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Effect of Ageing of Seminal Stains on Their Detection on Fabrics In Criminal Investigations

تأثير عُمر بقع السائل المنوي على اكتشافه على الأقمشة في التحقيق الجنائي

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Abstract

In sexual assaults the presence of semen on the crime scene, victim or suspect's belongings acts as crucial evidence for establishing the occurrence of the crime. Fabrics are a common piece of evidence obtained at such crime scenes. The detection of seminal stain on a fabric depends on various factors such as absorption by the fabric, the colour and texture of fabric, the age of the stain, environmental conditions, etc. The current study was conducted on three different types of fabrics: cotton, nylon and denim and were exposed to a temperature of 25˚C for four different time durations- 1 day (24 hours), 3 days (72 hours), 5 days (120 hours) and 7 days (168 hours). Three different tests were performed on the samples to be tested. Accordingly, fluorescence test, Barberio's test and Christmas tree staining test were carried out. It was determined that the fluorescence of the seminal stain increased with increasing time duration. The observations made in Barberio test was however, not seen to be affected by the time intervals. It was also observed that the sperm density was affected with increase of time of incubation of the seminal stains.

Keywords: Forensic Science, Forensic Serology, Seminal Stains, Porous Substrates, Ageing of Stains.

المستخلص

في حالات الاعتداء الجنسي، يعد وجود السائل المنوي في مسرح الجريمة، على متعلقات الضحية أو المشتبه به بمثابة دليل حاسم لإثبات وقوع الجريمة. وتعد الأقمشة دليل شائع يتم الحصول عليه في هذه النوعية من مسارح الجريمة. حيث يعتمد اكتشاف البقعة المنوية على القماش على عوامل مختلفة مثل امتصاص القماش، ولون القماش وملمسه، وعمر البقعة، والظروف البيئية، وما إلى ذلك. وقد أجريت الدراسة الحالية على ثلاثة أنواع مختلفة من الأقمشة: القطن، النايلون والجينز، حيث تعرضت هذه الاقمشة لدرجة حرارة 25 درجة مئوية لأربع فترات زمنية مختلفة - يوم واحد (24 ساعة)، 3 أيام (72 ساعة)، 5 أيام (120 ساعة) و 7 أيام (168 ساعة). وتم إجراء ثلاث اختبارات مختلفة على العينات المراد فحصها. ووفقًا لذلك، وتم إجراء اختبار الوميض الفلوريسي واختبار باربيريو واختبار صبغة شجرة عيد الميلاد. من الملاحظات التي تم الحصول عليها أثناء التجربة، وجد أن وميض البقعة المنوية يزداد مع زيادة المدة الزمنية. ومع ذلك، لم يُنظر إلى الملاحظات التي تم إجراؤها في اختبار باربيريو وذلك لتأثرها بالفترات الزمنية. كما تم ملاحظة أن كثافة الحيوانات المنوية تتأثر سلباً بزيادة وقت حضانة البقع المنوية.

الكلمات المفتاحية: علوم الأدلة الجنائية، علم الأمصال الجنائي، البقع المنوية، الركائز المسامية، تقادم البقع.

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1. Introduction

The current study focuses on the visualisation of fluorescence, crystal formation (here, spermine picrate) and spermatozoa in a given seminal stain that have been deposited on different fabrics under controlled conditions. The aim of the study was to study the effect of different time intervals on this examination and how similar conditions of time intervals could affect the examination procedure in a crime scene as well.

Physiological fluids and biological material are two customary types of physical evidences found on any violent crime scene. With the advent of DNA typing from these body fluid components, the possibility of individualization has been developed, thus increasing their evidentiary value. These body fluids such as semen initially aid in the establishing of the mentioned incident (such as sexual assault) and then go as further as the identification of the culprit (DNA typing from spermatozoa). The main objectives of biological evidence analysis are identification (or classification), individualization (DNA typing), and reconstruction of the crime. Classification of the evidence refers to determining whether it is blood, semen, or another bodily substance [1]. Individualization of biological evidence is based on DNA typing done from the cells present in the fluids, which is highly discriminating and has the power to attribute biological evidence to an individual with an extremely high degree of probability.

