRIPTION
General Information	You may be eligible to take part in a research study. This form will give you important information about the why this study is being done, what will happen during the study, and the possible risks and benefits. Please read it carefully. After you finish, you may want to talk with the researcher and ask questions. You may also want to talk to family, friends, your primary care doctor, or other health care provider about joining this study. If you decide that you would like to take part in the study, you will be asked to register on the study web site letting us know you consent to participate in the study and you will be able to print a copy of the form to keep.
General Information about the Study and the Researchers	Title: Post-coital DNA Recovery Sponsor: University of Tennessee Health Science Center[1] and University of Central Florida[2] Names of Researchers: Patricia M. Speck[1], DNSc, APN, FNP-BC, DF-IAFN, FAAFS, FAAN and Jack Ballantyne[2], PhD
Purpose of the Study	This study is being done to determine the timing for sperm recovery after coitus and to see if there are variables such as medication or monthly cycle to impact that recovery.
Information about study participants	You are being asked to participate in this study because you fit the necessary criteria needed to enroll in one of 2 groups. We will choose up to a total of 150 proxy couples. You may leave the study at any time. There will be no consequence to you and you always have the option to not be in this study. However, if you decide to stop participation in the study, please call Dr. Pat Speck to formally withdraw so another couple may be recruited. There is no medical treatment involved in this study. Data collection will be ongoing over a 3-year period, however, your participation will last less approximately 40 days. You will be notified of the study results and all study subjects will have their personal information protected in a separate data base and not available to other research members involved in data collection and analysis.
Information about study procedures	Your participation in this study will occur over 39-40 days. The procedures include pre- and post-coital sampling from the cervix and the posterior fornix of the vagina. The sampling will occur on the following time schedule:

Pre Coital	Coitus act	Post-coital Day 4	Post-coital Day 7	Post-coital Day 9

	Collect buccal standard from couple, vagina/ cervix samples from female	Without barrier method – no collection today; repeat after 10 days of abstinence and barrier use	Collect from vagina and cervix	Collect from vagina and cervix	Collect from vagina and cervix
	Condom use or abstain	No barrier used	Condom use or abstain	Condom use or abstain	Condom use or abstain
	Day 1-10, 11-20, 21-30, 31-39	Day 10	Day 14	Day 28	Day 39

Information about Risks and Benefits	There are few foreseeable risks to you for participation in this study. They may include collection injury (such as pinching, pulling or discomfort), reliving painful memories if you are a victim of sexual assault, partner anxiety, and/or domestic violence. To protect you, we will screen you for these risks and provide information about communication, collection techniques and self-protection. The PI is available to speak with all study participants for questions and concerns or to discuss adverse events and solutions while participating in this study. If you get sick or hurt or have other problems while in this study, you may continue by starting over. While you are participating in this study, you may participate in other studies, without the approval of people in charge of both studies. You will receive an incentive of $150 to participate in this study upon completion of questionnaires, collector descriptions, and collection of all samples, but there is no other direct benefit from participating in this study. However, we hope that the information learned from this study will benefit rape victims in the future. If any new information is learned at any time during the research, which might affect your participation in the study, we will contact you directly using the information provided in the confidential demographic data base.
Information about Ending the Study	If you wish to stop taking part in this study, there is no consequence to your withdrawal from the study but please call Dr. Speck. If you withdraw, we will use the information provided by you in the analysis, however you will not be paid the incentive of $150. The Core Expert Advisor Panel may advise that you not continue in the study particularly if it is believed that it is not in your best interest to continue. This might be because you failed to follow instructions, new information became available and your safety may be a concern, or other reasons the research investigators believe are important.
Information about the Costs	If you join this study, you may purchase the non-latex, non-lubricated barrier methods used or you may abstain from copulation but there should be no other costs. You will not be charged for the DNA transport or analysis, kits, or surveys that are part of this study. Your insurance company will not be billed for the costs associated with this study. You will, however, be paid $150 when

	you finish all questionnaires, collector forms and DNA collections according to the protocol, within 90 days of completion of the protocol. Dr. Speck and Ballantyne are members of a Technical Working Group for the National Institutes of Justice that suggests funding priorities for grantors but neither are paid in this capacity. This granting opportunity is in a separate section of the NIJ.
Information about Confidentiality	Your demographic information will remain confidential, seen only by the Principal Investigator, co-Principle Investigator Research Nurse and the finance administration. This is so you can be paid at the completion of the study protocol. You will be assigned a unique identifier for all other activities associated with the grant and data bases will be populated individually and then combined using this unique number assigned to you. After the study, your demographic information will be stored for 3 years and you will be sent the study results where all study participant information will be combined into one large data base. You will never be individually identified in this process. If you leave the study, the information collected will be used, and you will be notified of the study results, however you will not receive the incentive of $150 for completion of the protocol.
Contact Information	You may contact the following people about the study: Principle Investigator, Dr. Pat Speck, 901/448-6098 pspeck@uthsc.edu; Project Researcher, Dr. Wendy Likes, 901/448-6144 wlikes@uthsc.edu and the Research Nurse (to be hired) 901/448-1632 or the Office of Research and Grants Support, Dr. Mona Wicks at 901/448-6250 mwicks@uthsc.edu . Your rights are protected through an Institutional Review Board at the University of TN Health Science Center , Terrence F. Ackerman, Ph.D., Chair (901) 448-4824 tackerman@uthsc.edu or Donna Stallings, CIM IRB Analyst (901) 448-4824 dstallings@uthsc.edu

Table 2. Deviation from Initial Sampling Plan in 2010

	Submitted design proposal	Change to design	Rationale
Sample	300 couples divided into 3 age groups or 100 couples in each group (menstruating, peri-menopausal, menopausal)	150 couples in 2 groups (menstruating v. not)	Reduce variability among couples
Collection protocol	Self-collection proposed	Addition of an experienced collector and calendar, diary card, and aging scale tools	To achieve greater specificity in the specimen collection and description of the female cycle
Stipend	$100/couple	$150/per participant (male and female)	Couples now collect 4 samples over 4 periods of at least 10 days of abstinence from unprotected coitus, and males are recognized as participants.
Pilot 5 couples	No pilot	Added	Scientific team recommended Bayesian statistics to eliminate bias and insure statistical methods are sound and procedures provide appropriate lab samples
Eligibility questionnaire	Present	Elements changed following subject matter expert input	Richer data collection allowing for identification of all valid variables

Table 3. Allele Recovery Summary for 72 Post-Coital Sample Kits (Baseline, 4-, 7- and 9-day) with and without completion of the full protocol.

Interval	Treatment	Allele Recovery (out of 17 possible)									
		100P02		100P10		100P09		100P09 (2)		100P15	
		Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Baseline	Standard	NT	NT	NT	NT	13	17	0	0	0	0
	Enhanced	0	NT	7	5	17	17	2	2	0	0
4 day	Standard	NT	NT	NT	NT	0	0	--	--	0	0
	Enhanced	17	17	17	17	17	6	--	--	0	1
7 day	Standard	NT	NT	NT	NT	0	0	--	--	0	0
	Enhanced	17	9	17	0	1	5	--	--	0	0
9 day	Standard	NT	NT	NT	NT	NR	NR	0	0	0	0
	Enhanced	11	0	0	0	NR	NR	1	2	0	1

*100P09 (2) – 2nd baseline and 9 day sample

Interval	Treatment	Allele Recovery (out of 17 possible)									
		100P14		100P17		100P19		100P22		100P23	
		Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Baseline	Standard	0	0	3	3	1	0	0	0	0	0
	Enhanced	0	0	11	15	8	14	0	1	0	0
4 day	Standard	3	3	14	8	12	0	0	0	0	0
	Enhanced	17	17	17	17	17	17	0	0	0	11
7 day	Standard	11	0	16	11	0	0	0	0	0	0
	Enhanced	17	17	16	17	0	2	1	1	0	3
9 day	Standard	1	1	1	0	0	0	0	0	0	0
	Enhanced	10	3	4	7	7	10	3	15	0	7

Interval	Treatment	Allele Recovery (out of 17 possible)									
		100P25		100P24		100P26		100P21		100P16	
		Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Baseline	Standard	0	0	0	1	0	0	0	0	0	0
	Enhanced	4	0	1	1	0	0	15	15	0	0
4 day	Standard	0	3	0	0	0	0	0	0	0	7
	Enhanced	6	14	16	15	3	4	17	6	17	17
7 day	Standard	0	0	0	0	0	0	0	0	0	0
	Enhanced	0	0	15	10	0	2	13	15	14	11
9 day	Standard	0	0	2	0	0	0	0	0	0	0
	Enhanced	0	5	11	15	2	0	0	0	0	1

Interval	Treatment	Allele Recovery (out of 17 possible)									
		100P18		100P29		100P20		100P30		100P28	
		Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Baseline	Standard	2	0	0	0	0	0	1	1	0	0
	Enhanced	2	0	6	1	0	0	5	0	6	2
4 day	Standard	1	0	2	0	7	0	5	0	1	0
	Enhanced	0	0	8	17	17	17	16	0	17	17
7 day	Standard	0	0	0	0	1	0	1	0	0	0
	Enhanced	0	0	0	16	5	17	4	13	17	16
9 day	Standard	0	2	0	0	0	0	0	0	0	0
	Enhanced	0	1	0	0	3	1	8	1	8	2

Interval	Treatment	Allele Recovery (out of 17 possible)									
		100P31		100P13		100P35		100P34		100P27	
		Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Baseline	Standard	0	0	0	0	0	0	NA	NA	0	0
	Enhanced	4	17	0	0	0	0	NA	NA	2	1
4 day	Standard	2	0	0	0	7	4	0	6	17	17
	Enhanced	16	8	13	2	17	17	17	17	17	17
7 day	Standard	0	0	0	0	1	2	0	0	0	12
	Enhanced	1	0	0	1	14	14	6	9	6	17
9 day	Standard	0	0	0	0	2	0	0	0	7	3
	Enhanced	0	0	0	0	4	6	0	0	17	0

Interval	Treatment	Allele Recovery (out of 17 possible)									
		100P38		100P36-2		100P41		100P41 (2)		100P03	
		Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Baseline	Standard	0	0	0	0	0	0	0	0	0	0
	Enhanced	0	17	0	0	0	1	0	3	10	8
4 day	Standard	0	0	8	5	0	0	--	--	9	0
	Enhanced	0	17	17	17	4	1	--	--	17	17
7 day	Standard	5	2	0	0	0	0	--	--	3	5
	Enhanced	16	17	3	17	4	0	--	--	17	16
9 day	Standard	0	0	0	0	NR	NR	0	0	0	0
	Enhanced	6	4	0	0	NR	NR	0	1	6	6

Interval	Treatment	Allele Recovery (out of 17 possible)									
		100P43		100P48		100P56		100P58		100P59	
		Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Baseline	Standard	2	0	0	0	0	0	0	0	0	3
	Enhanced	14	10	16	9	2	0	0	0	8	17
4 day	Standard	0	2	15	8	0	0	0	3	10	13
	Enhanced	17	17	16	16	2	6	1	0	17	17
7 day	Standard	3	0	0	0	0	0	0	0	1	9
	Enhanced	17	17	17	17	0	2	0	0	12	17
9 day	Standard	0	0	0	0	0	0	0	0	0	0
	Enhanced	6	0	17	17	0	8	2	0	6	0

Interval	Treatment	Allele Recovery (out of 17 possible)									
		100P62		100P63		100P63-2		100P64		100P65	
		Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Baseline	Standard	0	0	NR	NR	0	0	0	0	0	0
	Enhanced	5	5	--	--	0	0	0	0	0	0
4 day	Standard	17	17	0	0	--	--	0	0	0	0
	Enhanced	17	17	6	12	--	--	5	2	0	0
7 day	Standard	14	17	NR	NR	0	0	0	0	0	0
	Enhanced	17	16	--	--	0	0	1	1	0	1
9 day	Standard	5	14	1	0	--	--	0	0	0	0
	Enhanced	17	17	8	6	--	--	0	0	4	2

