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Assessment of the vaginal residence time of biomarkers of semen exposure☆,☆☆,★

Andrea Thurman*, Terry Jacot, Johan Melendez, Thomas Kimble, Margaret Snead, Roxanne Jamshidi, Angie Wheeless, David F. Archer, Gustavo F. Doncel, Christine Mauck

Eastern Virginia Medical School, 601 Colley Avenue, Room 235, Norfolk, VA 23507, USA

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Abstract

Objective: The primary objective of this pilot study is to determine and compare the residence time in the vagina of biomarkers of semen exposure for up to 15 days post exposure. The biomarkers are prostate-specific antigen (PSA), Y chromosome DNA, the sex determining region of the Y chromosome (SRY) and testis-specific protein Y-encoded 4 (TSPY4). The secondary objectives are to determine if biomarker concentrations differed between intercourse and inoculation groups, to establish whether the sampling frequency post exposure affected biomarker concentrations and decay profile and to determine if biomarker concentrations in vaginal swabs obtained by the participant at home were similar to swabs obtained by the nurse in the clinic.

Study design: We randomized healthy women to unprotected intercourse ($n=17$) versus vaginal inoculation with the male partner's semen in the clinic ($n=16$). Women were then further randomized to have vaginal swabs obtained at either 7 or 4 time points after semen exposure, up to 15 days post exposure, either obtained at home by the participant or in the clinic by the research nurse.

Results: PSA and SRY were markers of recent semen exposure. TSPY4 was detectable in approximately 50% of participants at 15 days post exposure. Unprotected intercourse resulted in significantly higher concentrations of select biomarkers. Sampling frequency and home versus clinic sampling had no significant effect on biomarker concentrations.

Conclusions: Objective biomarkers of recent or distant semen exposure may have great utility for verifying protocol compliance in a variety of clinical trials.

Keywords: Semen; Biomarker; Y chromosome; Clinical trials

1. Introduction

Objective biomarkers of vaginal exposure to semen were developed for use in forensic medicine to provide evidence of sexual contact [1]. Ultimately they may be used as surrogates for risk of pregnancy and sexually transmitted infections (STIs) and alternative endpoints in contraceptive efficacy trials [2–9], the assessment of slippage/breakage of condoms [7,8,10–14], and as an objective biomarker to validate self-reports of condom use and/or sexual activity [15–23].

Prostate-specific antigen (PSA) is the best characterized marker of seminal fluid (reviewed in Ref. [24]). PSA is detectable in the vagina immediately post exposure until approximately 24–48 h post exposure [1,8,10,11,25,26]. Other objective biomarkers of sperm or male cell exposure are linked to Y chromosome DNA (YcDNA), present in Y-bearing spermatozoa, immature germ cells and nongerminal male cells (e.g., epithelial cells and leukocytes). Two genes on the Y chromosome that are of particular interest are the sex determining region of the Y chromosome (SRY) and

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* Corresponding author.

E-mail address: thurmaar@evms.edu (A. Thurman).

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the testis-specific protein Y-encoded 4 (TSPY4) gene [27–29]. These biomarkers were utilized in small clinical trials, from the cell pellet from cervicovaginal lavage specimens [17] and vaginal swabs [5,17,26,30].

For new biomarkers to be used in future contraceptive and other clinical trials, it is important to determine residence time in the vagina under various exposure and sampling conditions. Only a few published studies were structured this way, with measurement of PSA and YcDNA as endpoints [8,10,11,26], and none compared methodologies to assess these new semen biomarkers.

The primary objective of this study is to describe the decay of SRY and TSPY4 in the vagina, for up to 15 days, using two different polymerase chain reaction (PCR) platforms, multiplex PCR and quantitative PCR (qPCR). Utilizing exposure to semen through unprotected vaginal intercourse or inoculation with the partner's semen in the clinic, we measured and categorized biomarker detection post exposure and determined whether sampling frequency affects the decay of the markers. Finally, we compared concentrations of semen biomarkers obtained from vaginal swabs collected at home versus swabs obtained from the same participant by a research nurse in the clinic.

