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Prostate-specific antigen concentration in vaginal fluid after exposure to semen☆

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Abstract

Objective—Prostate-specific antigen (PSA) is the best established biomarker of semen exposure. PSA in vaginal fluid returns to pre-exposure concentrations within 24–48 h, but the speed of decay during the first 10 h is unknown. We sought to determine how fast PSA concentrations decline during the first 10 h after exposure to semen.

Study design—Women in the 50 enrolled couples were intravaginally inoculated with 10, 20, 100 and 200 μl of their partner's semen and then collected vaginal swabs immediately after, 30 min, 4 h and 10 h after exposure. Forty-seven sets of samples were tested for PSA. Mixed linear models for repeated measures examined the association between log-transformed PSA values and sampling time and semen exposure volume. Sensitivity analyses excluded data from nonabstainers. Fixed-effect estimates from the statistical models were graphed.

Results—PSA values were highest at 200 μl inoculation volumes and at earlier post-exposure time points, then decline steadily. The lowest inoculation volume (10 μl) corresponded to the smallest concentration of PSA throughout the post-inoculation time points. Average PSA levels return to clinically non-detectable levels within 10 h only at the lowest semen exposures. The PSA decay curve assumes a very similar profile across all time points and semen amounts.

Conclusions—The PSA decay curve is similar for varying semen exposure volumes, with average PSA concentrations remaining above clinical thresholds 10 h after exposure at all except the very smallest semen exposure levels. PSA is an objective marker of recent exposure to semen, permitting such detection with high accuracy.

Implications—This study clarifies how PSA values vary at different semen exposure levels and time points during the first 10 h post-exposure. Future contraceptive studies that use PSA as a

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semen biomarker will be better informed about PSA concentrations at different sampling times and exposure amounts.

1. Introduction

Biomarkers of semen exposure have been used in condom effectiveness [1–5], female condom [6], and microbicide trials [7], as well as other women's health research [8–13]. Most studies have used prostate-specific antigen (PSA), a robust biomarker for determining the presence of semen in vaginal fluid. PSA is secreted by the prostate, found in large amounts (0.2–5 mg/mL) in seminal fluid, and detectable in the vagina immediately post-exposure before returning to background levels within 24–48 h [1,2,14,15]. Little is known, however, about the shape of the PSA decay curve [16] during the first 10 h after semen exposure.

The primary objective of this study was to measure PSA in vaginal fluid collected immediately, 30 min, 4 h and 10 h after exposure to different amounts of semen (10, 20, 100 and 200 μl). The largest exposure evaluated in this study is about one-tenth the volume of a typical ejaculate [17]. The study therefore targeted very small volume semen exposures.

2. Materials and methods

2.1. Study population

Couples were recruited from two urban reproductive health clinics. Participants were initially pre-screened through a brief telephone interview. Inclusion criteria included: ≥19 years of age, in a mutually monogamous, heterosexual relationship for ≥1 year, no known history of STIs in the previous year, using effective non-barrier contraception or intending to conceive, willing to abstain from intercourse for 3 days before and for 10 h after each study visit and not enrolled in a full-time undergraduate program. Men also had to have intact prostate and seminal vesicles.

Of 54 couples who completed the screening interview, 50 were enrolled in the study, with samples returned at all four study time points for 39 couples (Fig. 1). During the enrollment visit, interested and eligible couples completed informed consent procedures and were provided educational materials regarding contraception and HIV/STI prevention. The clinic nurse also instructed the women how and when to take the vaginal swabs, how to properly package them, when and where to return them.

2.2. Study design

Women were asked to participate in four clinic visits for in-clinic insemination with their partner's semen under carefully controlled conditions. They were asked to abstain from any vaginal product use for 72 h prior to each visit. The four clinic visits could be scheduled any day of the week based on the availability of the participant and the clinic nurse practitioner. Women were not scheduled for visits during menstruation or for 3 days after their last clinic visit. During the study visits women took vaginal fluid samples before insemination and immediately after exposure to their partner’s semen. They also self-sampled at the clinic at 30 min and in their homes 4 and 10 h post-exposure. The intravaginal exposures were with

one of four measured, increasingly larger amounts of her partner's semen: 10 μl at clinic visit 1, 20 μl at visit 2, 100 μl at visit 3, and 200 μl at visit 4.

Men enrolled in the study were asked to produce a semen sample. For subsequent clinic visits where the woman was exposed to her partner's semen, they could either return or their partners could bring a semen sample to the clinic if it was obtained within 1 hour of the scheduled visit. To further preempt protocol nonadherence, women were asked if they had engaged in unprotected vaginal intercourse during the 72 h before each clinic visit, so that no semen remained in the vagina from previous acts of unprotected intercourse.