1.1 Examination of Evidences on Sexual Assault Crime Scenes

A sexual assault evidence usually but not always, consists of three types of evidences: evidence from the victim, evidence from the crime scene and evidence from the accused. The evidence from the victim and the accused are usually collected by a Medical Officer (M.O) and forwarded to the forensic laboratory. These evidences include vaginal swabs, oral swabs, anal swabs, nail clippings, hair samples, etc. The evidence from the crime scene is collected on reaching the site and they usually include clothing items of the victim and/or accused, soil samples and other samples depending on the case. The examination of the case also depends on what happened during the crime and how long the victim waited to come forward to report the same. As time lapse between the crime and its investigation increases, the chances of finding semen evidence on swabs and other similar evidences decreases. However, semen evidence on clothing items such as the panties of victim can still show positive result for semen. In many of the cases, the semen stains found on the clothing of victim and condoms are crucial evidences. Condoms are useful because they have the probability of providing semen as evidence in the inside and vaginal fluid on the outside.

1. 2 Examination Of Seminal Stains

The examination of stains and deposits is done by using presumptive tests for screening on the items of clothing where the biological material (such as semen) is suspected to be present. The presence of semen confirms sexual activity; but it fails to confirm whether the activity was consensual, non-consensual, or due to masturbation. The normal ejaculate volume of human males ranging from 2 to 6 ml contains a mean value of about 100 million sperm cells per millilitre.

1. 2. 1 Visual examination of semen stains

While locating semen stains on clothing it has been observed that the most common area for sampling is the inside crotch area of the underpants of the victim. It corresponds to the vaginal drainage from internal ejaculate. External drainage can also be seen on other clothing items such as sweaters,

pants, jackets, etc. Dried semen on clothing items usually appears as a yellowish white crust if it has not been diluted. On white coloured cotton garments, it usually has an off-white appearance. As the stain begins to age, it shows signs of stiffening and yellowing of the garment at that specific portion [1]. This can act as a guide to the examiner while locating semen stains. Along with this, it also starts seeping into the fibres of the fabric depending on the type of fabric. Fabrics such as denims usually tend to show low fluorescence as compared to the cotton fabrics [2]. Untreated and undiluted semen shows quite strong fluorescence and it can be located using suitable wavelengths of UV light. This fluorescence is seen due to the presence of molecules such as flavin and choline-conjugated proteins. This detection or screening technique is however very presumptive in nature as many molecules will fluoresce in a way similar to semen [1]. Additionally, not all semen stains will fluoresce, as exposure to different environments (temperature, humidity), different types of fabrics and different fabric treatments can affect its fluorescent activity. It has been understood through the researches that fluorescence could be detected on the older stains on fabrics to a comparatively greater extent than the fresh stains [3]. There are a number of studies that have been carried out to understand the effect of ageing of seminal stains on the spectroscopic analysis [4–8]. where other detailed information can also be obtained in one single measurement. Samples of human blood and semen were characterized utilizing Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR. There have been studies conducted previously with respect to ageing of other body fluids such as blood, urine, etc [4,8–10].

The current study however, focuses on the effect that could be perceived on the preliminary detection, presumptive test and a confirmatory test with regards to change in time since deposition and the nature of substrate.

An instrument, Video Spectral Comparator (VSC- 6000/HS) has been used in the current study to visualise the fluorescence, if any, of the seminal stains on the three different types of fabrics chosen (cotton, nylon and denim). The instrument is designed to detect the different optical properties of materials under examination. It is a non- destructive technique and does not alter the appearance of the concerned evidence. The instrument helps in irradiating different wavelengths of light along with different filters.

The instrument allows viewing and recording the response of the fabric on exposure of light of various wavelengths. This is useful for examining, locating and establishing a probability of the presence of some biological fluid on the fabric sample. In the current study, the fabric samples were exposed to different light conditions such as 365 nm, Transmitted UV (365nm) and a higher wavelength of 400-535 nm. The fluorescence for aged samples is easier to check on the absorbent surfaces as they retained the stain for a longer time. The semen samples show less fluorescence when in moist condition as compared to the dried condition of the stain [11]. The colour and nature of the fabric onto which semen was deposited also affects the detection of fluorescence of the seminal stain [12].