Interval	Treatment	Allele Recovery (out of 17 possible)									
		100P51		100P32		100P44		100P68		100P67	
		Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Baseline	Standard	0	0	0	0	0	0	0	0	0	0
	Enhanced	7	11	5	0	1	1	2	16	12	15
4 day	Standard	4	5	0	0	0	0	0	0	1	0
	Enhanced	17	17	17	17	13	16	1	0	12	4
7 day	Standard	13	0	0	0	0	0	0	0	0	0
	Enhanced	17	17	0	0	1	1	0	1	1	1
9 day	Standard	0	3	0	0	0	0	0	0	0	0
	Enhanced	17	17	0	1	0	1	0	1	4	3

Interval	Treatment	Allele Recovery (out of 17 possible)									
		100P49		100P06		100P42		100P71		100P72	
		Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Baseline	Standard	8	0	0	0	0	0	6	0	0	7
	Enhanced	17	17	1	0	1	2	17	10	2	17
4 day	Standard	0	17	0	0	0	0	0	0	0	0
	Enhanced	17	17	4	3	0	0	2	5	17	15
7 day	Standard	0	0	0	0	0	0	0	0	0	0
	Enhanced	17	16	1	1	6	15	0	3	0	1
9 day	Standard	3	0	0	0	0	0	0	3	0	0
	Enhanced	10	16	0	0	3	9	0	2	4	13

Interval	Treatment	Allele Recovery (out of 17 possible)									
		100P75		100P77		100P78		100P79		100P94	
		Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Baseline	Standard	0	0	2	0	0	0	0	0	0	0
	Enhanced	2	0	2	8	0	0	10	0	2	2
4 day	Standard	0	0	0	0	0	0	0	0	0	0
	Enhanced	8	17	1	10	14	4	7	7	5	17
7 day	Standard	0	0	0	0	0	0	0	0	3	6
	Enhanced	9	1	1	1	0	0	0	0	17	16
9 day	Standard	5	0	0	0	0	0	0	0	0	0
	Enhanced	2	0	0	1	7	0	5	0	1	0

Interval	Treatment	Allele Recovery (out of 17 possible)									
		100P69		100P80		100P83		100P73		100P93	
		Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Baseline	Standard	0	0	3	0	0	0	0	0	0	0
	Enhanced	0	0	9	14	1	0	0	0	0	1
4 day	Standard	3	2	8	0	0	0	0	0	10	0
	Enhanced	17	17	17	17	0	2	0	0	17	16
7 day	Standard	5	0	0	0	0	0	0	0	0	0
	Enhanced	15	11	8	1	1	0	0	0	0	0
9 day	Standard	0	0	0	3	0	0	0	0	0	0
	Enhanced	2	0	14	16	0	0	0	0	2	0

Interval	Treatment	Allele Recovery (out of 17 possible)									
		100P102		100P111		100P104		100P107		100P110	
		Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Baseline	Standard	0	0	0	0	0	0	0	0	0	0
	Enhanced	0	0	0	0	0	1	0	0	0	0
4 day	Standard	0	0	0	0	0	0	0	0	0	4
	Enhanced	1	1	0	2	3	5	0	4	17	17
7 day	Standard	0	0	0	0	0	0	0	0	0	0
	Enhanced	3	0	1	0	0	0	0	0	15	15
9 day	Standard	0	0	6	2	0	0	0	0	0	0
	Enhanced	0	0	11	16	2	0	0	0	0	0

Interval	Treatment	Allele Recovery (out of 17 possible)									
		100P105		100P101		100P81		100P103		100P85	
		Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Baseline	Standard	0	0	0	0	1	2	0	0	0	0
	Enhanced	2	2	16	1	0	0	3	1	2	1
4 day	Standard	0	0	0	0	2	0	0	0	3	0
	Enhanced	0	0	0	11	3	0	5	17	17	17
7 day	Standard	0	0	0	0	2	1	0	2	0	0
	Enhanced	0	0	0	0	2	0	16	17	4	1
9 day	Standard	1	6	0	0	0	0	0	5	0	2
	Enhanced	14	17	1	2	0	0	8	17	4	5

Interval	Treatment	Allele Recovery (out of 17 possible)									
		100P106		100P108							
		Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Baseline	Standard	0	2	1	0						
	Enhanced	5	5	11	0						
4 day	Standard	2	1	4	17						
	Enhanced	10	9	17	17						
7 day	Standard	0	0	2	0						
	Enhanced	0	1	9	8						
9 day	Standard	0	0	6	0						
	Enhanced	0	0	16	15						

The number of alleles recovered from each swab per collection site and time interval (baseline, 4 day, 7 day and 9 days) is shown. The shading represents the average RFU value of all alleles within in the profile (white – not detected; light grey 1-1000 RFUs; dark grey >1000 RFUs. NT = not tested.

(Source: Ballantyne, J. (2013). *DNA profiling of the semen donor in extended interval post-coital samples*. (241299). Washington DC: NCJRS.)

Table 4. Counts of DNA according to numbers of allele recovery by technique and timeline (n=66)

	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix	Cervix	Fornix
Time Technique	None	None	1 to 5	1 to 5	6 to 10	6 to 10	11 to 17	11 to 17	*	*
Base Standard	52	56	9	6	2	1	0	0	3	3
Base Enhanced	26	29	21	18	9	5	9	12	1	2
Day 4 Standard	40	47	13	8	6	4	5	5	2	2
Day 4 Enhanced	12	8	13	14	6	7	35	37	-	-
Day 7 Standard	49	54	11	5	0	2	4	3	2	2
Day 7 Enhanced	26	21	14	19	5	4	21	22	-	-
Day 9 Standard	53	53	8	9	3	1	0	1	2	2
Day 9 Enhanced	26	27	18	20	12	7	10	12	-	-

* Records with missing information for each time frame, technique and location

Table 5. Mean and standard deviation for allele recovery by collection day, site and method

Day	Variable	n	Mean	Std Dev
0	stc	63	0.48	1.39
	enc	65	3.92	5.09
	stf	63	0.30	1.07
	enf	64	4.14	6.03
4	stc	64	2.47	4.49
	enc	66	10.05	7.30
	stf	64	2.09	4.61
	enf	66	10.47	6.98
7	stc	64	1.23	3.34
	enc	66	6.08	7.11
	stf	64	1.05	3.18
	enf	66	6.23	7.14
9	stc	64	0.61	1.63
	enc	66	4.30	5.27
	stf	64	0.69	2.09
	enf	66	4.27	6.00

Note. stc=Standard Cervix; enc=Enhanced Cervix; stf=Standard Fornix; enf=Enhanced Fornix from SPSS analysis

Table 6. Adjusted Odds Ratio of DNA recovery (cervix or posterior fornix) by day using standard Y-STR method when menses during one of the time periods was reported

Day	OR	95%CI	p value
0	0.4947	(0.1105 to 2.2146)	0.3574
4	0.7863	(0.2155 to 2.8692)	0.7158
7	0.7640	(0.1989 to 2.9352)	0.6951
9	0.3393	(0.0751 to 1.5326)	0.1600

Table 7. Adjusted Odds Ratio of DNA recovery (cervix or posterior fornix) by day using the standard Y-STR method when hormone birth control use was reported

Day	OR	95%CI	p value
0	0.1791	(0.0347 to 0.9259)	0.0402
4	0.6090	(0.2090 to 1.7740)	0.3633
7	0.0617	(0.0074 to 0.5114)	0.0098
9	0.0579	(0.0069 to 0.4877)	0.0088

Table 8. Adjusted Odds Ratio of DNA recovery (cervix or posterior fornix) using repeated measures adjusting for occurrence of menstrual period and hormonal birth control using standard Y-STR method

	OR	95% CI	p value
Menstrual period	0.5412	(0.2974 to 0.9849)	0.0445
Hormonal contraceptive	0.2000	(0.0959 to 0.5047)	0.0004
day4 x day 0	3.0906	(1.4155 to 6.7482)	0.0046
day7 x day4	0.4391	(0.2321 to 0.8306)	0.0114
day9 x day7	0.9513	(0.43841 to 2.0636)	0.8994

Table 9. Adjusted Odds Ratio of DNA recovery (cervix or posterior fornix) by day using the enhanced Y-STR method when oral hormone use was reported

	OC		
Day	OR	95%CI	p value
0	0.8593	(0.2641 to 2.7962)	0.8011
4	0.6402	(0.0715 to 5.7291)	0.6900
7	1.0574	(0.2979 to 3.7531)	0.9312
9	0.2126	(0.0557 to 0.8107)	0.0234

Table 10. Adjusted Odds Ratio of DNA recovery (cervix or posterior fornix) by day using enhanced Y-STR method when menses during one of the time periods was reported

	Menses		
Day	OR	95%CI	p value
0	0.4354	(0.1290 to 1.4698)	0.1804
4	0.0637	(0.0059 to 0.6870)	0.0232
7	1.3899	(0.3710 to 5.2075)	0.9312
9	0.5198	(0.1380 to 1.9585)	0.3337

Table 11. Adjusted Odds Ratio of DNA recovery (cervix or posterior fornix) using repeated measures adjusting for occurrence of menstrual period and hormonal birth control using enhanced Y-STR methods

	OR	95% CI	p value
Menstrual period	0.5251	(0.2664 to 1.0350)	0.0628
Oral contraceptive	0.6254	(0.2977 to 1.3138)	0.2152
Day 4 x day 0	5.6401	(1.7522 to 18.1545)	0.0037
Day 7 x day4	0.278	(0.1016 to 0.7601)	0.0126
Day 9 x day7	0.9822	(0.3890 to 2.4802)	0.9854

