J Forensic Sci, Oct. 1978, Vol. 23, No. 4

J. H. Duenhoelter,[1] *M.D.; I. C. Stone,*[1] *Ph.D.;*
Rigoberto Santos-Ramos,[1] *M.D.; and D. E. Scott,*[1] *M.D.*

Detection of Seminal Fluid Constituents After Alleged Sexual Assault

Spermatozoa and prostatic acid phosphatase, identified in vaginal fluid after an alleged sexual assault, constitute important physical evidence useful in courts of law during rape trials. It has been recommended that the physician examining a victim of alleged sexual assault should promptly attempt identification of spermatozoa in a native preparation of vaginal fluid. Additionally, he should collect certain specimens that later can be evaluated in detail in a forensic pathology laboratory. The presence or absence of spermatozoa, prostatic acid phosphatase, and various blood group substances can be ascertained with a variety of laboratory methods. This study was undertaken to investigate the following items:

(1) the correlation of spermatozoa detected in native preparations of vaginal fluid and of results obtained by various forensic laboratory methods;
(2) the incidence of detection of seminal fluid constituents at certain time intervals following an alleged rape; and
(3) the possibility that acid phosphatase detection provides additional evidence when spermatozoa cannot be identified in native or fixed preparations of vaginal fluid.

Material and Methods

Three hundred consecutive records were reviewed of women who complained of sexual assault and were subsequently examined in the Parkland Memorial Hospital Emergency Room. Their ages ranged from 12 to 82 and all were postmenarcheal. Each was examined by a faculty member of the Department of Obstetrics and Gynecology; all faculty members were board-certified obstetricians and gynecologists. A brief history of the alleged attack was recorded and a physical examination performed. Fluid from the posterior vaginal fornix or cervical mucus, together with a drop of normal saline, was placed on a glass slide, covered with a cover slip, and searched microscopically for spermatozoa. Results were reported as (1) motile spermatozoa, (2) nonmotile spermatozoa, and (3) absent spermatozoa.

For identification of spermatozoa in the forensic science laboratory, liquid from the posterior vaginal fornix or cervical mucus was collected with a cotton-tip applicator or an Ayre spatulum, applied to a glass slide, and then sprayed with a fixative (Spray-Cyte).[2]

For detection of acid phosphatase in the forensic science laboratory, a cotton-tip appli-

Received for publication 23 Nov. 1977; revised manuscript received 11 Jan. 1978; accepted for publication 16 Jan. 1978.

[1]Members of the staff, The Departments of Obstetrics and Gynecology and Pathology, The Southwestern Institute of Forensic Sciences, University of Texas Health Science Center, Dallas, Tex.

[2]Clay, Adams Division of B-D Co.

cator was soaked in the fluid of the posterior vaginal fornix and placed into a screw-top culture tube containing 2 ml normal saline. All specimens were then placed into an envelope, which was sealed and submitted to the Institute of Forensic Sciences for identification of spermatozoa and detection of acid phosphatase.

Spermatozoa were identified after staining with nuclear fast red and picroindigocarmine according to the method of Oppitz [*1*]. Acid phosphatase was detected with an enzymatic reaction with alpha-naphthyl-phosphate according to the methods of Rupp [*2*] and Sivaram and Bami [*3*]. The results were reported as spermatozoa present, spermatozoa absent, acid phosphatase detected, and acid phosphatase not detected.

Results

From the records of 300 consecutive postpubertal women alleging recent sexual assault the data required for this study were available in 288 instances. Details concerning victims and events surrounding the alleged assault will be reported later.

In Table 1 the time intervals between the alleged sexual assault and the examination in the emergency room are reported. Approximately three fourths of the victims were examined within 6 h, 89.3% within 12 h, and 97.7% within 24 h after the event.

Table 2 contains the data that allow comparison of spermatozoa identified shortly after the examination by the gynecologist and those identified later in the forensic laboratory. In four instances in which spermatozoa were visualized in the fresh but not in the fixed and stained preparation a second slide, which had been retained, was stained and spermatozoa were detected.

In 70 of the 288 victims (24.3%) spermatozoa could not be identified in either the native or the fixed preparations of vaginal fluid nor was acid phosphatase detected in vaginal fluid samples. In 19.8% of cases physicians failed to identify spermatozoa in fresh preparations, although the investigators in the forensic laboratory identified one or more seminal fluid constituents. Among individual physicians the figures varied between 16.7 and 36.8%.

Among the 218 cases in which a seminal fluid component was identified the results were positive with all three methods of detection in 138 instances (63.3%). In 24 instances (4.0%) only one of the three methods provided evidence of recent intercourse. Only spermatozoa were demonstrated in the fresh preparation in 1 instance, in the fixed preparation in 18 instances, and in 5 instances acid phosphatase was the only seminal fluid component detected.

