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Vaginal Fluid Zinc Concentration as a Marker for Intercourse

REFERENCE: Rogers, C., Bernstein, G., Nakamura, R., Endahl, G., and Bhoopat, T., **"Vaginal Fluid Zinc Concentration as a Marker for Intercourse,"** *Journal of Forensic Sciences,* JFSCA, Vol. 33, No. 1, Jan. 1988, pp. 77-83.

ABSTRACT: Zinc is present in high concentration in semen, but in low concentration in vaginal fluid. We evaluated vaginal zinc levels as a marker for intercourse by measuring precoital (>11 h after intercourse) or postcoital (<5 h after intercourse) zinc and acid phosphatase levels in 26 specimens of vaginal fluid from 18 women.

The approximate 95% reference range for zinc in precoital vaginal fluid was 1.2 to 15 μg/mL (mean 4.5), and in postcoital vaginal fluid 4.0 to 135 μg/mL (mean 24). There is an overlap between the precoital and postcoital reference ranges. Provided that the vaginal fluid zinc level is less than approximately 4.0 μg/mL or greater than approximately 15 μg/mL, vaginal fluid zinc concentration may be useful as an indicator of intercourse.

KEYWORDS: pathology and biology, coitus, zinc, phosphatases

For the past 40 years, detection of spermatozoa and elevated acid phosphatase activity in vaginal fluid have provided important physical evidence in cases of sexual assault. Spermatozoa in the vaginal fluid are absolutely diagnostic of recent intercourse. Interference may arise, however, from spermatozoa remaining in the vagina from previous intercourse. The maximum time that spermatozoa can remain in the vagina after intercourse is debatable [*1,2*]. Conversely, false negative results may occur when vaginal cells or organisms obscure the spermatozoa or when mechanical factors such as exercise or douching remove spermatozoa from the vagina. In addition, rapists reportedly have a high incidence of oligospermia and azoospermia [*3*]. In clinical trials conducted on women presenting because of rape, spermatozoa were detectable in 52 to 93% of cases [*4-6*].

Measurement of vaginal fluid acid phosphatase activity may be valuable in cases where spermatozoa are not detected. The level of acid phosphatase in semen is 500 to 1000 times higher than in other body fluids [*7*]. The endogenous level of acid phosphatase in the vagina

Presented in part at the 39th Annual Meeting of the American Academy of Forensic Sciences, San Diego, CA, 16-21 Feb. 1987. Received for publication 13 March 1987; revised manuscript received 7 May 1987; accepted for publication 8 May 1987.

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is not zero, however, and one must set an arbitrary threshold between "negative" and "positive" acid phosphatase activities. False negative results may be due to loss of the enzymatic activity of acid phosphatase, which may occur to a significant extent within 24 h at 25°C [*8*]. In a clinical trial, determination of vaginal fluid acid phosphatase activity identified semen in only 1.4% more cases than a search for spermatozoa [*5*].

Several other techniques, such as detection of the seminal protein p30, have been suggested as methods of identifying semen. Experience with these techniques has not been as extensive as with detection of spermatozoa and measurement of acid phosphatase.

Human semen contains approximately 100 times the concentration of zinc present in other body fluids [*9*]. To determine whether the zinc concentration of vaginal fluid is useful as an indicator of intercourse, we compared zinc concentration and acid phosphatase activities in pre- and post-coital vaginal fluid specimens from 18 women.

Materials and Methods

The experimental subjects were healthy premenopausal women presenting for diagnostic postcoital testing at Los Angeles County–University of Southern California Medical Center. One specimen of vaginal fluid was obtained after at least 11 h of abstention from intercourse. With this limited number of specimens, there was no obvious relationship between the period of abstention and the vaginal zinc level. A second specimen was obtained at the time of postcoital examination, 1.0 to 4.8 h after intercourse. In eight cases, it was possible to obtain both pre- and post-coital specimens from the same subject ("paired specimens"). In seven cases, only a precoital specimen was obtained, and in three cases, only a postcoital specimen ("unpaired specimens").