Figures

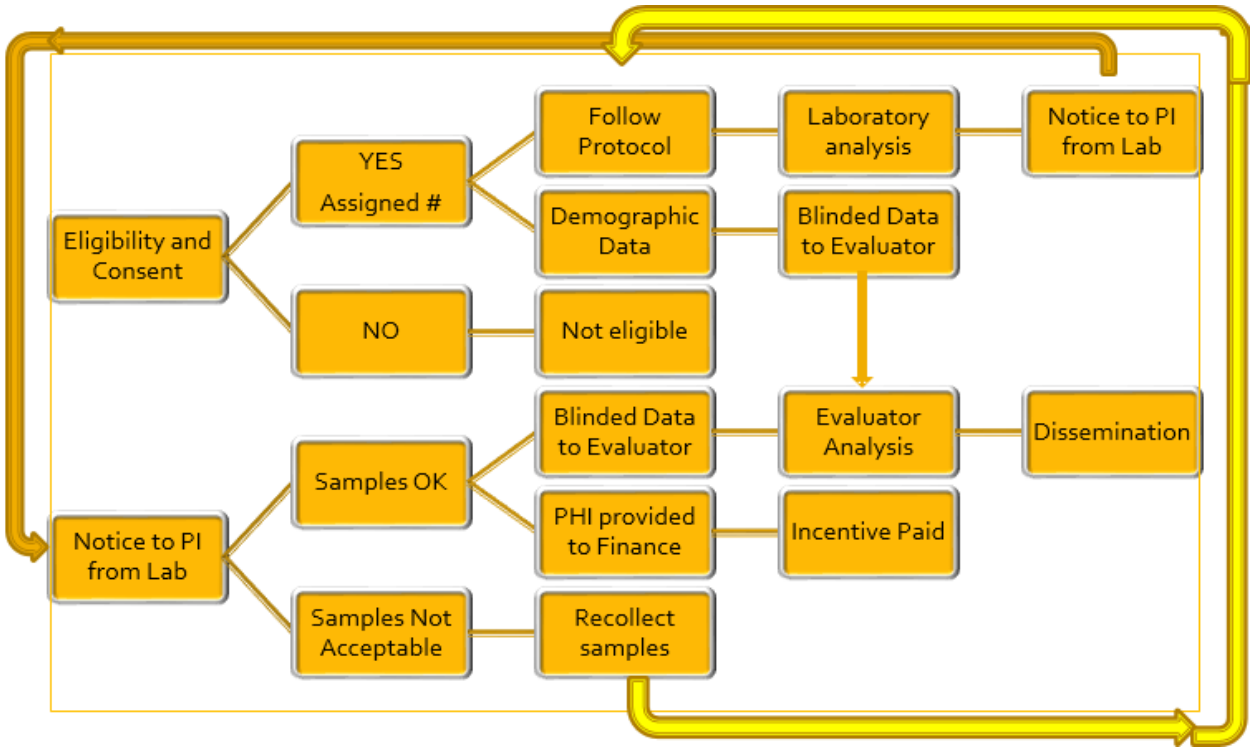

Figure 1. Post-coital DNA Recovery Protocol diagram

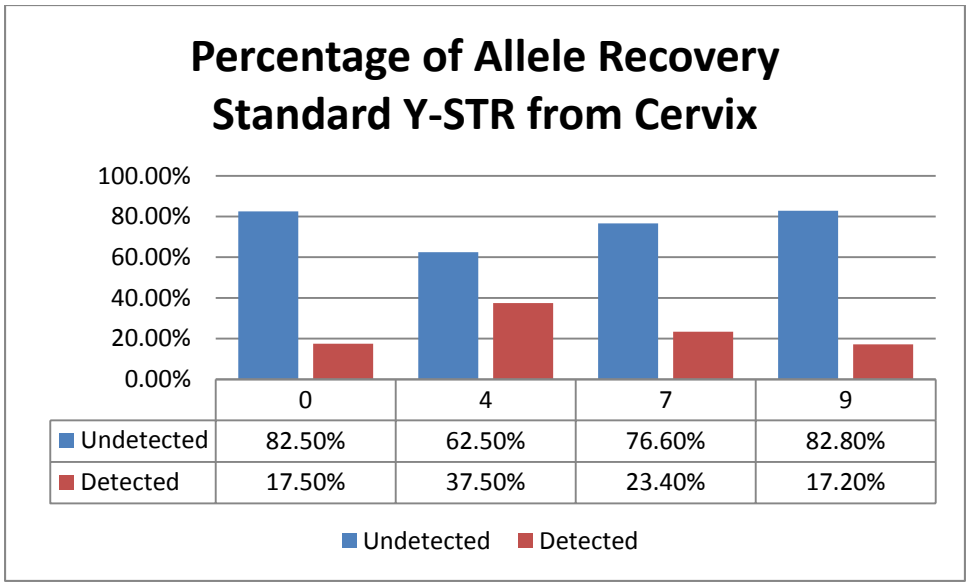

Figure 2. Percentage of allele recovery from cervix using Standard Y-STR

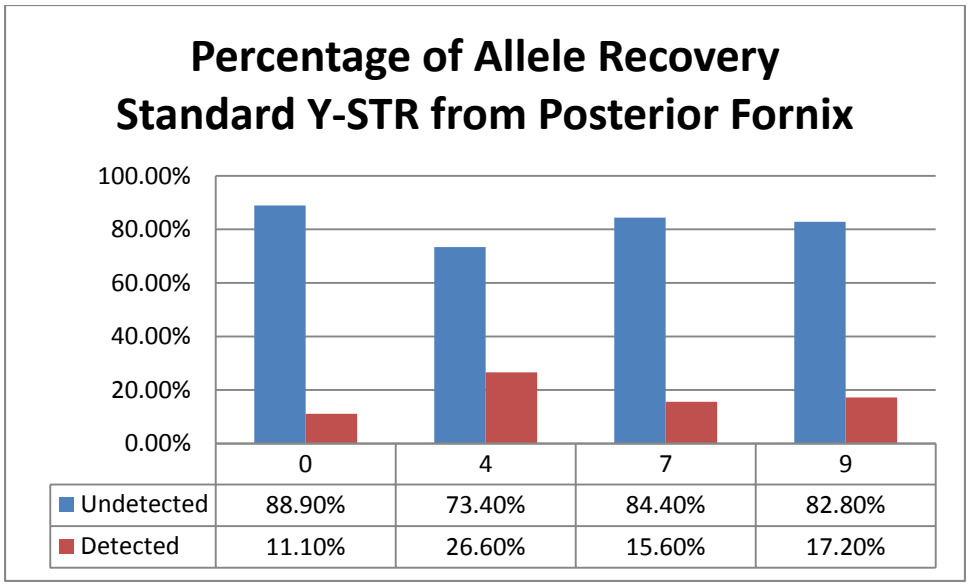

Figure 3. Percentage of allele recovery using Standard Y-STR from posterior fornix

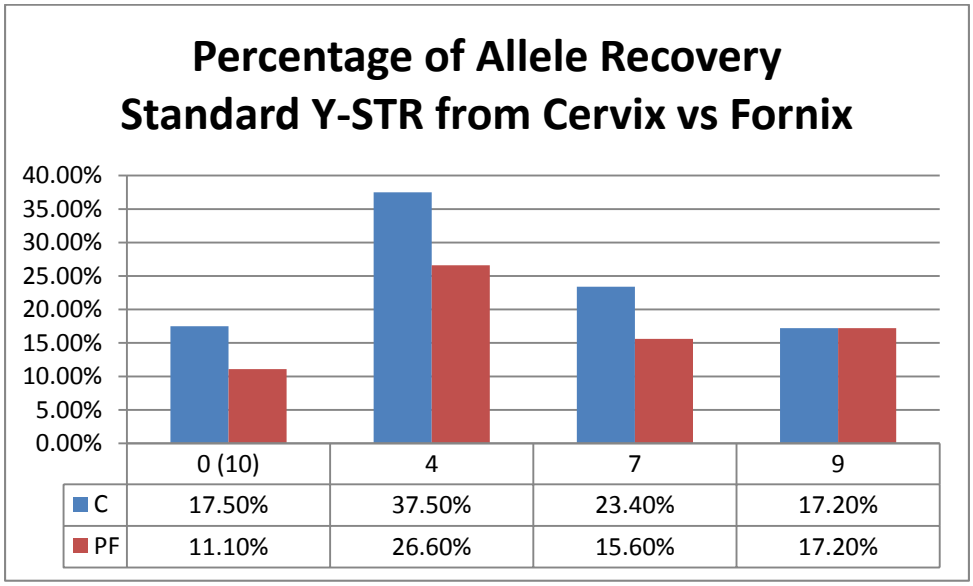

Figure 4. Percentage of allele recovery using Standard Y-STR from the cervix and posterior fornix

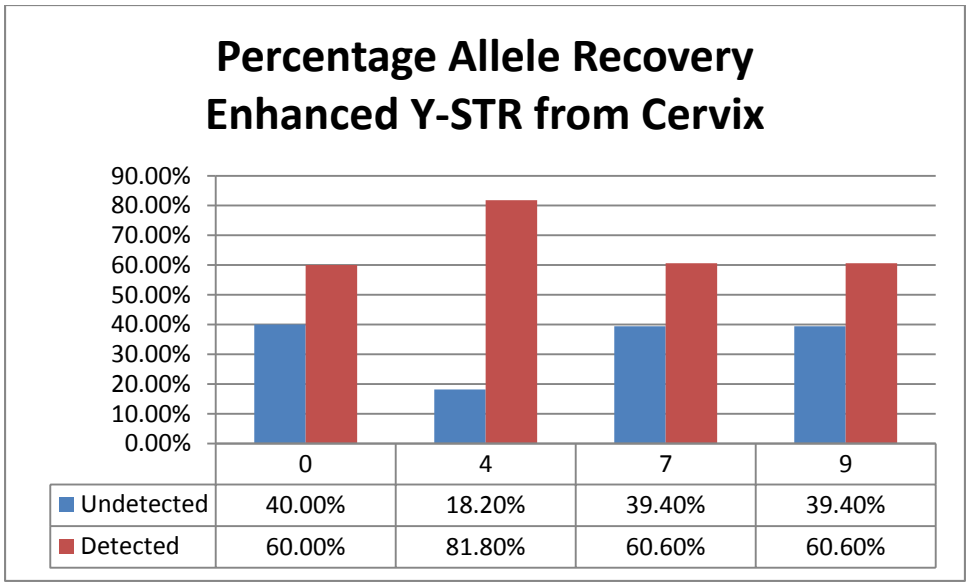

Figure 5. Percentage of allele recovery using Enhanced Y-STR from the cervix

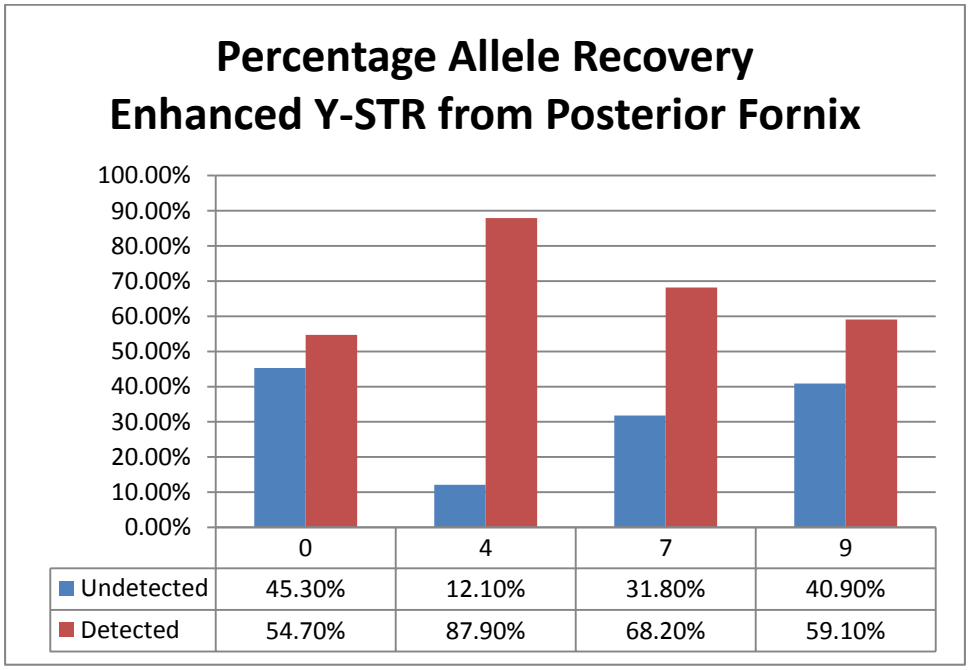

Figure 6. Percentage of allele recovery using Enhanced Y-STR from the posterior fornix

