Study On Persistence Of Spermatozoa And Prostatic Acid Phosphatase In Human Vaginal Tract

[1]Pramod Kumar Saini, [2]Sanjeev Buri, [3]Keshav Soni, [4]Asmita Nayak

[1&2]Assistant Professor, [3]3rd year Resident, Dept. of Forensic Medicine & Toxicology,

[4]Sr. Resident, Dept. Of Obstetrics & Gynaecology,

S. P. Medical College, Bikaner, Rajasthan

ABSTRACT

Background: The presence of spermatozoa and seminal fluid in the female genital tract after coitus is a matter of considerable academic and medicolegal importance. The aim of this study to evaluate the presence of spermatozoa and acid phosphatase in its various forms at various time intervals since intercourse in vaginal swabs taken from vaginal tract. **Material & Methods:** A Total 239 cases were studied, out of them 192 samples were collected from married women who were attending the Gynae OPD of SMS Hospital, Jaipur (Mahilla Chikitsalya) from June 2004 to July 2004 and subsequently 47 cases were studied at PBM Hospital, Bikaner, from March 2011 to May 2011 Total 239 cases were studied during this study. **Results:** The present study showed the maximum number of cases belonged to the age group of 20-30 yrs. (64.85%). Spermatozoa was detected in the husband's age group of 20-30 yrs. The comparison between the spermatozoa detection and Acid Phosphatase Positivity, in 228 cases percentile positivity for spermatozoa and in 239 cases for percentile Acid Phosphatase Positivity vis-a–vis time since intercourse. **Conclusion:** The women having a history of adoption of contraceptive method (IUCD, OCP, Tubectomy) did not affect the presence of spermatozoa. Acid phosphatase was detected mostly when the women had a history of intercourse around the ovulation time.

Key-words: Spermatozoa, Acid phosphatase, Vaginal fluid, Semen examination.

Corresponding Author: Dr. Pramod Kumar Saini, Asstt. Professor, Deptt. of Forensic Medicine & Toxicology, S.P Medical College, Bikaner, Rajasthan. Mail: drpramodkumarsaini@gmail.com Mob. 09352587597.

INTRODUCTION

The presence of spermatozoa and seminal fluid in the female genital tract after coitus is a matter of considerable academic and medicolegal importance. In case of rape, the accused is usually acquitted due to lack of evidence in support of rape. This acquittal of the accused because of lack of evidence causes much mental trauma to the alleged victim and explains why women are reluctant to report rape to the authorities. In sexual assault cases the amount of information available to forensic expert has increased significantly in the last few years. In case of rape the identification of given specimen as semen may provide objective evidence of a corroborative nature. Such specimen also retains their individuality over a long

period of time and so lengthens the permissible interval between their discovery and production for use as evidence.[1]

The presence of spermatozoa in a given specimen is confirmatory of seminal stains. The failure to find spermatozoa does not exclude the possibility of stain being seminal in nature. In such cases, if the chemical tests for seminal fluid are positive (particularly acid phosphatase), there is a strong presumption that the stain is a seminal stains. In the vasectomized male, though no sperms will be present, chemical and enzymatic test for semen may be positive. Presence of spermatozoa or seminal stain is important not only in living cases, but also in homicides due to sexual jealousy. Even in case of false virgin genital injuries may not be a good evidence of rape, but still rape may be suspected.[2]

There are widely varying estimates of the time that spermatozoa survive in the female genital tract. Most of the studies that have been conducted were on the specimens collected from the cervical canal, done mostly for fertility purpose. But in the medicolegal cases the samples are collected from the posterior fornix of the vagina.[1] Data collection is an important part of forensic expert so that he can assess the value of each test and indicate the significance of the results when giving1. evidence in the court of law. Data collection in case of rape is difficult and2. time consuming due to fewer numbers of cases and variability of reporting time. To generate a large number of data many3. years of the study would be required. To study a large number of cases in a short span of time it was thought proper to conduct the study on survival of spermatozoa and presence of seminal acid phosphatase in the genital tract of women attending the Out Patient Department (OPD) of Gynaecology Department of SMS Medical College and Associated Group of Hospitals, Jaipur and P.B.M. Hospital and Associated Group of Hospital, Bikaner. The aim of this study to evaluate the presence of spermatozoa & acid phosphatase in its various forms at various time intervals since intercourse in vaginal swabs taken from vaginal tract. The information so generated would prove useful in rape cases also, so this study was conducted.