Each woman was trained to use a vaginal self-sampling device to take swabs before and after being intravaginally exposed to her partner's semen. Two swabs were taken at each time point for later PSA evaluation. They were then placed in a resealable bag with two packets of desiccant. The dried swabs were temporarily stored at the clinic sites before shipment to the laboratory where they were eluted and assayed for PSA. Participants were reimbursed for research participation at each visit. The research protocol was reviewed and approved by the institutional review boards (IRBs) of the authors' institutions.

2.3. Laboratory methods

Swabs were stored at room temperature until processing. The 1 ml capacity rayon swabs were re-hydrated in 3 mL of phosphate-buffered saline (PBS) for 20 min and agitated by vortex. The swabs were then removed and disposed of, with the eluent frozen for later PSA testing. Frozen specimens were stored at −80 °C until testing, at which point they were left at room temperature until visibly thawed (approximately 10 min). A technician vortexed the thawed specimens (eluents in cryotubes) for 3–5 s and pipetted 200 μl of the specimen into a sample cup to test with the Abbott Architect Total PSA assay (Abbott Diagnostics, Abbott Park, IL, USA) [18]. Samples exceeding the upper limit of assay quantification (100 ng of PSA per mL) were diluted with PBS and re-tested to quantify the concentrations. The lower limit of detection for the assay is 0 ng/ml. All the samples of neat semen tested PSA positive and yielded concentrations above 100 ng/ml (the upper level of detection).

2.4. Statistical analysis

Descriptive statistics were expressed as mean and standard deviations for continuous variables and as frequency counts and percentages for categorical variables selected from the initial contact interview and enrollment forms. The primary outcome was detection of PSA in self-collected vaginal swabs, previously validated as reliable for PSA measurement [14,19]. PSA results were evaluated for any outliers. We generally selected first swab values which were systematically higher in general because with such low semen exposure levels as investigated, some of the exposure would be lost for the second swab. However, swab 2 results were substituted where swab 1 values seemed unreasonably low and un-evaluable. Examples of unevaluable swab sets include those taken at the same semen exposure cycle where the first swab was negative and second swab was positive (N=2), or if the first swab was low positive and the second swab was high positive (N=8). PSA results were evaluated for evidence of not following the study protocol (nonabstainers) by identifying PSA-positive results (>1 ng/mL) prior to exposure (N=5), an accepted standard for PSA measurement

indicating positive semen exposure [2]. Descriptive statistics for the outcome variable include means and standard deviations of PSA measured in ng/ml for each time point post-exposure, for each semen exposure volume with and without adjustment for deletion of non-adherers. To further evaluate the proportion of PSA positive results, the percent of vaginal fluid specimens positive for PSA for >1 and >5 ng/ml were calculated according to semen exposure amount and time since exposure.

The natural logarithm of the PSA value in ng/ml was used as the dependent variable in mixed linear models for repeated measures (SAS version 9.4 procedure MIXED), which indicated the association of PSA level and sampling time for each semen exposure volume. We added 0.001 ng/ml to each PSA value to avoid natural log transformations of zero values. We modeled the natural log-PSA as a continuous outcome with random subject and semen exposure volume effects and with repeated effects for subject and semen exposure volumes with an autoregressive covariance structure. The residual (restricted) maximum likelihood (REML) estimation method was used for covariance parameters. Sampling time values were the number of hours since semen exposure.

Alternative models evaluated the independent variable sampling time as a categorical variable, a continuous variable, and as a continuous variable with a quadratic term. We tested using F-tests for fixed effects for the addition of an interaction term for sampling time with the semen exposure volume and also for the sampling time quadratic term with the semen exposure volume. F-tests for fixed effects and fit statistics (Akaike's Information Criterion, AIC, and Bayesian Information Criterion, BIC) were compared across models. In the set of tested alternative models, AIC and BIC were concordant in identifying the best model. We fitted models including all semen exposure volumes for all participants, and then again with nonabstainers for a particular semen exposure visit removed for sensitivity analyses.

2.5. Graphical analysis

Graphs display the individual PSA results and the mean values estimated using mixed effects models (Figs. 2–3) for each semen exposure level (10, 20, 100, and 200 μl) during the 0–10 h timeframe (immediately after exposure, 30 min, 4 h, and 10 h) (SAS version 9.4 procedure SGPLOT). Each plot consists of a scatter plot of the natural log-transformed PSA values for individual samples and a fitted line curve for the mean values estimated using the mixed linear models, compared with a penalized basis smoothed cubic spline curve (with 4 equally spaced knots) based on the individual PSA values. The cubic spline curves display a more flexible fit to the data than can be obtained with the regression model, and provide a visual check on the goodness of fit of the regression models.