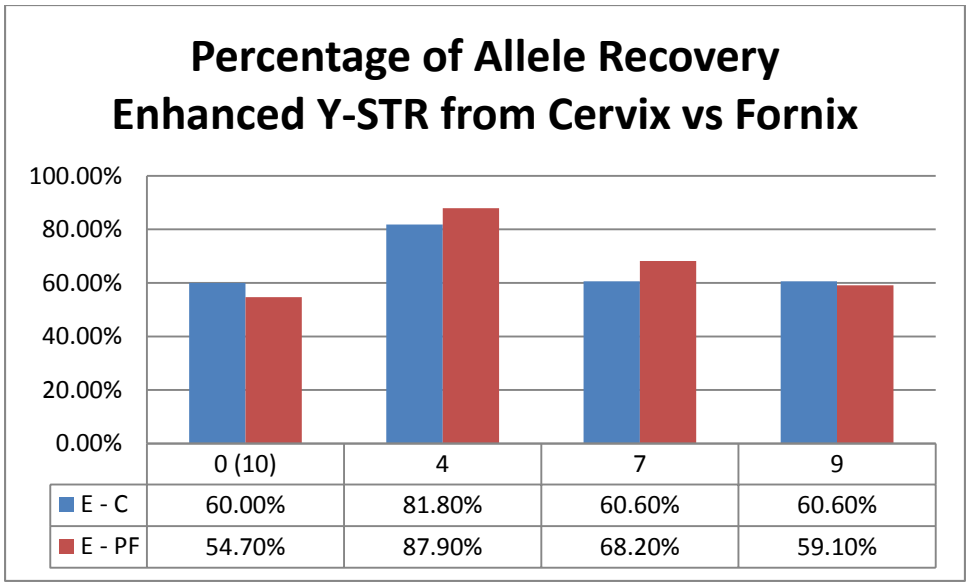

Figure 7. Percentage of allele recovery across days using Enhanced Y-STR from the cervix and posterior fornix

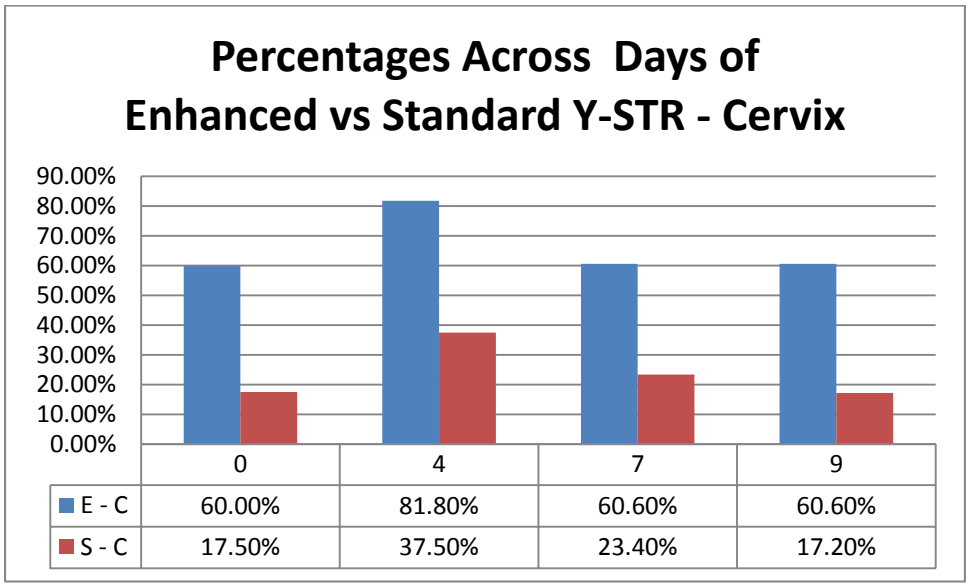

Figure 8. Percentage of allele recovery across days comparing Enhanced Y-STR to standard Y-STR using cervix samples

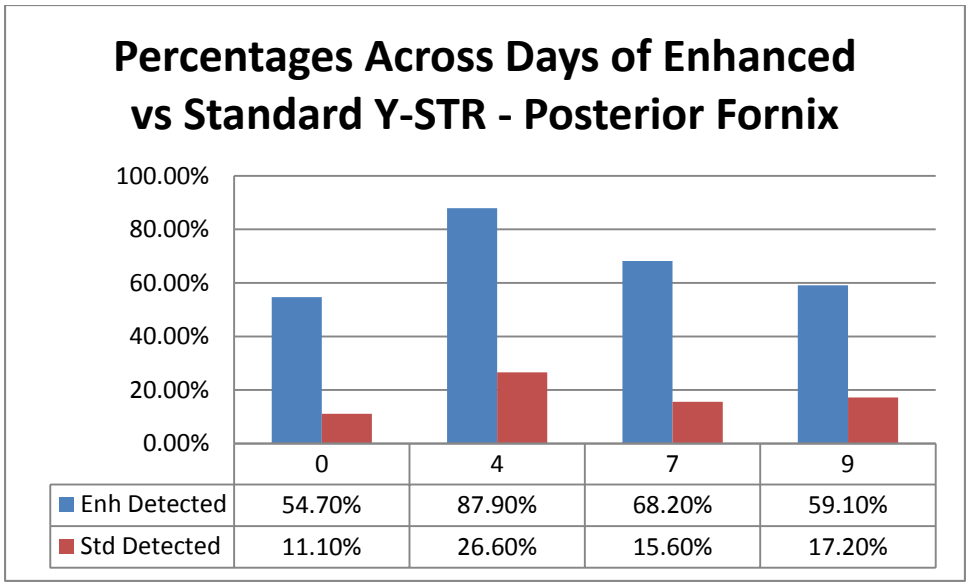

Figure 9. Percentage of allele recovery across days comparing enhanced to standard Y-STR methods using posterior fornix samples

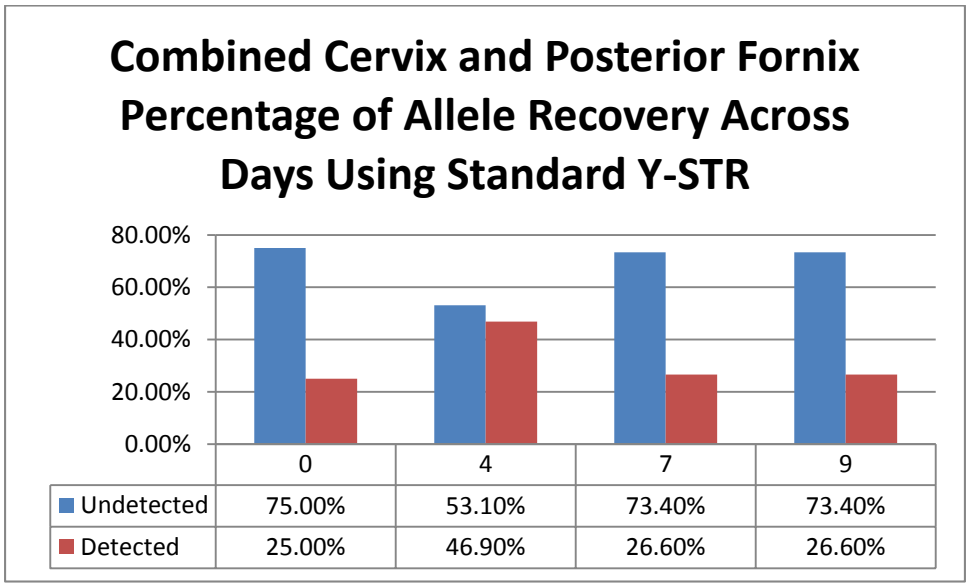

Figure 10. Combined cervix and posterior fornix samples reflecting the percentage of allele recovery across days using the standard Y-STR method

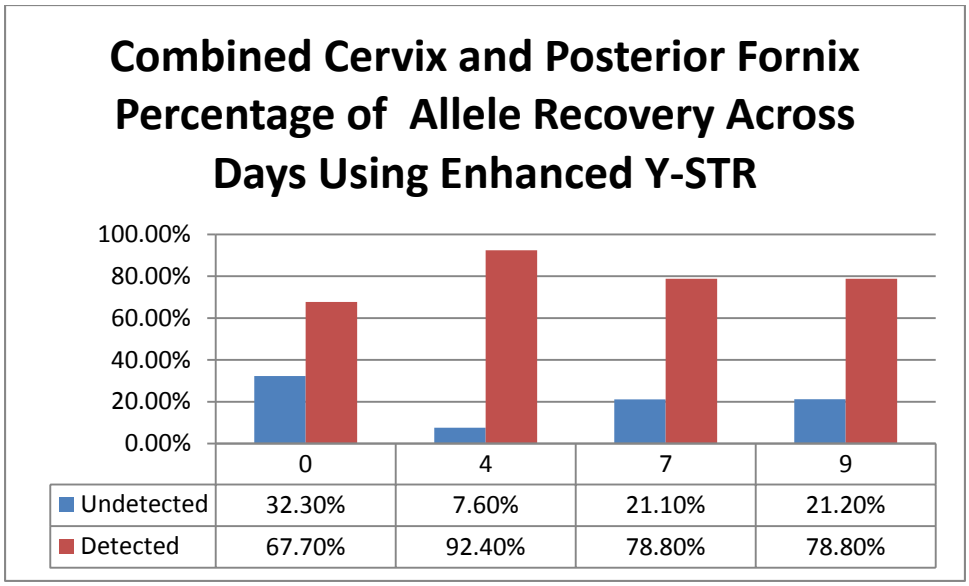

Figure 11. Combined cervix and posterior fornix samples reflecting the percentage of allele recovery across days using the enhanced Y-STR method

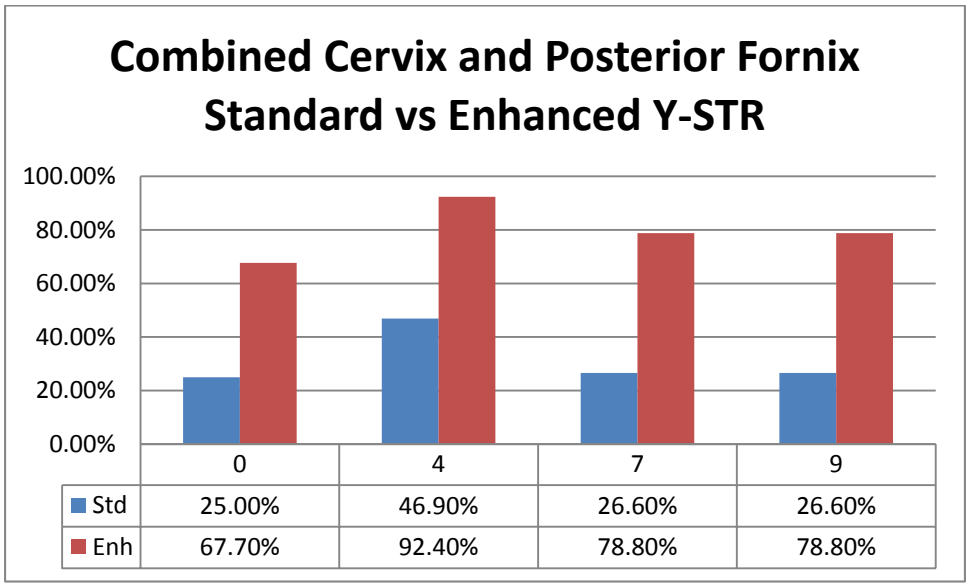

Figure 12. Comparing standard Y-STR to enhanced Y-STR from combined cervix and posterior fornix samples reflecting recovery in percentages across days

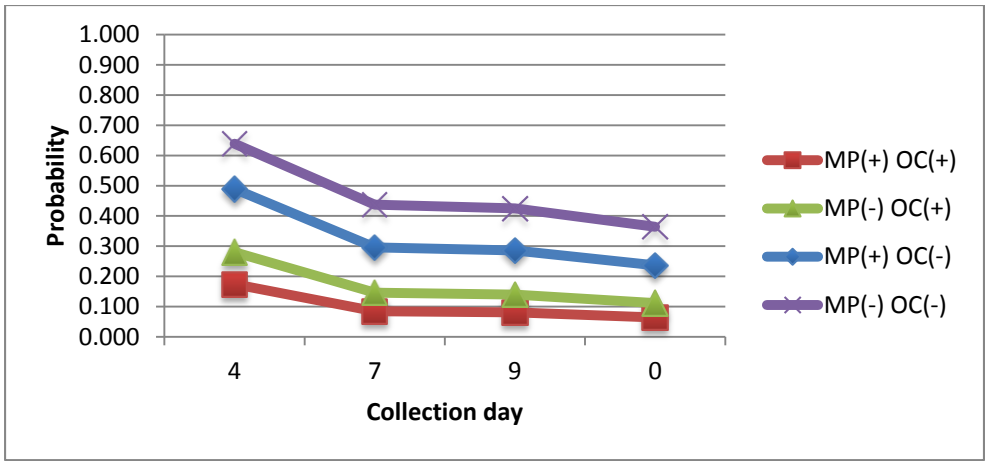

Figure 13. Generalized Estimating Equation (GEE) modeling for estimating the probabilities of DNA detection with the use of the **standard Y-STR** DNA detection methods. In this graph, the baseline, which is Day 10, is moved to reflect the gradual and expected fall of DNA recovery with the use of standard Y-STR methods.