Once the visual examination of stain has been done, it helps in establishing the presence of a stain and also in locating the stain on the piece of fabric. To further test for the possibility of the stain being that of semen, it needs to be extracted from the fabric. In case of biological products, the solvent used in the process of extraction is usually distilled water. It was found that for chemical purposes, distillation furnished a convenient means for ridding

water from a great part of its impurities, therefore enabling easy extraction of the body fluid without any interference being caused by the solvent used [13]. The extraction procedure involves an overnight process of soaking the stained area of the fabric in distilled water. The extract obtained can then be used for further presumptive and confirmatory assays being conducted by the experimenter.

1. 2. 2 Microscopic examination for human seminal extract:

Barberio test:

Barberio test is one of the microscopic presumptive assays that can be done to check for the presence of semen. The test included the addition of alcoholic solution of picric acid to the seminal stain or a watery extract of the stain. On addition of this solution, within a few minutes the formation of yellow coloured rhombic needle shaped crystals was seen. The crystals formed are due to the reaction of spermine with picric acid leading to spermine picrate crystals. While examining a suspected stain, a small piece of the stained fabric is dipped in distilled water to allow the extraction. The extract is then taken onto a microscope slide and a drop of the reagent is added to it by means of a platinum loop. After waiting for a minute or two, a cover- glass is placed over it and the preparation is then examined under a compound microscope. For a positive reaction, the crystals will be seen at once or appearing in the course of a minute or so. They are usually present only in certain areas of the specimen but are abundant and distributed throughout the prepared slide. The crystals vary considerably in shape and size, varying from 5 to 20μm, also subject to more variation. When fully developed, the crystals have the form of sharp-ended needles, or of rhombic prisms [14]. Figure 1 shows the spermine picrate crystals seen in a reference sample of seminal fluid. There

Figure 1- *Spermine picrate crystals seen in reference seminal fluid sample.*

have been a number of studies which have been carried out for the detection of seminal fluid using presumptive tests under various conditions that could be encountered at the crime scene as well as in the laboratories [15–17].

1. 2. 3 Microscopic examination for extracted human spermatozoa:

Christmas tree staining:

The Christmas tree staining is a slide staining technique used to microscopically identify spermatozoa. The test consists of two dyes- Nuclear Fast Red and Picroindigocarmine. Nuclear Fast Red is an acidic stain that stains the nuclear material present in the head of the sperm. The sperm heads are stained red and can be well differentiated from the acrosome, as the acrosome is comparatively less densely stained and can be seen as a pinkish or transparent region in contrast to the remaining portion of the head. The sperm tails and epithelial membranes are stained green by picroindigocarmine [18]. The sperms have been found to be present for upto 18 hours and the heads have been detected upto 24 hours after intercourse [19]. Figure 2- depicts the sperms visualized with the Christ-

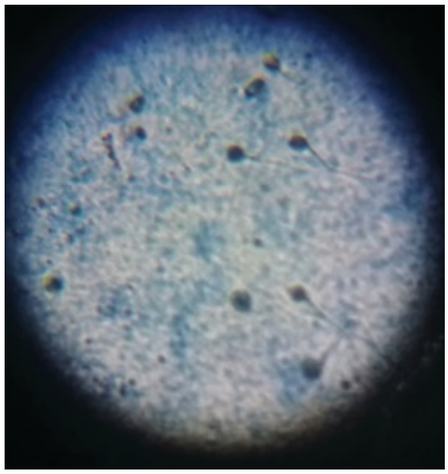


Figure 2- *Spermatozoa seen under 40X magnification of compound microscope after Christmas tree staining.*

mas tree staining method for a reference sample under a magnification of 40X of a compound microscope. Christmas tree staining, however is not the only test that could be used as a confirmatory test for semen. Studies have been conducted focussing on other components of semen that could be used to identify the body fluid [20].