3. Results

3.1. Participant characteristics

Of the 50 couples enrolled at baseline, 48 provided essential questionnaire data, and 47 gave full sets of specimens; at visit 4, there were 37 sets of specimens (Fig. 1). Ten couples were lost to follow-up. At each clinic visit, we examined to see if there were any nonabstainers (PSA positive prior to exposure). Although no participants reported protocol non-adherence,

five couples were considered nonabstainers for 1 of their 4 semen exposure cycles, and 1 couple was considered a nonabstainer for 2 of 4 semen exposure cycles. Overall, there were 668 usable specimens in the analyses, of which 640 were included after exclusion of nonabstainers.

Female participants were on average 33 years and males 36 years old. Most participants reported their race as white (>70%), their marital status as married/cohabitating (83%), and used some form of contraception or were no longer at risk of pregnancy (short-acting hormonal, IUD/Implant, or hysterectomy/menopause) (81%). Ten percent of women and 23% of men reported smoking, and about half of men and women had 2+ alcoholic drinks/week (Table 1).

3.2. Mean PSA values at different sampling time points after semen exposure

For samples taken immediately after exposure at clinic visit 1 (the lowest semen exposure volume, 10 μl), the mean PSA concentration (ng/ml) was 290.4 for all couples and 294.1 when nonabstainers were removed (Table 2). By 30 min, PSA had decayed to 49.5 for all couples and 48.8 when nonabstainers were removed. At 10 h, the mean PSA was 0.6 for both groups, considered clinically in the undetectable range (less than 1 ng/ml). After the fourth clinic inoculation (exposure to the largest volume of semen, 200 μl), immediately after exposure the mean PSA was 4754.5 for all couples and 5010.2 when nonabstainers were removed. By 30 min it had decayed to 541.6 for all couples, and 563.8 when nonabstainers were removed. By 10 h, the mean PSA was 5.4 and 5.0, respectively, for both groups.

3.3. Proportion of vaginal fluid specimens tested positive for PSA

The proportion of specimens that were PSA positive at either >1 ng/ml or >5 ng/ml varied by exposure volume and time since exposure (Table 3). Depending on the threshold used to define PSA-positive results and whether nonabstainers data were excluded from the analysis, the proportion with positive PSA immediately after exposure ranged 48–49%after exposure to 10 μl, 54–67% after 20 μl, 74–80% after 100 μl, and 82–84% after 200 μl. The larger the exposure, the greater the proportion of PSA-positive samples detected immediately after exposure. Fewer samples were PSA positive at 10 h post exposure, but this also was a function of the exposure volume. Exposure to 10 μl yielded only 4–7% PSA-positive samples at 10 h post exposure. This proportion was only moderately increased for exposure to larger semen volumes.

3.4. Mixed linear effects model selection

Simultaneous modeling of the effect of semen volume and time since exposure showed that both factors were significant predictors of log-transformed PSA concentrations: F-tests for fixed effects yielded $p<.05$ for sampling time, semen exposure volume and their interaction when modeling sampling time as a categorical or as a continuous variable. In models evaluating time since exposure as a continuous variable, we tested departure from a simple log-linear relation with PSA levels by adding polynomial functions of sampling time. In these models, a quadratic term for sampling time was statistically significant (F-test: $p<.0001$), suggesting that the PSA decay is faster than predicted by a simple exponential curve

shortly after exposure, and tapers off thereafter. There was no significant interaction of the quadratic term with semen exposure volume (F-test: p=.89), indicating that this feature of the decay curve could be independent from the initial semen exposure volume. Model fit statistics indicated that the model fit the data well, although the data were fit best by a fully categorical model with terms for each exposure volume and specimen collection time (results not shown). This same model was applied to all 37 couples who completed all four clinic study visits and negligible changes in parameter estimates were observed. Thus, we conclude that those who were lost to follow-up were not different in any significant way from couples who completed the study (results not shown).

3.5. Graphical analysis of the 10 h decay curves

Natural log-transformed PSA values and a penalized basis smoothed cubic spline through these points, as well as fitted lines for the fixed effects parameter estimates from the model (described above) are presented as graphs for each semen exposure volume (Fig. 2, graphs 1–4). Graph 1 illustrates the situation with exposure to 10 μl for the 0–10 h timeframe (immediate, 30 min, 4 h, and 10 h), with graphs 2–4 showing the 20, 100, and 200 μl exposure volumes, respectively. Fig. 3 (graphs 5–8) follows the same characterization but removed nonabstainers as a sensitivity analysis.