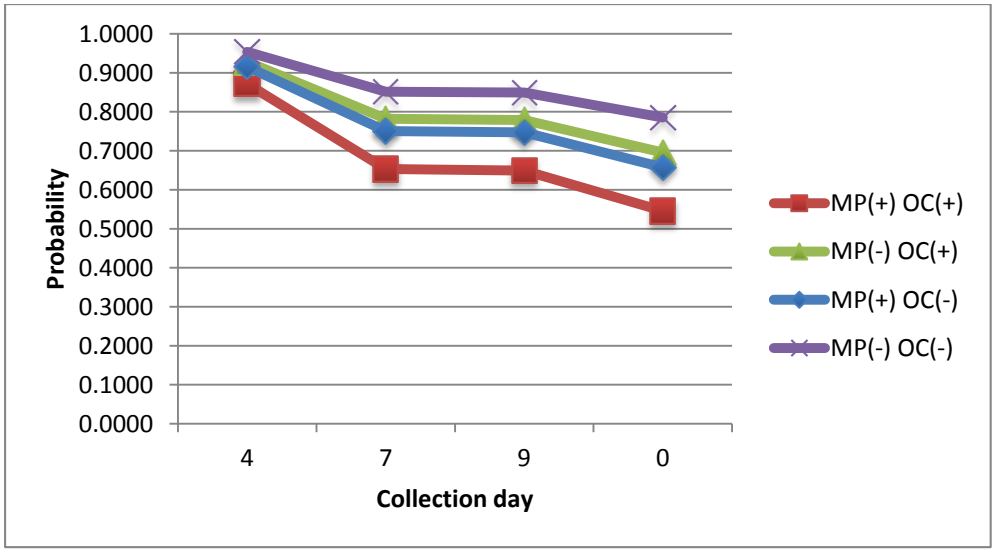

Figure 14. Generalized Estimating Equation (GEE) modeling for estimating the probabilities of DNA detection with the use of the **enhanced Y-STR** DNA detection methods. In this graph, the baseline, which is Day 10, is moved to reflect the gradual and expected fall of DNA recovery with the use of enhanced Y-STR methods.

CONCLUSIONS

Discussion of Findings

The Post-Coital DNA Recovery study (NIJ 2009-DN-BX-0023) is largest in vivo study to measure DNA detection and recovery using Y-STR methods. The research attempted to answer two research questions: What is the period for DNA recovery in proxy couples from the cervix and posterior fornix using Y–STR laboratory methods? [and] What are the common physiological conditions that may influence DNA recovery in proxy post-coital couples?

This research adds impetus to the growing body of literature supporting the expansion of timing of collection in post-coitus samples from the posterior fornix and cervix for DNA recovery using Y-STR laboratory methods. With the objective of eliminating obvious deficiencies and limitations in former studies, another goal was to identify heretofore promoted and published conditions and environments as empirical explanations, not yet supported by generalizable research, that influence the recovery of DNA in post-coital samples. As such, this research identified gaps in evidence to support current policy.

Newer developments in forensic laboratory studies in 2009 along with synergistic interprofessional meetings provided the motivation to question the current time-limiting protocols in use by jurisdictions nationally and the influences in recovery of DNA. In a recent publication report, the quantity of DNA drops substantially in the extended time intervals of 7 and 9 days (Ballantyne, 2013, p. 11). However, this research study demonstrated dramatic differences in DNA recovery between standard Y-STR and enhanced Y-STR, improving DNA detection and recovery across all sampling times (4, 7, 9 and baseline or 10 days).

Findings detail a homogenous population of proxy couples, primarily white, college educated and motivated to follow the difficult and complex protocol. In some cases, re-collection was necessary which meant a fifth 10-day abstinent period and recollection on the

specified date. Additionally, female members of the participating couple kept diary cards of menses, medication, stresses, coital activities with and without condoms, and other variables identified in the literature by subject matter experts as influencing DNA recovery. The results reflected a 30% dropout rate for reasons unique to the couple, despite the continual outreach by researchers.

As expected, the DNA recovery dropped for most with each timed collection regardless of standard or enhanced Y-STR method where statistical differences in DNA recovery between timings (0-4 days, 4-7 days, and 7-9 days) (Table 7 and Table 10) using standard and enhanced Y-STR methods was expected and explained. For those with increased alleles detected on Day 10, an assumption that each couple is unique, but control of couple 'start and stop' of protocol and increased numbers of protected coitus between deposit and collection is one explanation for increases or decreases in allele recovery for some couples. Regardless, recovery of DNA improved considerably on all timed collections with enhanced Y-STR compared to standard Y-STR in the cervix (Figure 8) and posterior fornix (Figure 9), with enhanced Y-STR methods out-performing standard Y-STR methods on all timed collections respectively. When combined posterior fornix and cervix samples with comparison of enhanced Y-STR to standard Y-STR methods, there is substantial increase in DNA detection (Figure 12), specifically 92.4% to 46.9% on Day 4, 78.8% to 26.6% on Day 7, 78.8% to 26.6% on Day 9, and 67.7% to 25.0% on Day 10 (baseline) respectively.

Using standard Y-STR across all times, this research revealed recovered DNA in the cervix more often than in the posterior fornix (Figure 4). Surprisingly, in this study, using results from the enhanced Y-STR, DNA detection occurred more frequently in the posterior fornix on days four and 7 (Figure 7). Supporting this finding is descriptive data (Table 3) revealing that

DNA detection using both standard or enhanced Y-STR method from the posterior fornix occurred in 53.7% (N=72) in at least one of four timed collections when it was not present in the cervix. Conversely, DNA detection from the cervix (Table 3) occurred in 56.9% (N=72) in at least one of four timed collections when it was not present in the posterior fornix, where the differences between the posterior fornix and cervix are not significant. Complicating the location of DNA, of the couples, 31.9% had one of four collections with DNA detection from the posterior fornix but not the cervix and one of the remaining three collections from the cervix but not the posterior fornix. These results imply that while present on any given day, DNA location in a post-coital environment is unpredictable for an individual. To help solve this dilemma, statistical analysis of the Y-STR methods revealed that DNA detection increased by combining samples taken from the posterior fornix and the cervix (Figure 12) rather than from either the posterior fornix (Figure 3, 6) or the cervix (Figures 2, 5) singly.

The diary card documented physical changes and medication use throughout the study period. These self-reported diary cards revealed significant variables influencing DNA recovery. Through literature review, menses and hormonal birth control changes to the genital track promoted a closer look at these two variables. The analysis of the study data revealed that the odds of DNA recovery is significantly lower with the standard Y-STR methods when menses is reported (OR: 0.5412; p=0.0445), and when hormonal birth control is used (OR: 0.2000; p=0.0004) (Table 7). With GEE modeling, data demonstrated the lowest recovery (but not absence) of DNA using the standard Y-STR method occurred when both menses and hormonal birth control are present (Figure 13). This significant finding was not duplicated using enhanced Y-STR, although there was an association.

Implications for Policy and Practice

In the majority of jurisdictions, collection of sexual assault evidence kits is limited to 72 hours after a report of sexual assault. In this research, we have successfully demonstrated the ability to obtain full and probative partial DNA profiles from enhanced Y-STR methods from the cervix, posterior fornix and the combined samples of the cervix and posterior fornix samples collected up to 9 days after intercourse in 78.8% of participating couples. Surprisingly, after 10 days of abstinence, there was recovery in 67.7% of participating couples. Detection does not drop to zero on day 11. When measured, there were no differences found between those with and without DNA detection at Day 10. Therefore, no one can definitively say that something, or nothing, will be found after some defined post-coital interval. This research indicates that failure to collect samples from victims with an extended post-coital interval may result in the potential loss of probative evidence that could be crucial to the investigation and prosecution of sexual crimes. Even with menstruating women on hormone birth control and using enhanced Y-STR, DNA detection is greater than 50% on Day 10. This study supports evidence collection consideration of 'until a completed menstrual period' in women having periods. Additionally, this research supports the practice change of combining the sample collection from the cervix with the posterior fornix. Given the legal needs for two swabs, this can be accomplished with a double-headed swab and one simultaneous collection, beginning first with the cervix followed by the posterior fornix. To avoid injury and dilution complications related to bleeding from the cervix, scrapping and twirling, as well as touching the cervix with the speculum blade, should be avoided.

The results from this study should enhance the policy debate about evidence collection timing and collection methods among SART members, including forensic medical health care, law enforcement, prosecution, forensic laboratories, advocates, public health promotion

specialists, and community activists. The policy debates should result in expedited changes to protocols and practices for rapid dissemination in support of evidence collection timing expansion through menses and implementation of the recommended increase in timing a victim can seek evidence collection and forensic medical care following rape in all jurisdictions throughout the U.S. and globally.

Policy should reflect and reinforce patient-centered and therapeutic trauma-informed care with financially supported national cross-training opportunities for advocates, law enforcement, forensic medical providers (SAFE/SANE), laboratory personnel, and prosecution. These policy recommendations should inform practice for the SART and local specialists in communities promoting health or responding to the increasing demands for services from victims and accused.

The results of this study informs policy which influences practice and triage protocols that currently exist among SART member organizations, whether forensic medical, law enforcement, prosecution, advocacy and forensic laboratory systems. This study also challenges each SART member agency's internal policies and protocols. For instance, in forensic medical communities, health histories that include menses and use of oral hormones challenge the specimen timing related to DNA recovery guidelines and medical treatment recommendations. The study results also challenge current thinking about medical risk and exposure from remaining and discoverable DNA particulate, whether epithelial or squamous cells and sperm fragments with white blood cells in seminal products, not to mention the viral particulate remaining in the closed environment. This research poses questions about continued exposure to disease from the detectable DNA and informs the prophylaxis verses treatment discussion among medical forensic providers and researchers.

With this research, the forensic laboratory community challenge is to question policy that supports the use of only one demonstratively insufficient standard Y-STR method for DNA detection in extended interval collections. The community challenge is to form partnerships and create collaborative triage protocols with resourced forensic laboratories using enhanced or other novel methods for DNA detection when there are negative or ambiguous results with CODIS approved methods.

Law enforcement will need to reflect the new evidence from this study in internal policies and standard operating procedures, and plan for increasing demands for services from crime reporters and the accused requesting an extended interval collection intervention and testing during an investigation.

All SART member organizations should plan policy for the increased economies of scale, specifically, cost reductions in light of increasing demand for services as awareness in communities spreads, by developing and testing new processes, procedures, and methodologies to achieve justice for victims and accused.

The debates internal to SART organization administrative policy maker members should focus on collaborations and cross training on topics such as case triage, evidence collection and medical treatment opportunities, workforce burden, prosecutor, law enforcement and laboratory capacity, as well as economic impact planning, particularly with extended interval complainants. The implications for criminal justice professionals working other types of crime are also challenged, as DNA detection improves and the science evolves. As DNA detection improves, planning that considers future advances in DNA detection in policy decisions today are protective from the seismic changes necessary following the dissemination of these research results. With policy and procedure changes associated with the recommendations from this

study, these researchers predict there is a corresponding expectation for increased rates of successful sexual assault convictions and exonerations with informed policies and procedures reflecting economies of scale with increased reporting.

