TECHNICAL NOTE

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A Demonstration of Spermatozoa on Vaginal Swabs after Complete Destruction of the Vaginal Cell Deposits

REFERENCE: Iwasaki, M., Kubo, S., Ogata, M., and Nakasono, I., **"A Demonstration of Spermatozoa on Vaginal Swabs after Complete Destruction of the Vaginal Cell Deposits,"** *Journal of Forensic Sciences*, JFSCA, Vol. 34, No. 3, May 1989, pp. 659-664.

ABSTRACT: The proteolytic enzyme, proteinase K, has been found to destroy all vaginal cells though it does not have the same effect on spermatozoa. In cases of sexual offenses, in which a swab has been used to wipe out the vagina, the female cells and their nuclei on that swab may also contain the heads of spermatozoa. After as short a time as 30 min of proteinase K treatment, the spermatozoa that had separated from the enzymatically destroyed vaginal cells were recovered. This proteinase destruction furnishes some spermatozoa with deformed heads and a somewhat greater number of isolated tails though a sufficient number of spermatozoan heads still remain for a reliable diagnosis. For detection of spermatozoa from a vaginal swab after proteinase K pretreatment, the heads of the spermatozoa are distinctly stained by Oppitz's method. Further, on prior treatment with proteinase K, the ABO blood grouping of the spermatozoa could also be determined on the vaginal swab by using the absorption-elution technique. The resistance of the spermatozoa to proteinase K is the basis for this method.

KEYWORDS: criminalistics, spermatozoa, vaginal swabs, criminal sex offenses, proteinase K

The detection of spermatozoa in the vaginal contents is an important consideration in the medicolegal examination of sexual offenses, and a substantial number of research projects have been conducted to identify such spermatozoa. For the detection of this spermatozoa, the Baecchi's [*1*] and Oppitz's staining methods [*2*] have been used in our laboratory since these methods make it possible for the spermatozoa and other cellular elements of the vagina to be determined from the substances collected by a vaginal swab.

In 1985, Gill et al. [*3*] reported that the vaginal cells from semen-contaminated swabs were preferentially lysed by incubation using a proteinase K (EC 3.4.21.14) mixture that was used to determine deoxyribonucleic acid (DNA) fingerprints. In an attempt to identify the spermatozoa separated from the vaginal cells, we tried using this proteinase K pretreatment technique and found that though the vaginal cells were completely destroyed the heads of the

Received for publication 20 May 1988; revised manuscript received 29 July 1988; accepted for publication 1 Aug. 1988.

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spermatozoa were stained distinctly. In addition, on treatment with proteinase K, the ABO blood grouping of the sperm atozoa also could be detected on the vaginal swab by using the absorption-elution technique.

Consequently, our results suggest that proteinase K pretreatment is a useful method to identify the separated spermatozoa from other cellular elements and also to detect their ABO blood grouping.

Materials and Methods

Light Microscopic Examination of Spermatozoa

Semen was collected from healthy, young adults. Semen, which had been allowed to liquefy at room temperature, was diluted 1:100 with 0.01*M* phosphate-buffered saline (PBS), pH 7.2. Vaginal swabs were prepared from autopsied cases by placing vaginal cells onto cotton cloth. The vaginal swabs, which had been stored at room temperature, were cut into pieces (1.0 by 1.0 cm in size). The vaginal cells were extracted from each piece of the swabs with 1 mL of PBS. Then, 0.1-mL aliquots of the diluted semen and 0.1-mL aliquots of the extract of vaginal swabs were mixed and incubated for 30 min at 37°C in a 0.01*M* ethylenediaminetetraacetate (EDTA), 0.1*M* sodium chloride (NaCl), 0.01*M* Tris-hydrochloric acid (HCl) buffer (pH 8.0) containing 0.2 mg/mL of proteinase K. Pellets were obtained by centrifugation (3000 rpm, 10 min) and washed with PBS after which the pellets were smeared on a glass slide and fixed with methyl alcohol. After fixation, the slides were stained according to Oppitz's staining method.

Electron Microscopic Observation of the Spermatozoa

Electron microscopic observation of the spermatozoa was performed in the standard following manner. Both the proteinase K-treated and the untreated semen were fixed with 4% paraformaldehyde and 1% glutaraldehyde in a 0.1*M* phosphate buffer (pH 7.4) for 2 h at 4°C. After washing with the same buffer, the specimens were postfixed with 1% osmium tetroxide in a phosphate buffer for 30 min at 4°C. After washing with the same buffer that contained 7.5% sucrose, the specimens were dehydrated in graded ethanol and embedded in Epon 812. Ultrathin sections were examined with a JEOL-100B electron microscope.