The PSA decay curve demonstrates similar profiles across all time points and semen exposure volumes. PSA values were highest immediately post-exposure, declined sharply in the first 30 min thereafter and more slowly subsequently. PSA returns to clinically non-detectable levels within 10 h only at the lowest semen exposures (10 μl exposure volume). Although the lowest volumes have similar trajectories to higher volumes, the starting and ending points of the slopes are dependent upon semen exposure volumes; when the semen exposure volumes are lower (10 and 20 μl), the model-fitted lines are closer to the smoothed lines.

The scatter plots yielded by the sensitivity analyses show less variation at each time point after exclusion of nonabstainers (Fig. 3). However, the slopes and the distances between the fitted and smoothed lines are very similar to Fig. 2.

4. Discussion

This study provides a detailed longitudinal assessment of PSA in vaginal fluid during the first 10 h after exposure to measured volumes of semen. Earlier work evaluated PSA levels immediately after exposure and 1 h later, with subsequent samples collected at 24 h and 48 h [2]; more recent studies showed that PSA values tend to decrease sharply during the first 24 h [15,16]. We used mixed models to assess how mean PSA levels vary by intensity (volume) of semen exposure and by time since exposure, and estimated the fixed effects of exposure and time since exposure while controlling for within-subject correlation of measurements and potential between-subject variation in the signal decay rate.

PSA values were highest at the largest (200 μl) exposure and at earlier post-exposure time points. The lowest exposure volume (10 μl) showed the smallest PSA concentration throughout and cleared rapidly. Overall, the PSA decay curve during the first 10 h has a very

similar profile across the range of semen exposure volumes, with a high rate of decline in the first hour and a slower decline subsequently. This confirms that PSA is a sensitive indicator of exposure to semen if samples are collected shortly after exposure, and can detect low-level exposures at a semen exposure level that may result from a condom leak, breakage, slippage or incorrect use (in this study, as little as 20 μl). The sensitivity of the sampling system, however, is clearly a function of the semen exposure, and does not guarantee that all exposures to small volumes of semen are detectable even if vaginal fluid is sampled immediately after exposure: in this study, only about 50% of samples taken immediately after exposure to 10–20 μl were PSA-positive, whereas sensitivity was high (80% or more) immediately after exposure to 100–200 μl. Further, these features confirm the validity of the sampling and assay system adopted in studies of condom functionality [3,6,20], based on comparing PSA measurements in samples obtained before and after protected intercourse.

Clearly, sensitivity of PSA is high for exposure to larger semen volumes, as after unprotected intercourse or a complete condom failure, when it may be high even after 10–12 h. Previous studies using 1 ml of semen as a proxy for exposure to an ejaculate yielded low rates of PSA detection at 24 h [2,15], but a recent study using exposure to 2 ml of semen or to unprotected intercourse showed PSA detectable in up to 70% of the samples collected at 24 h [16]. Collectively, these findings confirm that PSA is a sensitive marker of semen exposure: PSA detection is compatible with semen exposure due to unprotected intercourse or complete condom failure within the previous 12–24 h, or with lower semen exposure levels compatible with functional condom failure within the previous few hours.

5. Strengths and limitations

Our analysis has several limitations. First, characterization of the decay curve would benefit from testing with a larger number of participants to examine other time points and reduce the uncertainty margins for those considered. There was large variability between subjects in the PSA value obtained immediately after exposure and in subsequent measurements, although most individual trajectories followed the pattern described by the regression models. However, our sample size was not small compared to other vaginal exposure studies and proved sufficient to clearly assess the average trajectory of PSA values. Second, our study was restricted to the relatively short timeframe of the first 10 h post-exposure to semen. However, this is the critical window for which additional data on mean PSA values were most needed. More sampling times could be chosen [16], but it would be difficult to ensure the full cooperation of couples to accommodate further sampling time points because of the likely high inconvenience and non-adherence.

A potential source of bias is the storage of dry swabs at room temperature. The PSA measurements in this and other research that has not provided for immediate freezing of the samples [21] may underestimate the actual PSA concentration in the original samples, and the proportion of samples positive for PSA is likely to underestimate the sensitivity of PSA as a marker of semen exposure. However, the direction of the bias is such that our interpretation of the findings and overall conclusions are unlikely to change.