The null hypothesis of the current study states that there is no correlation between the variables fluorescence of the seminal stain and sperm density with the ageing of the stain (time duration for which the stain was incubated). This suggests that an increase/ decrease in the time interval of the incubation of the stain would not have any effect on the fluorescence and sperm density of the stain.

The alternate hypothesis of the current study states that there is a correlation between the variables, namely fluorescence of the seminal stain and sperm density with the ageing of the stain (time duration for which the stain was incubated). This means that an increase/ decrease in the time period of the incubation of the stain would affect the fluorescence and sperm density of the stain either positively or negatively.

2. Materials And Methodology

The materials required for conducting the experiment included the following: **Glasswares;** Beakers, eppendorfs, micropipettes, glass slides, coverslips, petri dishes, watch glass, glass stirrer, forceps, etc., **Instruments;** Incubator, Video Spectral Comparator- 6000/HS, Compound microscope, vortex mixer, **Chemicals;** Semen sample, Picric acid, Neutral red, Picro-indigo carmine and distilled water, **Miscellaneous;** Fabric samples (48 in number, 8 cm * 8 cm), brush, nichrome loop etc. The experiment was conducted using three types of fabric samples namely cotton (white), nylon (white) and denim (blue) of dimensions 8 cm * 8 cm. The number of fabric samples taken were 4 (1 negative control and 3 replicates) of each type for carrying out the experiment in one condition. A subject was voluntarily asked to provide the semen sample and the semen sample was then deposited onto the different fabric samples using micropipettes. 200 µl of semen sample was deposited onto the centre of each of the fabric samples which were then kept for incubation in controlled environment within an incubator for varying time intervals at a constant temperature of 25°C. The different time intervals chosen for the experiment included 1 day, 3 days, 5 days and 7 days. On completion of incubation, the samples were then removed from the incubator and observed for fluorescence and diffusion of the stain using Video Spectral Comparator (VSC) - 6000/HS (High Spectra). The fabric samples were observed under alternate light sources such as Fluorescent light (400-535 nm) (using red filter), Ultraviolet light (365 nm) and Transmitted Ultraviolet light (365 nm). Figure 3 shows the arrangement of the fabric samples kept for incubation under controlled condition of temperature.

Figure 3- *Fabric samples deposited with seminal fluid kept for incubation at 25°C.*

2.1 Fluorescence detection of sample on fabric:

The fabric samples were visualised under Foster and Freeman's Video Spectral Comparator (VSC) - 6000/ HS. The samples were visualised under three different ranges of wavelengths- 400 to 535 nm, 365 nm (Ultraviolet light) and 365 nm (Transmitted Ultraviolet light).

2. 2 Extraction procedure:

After checking for fluorescence, the samples had to undergo extraction procedure to conduct the further analysis that included Barberio test and Christmas tree staining. The extraction procedure was carried out by cutting 1 cm * 1 cm from the centre of each fabric and immersing it in 500 µl of distilled water in eppendorf tubes. The eppendorf tubes were then subjected to vortexing at 500 rpm for 2 minutes. The tubes were then allowed to stay overnight to allow the extraction of semen from the fabrics to occur successfully.

2.3. Microscopic examination of crystals using Barberio test

The extracted liquid was then used for the further analysis.

1. About 5 µl of the liquid was pipetted onto the glass slide and one drop of Picric acid was added to it, covered with a cover slip and then allowed to dry.
2. The slides were then observed under 40X of a compound microscope to detect for the presence of any yellow-coloured needle shaped crystals of spermine picrate.
3. Once the crystals were seen, the given sample could help us in establishing the presence of semen in it.

The possible outcome for the Barberio's test could be that spermine picrate crystals are either present or absent for the individual slides with an exception for the negative controls which would show an absence of the crystals.

2. 4 Microscopic examination of spermatozoa using Christmas tree staining:

Once Barberio test was completed, the further confirmatory test of Christmas tree staining was conducted.

