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ORIGINAL COMMUNICATION

Rapid detection of sperm: comparison of two methods

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SUMMARY. Sperm detection can be an important factor in confirming sexual assault in cases of rape. This paper compares two biochemical methods used in forensic medicine: the first detects the presence of zinc, the second detects acid phosphatase activity. The population studied was composed of 174 consenting women seen at the Male Infertility Center in Toulouse, France. The date of their last sexual intercourse was known accurately. Cytology was the reference test to confirm the presence of sperm in the vaginal samples. We studied the sensitivity, specificity, and positive and negative predictive value of the two biochemical methods. Acid phosphatase detection was the most valuable technique, but its use is limited in time. The zinc test gave disappointing results in our study and does not seem to be a useful reference method for the forensic physician.

Keywords: Sperm; Spermatazoa; Detection of sperm; Zinc; Acid phosphatase; Sexual intercourse; Sexual assault; Rape

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INTRODUCTION

Rape is usually an unwitnessed crime and evidence of semen can play a crucial role in corroborating the victim's allegations during the initial phase of the investigation.[1] The medical examination provides the opportunity to collect physical evidence and biological samples to confirm the presence of sperm and convict an assailant.[2–6] The presence of spermatozoa is the current criterion confirming sexual intercourse. This biological evidence is accepted both by the medical forensic teams, for whom cytology is a gold standard even if other methods are also used,[1,5,7–13] and by the judicial authorities.[14]

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Other biological methods can help the forensic physician to detect the presence of sperm:

- Measurement of free choline, but as it is rapidly degraded (<24 h) in the vaginal environment this method is of little value in routine forensic practice.[2]
- Detection of prostatic acid phosphatase.[10,12,13,15] Measurement of this activity[1,12] seems to be an interesting line for research because the enzyme is highly specific.
- The zinc test has been used by some teams to reveal the zinc which is contained in sperm;[10–13,16] zinc can apparently be detected in 20-year-old sperm stains.[16]
- Specialized immunologic methods have been proposed detecting specific substances, such as lactate dehydrogenase isoenzyme C4,[17] prostate-specific antigen,[18] seminal vesicle-specific antigen,[4,19] spermatozoid wall-specific antigen,[20], and there is even a chromatographic method of prostaglandin E extraction,[21] but their complex technology limits their routine use in forensic medicine.

Because of their ease of use, we chose two of the methods mentioned above: the acid phosphatase test and the zinc test. The first technique, acid phosphatase detection, is the simplest and has received the

most attention, but its use in forensic medicine is limited by the short life of the protein. The enzymatic activity of acid phosphatase generally lasts 48 h.[1] The zinc test has been little mentioned in the literature but in theory its use is interesting in forensic practice because detection of zinc does not seem to be subject to a time limit.

MATERIALS AND METHODS

Population studied

The authors studied 174 cervicovaginal samples from consenting women seen at the Male Infertility Center of the University Hospital of Toulouse, France, for in vitro or postcoital tests. The mean age of the men was 32 years (range 23–48) and of the women 30 years (range 21–40). The date of the last sexual intercourse for each patient and time of sampling were noted to evaluate the interval between intercourse and swab taking.

Cytology

A glass slide was prepared from each swab. It was air dried, fixed in alcohol and ether, and stained with nuclear fast red and picroindigocarmine (Christmas tree stain). All slides were screened microscopically with a magnification 40× and the presence of spermatozoa was confirmed or not. Cytology was the reference test to confirm the presence of sperm in the samples.[22]

Biochemistry

For the acid phosphatase test, a commercially available test paper strip was used (Phosphatesmo KM, Macherey–Nagel, Düren, Germany). The swab is applied directly on to the paper strip and the color change is immediately read by the technician.

For the zinc text, we used Hooft's improved reagent.[11] Two drops are applied directly on to the vaginal sample swab and again the color change is immediately read by the technician.

Statistical analysis

The slides prepared with the cytological stain were read blind by two observers. The value of the biochemical methods in comparison with the cytological reference method was estimated by calculating sensitivity, specificity, and positive and negative predictive values. Quantitative variables were compared using Student's t test and the threshold of 5% was considered as significant. All 174 samples were suitable for use with the acid phosphatase test and 171 with the zinc test.