In Table 3 results are listed for spermatozoa and acid phosphatase detection after several time intervals following the alleged assault. Recorded in Table 4 are data concern-

TABLE 1—*Time intervals between alleged assault and examination in the emergency room.*

Hours After Assault	Cases, *n*	Per Time Period, %	Cumulative Per Time Period, %
<2	30	10.4	10.4
3	57	19.8	30.2
4	52	18.1	48.3
5	42	14.6	62.9
6	29	10.1	73.0
7-12	47	16.3	89.3
13-24	24	8.3	97.6
>24	4	1.4	99.0
Unknown	3	1.0	100.0
Total	288	100.0	100.0

TABLE 2—*Identification of spermatozoa in a native preparation and later forensic laboratory identification of spermatozoa and acid phosphatase in 288 cases of alleged sexual assault.*

Forensic Laboratory Examination	Identification of Spermatozoa in Native Preparation					
	Present, Motile		Present, Nonmotile		Absent	
	n	%	*n*	%	*n*	%
Spermatozoa present, acid phosphatase detected	54	18.8	86	29.9	34	11.8
Spermatozoa present, acid phosphatase not detected	5	1.7	11	3.8	18	6.3
Spermatozoa absent, acid phosphatase detected	1	0.3	0	0.0	5	1.7
Spermatozoa absent, acid phosphatase not detected	1	0.3	3	1.0	70	24.3

TABLE 3—*Identification of seminal fluid constituents as a function of time after alleged assault.*

Time After Alleged Assault, h	Cases with Identification of at Least One Seminal Fluid Constituent, *n*	Cases in Which Spermatozoa Were Identified				Cases in Which Acid Phosphatase Was Identified	
		Native Preparation		Fixed Preparation			
		n	%[a]	*n*	%[a]	*n*	%[a]
<2	25	17	68.0	25	100.0	16	64.0
3	46	28	60.9	41	89.1	41	89.1
4	42	35	83.3	41	97.6	36	95.7
5	33	22	66.7	32	97.0	31	93.9
6	18	15	83.3	18	100.0	16	88.9
7-12	39	24	61.5	38	97.4	29	74.4
13-24	11	8	72.7	10	90.9	8	72.7
>24	2	2	100.0	2	100.0	1	50.0
Unknown	2	0	0.0	1	50.0	2	100.0
Total	218	161	73.9	208	95.4	180	82.6

[a] Percentage of cases in which at least one seminal fluid constituent was identified.

TABLE 4—*Identification of spermatozoa during two time periods in cases in which at least one seminal fluid constituent was detected.*

Time After Alleged Assault, h	Cases with Identification of at Least One Seminal Fluid Constituent, *n*	Cases in Which Spermatozoa in the Native Preparation Were Identified			
		Motile		Nonmotile	
		n	%[a]	*n*	%[a]
≤6	164	52	31.7	78	47.6
7-24	50[b]	9[b]	18.0	22	44.0
Total ≤24 h	214	61	28.5	100	46.7

[a] Percentage of cases during this time period in which at least one seminal fluid constituent was identified.

[b] In three instances specimens were obtained from cervical mucus.

ing presence of spermatozoa in vaginal smears of victims who were examined within 6 h and after 6 h of the alleged sexual assault and in whom at least one seminal fluid constituent was detected. In three instances motile spermatozoa were found in cervical mucus although the alleged assault occurred more than 6 h before the victim was seen and examined.

Discussion

Since Dallas County and its several law enforcement agencies cooperate with the Department of Obstetrics and Gynecology at the University of Texas Health Science Center at Dallas, female victims of sexually related crimes are brought into the Parkland Memorial Hospital Emergency Room expeditiously and shortly thereafter are examined by a member of the faculty. Most women in this study were seen within 5 h and 97.6% were examined within 24 h after the incident allegedly occurred. A review of previous investigations concerning identification of seminal fluid constituents indicates that it should be possible to demonstrate one of these constituents if the patient is examined within this time period [*4-6*].

Among a total of 288 women, no evidence of recent intercourse was found in 70 cases with the following three laboratory methods: (1) demonstration of spermatozoa in a native preparation, (2) demonstration of spermatozoa in a stained preparation of vaginal fluid, and (3) detection of acid phosphatase in the vaginal fluid specimen. From the history of several of these victims it could be ascertained that ejaculation had not taken place. In the remaining instances ejaculation, unknown to the victims, may not have occurred. The possibility also exists that we failed with all three methods to detect a seminal fluid component when ejaculation took place.