ABO Blood Grouping of Spermatozoa

Semen was collected from four healthy, young adults of known ABO blood groups. Semen-free vaginal swabs were obtained aseptically from four volunteer donors of known ABO blood groups. The swabs, which had been dried and stored at −20°C, were cut into pieces (1.0 by 1.0 cm in size). Vaginal cells were extracted from each piece of the swabs with PBS and collected by centrifugation. Then, to 0.1-mL aliquots of vaginal cells with erythrocyte type A, B, O, and AB, 0.1-mL aliquots of semen with different ABO types were added. The mixtures were incubated for 30 min at 37°C in a 0.01*M* EDTA, 0.1*M* NaCl, 0.01*M* Tris-HCl buffer (pH 8.0) containing 0.2 mg/mL of proteinase K. Pellets were obtained by centrifugation (3000 rpm, 10 min) and washed three times with PBS. The stains then were prepared by placement onto cotton cloth. The elution tests were performed following Yada's method [*4*]. For each specimen, a 5-mm length of thread was placed into a test tube and one drop of antiserum then was added. Following incubation at 4°C overnight, these specimens were cautiously rinsed five times in a cold physiological saline solution to ensure the complete removal of the unabsorbed antibody. At the end of these rinsings, one drop of the saline solution was added, and the deposit mixture then was incubated in a water bath at 55°C for 10 min. After this incubation, one drop of a 0.5% suspension of group A, B, and O cells was

added to each tube, after which the tube was centrifuged at 1000 rpm for 1 min. Then, by holding each tube gently over a microscopic concave mirror, the presence or absence of agglutination was ascertained.

Results and Discussion

The identification of human spermatozoa from the vaginal contents is a very important consideration in the forensic science analysis of sexual offenses. In our laboratory, Oppitz's method is usually used to stain the spermatozoa, since we have found this staining method to be very convenient in distinguishing the spermatozoa from the vaginal cells. Yet, we often have encountered difficulty in detecting the spermatozoa in semen-contaminated vaginal swabs, since the head of spermatozoa and the female components are stained simultaneously (Fig. 1). Thus, many hours have been spent searching for a single spermatozoa among a lot of vaginal cells.

In 1985, Gill et al. [*3*] reported on the forensic science application of DNA fingerprinting, having discovered that they could separate sperm nuclei from vaginal cellular debris by a technique that uses proteinase K treatment. Thus, we have tried to apply this same technique to detect the spermatozoa that might be present in vaginal swabs. Figure 2 shows that after proteinase K pretreatment, the heads of the spermatozoa are distinctly stained scarlet by Oppitz's staining method. In as short a time as 30 min of proteinase K treatment, the spermatozoa that separated from the enzymatically destroyed vaginal cells were recovered. Though this proteinase K destruction does furnish some spermatozoa with deformed heads and a somewhat greater number of isolated tails, a sufficient number of viable heads still remain for a reliable diagnosis. Figure 3 shows the presence of human spermatozoa, from a criminal case, on a vaginal swab that had been kept at room temperature for two months. After proteinase K treatment, the female components were completely lysed though the heads of spermatozoa were distinctly stained by Oppitz's method. Though the tails of spermatozoa were separated from the heads by proteinase K pretreatment, it is easy to identify the human spermatozoa from the vaginal swabs, since the size of the heads of spermatozoa (2 to 5 μm) is smaller than that of the nuclei of other human cells. Also, squamous epithelium cells such as vaginal cells might be lysed by proteinase K treatment. And there is a distinct difference between the size of human sperm cells and that of other cellular elements such as bacteria and yeast. Further, observation of morphology of heads of spermatozoa was not prevented by proteinase K treatment. Therefore this method can be applied to detect human sperm cells.

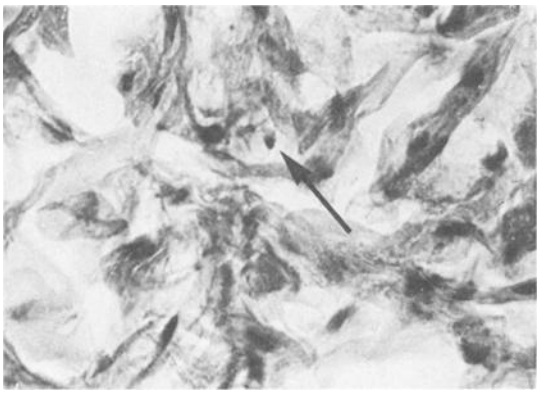


FIG. 1—*Semen-contaminated vaginal swab (Oppitz's stain [×400]). Spermatozoa* (arrow) *can be distinguished from vaginal cells.*