Implications for Further Research

The Post-coital DNA Recovery study generated useful outcomes and revealed an ability to obtain full and probative partial DNA Y-STR profiles from cervix, posterior fornix, and combined cervical-vaginal samples recovered 9 and 10 days after DNA deposition of seminal and sperm products using standard and enhanced Y-STR methods. The ripple from these research findings will touch all criminal justice and health care systems, which demonstrates the need for further research on many fronts.

In the forensic medical health care community, vulnerable populations of women subjected to the highest rape rates necessitate research that confirms or denies similar post-coital DNA recovery timing. The volunteer population of primarily white college-educated females in this study limits generalizability to minorities. Therefore, future research should concentrate on this vulnerable population of minority women by following the existing complex post-coital DNA recovery protocol validated in this study. Other populations not studied but meriting research under this protocol include the growing number of older women experiencing menopause (with implications for child sexual assault with similar basic vaginal environments), digital penetration of women in all age groups, and DNA recovery from suspects and victims' oral cavities following cunnilingus or fellatio. The population of females with vasectomized male partners, which is a popular growing method of birth control, will yield a comparison group for touch DNA detection and detection of seminal products.

Further research is necessary to substantiate the recommended practice change for a single swabbing technique, given the legal challenges that await this evidence-based practice change recommendation.

Decisive forensic laboratory research is necessary to delineate clearly a standardized process for application of enhanced Y-STR methods, including the threshold for individual identification after DNA recovery, and development of newer novel methods for use on extended post-coital timing samples. With the success of the 9 and 10-day profiles at 25.5-67.7% or greater with standard and enhanced Y-STR methods respectfully, it might even be possible to obtain profiles from samples collected beyond menses, the recommended evidence-based timing for collection from this research. We have concentrated on use of standard and enhanced Y-STR methods in this study, demonstrating substantial DNA detection rates at 9 and 10 days with enhanced Y-STR methods. It is likely the final detection limit is unknown, nor has science reached the limits of discovery of post-coital DNA. Therefore, additional testing targeting samples collected 14 or more days after coitus may provide additional insight about timing of collections. While more challenging and possibly controversial, research should test the extension of the post-coital interval, from which obtaining a standard autosomal STR-typing of the semen donor might occur.

All systems and agencies responding to victims of sexual assault and rape should implement formative and summative program evaluations to study the impact of the evidence-based recommendations arising from this research. Systematic program evaluation with summative and formative outcomes and outputs assists in the implementation of yet unknown best practices and processes that will affect the economies of scale for all.

Recommendations

This research provides the evidence base for a practice change in timing for evidence collection in cases of rape from SART members who respond to all raped complainants. The recommended timing is through the first post-rape menses in menstruating females. The recommendation is that SART members provide a thorough investigation and medical forensic evaluation for evidence of injury, pregnancy and illness exposure, as well single swabbing from the cervix followed by the posterior fornix. When delivered to forensic laboratories, the recommendation is to apply the evidence from this study to collected extended interval samples to detect DNA using enhanced Y-STR methods.

The research provides the evidence base necessary to extend the timing for sample collection through menses using one swabbing – first from the cervix, followed by the posterior fornix for the purposes of recovering DNA areas using Y-STR methods. This practice change for SANE/SAFE comes with a cautionary note to avoid scraping or twirling the swabs to avoid iatrogenic injury. If the speculum scrapes the cervix, you have contaminated the area with external materials and possibly diluted the sample with blood from the cervical injury or even scraped the available DNA off the cervix. Another recommendation to avoid injuring the cervical site is to place the swabs in the cervix for a period of approximately 15 seconds while the swabs absorb cervical fluid, and then sweep the posterior fornix fluid pool, avoiding contamination from the vaginal sidewall encroachment, which has external debris and fluid contamination via the speculum insertion.

Future research should include quantification studies about laboratory analysis of parts of a single swab and determination of differences between the first cut sample and the remaining second sample. Policy recommendations include collaborative discussions among SART members about the legal ramifications of the ‘one swabbing’ recommendation, since defense

attorneys typically want and often receive a court order for a separate swab to testing in an independent laboratory.

This research also supports including last menstrual period and type of birth control in the medical forensic history, specifically to understand and anticipate that hormone birth control with menses is significantly associated with a reduction in (not absence of) DNA detection and recovery using standard Y-STR methods. Of note, duplication of the standard Y-STR significant finding did not occur using enhanced Y-STR, although there was a small association. Therefore, since population research can only inform practice, the individual who has both menses and is on hormone birth control or has an extended post-coital interval extending through the first period should be advised of these study findings. The findings include significant reduction of DNA detection with standard Y-STR methods for the population of participants with intervening menses, hormone birth control and extended post-coital recovery. Advice should include that this study's findings also include that there is improved recovery of DNA in the population of participants with intervening menses, hormone birth control and extended post-coital recovery when using enhanced Y-STR methods. Population research informs providers, and cannot predict an individual's DNA recovery outcome using either standard or enhanced Y-STR laboratory methods. Laboratories, with notification of these two physical events and/or extended post rape intervals, should implement policies that triage all samples to enhanced Y-STR methods if the standard Y-STR method or other methods are negative for DNA detection and recovery. Additional research is necessary to determine the outer limits of timing post-coital recovery.

These practice recommendations are evolutionary based on a number of small studies, confirmed by this larger research validating expanded post-coital interval timing. However, the

ramifications of this recommended practice change is not without economies of scale impact and potential for increased reporting. Therefore, these authors recommend a common model for systems evaluation with validated tools measuring quality and processes, which should be available to all SART members in a widely disseminated online toolbox to promote standardization of best practices within disciplines and between members of the SART. All the recommendations from this study, when implemented, could increase therapeutic interventions, improve DNA detection, increase plea agreements through improved prosecution, and result in conviction or exoneration of the accused, all while creating an economies of scale offsetting the costs of a predicted increase in reporting.

Change to standard protocols that currently instruct health care providers to gather multiple swabs samples from the vaginal vault is necessary to reflect the evidence from this study, which is one swabbing increases DNA recovery across all timed locations. The specimen collection procedure for medical reasons requires avoidance of iatrogenic injury to the vascular cervix, whereby the provider should first locate the cervix (without scraping with the speculum or swab), and use a swab to absorb the cervical fluid into the cotton tipped applicator, where wicking may take a few seconds. To avoid injury and possible disease transmission, the cervix should not be penetrated with the swab. Following the absorption of cervical moisture, the swab should sweep the fluids in the posterior fornix to yield a larger quantity of DNA, particularly if the person's case is an extended time interval collection.

In summary, DNA recovery has advanced significantly, creating gradual changes in policy, protocols, and practice over the last 25 years. The evidence from this research will promote rapid changes in practice related to timing of collections and improvement in understanding the variables affecting DNA recovery are more complex than previously known.

For the SART members and their organizations, resourced organizations will make changes quickly, but for others, these practice changes will occur slowly, building over time and are dependent on economies of scale. In the short term, the practice changes may be disruptive, but in the long term, through ingenuity, resource development, and collaboration, the practice changes supported by this research will benefit the victim and the accused by improving criminal justice outcomes.

REFERENCES

Agarwal, A., Durairajanayagam, D., Halabi, J., Peng, J., & Vazquez-Levin, M. (2014). Proteomics, oxidative stress and male infertility. *Reprod Biomed Online*. doi: 10.1016/j.rbmo.2014.02.013

Ahlgren, M. (1975). Sperm transport to and survival in the human fallopian tube. *Gynecol Invest, 6*(3-4), 206-214.

Andrikopoulou, M., Michala, L., Creighton, S. M., & Liao, L. M. (2013). The normal vulva in medical textbooks. *J Obstet Gynaecol, 33*(7), 648-650. doi: 10.3109/01443615.2013.807782

Anxiety and Depression Association of America. (2014). Body dismorphic disorder. Retrieved June 20, 2014, from http://www.adaa.org/understanding-anxiety/related-illnesses/other-related-conditions/body-dysmorphic-disorder-bdd

Archambault, J. (2000, September, September 15-17, 1999). *Forensic DNA*, Denver, CO.

'Badly fragmented' forensic science system needs overhaul. (2009). *Medical News Today*. http://www.medicalnewstoday.com/articles/139657.php

Baker, R. R., & Bellis, M. A. (1993). Human sperm competition: ejaculate manipulation by females and a function for the female orgasm. *Animal Behavior, 6*, 887-909.

Ballantyne, J. (2013). *DNA profiling of the semen donor in extended interval post-coital samples*. (241299). Washington DC: NCJRS.

Ballantyne, J., van Daal, A., & Lubenow, H. (2013). *Improved detection of male DNA in post coital samples*. (241298). Washington DC: NSJRC.

Beckmann, C. R. B., Casanova, R., Ling, F. W., Chuang, A., Herbert, W. N. P., Goepfert, A. R., . . . Weiss, P. M. (2014). Infertility *Obstetrics and Gynecology* (7 ed.). Baltimore MD: Lippincott Williams & Wilkins.

Berger, B., Niederstatter, H., Kochl, S., Steinlechner, M., & Parson, W. (2003). Male/female DNA mixtures: a challenge for Y-STR analysis. *Progress in Forensic Genetics, 9*, 295-299.

Bouvet, J. P., Gresenguet, G., & Belec, L. (1997). Vaginal pH neutralization by semen as a cofactor of HIV transmission. *Clinical Microbiology and Infection, 3*(1), 19-23.

Boykins, A. D., & Mynatt, S. (2007). Assault history and follow-up contact of women survivors of recent sexual assault. *Issues Ment Health Nurs, 28*(8), 867-881. doi: 10.1080/01612840701493394

Bromfield, J. J. (2014). Seminal fluid and reproduction: much more than previously thought. *J Assist Reprod Genet, 31*(6), 627-636. doi: 10.1007/s10815-014-0243-y

Budowle, B., Bottrell, M. C., Bunch, S. G., Fram, R., Harrison, D., Meagher, S., . . . Stacey, R. B. (2009). A perspective on errors, bias, and interpretation in the forensic sciences and direction for continuing advancement. *Journal of Forensic Sciences, 54*(4), 798-809. doi: 10.1111/j.1556-4029.2009.01081.x

Burgess, A. W., Fehder, W. P., & Hartman, C. R. (1995). Delayed reporting of the rape victim. *J Psychosoc Nurs Ment Health Serv, 33*(9), 21-29.

Butler, J. M. (2006). Genetics and genomics of core short tandem repeat loci used in human identity testing. *Journal of Forensic Science, 51*, 253-265.

Campbell, R., Patterson, D., Bybee, D., & Dworkin, E. R. (2009). Predicting sexual assault prosecution outcomes: the role of medical forensic evidence collected by SANEs. *Criminal Justice and Behavior, 36*(7), 712-727. doi: 10.1177/0093854809335054

Cerri, N., Ricci, U., Sani, I., Verzeletti, A., & De, F. F. (2003). Mixed stains from sexual assault cases: autosomal or Y-chromosome short tandem repeats? *Croatia Medicine Journal, 44*, 289-292.

Chavarro, J. E., Toth, T. L., Wright, D. L., Meeker, J. D., & Hauser, R. (2009). Body mass index in relation to semen quality, sperm DNA integrity, and serum reproductive hormone levels among men attending an infertility clinic. *Fertility and Sterility*.