During rape trials, inquiry often is made whether the identified seminal fluid components were deposited during a previous voluntary intercourse or during the alleged event. Previous investigators who attempted to identify motile and nonmotile spermatozoa in vaginal fluid disagree for how long after intercourse motility can be demonstrated. Sharpe [*7*] concluded from his own experiences and a review of the literature that motile spermatozoa "are found in the vagina ½ to 6 hours after coitus, the average time being 3 hours." The same author states that under exceptional circumstances nonmotile spermatozoa can be found 18 to 24 h after intercourse. We observed motile spermatozoa in 17% and nonmotile spermatozoa in 51% of fresh and unstained vaginal fluid specimens if intercourse allegedly took place 7 to 24 h prior to examination. This contrasts with the statements of Sharpe [*7*]. Davies and Wilson [*8*] were able to detect spermatozoa in 14 of 41 specimens (34%) collected 90 to 156 h after an index intercourse. Occasionally, the possibility that the seminal fluid was deposited during a prior voluntary intercourse can be ruled out through identification of blood group antigens.

From the results of the present study, it can be concluded that the detection of spermatozoa in vaginal fluids is possible in 75% of cases in the fresh specimen and in 93% in the preserved and stained specimen. Thus, identification of spermatozoa by the technique used in our forensic laboratory was least likely to give a negative result. Unfortunately, we cannot conclude from the data in how many instances spermatozoa were identified when, in fact, intercourse had not taken place. However, it appears highly unlikely that these trained observers falsely identified spermatozoa.

Motile spermatozoa were observed in 31.7% of victims examined within 6 h of the alleged event and in 9 out of 50 (18%) who were examined between 7 and 24 h. Unfortunately, in 3 of the 9 instances in the latter group motile spermatozoa were searched for and identified only in cervical mucus, where they are known to remain motile for longer periods of time than in vaginal fluid. We do not know if motile spermatozoa were also present in the vaginal fluid of these 3 victims. The arbitrary separation of vaginal fluid

and cervical mucus does not seem justified in this context, since cervical mucus containing spermatozoa may very well contaminate the vaginal fluid. Since there are no good prospective studies published, we plan an investigation in which the duration of spermatozoal motility in vaginal fluid and cervical mucus, as well as the presence of nonmotile spermatozoa, will be ascertained sequentially over a period of several days.

Spermatozoa were identified in the fixed and stained preparation in 96% of victims who had some evidence of recent intercourse and who were examined within 24 h of the alleged event. Thus with the fixed preparations it was least likely to obtain a falsely negative result, especially if they were reevaluated when an additional seminal constituent was identified with another method.

Identification of acid phosphatase in vaginal fluid in which spermatozoa cannot be detected is especially important because oligospermia and azoospermia seem to be more prevalent among rapists than among adult males in general [*9*]. In our study there were five cases in which only acid phosphatase but no spermatozoa could be demonstrated. This finding may indicate oligospermia or azoospermia as a consequence of an abnormality or following a vas deferens ligation of the alleged rapist. Another remote possibility is the false identification of acid phosphatase when no intercourse had taken place.

In victims whose vaginal fluid contained spermatozoa, acid phosphatase was not detected in 9 of 25 or 36% when an examination was performed within 2 h or less of the alleged assault. If the examination took place between 3 and 12 h after the event, we failed to identify acid phosphatase in only 24 of 178 women (13.5%). This difference is statistically significant ($P < 0.02$). Apparently, the qualitative method of acid phosphatase detection used in this study showed more frequently false-negative results if victims were examined shortly after intercourse than if they were examined between 3 and 12 h after the event.

Conclusion

The seminal fluid components collected from 300 consecutive postmenarcheal women who were examined at Parkland Memorial Hospital after an alleged sexual assault were analyzed to ascertain the relative value of acid phosphatase determination and spermatozoal detection in fresh unstained and in fixed stained specimens of vaginal fluid. In the majority of cases in which there was some laboratory evidence of recent intercourse all three methods of seminal fluid detection were positive (63.3%). In 27 instances (12.4%) only one of the three methods provided evidence of recent intercourse: spermatozoa only were demonstrated in the fresh preparation in 4 instances, in the fixed preparation in 18 instances, and in 5 instances acid phosphatase was the only seminal fluid component detected. Spermatozoa in the unstained specimen of vaginal fluid were detected in 75% and in the preserved and stained specimen in 93% of those cases in which one laboratory method showed evidence of recent intercourse. Motile spermatozoa were identified in the native preparation of vaginal fluid or cervical mucus in 31.7% of cases in which at least one seminal fluid constituent was detected if patients were examined within 6 h. If the examination was delayed between 7 and 24 h after the alleged assault, this figure decreased to 18%. It is concluded that determination of acid phosphatase and the detection of spermatozoa, both in the fresh and unstained specimen and in the preserved and stained specimen, are of value when evidence of recent sexual intercourse is collected.

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Address requests for reprints or additional information to
Johann H. Duenhoelter, M.D.
Department of Obstetrics and Gynecology
The Southwestern Institute of Forensic Sciences
University of Texas Health Science Center
5323 Harry Hines Blvd.
Dallas, Tex. 75235