Pelvic examinations were performed using stainless steel vaginal specula, which do not contain measurable quantities of zinc as shown by washing the specula in 2.0*N* nitric acid and assaying the wash fluid. Specimens were collected on cotton swabs[4] from the posterior fornix or from any obvious pool of semen, and were stored in screw-cap glass vials[5] at −20°C. These glass vials were not contaminated with appreciable amounts of zinc. The swabs contained nearly constant amounts of zinc. The zinc assay was standardized so that this zinc content was included in the blanks (see below). The period of storage ranged from 2 to 29 days. Previous work has shown acid phosphatase to be stable for up to 4 months when frozen [*10*]. We have confirmed this observation with control sera.

At the time of assay, the specimens were weighed by difference, and results were later corrected for the weight of the specimens. Two millilitres of water were added to each tube, and the tubes were shaken on a mechanical platform shaker at 160 oscillations per minute for 30 min. The fluid was decanted and centrifuged at 1600 × *g* for 20 min at 4°C. The supernatant was frozen at −20°C for use in the zinc and acid phosphatase assays.

Zinc levels were measured on a Perkin-Elmer Model 2380 atomic absorption spectrophotometer, using a wavelength of 214 nm. Standard curves were constructed by adding known concentrations of standard zinc solutions[6] to the glass tubes with cotton swabs, and shaking and centrifuging as described above. Of 26 specimens examined in this way, 7 gave results below the linear range of the instrument, 0.1 to 1.0 μg/mL. Six of these seven specimens were precoital. These results were excluded from the analysis.

The total acid phosphatase activity was determined by an established procedure using a *p*-nitrophenol substrate [*11*]. Enzyme activities are reported in International Units (IU) per litre of vaginal fluid, where one unit is defined as the quantity of enzyme that hydrolyzes one micromole of substrate per minute at 37°C. Specimens were diluted to fall within the linear

[4]Solon Company, Solon, ME.
[5]American Scientific Products, Irvine, CA.
[6]Sigma Chemical Company, St. Louis, MO.

range of the assay, 1.7 to 46.8 IU/L. No values were below the lower limit of detection of the acid phosphatase assay.

For precoital specimens, the sediment remaining after centrifugation was spread on glass slides and air-dried. Following brief fixation in methanol, slides were stained with hematoxylin and eosin and then examined for spermatozoa. No precoital specimens contained spermatozoa.

Precoital and postcoital distributions of measurements were compared by the Wilcoxon signed rank test for paired specimens and by a one-tailed Mann-Whitney U-test for unpaired specimens. Each measurement was assigned a rankit value, and the logarithms of the measurements were plotted against the rankit values to estimate the ranges containing 95% of precoital or postcoital values.

The precision of the vaginal fluid assays could not be determined directly because of the difficulty in reproducibly obtaining multiple specimens of vaginal fluid. However, precision of the assays was estimated in several ways. Using aqueous zinc standards or serum acid phosphatase standards, the within-run coefficients of variation were 2.3% for the zinc assay and 3.4% for the acid phosphatase assay. The respective between-run coefficients of variation were 4.2 and 10.7%. Using swabs without any added zinc, there was a 5.9% coefficient of variation in the zinc content of the swabs. Using the aqueous zinc standard absorbed on swabs and then re-eluted, the within-run coefficient of variation was 8.3%. Finally, using a specimen of semen absorbed on swabs and then re-eluted, the within-run coefficients of variation were 42% for zinc and 41% for acid phosphatase. Average recovery for the assay using standard zinc solution added to semen was 93%.

This study was conducted under the supervision of the Research Committee, Los Angeles County Hospital.