Our analysis adds to the limited evidence base on the shape of the PSA decay curve over time and in relation to semen volume. This is important because objective markers of semen exposure can effectively complement and correct possible bias in self-report, as women's (and men's) self-report of condom use (and sexual behaviors) is prone to bias [22,23]. Another strength involved the careful nature of the study design and its execution, navigating numerous patient recruitment difficulties stemming from the added burden of scheduling couples (instead of individuals) for clinic visits; maintaining a taxing adherence regimen; the demanding nature of requesting participants to provide swabs in-clinic or at home, and use of a quantitative laboratory assay confirmed recently as the most effective for such work [13].

6. Conclusion

Our findings add evidence that PSA is a sensitive marker of recent exposure to semen and document that PSA concentrations decline rapidly during the first few hours, with a higher proportion of samples yielding PSA-positive results after larger semen exposure volumes. PSA can be detected in vaginal samples taken within a few hours after exposure levels compatible with functional failure of a condom, and up to 10 h after exposure levels compatible with unprotected intercourse or complete condom failure.

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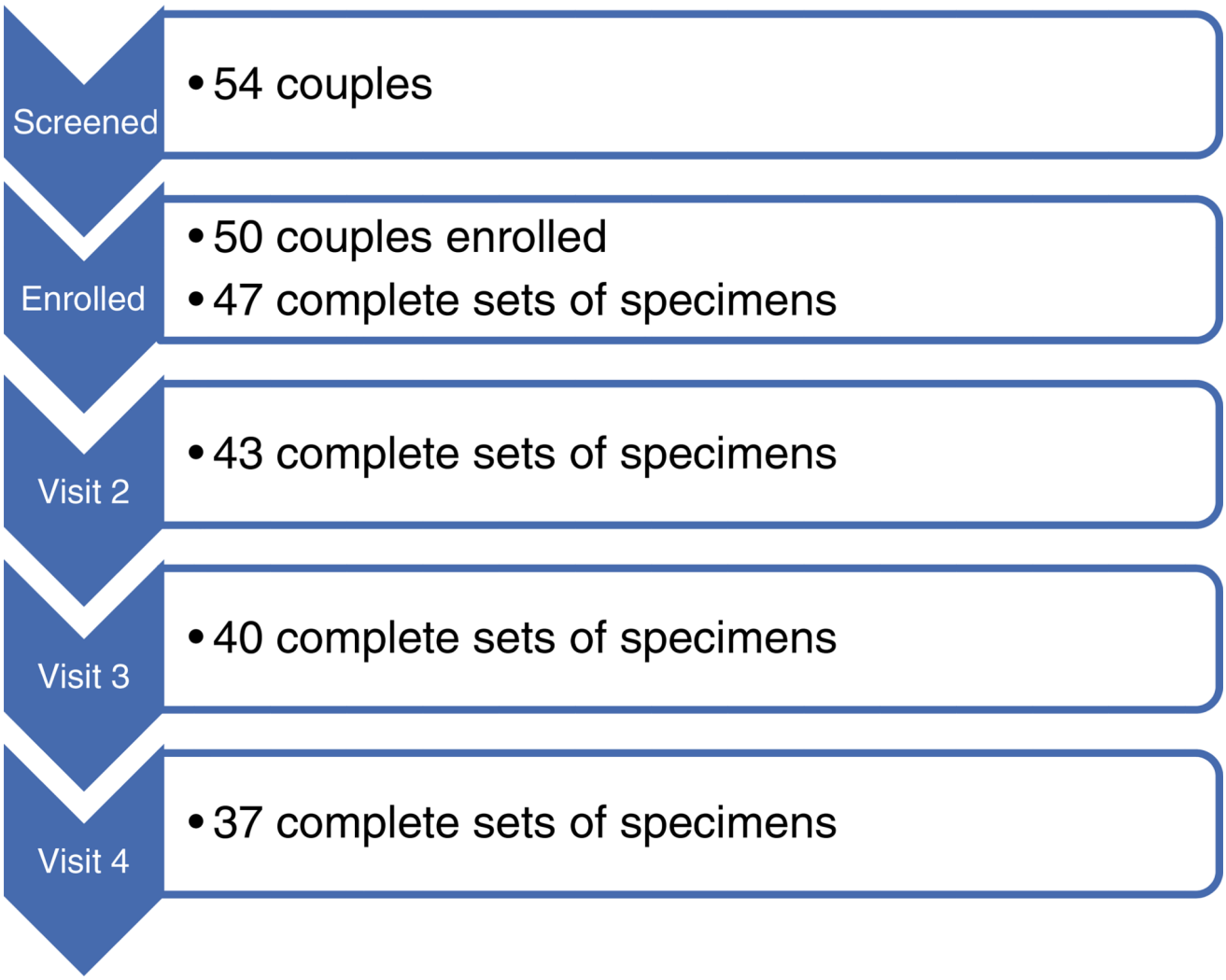

Fig. 1.
Study screening, enrollment and continuation experiences of participants, with numbers of evaluable specimens at each clinic visit.