2. Materials and methods

This is a single-blinded, randomized, outpatient trial conducted at the Johns Hopkins University (JHU) Bayview Medical Center (Baltimore, MD) and the CONRAD Clinical Research Center (CRC) at the Eastern Virginia Medical School (EVMS) in Norfolk, VA. The institutional review board (IRB) (Approval Number NA_00016471) at the JHU (for the Bayview site) and the Chesapeake IRB (Protocol #Pro0003114) for the CONRAD CRC site approved this protocol. We screened monogamous couples at low risk of acquiring STIs, including a healthy, normally menstruating woman, aged 18–50 years, who was not at risk for pregnancy due to surgical sterilization or who was willing to become pregnant, and her male sexual partner, aged 18–55 years.

Women and men were interviewed and consented separately. Men enrolled in the study had to be in good health and had to have screening semen sample parameters as follows: volume: ≥1.8 mL (or ≥1.0 mL with a total sperm count of ≥120 million), total sperm count: 100–400 million, sperm concentration: ≥35 M/mL, total sperm motility (progressive motility+ nonprogressive motility): ≥40%, viability: ≥70% vital cells, round cells: ≤5 M/mL and leukocytes: ≤1 M/mL.

We used the random permuted blocks method to generate allocation sequences assigning the couples to one of four subgroups (A1, A2, B1 and B2) in a 1:1:1:1 ratio. The sequences were created by a randomization manager, not otherwise involved in the statistical analyses of this study using a verified program based on the random function RANUNI in the SAS(r) System (SAS Institute, Cary, NC). The randomization groups and interventions are outlined in Table 1.

Women were given the choice of having all the vaginal swabs collected in the clinic by a nurse ("in-clinic sampling") or having some samples collected in the clinic and collecting some at home ("mixed sampling"). For those opting for "mixed sampling", all samples taken at 6 and 48 h were collected by the participant at home; a nurse in the clinic collected the remaining samples. Participants were instructed to abstain from vaginal or receptive oral intercourse during the 15 days of follow-up.

Up to two women in each of the four groups (A1, A2, B1 and B2) were invited to participate in a substudy. These women were asked to go through the study visits a second time, in their same group assignment, using the type of sampling that they did not use during their first time through to test the hypothesis that samples collected by participants yielded results similar to those collected in the clinic by the nursing staff.

Participants' swabs were immediately placed in 1 mL of cold phosphate buffered saline (PBS). Next, the solution was spun for 5 min at 14,000 rpm. We sent the supernatant to the Centers for Disease Control and Prevention for PSA measurements, as previously described [31]. The pellet was resuspended in 700 μL PBS, and half was used for SRY qPCR (labeled "YcDNA") analysis, and the other half was used for SRY-TSPY4 multiplex PCR and TSPY4 qPCR analysis, both performed as previously described [32,33].

We developed the TSPY4 qPCR using the Universal Probe Library system in combination with the Lightcycler TaqMan Master kit for the Lightcycler 2.0 (Roche Diagnostics, Indianapolis, IN). The method detected that TSPY4 amplified from sperm DNA as low as 1 pg and, in semen samples with DNA, as low as 2 pg. We used both qPCR and multiplex PCR to assess both Y chromosome biomarkers to measure semen exposure. Although both assays detect the SRY region of the Y chromosome, we use the terms SRY for the multiplex system and YcDNA for the qPCR system, to maintain consistency with previously published data [26,32,33].

Statistical analyses were performed using SAS version 9.3 (Carey, NC). Descriptive statistics were expressed as mean, median and standard deviation. Independent group comparisons for normally distributed data were compared with parametric methods and for nonnormally distributed data with nonparametric methods, as appropriate. Paired comparisons were performed to compare the quantity of biomarker detected on vaginal swabs obtained at home and in the clinic, from the same woman. Repeated-measures analysis was used to compare in clinic versus at home sampling among participants enrolled in the substudy. Statistical significance was detected at a p value of $<.05$.

3. Results

We screened 71 women and enrolled 33 couples (Table 1). Two women from the EVMS site withdrew