MATERIAL & METHODS

A prospective and comparative study was undertaken to evaluate the presence of spermatozoa & acid phosphatase in its various forms at various time intervals since intercourse in vaginal swabs. A total 239 cases were studied, out of them 192 samples were collected from married women who were attending the Gynae OPD of SMS Hospital, Jaipur (Mahilla Chikitsalya) from June 2004 to July 2004 and subsequently 47 cases were studied at PBM Hospital, Bikaner, from March 2011 to May 2011 Total 239 cases were studied during this study.

Inclusion Criteria

Married women in the age group of 18 to 45 years with active sexual life.

Women who were not using barrier contraceptive and spermicidal contraceptives.

Women reporting during the 5th day of the menstrual cycle to the beginning of the next cycle.

Exclusion Criteria

1. Women using barrier contraceptive and spermicidal contraceptive.
2. Women during day 1st to 4th day of the menstrual cycle.

Collection of Material

Using a clean speculum, posterior fornix of the vagina was visualized and 0.2 ml- 0.3 ml of normal saline was introduced into the vagina around the cervix and using a cotton swab on a wooden stick the samples were collected from posterior fornix. Immediately after taking the swabs slides were prepared by swiping the swab on a glass slide. One slide was wet mounted using a cover slip for visualizing motility of sperms. One slide was dried, fixed and stained to detect the presence of spermatozoa.

1. **Slides:** Post-coital slides were prepared from the posterior vaginal fornix for the detection of motility of spermatozoa and spermatozoa after staining with double stain.
2. **Swabs:** Cotton wool swabs were taken from the posterior vaginal fornix for the detection of acid phosphatase.
   In this study, double stain (Aniline blue and Eosin blue) was used as it gives contrast between the head and the tail of spermatozoa.

Method

a. Using a clean speculum, posterior fornix of the vagina was visualized and 0.2ml- 0.3ml of normal saline was introduced into the vagina around the cervix.
b. Using a cotton swab on a wooden stick the sample was collected from the posterior fornix
c. Immediately after taking the swab slide was prepared by swiping the swab on a glass slide
d. The slide prepared was air dried.
e. The dried slide was fixed by fixative solution.
f. Then the slide was stained by double stain (for contrast)
g. The stained slide was examined under oil immersion in light microscope (100x) with fully open aperture. The head of the spermatozoa was identified by its oval shape and it appears pink while the tail appears blue.

Demonstration of Acid Phosphatase Activity[2]

Method

a. Using a clean speculum, posterior fornix of the vagina was visualized and 0.2ml- 0.3ml of normal saline was introduced into the vagina around the cervix.
b. Using a cotton swab on a wooden stick the sample was collected from the posterior fornix
c. The swab was placed on clean white filter paper.
d. Two drops of buffer solution were added to swab and was squeezed to extract its contents on the filter paper.
e. Then a drop of the substrate solution that was solution of sodium 1-Naphthyl phosphate was added.
f. Finally a drop of Fast Blue B solution was added.

Positive test was indicated by the development of deep purple color immediately that was within 5 seconds. A reaction time of less than 30 seconds was considered a very good indication of presence of semen as no vaginal acid

phosphatase react within this time. The light color may be due to weak semen stains or endogenous acid phosphatase level and was considered as negative. **Negative test**: was considered when there was no color change or light color change or color changed after 30 seconds.