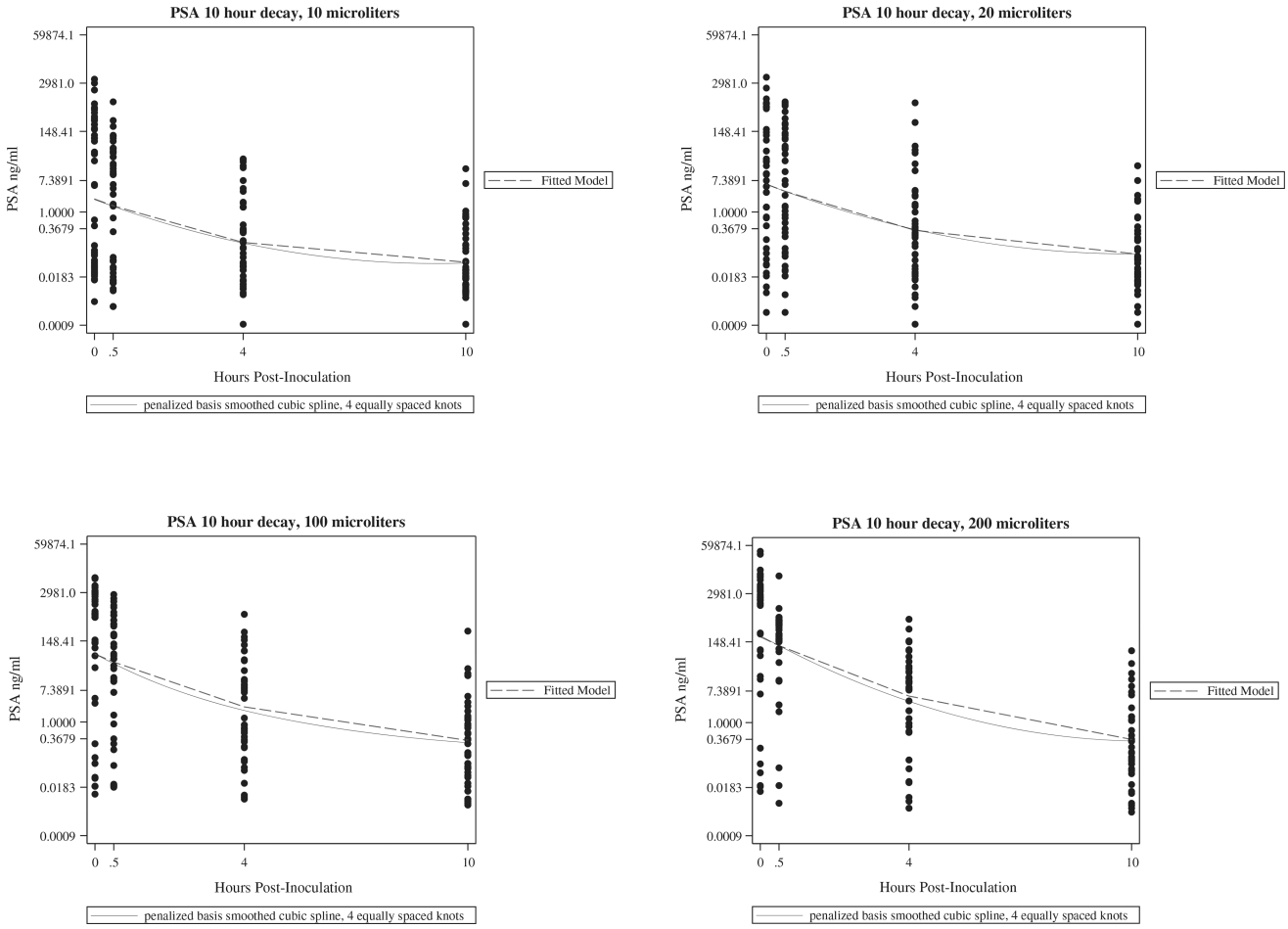

Fig. 2.

PSA decay by time since exposure (0–10 h) and exposure volume (10–200 μl).[1]

[1]Graphs show scatter plots of the natural log transformed PSA actual data and a penalized basis smoothed cubic spline curve with 4 equally spaced knots for this data. Results of the mixed linear models were used to derive a fitted line curve for the fixed effects parameter estimates.