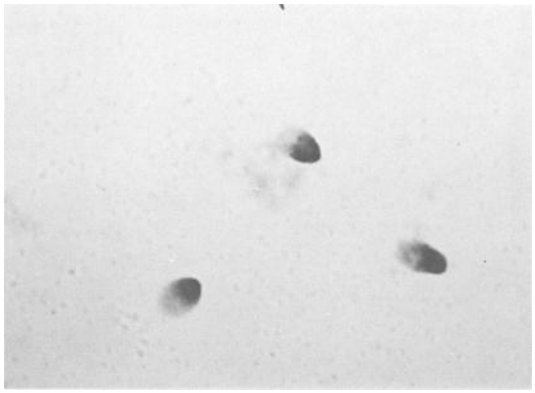
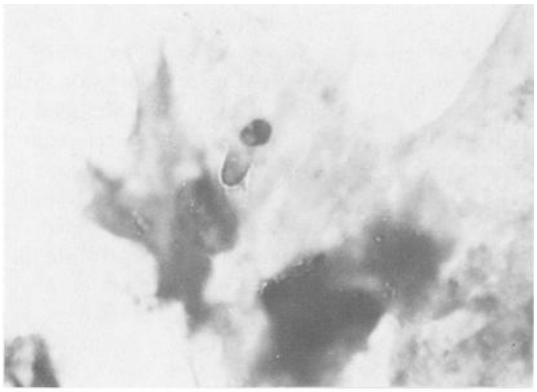
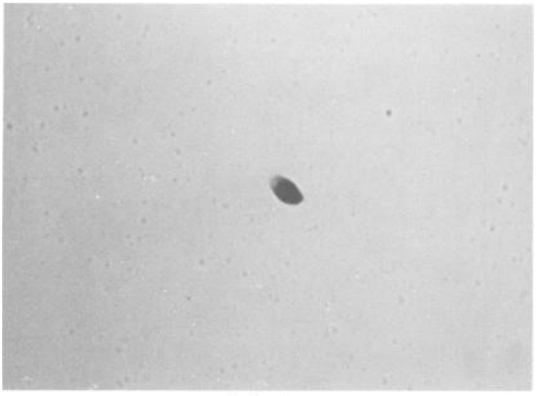


FIG. 2—*A proteinase K-treated, semen-contaminated vaginal swab (Oppitz's stain [×1000]). Vaginal cells were almost all destroyed, but the heads of the spermatozoa were stained scarlet.*



(a)



(b)

FIG. 3—*Demonstration of human spermatozoa on a vaginal swab kept at room temperature for two months (Oppitz's stain [×1000]):* (a) *an untreated vaginal swab and* (b) *a proteinase K-treated vaginal swab.*

Electron microscopic studies of both proteinase K-treated and untreated semen have been pursued in the usual manner, and examples of both normal spermatozoa and proteinase K-treated spermatozoa are shown in Fig. 4*a* and 4*b*, respectively. After the spermatozoa had been treated with proteinase K, its cytomembranes were found to turn upwards near the joint of acrosome. Cytolysis was found to have occurred to the neck and middle piece, which were almost completely destroyed, though the nucleus remained unaffected (Fig. 4*b*). The sperm nuclei are known to be ramified with cross-linked, thiol-rich proteins [5], so it may be that the specific structure of the sperm nucleus might be responsible for making the nucleus resistant to proteinase K treatment.

We further examined whether the spermatozoan ABO blood group substances could be detected after proteinase K pretreatment and found that the ABO blood grouping of the spermatozoa could be detected from vaginal swabs by using the absorption-elution technique (Table 1). The blood group ABH substances of the vaginal cells were found to disappear completely after proteinase K pretreatment, while the spermatozoan ABH substances could be detected by the absorption-elution technique. These results suggest that the ABO blood grouping of the spermatozoa on the vaginal swabs can be determined with accuracy by use of proteinase K treatment.

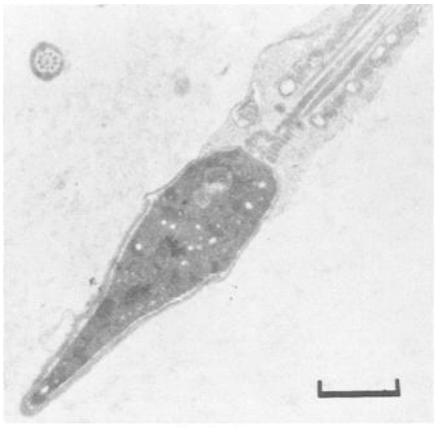


FIG. 4—*Transmission electron micrograph of spermatozoa (×6700):* (a) *an untreated spermatozoa and* (b) *proteinase K-treated spermatozoa.*

TABLE 1—*The ABO blood grouping of the mixtures containing spermatozoa and vaginal cells using the absorption-elution technique[a] after proteinase K treatment.*

Blood Group		Proteinase K Treatment			No Treatment		
Spermatozoa	Vaginal Cells	Anti-A	Anti-B	Anti-H	Anti-A	Anti-B	Anti-H
A	B	++	−	−	+++	+++	−
A	O	++	−	−	+++	−	+++
A	AB	++	−	−	+++	+++	−
B	A	−	++	−	+++	+++	−
B	O	−	+	−	−	+++	+++
B	AB	−	++	−	+++	+++	−
O	A	−	−	++	+++	−	+++
O	B	−	−	++	−	+++	+++
O	AB	−	−	++	+++	+++	+++
AB	A	++	++	−	+++	+++	−
AB	B	++	++	−	++	+++	−
AB	O	++	+	−	+++	+++	+++

[a]The strength of agglutination graded from none (−) to intense (+++).

In conclusion, our results have demonstrated that the proteinase K pretreatment technique has been found a useful method to aid in the identification of spermatozoa separated from other cellular elements and to enhance the detection of the ABO blood group substances of spermatozoa.

References

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