Christian, C. W., Lavelle, J. M., De Jong, A. R., Loiselle, J., Brenner, L., & Joffe, M. (2000). Forensic evidence findings in prepubertal victims of sexual assault. *Pediatrics, 106*(1 Pt 1), 100-104.

Colagar, A. H., Marzony, E. T., & Chaichi, M. J. (2009). Zinc levels in seminal plasma are associated with sperm quality in fertile and infertile men. *Nutrition Research, 29*(2), 82-88.

Committee on Identifying the Needs of the Forensic Sciences Community National Research Council. (2009). *Strengthening forensic science in the United States: A path forward*. Washington, DC: The National Academies Press.

Cooper, T. G., Noonan, E., von Eckardstein, S., Auger, J., Baker, H. W., Behre, H. M., . . . Vogelsong, K. M. (2010). World Health Organization reference values for human semen characteristics. *Human Reproduction Update, 16*(3), 231-245. doi: 10.1093/humupd/dmp048

Crandall, C. S., & Helitzer, D. (2003). *Impact evaluation of a SANE program*. (203276). Washington DC: NCJRS.

Daniels, D. L., Hall, A. M., & Ballantyne, J. (2004). SWGDAM developmental validation of a 19-locus Y-STR system for forensic casework. *Journal of Forensic Science, 49*, 668-683.

Davies, A., & Wilson, E. (1974). The persistence of seminal constituents in the human vagina. *Forensic Sci, 3*(1), 45-55.

Davis, K. C., Kiekel, P. A., Schraufnagel, T. J., Norris, J., George, W. H., & Kajumulo, K. F. (2012). Men's alcohol intoxication and condom use during sexual assault perpetration. *J Interpers Violence, 27*(14), 2790-2806. doi: 10.1177/0886260512438277

Dohrenwend, A. (2011). Relinquishing the truth. *Fam Med, 43*(6), 439-441.

Elliott, K., Hill, D. S., Lambert, C., Burroughes, T. R., & Gill, P. (2003). Use of laser microdissection greatly improves the recovery of DNA from sperm on microscope slides. *Forensic Sci Int, 137*(1), 28-36.

Enos, W. F., & Byer, J. C. (1977). Spermatozoa in the anal canal and rectum and in the oral cavity of female rape victims. *Journal of Forensic Science, 23*, 231-233.

Eskenazi, B., Kidd, S. A., Marks, A. R., Sloter, E., Block, G., & Wyrobek, A. J. (2005). Antioxidant intake is associated with semen quality in healthy men. *Human Reproduction, 20*(4), 1006-1012.

Faugno, D. K., & Speck, P. M. (2011). Basic anogenital and oral anatomy. In A. B. L. Ledray, & A. P. Giardino (Ed.), *Medical Response to Adult Sexual Assault*. St. Louis, MO: STM Medical Publishing.

Ferramosca, A., & Zara, V. (2014). Bioenergetics of mammalian sperm capacitation. *Biomed Res Int, 2014*, 902953. doi: 10.1155/2014/902953

Finkel, M., & Giardino, A. (Eds.). (2002). *Medical Evaluation of Child Sexual Abuse: A Practical Guide* (2nd ed.). Thousand Oaks: Sage Publications.

Fisch, H. (2009). Older men are having children, but the reality of a male biological clock makes this trend worrisome. *Geriatrics, 64*(1), 14-17.

Flanagan, N., & McAlister, C. (2011). The transfer and persistence of DNA under the fingernails following digital penetration of the vagina. *Forensic Sci Int Genet, 5*(5), 479-483. doi: 10.1016/j.fsigen.2010.10.008

Garvin, A. M., Bottinelli, M., Gola, M., Conti, A., & Soldati, G. (2009). DNA preparation from sexual assault cases by selective degradation of contaminating DNA from the victim. *J Forensic Sci, 54*(6), 1297-1303. doi: 10.1111/j.1556-4029.2009.01180.x

Giusti, A., Baird, M., Pasquale, S., Balazs, I., & Glassberg, J. (1986). Application of deoxyribonucleic acid (DNA) polymorphisms to the analysis of DNA recovered from sperm. *J Forensic Sci, 31*(2), 409-417.

Groth, A. N., & Burgess, A. W. (1977). Sexual dysfunction during rape. *New England Journal of Medicine, 297*, 764.

Hale, G. E., Robertson, D. M., & Burger, H. G. (2014). The perimenopausal woman: endocrinology and management. *J Steroid Biochem Mol Biol, 142*, 121-131. doi: 10.1016/j.jsbmb.2013.08.015

Hall, A., & Ballantyne, J. (2003). The development of an 18-locus Y-STR system for forensic casework. *Anal Bioanal Chem, 376*(8), 1234-1246. doi: 10.1007/s00216-003-2039-2

Hall, A. M., & Ballantyne, J. (2003a). The development of an 18-locus Y-STR system for forensic casework. *Annals of Bioanalytical Chemistry, 376*, 1234-1246.

Hall, A. M., & Ballantyne, J. (2003b). Novel Y-STR typing strategies reveal the genetic profileof the semen donor in extended interval post-coital cervicovaginal samples. *Forensic Science International, 136*, 58-72.

Hall, A. M., & Ballantyne, J. (2003c). Strategies for the design and assessment of Y-STR multiplexes for forensic use. *Forensic Science Review, 15*, 137.

Hanson, E. K., & Ballantyne, J. (2004). A highly discriminating 21 locus Y-STR "megaplex" system designed to augment the minimal haplotype loci for forensic casework. *Journal of Forensic Science, 49*, 40-51.

Hanson, E. K., Berdos, P. N., & Ballantyne, J. (2006). Testing and evaluation of 43 "noncore" Y chromosome markers for forensic casework applications. *Journal of Forensic Science, 51*, 1298-1314.

Harris-Glocker, M., & McLaren, J. F. (2013). Role of female pelvic anatomy in infertility. *Clin Anat, 26*(1), 89-96. doi: 10.1002/ca.22188

Hatsch, D., Amory, S., Keyser, C., Hienne, R., & Bertrand, L. (2007). A rape case solved by mitochondrial DNA mixture analysis. *J Forensic Sci, 52*(4), 891-894. doi: 10.1111/j.1556-4029.2007.00473.x

Hawkes, W. C., & Turek, P. J. (2001). Effects of dietary selenium on sperm motility in healthy men. *Journal of Andrology, 22*(5), 764-772.

Hess, R., Austin, R. M., Dillon, S., Chang, C. C., & Ness, R. B. (2008). Vaginal maturation index self-sample collection in mid-life women: acceptability and correlation with physician-collected samples. *Menopause, 15*(4), 726-729.

Hornor, G., Thackeray, J., Scribano, P., Curran, S., & Benzinger, E. (2012). Pediatric sexual assault nurse examiner care: trace forensic evidence, ano-genital injury, and judicial outcomes. *J Forensic Nurs, 8*(3), 105-111. doi: 10.1111/j.1939-3938.2011.01131.x

Jensen, T. K., Swan, S., Jorgensen, N., Toppari, J., Redmon, B., Punab, M., . . . Andersson, A. M. (2014). Alcohol and male reproductive health: a cross-sectional study of 8344 healthy men from Europe and the USA. *Hum Reprod*. doi: 10.1093/humrep/deu118

Johnson, C. L., Giles, R. C., Warren, J. H., Floyd, J. I., & Staub, R. W. (2005). Analysis of non-suspect samples lacking visually identifiable sperm using a Y-STR 10-plex. *J Forensic Sci, 50*(5), 1116-1118.

Jordan, G. H. (1999). Erectile function and dysfunction. *Postgraduate Medicine, 105*(2), 137-147.

Jouannet, P., Ducot, B., Feneux, D., & Spira, A. (1988). Male factors and the likelihood of pregnancy in infertile couples. I. Study of sperm characteristics. *International Journal of Andrology, 11*(5), 379-394. doi: 10.1111/j.1365-2605.1988.tb01011.x

Katz, D. F., Slade, D. A., & Nakajima, S. T. (1997). Analysis of pre-ovulatory changes in cervical mucus hydration and sperm penetrability. *Advances in Contraception, 13*(2-3), 143-151.

Kuiri-Hanninen, T., Haanpaa, M., Turpeinen, U., Hamalainen, E., Seuri, R., Tyrvainen, E., . . . Dunkel, L. (2013). Postnatal ovarian activation has effects in estrogen target tissues in infant girls. *J Clin Endocrinol Metab, 98*(12), 4709-4716. doi: 10.1210/jc.2013-1677

Kuiri-Hanninen, T., Kallio, S., Seuri, R., Tyrvainen, E., Liakka, A., Tapanainen, J., . . . Dunkel, L. (2011). Postnatal developmental changes in the pituitary-ovarian axis in preterm and term infant girls. *J Clin Endocrinol Metab, 96*(11), 3432-3439. doi: 10.1210/jc.2011-1502

Kurita, T. (2011). Normal and abnormal epithelial differentiation in the female reproductive tract. *Differentiation, 82*(3), 117-126. doi: 10.1016/j.diff.2011.04.008

Larsen, L., Scheike, T., Jensen, T. K., Bonde, J. P., Ernst, E., Hjollund, N. H., . . . Danish First Pregnancy Planner Study Team. (2000). Computer-assisted semen analysis parameters as predictors for fertility of men from the general population. *Human Reproduction, 15*(7), 1562-1567.

Ledray, L. E. (1999). *SANE SART development and operations guide* Retrieved from http://www.ojp.usdoj.gov/ovc/publications/infores/sane/saneguide.pdf

Lilja, H., & Lundwall, A. (1992). *Molecular cloning of epididymal and seminal vesicular transcripts encoding a semenogelin-related protein. .* Paper presented at the National Academy of Sciences.

Ludes, B., & Clisson, I. (2000). Short tandem repeat locic analysis in forensic casework. *Anthropology Annals, 58*, 23-27.

Maier, S. L. (2008). "I have heard horrible stories . . .": rape victim advocates' perceptions of the revictimization of rape victims by the police and medical system. *Violence Against Women, 14*(7), 786-808. doi: 10.1177/1077801208320245

Managing common vulvar skin conditions. (2008). *Harvard Women's Health Watch, 16*(3), 3-5.

Marthol, H., & Hilz, M. J. (2004). [Female sexual dysfunction: a systematic overview of classification, pathophysiology, diagnosis and treatment]. *Fortschr Neurol Psychiatr, 72*(3), 121-135. doi: 10.1055/s-2004-818357

Masse, J., Watrin, T., Laurent, A., Deschamps, S., Guerrier, D., & Pellerin, I. (2009). The developing female genital tract: from genetics to epigenetics. *Int J Dev Biol, 53*(2-3), 411-424. doi: 10.1387/ijdb.082680jm

Masters, W. H., & Johnson, V. E. (1966). *Human sexual response*. Toronto: Bantam Books.

Mayntz-Press, K. A., & Ballantyne, J. (2007). Performance characteristics of commercial Y-STR multiplex systems. *Journal of Forensic Science, 52*, 1025-1034.

Mayntz-Press, K. A., Sims, L. M., Hall, A. M., & Ballantyne, J. (2008). Y-STR profiling in extended interval (> or = 3 days) postcoital cervicovaginal samples. *Journal of Forensic Science, 53*, 342-348.

Maze, M., Stagnara, D., & Fischer, L. P. (2007). [Dr. Edmond Locard (1877-1966), the Sherlock Holmes of Lyons]. *Histoire des Sciences Médicales, 41*(3), 269-278.

McKnight, K., Hoang, H. D., Prasain, J. K., Brown, N., Vibbert, J., Hollister, K. A., . . . Miller, M. A. (2014). Neurosensory perception of environmental cues modulates sperm motility critical for fertilization. *Science, 344*(6185), 754-757. doi: 10.1126/science.1250598

Meeker, J. D., Ehrlich, S., Toth, T. L., Wright, D. L., Calafat, A. M., Trisini, A. T., . . . Hauser, R. (2010). Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic. *Reprod Toxicol, 30*(4), 532-539. doi: 10.1016/j.reprotox.2010.07.005

Mnookin, J. L. (2009, February 25). How scientific is forensic science? *UCLA Today*.