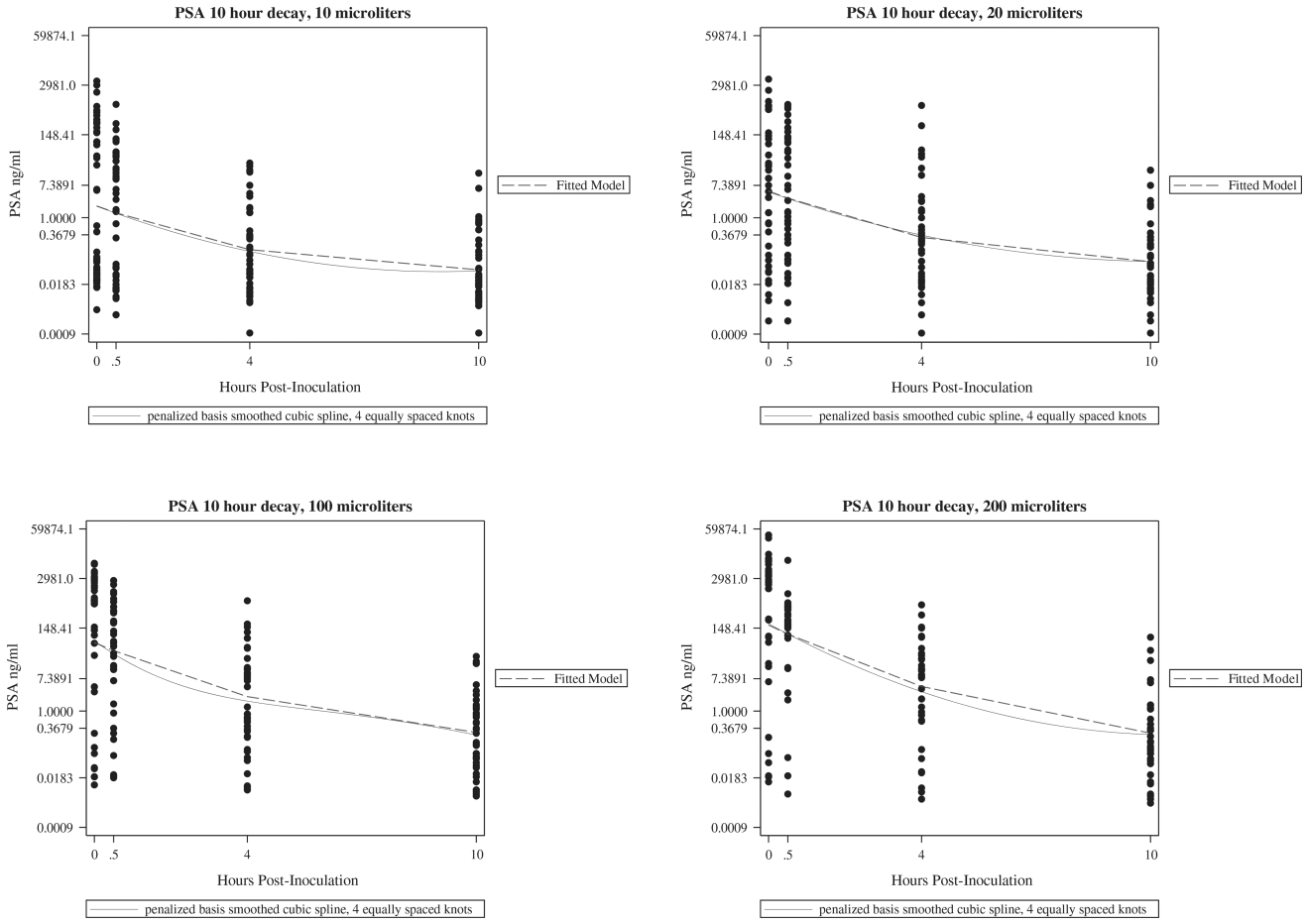

Fig. 3.

Sensitivity analysis (excluding non-compliant cycles) PSA decay by time since exposure (0–10 h) and exposure volume (10–200 μl).[1]

[1]Graphs show scatter plots of the natural log-transformed PSA actual data and a penalized basis smoothed cubic spline curve with 4 equally spaced knots for this data. Results of the mixed linear models were used to derive a fitted line curve for the fixed effects parameter estimates.