RESULTS

The present study showed the maximum number of cases belonged to the age group of 20–30 yrs. (64.85%) (Table 1). The Presence of spermatozoa was detected mostly within 24hrs since intercourse. Whole spermatozoa were detected up to 24 hrs, except in one case where time since intercourse was 64 hrs (Table 2). Spermatozoa positivity was consistently higher in the age group of 20–30yrs. (24.67%). After 30 years, there was a significant reduction in detection of spermatozoa. Spermatozoa positivity was high in the 41-45yrs age group, which is attributable to a statistically lower number of cases in this age group (Table 3). The high number of spermatozoa was detected in the husband's age group of 20-30 yrs (Table 4). The comparison between the spermatozoa detection and Acid Phosphatase Positivity, in 228 cases percentile positivity for spermatozoa and in 239 cases for percentile Acid Phosphatase Positivity vis-a-vis time since intercourse (Table 5).

DISCUSSION

The present study showed that most of the samples were collected in the younger age groups. This also reflects the proportion of the age group attending the outpatient department within the selection criteria of this study. The spermatozoa after staining with double stain were studied in their different form, that its whole spermatozoa or only head after losing a tail, with relation to the time since intercourse. Whole spermatozoa were detected up to 24 hrs., except in one case where time since intercourse was 64 hrs. The findings are in accordance with Michael R. Soules (1978)[3] et al who reported that whole spermatozoa were present up to 18 hrs and heads were present for 24-48 hrs, but not consistent with his observation that at 72hrs, spermatozoa were present in nearly 50% volunteers. The findings are also in agreement with Marry Jean Wallace et al (1975)[4] who observed that within 12 hrs after intercourse, spermatozoa were recovered in 73% of samples. The findings are also consistent with Rolando R. Gomez (1975)[5] et al, who observed that cases having sexual intercourse within 15 hrs, had positive spermatozoa. The observations in our study are also in agreement with Willott and Allard (1982)[6] for detection of whole spermatozoa but not for detection of head who reported the longest time for the head detection 120 hrs and for whole spermatozoa 26 hrs in their study of 1332 vaginal swabs. The findings are consistent with Silverman and Silverman (1978)[7] who studied 675 volunteers and observed 64% cases positive for spermatozoa within 24 hrs, of intercourse. The findings are also in agreement with the observation of Davies and Wilson (1974)[8] who observed that spermatozoa were found usually up to 3 days & smears without spermatozoa were obtained as early as 28 hrs. The findings are consistent with Parikh (1999)[9] & Dixit[10] who stated that non-motile spermatozoa are present for 24hrs. Findings are consistent with Glaister and

Rentoul (1966)[11] who stated that spermatozoa may survive for 2.0 hours in the vagina but they live as long as 43 hours in cervix and vagina. Glaister has mentioned that spermatozoa can be detected up to 85 hours and in our study, they are detectable up to 64 hours.

The findings are inconsistent with Sharpe N. (1963)[12] who stated that non-motile forms are usually found for 7 to 12 hours, but except for 18-24 hours while in our study the detection rate between 13-24hours was 33%. The findings of this study are not in agreement with Gordon and Shapiro (1988),[13] according to whom spermatozoa can be recovered from the vagina up to 3-4 days. Morrison (1972)[14] who stated that spermatozoa could be present up to 9 days and Pollak (1943)[15] who stated that spermatozoa could be present from 30min to 17days. The findings are inconsistent with Dongre & Shrigiriwar (2000),[16] who examined 300 female, attending Gynae OPD and observed that intact spermatozoa were found up to 12hours, and head up to 24hours. The observations are not in agreement with Reddy (2000)[17] who stated that complete spermatozoa present up to 48-72 hours and head up to 4 days.