RESULTS

The mean number of spermatozoa per microscopic field in acid phosphatase-negative samples was 0.0006 (maximum 0.04) and 15.24 (SD 39.10) in positive samples. This difference was statistically significant ($t = 3.05$, $p = 0.003$).

Compared with the reference cytological test, sensitivity was 95%, specificity 96%, positive predictive value 93%, and negative predictive value 98% (Table 1).

In samples taken within 24 h, two of 63 samples (3.2%) which were positive on cytology were acid phosphatase-negative, a detection rate of 96.8%. In samples taken within 48 h, the detection rate was 88.9%.

The time limit for detection of enzymatic activity was 106 h.

Individual study of the four samples which were acid phosphatase-positive and -negative on cytology (false positives) showed that:

- These four samples were taken early, between 10 and 36 h after intercourse, and therefore contained acid phosphatase which was still active and so could theoretically have yielded a positive result in the enzyme assay.
- The men taking part in this special sample had azoospermia or oligospermia (<40 millions of spermatozoa per ejaculate) and this could account for the absence of sperm cells on cytology.

The mean number of spermatozoa detected per microscopic field differed significantly in relation to the results of the zinc test ($t = 3.05$, $p = 0.003$). When the zinc test was negative, 3.60 spermatozoa were observed per field (SD 24.55) compared with 6.71 (SD 24.58) when the zinc test was positive. Compared with the cytological test, sensitivity was 81%, specificity 54%, positive predictive value 49%, and negative predictive value 84% (Table 2).

Table 1 Detection of sperm with the acid phosphatase test and cytology

	Cytology − ($n = 111$)	**Cytology + ($n = 63$)**
Acid phosphatase test − ($n = 110$)	107	3
Acid phosphatase test + ($n = 64$)	4	60

Table 2 Detection of sperm with the zinc test and cytology

	Cytology – ($n = 109$)	Cytology + (n = 62)
Zinc test – ($n = 70$)	59	11
Zinc test + ($n = 101$)	50	51

DISCUSSION

Our study showed that the acid phosphatase test has good sensitivity and specificity, and above all that it has a negative predictive value of 98%. It is thus valuable as a screening test and not as a confirmatory test. Study of samples which were acid phosphatase-positive but negative on cytology showed that the men had azoospermia or oligospermia, raising the question as to whether the acid phosphatase test is not more sensitive than cytology in such cases.

On the other hand, acid phosphatase detection and measurement techniques are hampered by the limited duration of enzyme activity. In our study, the longest timespan after which detection was possible was 106 h between intercourse and sampling (Fig. 1).

The zinc method appears to allow sperm detection over a long period.[16] While it seemed interesting in theory, we found it disappointing in practice: there is only a 50–50 chance that a zinc-positive sample actually contains sperm (positive predictive value 49%) and its negative predictive value was too low for a screening test (84%).

CONCLUSION

Our study confirmed that the acid phosphatase test is a useful screening test for routine forensic use. Because it is simple to use, gives a rapid result, and has a high negative predictive value, it appears to be the most useful test for investigators during the hours following a complaint of rape. The zinc test, as it is presently described in the literature, is not satisfactory for sperm detection.

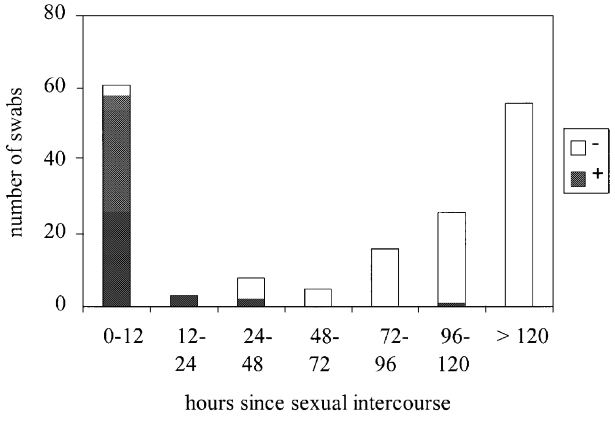

Fig. 1 Internal vaginal swabs. Frequency of positive swabs tested with the acid phosphatase technique according to time since intercourse.

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