Table 1

Demographic, contraceptive and behavioral characteristics of study participants (N=48 couples)

	Females ***n*** **(%)**	**Males** ***n*** **(%)**
Age (years), mean (SD)	33.1 (7.9)	36.3 (8.6)
Race		
Black/African American	10 (21)	11 (23)
White	36 (75)	34 (71)
Asian/Other	2 (4)	3 (6)
Education (years), mean (SD)	16.4 (2.8)	16.5 (3.4)
Marital status		
Single	8 (17)	7 (15)
Married/living with partner	40 (83)	40 (83)
Divorced/widowed	0	1 (2)
Years with partner (mean, SD)	6.7 (4.5)	7.3 (5.6)
Desires pregnancy	8 (17)	8 (17)
Contraceptive use at enrollment		
Not using	9 (18.8)	9 (18.8)
Hormonal methods[a]	21 (43.8)	21 (43.8)
IUD/implant	3 (6.3)	3 (6.3)
Permanent methods/not fecund[b]	15 (31.3)	15 (31.3)
Current cigarette smoker	5 (10)	11 (23)
Alcohol consumption (drinks per week)		
0	18 (37.5)	16 (33.3)
1	8 (16.7)	5 (10.4)
2+	22 (45.8)	27 (56.3)

N=48 with complete questionnaire data and 1 couple did not return specimens pertaining to the first clinic study visit, but completed the study protocol thereafter.
Percentages may not add up to 100 due to rounding.

[a]Hormonal methods included oral contraceptive pills, contraceptive patch, vaginal ring, and injectables.

[b]Permanent methods included tubal ligation and vasectomy; not fecund include one woman who had a hysterectomy and two menopausal women.

Table 2

Semen inoculation volumes and mean PSA values for vaginal swab time points (adjusted for nonabstainers)

		Mean (SD) ALL*	95% CL		Mean (SD) adjusted**	95% CL	
Volume	**Time of swab**	**ng/ml**			**ng/ml**		
10 µl (N=47)	immediate	290.4 (737.4)	73.8	506.9	294.10 (745)	72.8	515.4
	30 min	49.5 (147.5)	6.2	92.8	48.79 (149)	4.5	93.1
	4 h	2.9 (7.0)	0.9	5.0	2.43 (6)	0.6	4.2
	10 h	0.6 (2.3)	−0.1	1.2	0.58 (2)	−0.1	1.3
20 µl (N=43)	immediate	301.3 (761.5)	67.0	535.7	300.96 (777)	55.6	546.4
	30 min	113.2 (223.9)	44.3	182.2	111.48 (228)	39.4	183.5
	4 h	31.8 (141.9)	−11.8	75.5	33.30 (145)	−12.5	79.1
	10 h	1.3 (4.0)	0.1	2.5	1.33 (4)	0.03	2.6
100 µl (N=40)	immediate	1517.5 (2093.7)	847.9	2187.1	1552.85 (2109)	869.2	2236.5
	30 min	374.4 (622.4)	175.4	573.5	340.07 (591)	148.5	531.6
	4 h	47.6 (132.4)	5.2	90.0	42.04 (129)	0.1	84.0
	10 h	9.5 (43.3)	−4.4	23.4	2.71 (6)	0.6	4.8
200 µl (N=37)	immediate	4754.5 (8641.6)	1873.3	7635.8	5010.19 (8979)	1877.1	8143.2
	30 min	541.6 (1454.5)	56.7	1026.6	563.78 (1516)	34.8	1092.8
	4 h	48.4 (116.0)	9.8	87.1	50.05 (121)	7.9	92.2
	10 h	5.4 (15.8)	0.1	10.6	5.04 (16)	−0.7	10.7

Values of N refer to couples with complete specimens at all time points.

*Two swabs were taken at each time point. Substitutions were made with swab 2 from the same time point if the first swab was unevaluable.

**In addition to the swab 2 substitutions, results were removed for nonabstainers.

Table 3

Proportion (%) of vaginal fluid specimens positive for PSA (>1 and >5 ng/ml) according to amount of semen inoculation and time since exposure

Volume	Time of Swab	PSA + (ALL)*		PSA + (Adjusted)**	
		% >1 ng/ml	% >5 ng/ml	% >1 ng/ml	% >5 ng/ml
10 µl (N=47)	immediate	49	49	48	48
	30 min	55	47	54	46
	4 h	28	13	26	11
	10 h	6	4	7	4
20 µl (N=43)	immediate	67	56	66	54
	30 min	60	51	59	49
	4 h	35	16	34	17
	10 h	14	7	15	7
100 µl (N=40)	immediate	80	75	79	74
	30 min	77	75	77	74
	4 h	52	47	51	46
	10 h	30	15	28	13
200 µl (N=37)	immediate	84	84	82	82
	30 min	92	86	91	85
	4 h	70	59	68	56
	10 h	38	19	32	15

Values of N refer to couples with complete specimens at all time points.

*Two swabs were taken at each time point. Substitutions were made with swab 2 from the same time point if the 1st swab was un-evaluable.

**In addition to the swab 2 substitutions, results were removed for nonabstainers.