The detection of spermatozoa and age of women and the husband was compared. It was observed that there was a gradual decrease in the detection rate of spermatozoa as the age advances which is in agreement with Sharon A Kidd(2001)[18] who observed a decrease of 3-22%in semen volume, a decrease of 3-37% in sperm motility and a decrease of 4-18% in normal sperm morphology when compared 30years men with 50 years old men. The acid phosphatase positivity is influenced by the hormonal changes as just before the predicted time of ovulation there is maximum estrogen secretion unopposed by progesterone resulting in copious and clear mucus. The acid phosphatase being an enzyme acts optimally at pH 4.9; the vaginal pH is highest that is acidic level lowest just before and during menstruation. The Acid Phosphatase Positivity was 70.91% as compared to 64.81% of spermatozoa detection within 12 hours of intercourse. The Acid Phsophatase Positivity was 59.26% as compared to 33.33% of spermatozoa detection within 13-24 hours of intercourse. Overall Acid Phosphatase Positivity was 28.03% as compared to 22.81% of spermatozoa detection. The Acid Phosphatase positivity was observed to be higher as compared to spermatozoa detection, but where the detection of spermatozoa is confirmatory of stain being seminal in origin, the Acid Phosphatase Positivity is only strongly suggestive of the sample being seminal in origin.

Reported duration of motility, detection of spermatozoa and acid phosphatase activity of seminal fluids by different workers

No.	Worker	Observation		
		Motility	Spermatozoa Detected	Acid Phosphatase Positivity
1	Rupp (1969)	Within 8 hours., equal chance of finding motile and non – motile spermatozoa	14 hours. (Longest time)	Up to 24 hours.
2	Davies & Wilson (1974)	-	Usually upto 3 days, occasionally upto 6 days, longest time144 hours.	Usually upto one day, often upto 2 days and longest time 3 days
3	Rolando R. Gomez (1975)	-	Within 15 hours, detected in all cases	Within 15 hours., positive
4	McCloskey (1975)	-	-	Usually upto 18 to 24 hours, and occasionally upto 72 hours.,
5	Soules, Pollard, Brown (1978)	-	Complete spermatozoa upto 18 hours, had upto 24 to 48 hours, 50% of cases positive after 72 hours,	50% of cases positive upto 9 hours.,
6	Annie Davies (1982)	-	-	60% of cases positive within 40 hours.,
7	Allard and Davies (1979)	-	-	67% of cases positive within 48 hours.
8	Willott and Allard (1982)	-	Whole spermatozoa upto 26 hours., and head upto 120 hours.	Upto 2 days
9	Dongre AP (1998)	-	Whole spermetoza up to 12 hours., and head upto 24 hours.	Upto 24 hours. too high, persistent upto 36 hours, and declined by 48 hours.,
10	Allery Blanc (2003)	-	-	96.8% of cases within 24 hours., and 88.9% cases within 48 hours., (Spermatozoa presence confirmed by microscopy in all cases)

TABLE-1: Age Wise Distribution of Cases

Age Group (yrs.)	Number of Cases	Percentage of cases
20-25	87	36.40
26-30	68	28.45
31-35	35	14.64
36-40	40	16.74
41-45	9	3.77
Total	**239**	

TABLE-2: Presence of Spermatozoa in Terms of Time Since Intercourse

Time since intercourse (Hours.)	No. of cases	Whole Sperma-tozoa	Head of Sperma-tozoa	Total	Percentage (%)
0 – 12	54	34	1	35	64.81
13 – 24	27	6	3	9	33.33
25 – 36	30	-	5	5	16.67
37 – 48	24	-	2	2	8.33
49 – 60	26	-	-	-	0.00
61 – 72	20	1	-	1	5.00
73 – 84	14	-	-	-	0.00
85 – 96	21	-	-	-	0.00
97 – 108	6	-	-	-	0.00
109 – 120	6	-	-	-	0.00
Total	**228**	**41**	**11**	**52**	**22.81**

TABLE-3: Women's Agewise Distribution of Cases and Spermatozoa Positivity

Age of women (yrs.)	No. Of cases	Spermatozoa Detected	Percentage (%)
20 – 25	87	24	27.59
26 – 30	67	14	20.90
31 – 35	33	4	12.12
36 – 40	32	6	18.75
41 – 45	9	4	44.44
TOTAL	**228**	**52**	**22.81**