Results

Table 1 gives the results of the zinc and acid phosphatase assays for paired specimens. For both tests, there was a significant difference between pre- and post-coital distributions of

TABLE 1—*Pre- and post-coital zinc and acid phosphatase (paired specimens).*

Subject	Sample	Postcoital Time, h	Zinc, μg/mL	Acid Phosphatase, IU/L
1	precoital		5.2	134
	postcoital	4.0	34.0	246 000
2	precoital		...[a]	200
	postcoital	4.8	9.0	5 340
3	precoital		2.6	1 590
	postcoital	2.3	13.0	29 900
4	precoital		...[a]	752
	postcoital	1.0	76.0	30 400
5	precoital		...[a]	50
	postcoital	1.8	31.0	203 000
6	precoital		3.2	100
	postcoital	2.3	32.0	23 400
7	precoital		6.8	117
	postcoital	2.0	58.0	34 900
8	precoital		4.6	301
	postcoital	3.0	6.8	4 140

[a] Below lower limit of detection of instrument.

measurements ($p < 0.01$). The analogous data for unpaired specimens are given in Table 2. Again, the precoital and postcoital results were significantly different for both zinc ($p < 0.05$) and acid phosphatase ($p < 0.01$).

Figure 1 shows the zinc values for all precoital specimens ($n = 9$) and all postcoital specimens ($n = 10$) plotted against their respective rankit values. The data approximately follow a log normal distribution. Seven specimens were excluded from this analysis because their zinc concentrations were below the lower limit of detection of the instrument. Plotting paired

TABLE 2—*Pre- and post-coital zinc and acid phosphatase (unpaired specimens).*

Subject	Zinc, μg/mL	Acid Phosphatase, IU/L
	PRECOITAL SPECIMENS	
9	...[a]	618
10	...[a]	67
11	1.9	200
12	11	217
13	6.3	167
14	2.8	451
15	...[a]	685
	POSTCOITAL SPECIMENS	
16	...[a]	13 600
17	16	70 900
18	21	81 300

[a]Below lower limit of detection of instrument.

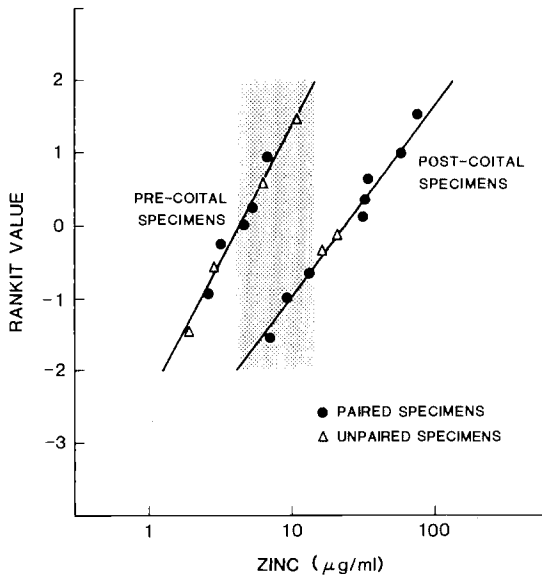

FIG. 1—*Distribution of vaginal fluid zinc levels for pre- and post-coital populations. Shaded area indicates overlap.*

and unpaired specimens on the same graph resulted in a single line, suggesting that there was no difference between the paired and unpaired specimen populations. With this relatively small number of specimens, there was no clear relationship between postcoital time and zinc level.

The 95% reference intervals, estimated by extrapolation, are 1.2 to 15 μg/mL (mean 4.5) for precoital specimens and 4.0 to 135 μg/mL (mean 24) for postcoital specimens. There is an overlap between the pre- and post-coital ranges from approximately 4.0 to 15 μg/mL, as shown by the shaded area in Fig. 1.

Figure 2 displays acid phosphatase activities for all precoital specimens (n = 15) and postcoital specimens (n = 11). The estimated 95% reference ranges are 35 to 1 800 IU/L (mean 250) for precoital specimens, and 2 000 to 500 000 IU/L (mean 32 000) for postcoital specimens. In contrast to Fig. 1, there is no overlap between the two ranges.