Morgan, J. A. (2008). Comparison of cervical os versus vaginal evidentiary findings during sexual assault exam. *JEN: Journal of Emergency Nursing, 34*(2), 102-105.

Nicholson, R. (1965). Vitality of spermatozoa in the endocervical canal. *Fertility and Sterility, 16*(6), 758-764.

O'Neal, E. N., Decker, S. H., Spohn, C., & Tellis, K. (2013). Condom use during sexual assault. *J Forensic Leg Med, 20*(6), 605-609. doi: 10.1016/j.jflm.2013.03.023

Oien, C. T. (2009). Forensic hair comparison: background information for interpretation. *Forensic Science Communications, 11*(2). http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/review/2009_04_review02.htm

Pacey, A. A., Povey, A. C., Clyma, J. A., McNamee, R., Moore, H. D., Baillie, H., & Cherry, N. M. (2014). Modifiable and non-modifiable risk factors for poor sperm morphology. *Hum Reprod*. doi: 10.1093/humrep/deu116

Parson, W., Niederstatter, H., Brandstatter, A., & Berger, B. (2003). Improved specificity of Y-STR typing in DNA mixture samples. *International Journal of Legal Medicine, 117*, 109-114.

Prinz, M., Boll, K., Baum, H., & Shaler, B. (1997). Multiplexing of Y chromosome specific STRs and performance for mixed samples. *Forensic Science International, 85*, 209-218.

Puppo, V. (2013). Anatomy and physiology of the clitoris, vestibular bulbs, and labia minora with a review of the female orgasm and the prevention of female sexual dysfunction. *Clin Anat, 26*(1), 134-152. doi: 10.1002/ca.22177

Ranjbar, V., & Speer, S. A. (2013). Revictimization and recovery from sexual assault: implications for health professionals. *Violence Vict, 28*(2), 274-287.

Roman, J., Walsh, K., Lachman, P., & Yahner, J. (2012). *Post-conviction DNA testing and wrongful conviction*. (238816). Washington DC: NCJRS Retrieved from https://www.ncjrs.gov/pdffiles1/nij/grants/238816.pdf.

Sargeant, H. A., & O'Callaghan, F. V. (2007). The impact of chronic vulval pain on quality of life and psychosocial well-being. *Australian and New Zealand Journal of Obstetrics and Gynaecology, 47*(3), 235-239.

Schisterman, E. F., Mumford, S. L., Browne, R. W., Boyd Barr, D., Chen, Z., & Buck Louis, G. M. (2014). Lipid Concentrations and Couple Fecundity: The LIFE Study. *J Clin Endocrinol Metab*, jc20133936. doi: 10.1210/jc.2013-3936

Schisterman, E. F., Mumford, S. L., Chen, Z., Browne, R. W., Boyd Barr, D., Kim, S., & Buck Louis, G. M. (2014). Lipid concentrations and semen quality: the LIFE study. *Andrology, 2*(3), 408-415. doi: 10.1111/j.2047-2927.2014.00198.x

Schonbucher, V., Maier, T., Mohler-Kuo, M., Schnyder, U., & Landolt, M. A. (2014). Adolescent perspectives on social support received in the aftermath of sexual abuse: a qualitative study. *Arch Sex Behav, 43*(3), 571-586. doi: 10.1007/s10508-013-0230-x

Sensabaugh, G. F. (1978). Isolation and characterization of a semen-specific protein from human seminal plasma: a potential new marker for semen identification. *Journal of Forensic Sciences, 23*(1), 106-115.

Sensabaugh, G. F. (1979). The quanitative acid phosphatase test. A statistical analysis of endogenous and postcoital acid phosphatase levels in the vagina. *Journal of Forensic Sciences, 24*(2), 346-365.

Sibille, I., Duverneuil, C., Lorin de la Grandmaison, G., Guerrouache, K., Teissiere, F., Durigon, M., & de Mazancourt, P. (2002). Y-STR DNA amplification as biological evidence in sexually assaulted female victims with no cytological detection of spermatozoa. *Forensic Sci Int, 125*(2-3), 212-216.

Sievers, V., Murphy, S., & Miller, J. J. (2003). Sexual assault evidence collection more accurate when completed by sexual assault nurse examiners: Colorado's experience. *J Emerg Nurs, 29*(6), 511-514. doi: 10.1016/j.jen.2003.08.010

Speck, P. M., & Patton, S. B. (2007, October 19). *Hymen morphology updated: Classifying estrogen effect across the life span*

Paper presented at the International Association of Forensic Nurses, Salt Lake City, UT.

Su, E. J., Xin, H., & Monsivais, D. (2012). The emerging role of estrogen receptor-beta in human reproduction. *Semin Reprod Med, 30*(1), 62-70. doi: 10.1055/s-0031-1299598

Suarez, S. S., & Pacey, A. A. (2006). Sperm transport in the female reproductive tract. *Human Reproduction Update, 12*(1), 23-37.

Suresh, A., Rajesh, A., Bhat, R. M., & Rai, Y. (2009). Cytolytic vaginosis: A review. *Indian J Sex Transm Dis, 30*(1), 48-50. doi: 10.4103/0253-7184.55490

Tamaki, K., & Jeffreys, A. J. (2005). Human tandem repeat sequences in forensic DNA typing. *Legal Medicine (Tokyo), 7*, 244-250.

Trisini, A. T., Singh, N. P., Duty, S. M., & Hauser, R. (2004). Relationship between human semen parameters and deoxyribonucleic acid damage assessed by the neutral comet assay. *Fertility and Sterility, 82*(6), 1623-1632.

Tucker, S., Claire, E., Ledray, L. E., Werner, J. S., & Claire, E. (1990). Sexual assault evidence collection. *Wisconsin Medical Journal, 89*(7), 407-411.

Voskoboinik, L., & Darvasi, A. (2011). Forensic identification of an individual in complex DNA mixtures. *Forensic Sci Int Genet, 5*(5), 428-435. doi: 10.1016/j.fsigen.2010.09.002

Waugh, A., & Grant, A. (2006). *Anatomy and Physiology in Health* Edinburgh: Churchill Livingstone.

WHO/Department of Reproductive Health and Research. (2010). *WHO laboratory manual for the examination and processing of human semen* (5 ed.).

Yin, Y., & Ma, L. (2005). Development of the mammalian female reproductive tract. *J Biochem, 137*(6), 677-683. doi: 10.1093/jb/mvi087

Young, K. L., Jones, J. G., Worthington, T., Simpson, P., & Casey, P. H. (2006). Forensic laboratory evidence in sexually abused children and adolescents. *Archives of Pediatrics & Sdolescent Medicine, 160*(6), 585-588.

Zinaman, M. J., Brown, C. C., Selevan, S. G., & Clegg, E. D. (2000). Semen Quality and Human Fertility: A Prospective Study With Healthy Couples. *Journal of Andrology, 21*(1), 145-153. doi: 10.1002/j.1939-4640.2000.tb03284.x

DISSEMINATION OF RESEARCH FINDINGS

Publications

1. Upcoming publication (submission November 2013): Y-STR Specific DNA Enhancement Strategies to Aid in the Analysis of Late Reported Sexual Assault Cases (title subject to change), Hanson, E and Ballantyne, J. Book chapter in Forensic DNA Typing Protocols, Methods in Molecular Biology Series. Humana Press (Springer publishing group). Expected publication 2014.
2. Enhancing the Sexual Assault Workflow: Testing of Next Generation DNA Assessment and Y-STR Systems. Ballantyne, J., Hanson, E., Green, R., Holt, A. and Mulero, J. Forensic Science International Genetics Supplement Series (October 30, 2013). Found at http://www.slideshare.net/Lifetech_HID/enhancing-the-sexual-assault-workflow-testing-of-next-generation-assessment-and-ystr-systems

Presentations

Specific to current study:

1. Post-coital DNA recovery – global transformation in forensic nursing health care. American Academy of Nursing (October 2014). Speck PM et al. American Academy of Nursing's 2014 Transforming Health, Driving Policy Conference, Washington DC
2. Post-coital DNA recovery (March 2014). Speck PM. SAFER Working Group. National Institutes of Justice, Washington DC.
3. Post-coital DNA recovery study results: A SART paradigm shift and a global forensic nursing practice change (October 2013). Speck PM et al. International Conference on Forensic Nursing on Science and Practice, Anaheim CA.
4. Enhancing the Sexual Assault Workflow: Testing of Next Generation DNA Assessment and Y-STR Systems. Ballantyne J, Hanson, E, Green R, Holt A, and Mulero, J. 25th World Congress of the International Society of Forensic Genetics, Melbourne, Australia.
5. Robust Methods for Improving Challenging DNA Workflows. Life Technologies Lunch Symposium. Ballantyne J. 25th World Congress of the International Society of Forensic Genetics, Melbourne, Australia.

Related/Relevant presentations

1. Extending the Post-coital Time Interval for DNA Profile Recovery. Orange County SART (Sexual Assault Response Team), Orlando, FL
2. From the Bed to the Bench: Defining the Vaginal and Cervical Environment for Post-Coital DNA Recovery. Speck, P., Faugna, D. and Ballantyne, J. AAFS Annual Meeting, Chicago, IL.
3. The Effects of Y-STR Research on Practice and Policy. The Annual NIJ Conference, Washington, DC.
4. Enhanced DNA Profiling of the Semen Donor Can Assist the Investigation of Late Reported (> 5days) Sexual Assaults. Hanson, E. and Ballantyne, J. American Society of Crime Lab Directors (ASCLD) Annual Symposium, Denver, CO.
5. Y-Chromosome Specific Nested PCR Pre-Amplification Method for Improved Detection of Male DNA. Hanson, E., Solivan, M., Strauss, S., Di Pasquale, F., Engel, H. and Ballantyne, J. 22nd Annual Symposium on Human Identification, National Harbor, MD.

6. Enhanced DNA Profiling for Detection of the Male Donor in Trace DNA Samples. Hanson, E and Ballantyne, J. European National Forensic Science Institutes (ENFSI) DNA Working Group Meeting, Athens, Greece
7. Current Status and Future of Y-STR Analysis Workshop. Association of Forensic DNA Administrators and Analysts (AFDAA), San Antonio, TX.

DEFINITION OF TERMS

Abstinence is absence of coitus (sexual intercourse), digital penetration, or cunnilingus; for the purposes of this study abstinence includes protected coitus, which includes condom use where the condom and vulva are not touched by the male partner externally

Coitus is sexual intercourse, specifically male penile penetration of the vagina of a female

Digital penetration is the insertion of any digit into the vulva or vagina of a female or the anus or rectum of a male

DNA is deoxyribonucleic acid, the genetic material of life

Enhanced Y-STR (nested) is a method to eliminate the problem small amounts of Y chromosome (see Y-STR)

Intercourse is male penile penetration of the vagina of a female

Post-coital is 'after' sexual intercourse

Protected Coitus is male penile penetration of the vagina of a female with a condom that the male did not touch

Rape is forcible male penile penetration of the vagina of a female

Sexual Assault is an incident that involves sexual contact that is forced (without consent)

Sexual intercourse is male penile penetration of the vagina of a female

Unprotected Coitus is male penile penetration of the vagina of a female without condom

Y-STR is a short tandem repeat (STR) on the Y-chromosome results in copies of short repeating sequence bases on the Y chromosome; useful in forensics; method based on Polymerase chain reaction (PCR) which allows for easy comparison of small amounts of male DNA