TABLE-4: Husband's Agewise Distribution of Cases and Spermatozoa Positivity

Age of Husband (yrs.)	No. of Cases	Spermatozoa	Percentage
20 – 25	34	10	29.41
26 – 30	70	19	27.14
31 – 35	55	10	18.18
36 – 40	30	5	16.67
41 – 45	28	4	14.29
46 – 50	11	4	36.36
51 – 55	-	-	-
TOTAL	**228**	**52**	**22.81**

TABLE–5: Comparison Between Spermatozoa Detection and Acid Phosphatase Positivity

Time since Intercourse (Hrs.)	Spermatozoa detection in percentile (%)	Acid phosphatase positivity in percentile (%)
0-12	64.81	70.91
13-24	33.33	59.26
25-36	16.67	13.33
37-48	8.33	20.83
49-60	0	6.67
61-72	5.0	4.76
73-84	0	0
85-96	0	0
97-108	0	0
109-120	0	0
Total	**22.81**	**28.03**

Conflicts of Interest : None.
Source of Funding : Nil.

References:

1. Vij K. Text Book of Forensic Medicine and Toxicology. 2nd Ed. Churchill Livingstone; 2002. 604-12.
2. Adams GE, Wraxall GB. Phosphatase in body fluids: The differentiation of semen & vaginal secretion. Forensic Sci 1974; 3: 57-62.
3. Soules RM, Albert A,Pollard, Kathren M, Brown, Verma M. The forensic laboratory evaluation of evidence in alleged rape. Am J of Obs and Gynaecol 1978; 130(2): 142-47.
4. Wallace MJ, Haagens, Holtrop HR. Recovery of spermatozoa from human vaginal washings. Fert and Steril 1975; 26 (2): 175-79.
5. Gomez RR, Wunsch CD, Davis JH, Hicks DJ. Qualitative and quantitative determinations of acid phosphatase activity in vaginal washings. AJCP 1975; 64(4):423-32.

6. Willott GM, Allard JE. Spermatozoa–their persistence after sexual intercourse. Forensic Sci Int, 1982; 19: 135-54

7. Silverman EM, Silverman AG. Persistence of Spermatozoa in the Lower Genital Tract of Women. JAMA 1978; 240:1875-77.

8. Davis A Wilson E. The persistence of seminal constituents in the human vagina. Forensic Sci Int 1974; 3: 45-55.

9. Parikh CK. A simplified Text Book of Medical Jurisprudence and Toxicology. 6th ed: CBS Publishers; 1999; 7.25-7.30.

10. Dixit PC. Textbook of Forensic Medicine And Toxicology. 1st Ed. PEEPEE; 2007; 391-94.

11. Glaister J, Rentoul E. Medical Jurisprudence and J Toxicology. 12th Ed. E. & S. Livingstone; 1966; 418 –23.

12. Sharpe N. The significance of spermatozoa in victims of sexual offences. Can Med Assoc J. 1963; 89:513-14.

13. Gordon I, Shapiro HA. Forensic medicine A guide to principles. 3rd Ed. Churchill Livingstone. 1988; 314-16.

14. Morrison AI. Persistence of Spermatozoa in the Vagina and Cervix. Br J Vener Dis 1972; 49:141-43.

15. Pollak OJ. Semen and Seminal Stains. Arch Path 1943; 35: 140-84.

16. Dongre AP, Shrigiriwar MB. Detection of spermatozoa & acid phosphatase level in vaginal fluid. JFMT 2000; 17 (1):1-5.

17. Reddy KSN. The Essentials of Forensic Medicine and Toxicology. 19th Ed. K. Suguna Devi; 2000. 347-50.

18. Sharon AK, Brenda E, Andrew JW. Effect of male age on semen quality and fertility: A review of literature. Fert and Steril 2001; 75 (2): 237–48.