Discussion

As shown in Fig. 2, acid phosphatase activity appears to be useful in distinguishing between pre- and post-intercourse vaginal fluid specimens. The number of false negative tests in postcoital specimens should increase, however, in circumstances where high temperature or other unfavorable conditions diminish the enzymatic activity of acid phosphatase.

The major advantage of using vaginal fluid zinc levels as an indicator of intercourse is that zinc is not subject to the environmental factors which degrade organic substances. However, if zinc levels are to be used for this purpose, caution is necessary in interpreting results.

First, 1 of 11 postcoital zinc specimens in this study was excluded because the level was below the instrument range. This may be an artifact associated with the method of specimen processing used in this study. An alternative explanation is that some semen has very low zinc levels; in a study of seminal plasma from 809 normal men, the zinc concentration ranged from 1 to 621 μg/mL with a median value of 133 μg/mL [*12*]. Other studies have reported high mean seminal zinc concentrations in normal men (mean 195 to 197 μg/mL), with lower concentrations in oligospermic (mean 132 to 140 μg/mL) or azoospermic men (mean 85 μg/mL) [*13,14*]. Semen with very low levels of zinc is probably undetectable by

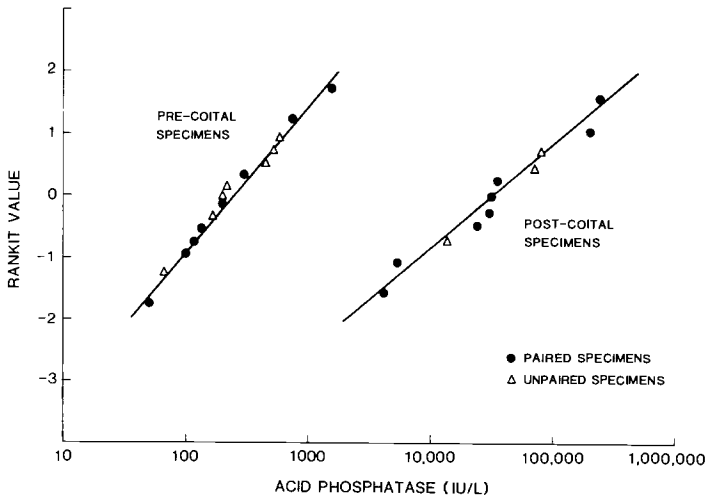

FIG. 2—*Distribution of vaginal fluid acid phosphatase activities for pre- and post-coital populations.*

atomic absorption spectrophotometry no matter how specimens are processed. Thus, some false negative results would be expected.

In addition, there is a range of zinc concentrations where it is not possible to distinguish precoital from postcoital specimens. The 95% reference ranges determined in this study cannot be regarded as definitive, because they are based on a relatively small number of specimens. However, there does appear to be an overlap between precoital and postcoital ranges of vaginal zinc at approximately 4 to 15 μg/mL. Thus, only values outside this range are clinically useful.

Finally, the substances which may interfere with the zinc method are not as well defined as for acid phosphatase. As shown in this study, there does not appear to be an appreciable quantity of zinc in metal vaginal specula. Suzuki et al. [*15*] have also shown, at least by qualitative analysis, that zinc levels in various body fluids and vegetable or fruit extracts are lower than in semen. Nevertheless, many substances contain zinc, and if specimens should become contaminated, false positive tests may result.

Conclusion

This study has shown that, when interpreted with due caution, vaginal fluid zinc levels may aid in detection of recent intercourse. In most cases of sexual assault, it is advantageous to use the standard laboratory tests on vaginal fluid. However, in certain exceptional cases, particularly those involving extremes of temperature, measurement of vaginal zinc levels may be a useful alternative.

Acknowledgment

The authors are grateful to Theophilus Boyd, M.S., M.T., and Virginia Bailey, M.T., for their technical assistance and to Thomas T. Noguchi, M.D., and David Endres, Ph.D., for suggestions and comments. One of the authors (T. B.) was supported by a fellowship from the King of Thailand.

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