1. The same amount of sample (i.e. 5 µl) was takₘen onto a glass slide; a smear was prepared using a nichrome loop and heat fixed.
2. One drop of Neutral red solution was added to it and allowed to dry for 15 minutes at room temperature. On completion of the stipulated time, the excess stain was washed off using distilled water.
3. One drop of Picroindigocarmine was then added onto the stain and allowed to stay for 15 seconds. The excess stain was then washed off using ethanol.
4. On drying, a cover slip was placed onto the stain and observed under 40X of compound microscope to check for the presence of any spermatozoa (whether intact or disintegrated).

Table 1- *Scoring for the tests conducted.*

Fluorescence (0/ 1/ 2/ 3)	Fabric type	Wavelength of light (nm)
Undetected/ Weak/ Moderate/ Strong	Cotton/ Nylon/ Denim	400 - 535
Undetected/ Weak/ Moderate/ Strong	Cotton/ Nylon/ Deinim	365 (UV)
Undetected/ Weak/ Moderate/ Strong	Cotton/ Nylon/ Denim	365 (Transmitted UV)
Fabric type	Scoring on the basis of spermatozoa seen	Description of the score based on sperm density
Cotton/ Nylon/ Denim	0	Negative
	1	Hard to find (1- 5 sperms in one field)
	2	Easy to find; Some sperms in some fields (approximately 5- 15 sperms in 3 fields each)
	3	Many sperms (5- 15 or greater) in most fields
	4	Many sperms in every field (greater than 20)

3. Observations

The three types of fabrics that were used to conduct the study were white cotton, white nylon and blue denim. On making physical examination of the fabrics after specifc intervals of time, a yellowish or off-white crust could be seen on the white cotton and white nylon fabrics, and such appearance was not to be seen in denim. It was also observed that as the stain begins to age or dry on the fabric, the area of the stain increases in size as it is seen to have diffused to the surrounding areas of the fabric from where it was deposited. The diffusion of the stain was seen to be the least in denim; on nylon, the diffusion was such that irregular margins of the stain could be observed. Table -2 indicates the scores obtained during the observation of the seminal stains deposited on the different fabrics when subjected to different wavelengths of light. As the stain begins to age, its starts diffusing and drying on the fabric itself and this led to a comparative increase in the efficiency of detection of the stain using lights of different wavelengths. As the stain starts drying on the fabric, its fluorescence was seen to have increased. In case of denim, the fluorescence detected was the least. This might have occurred due to the masking of fluorescence of the stain due to the fluorescent properties of the background (here, the fabric, denim). The fluorescence was seen to be increasing as the wavelength of light used increases upto 535 nm. Accordingly, the fluorescence was easily visible under the wavelengths 400- 535 nm and not very easily detected under 365 nm. The fluorescent semen stains often show banded structure (with certain regions showing more fluorescence in comparison to others). This structure can be observed more clearly when the stain diffuses on the fabric. It also means that there are multiple components in semen that experience chromatographic separation as they migrate through the fabric. The Barberio test showed no effect of the ageing of the stain on spermine picrate crystal formation. This suggests that the component, spermine of the seminal fluid does not get affected when the stain is kept at standing for a maximum duration of 1 week. It showed no increase or decrease or any other significant difference in the shape and size of the crystals formed. The Christmas tree staining helped in understanding that as the stain begins

Table 2- *Observations for fluorescence detection of sample*

TIME (hrs)	FABRIC TYPE								
	COTTON			NYLON			DENIM		
	fluorescent (400-535nm)	UV (365nm)	TUV (365nm)	fluorescent (400-535nm)	UV (365nm)	TUV (365nm)	fluorescent (400-535nm)	UV (365nm)	TUV (365nm)
24 (1 day)	Strong (3)	Weak (1)	Weak (1)	Moderate (2)	Undetected (0)	Undetected (0)	Weak (1)	Undetected (0)	Undetected (0)
72 (3 days)	Moderate (2)	Weak (1)	Moderate (2)	Strong (3)	Undetected (0)	Undetected (0)	Weak (1)	Undetected (0)	Undetected (0)
(days 5) 120	Moderate (2)	Moderate (2)	Strong (3)	Strong (3)	Undetected (0)	Undetected (0)	Moderate (2)	Undetected (0)	Undetected (0)
(days 7) 168	Moderate (2)	Moderate (2)	Strong (3)	Strong (3)	Undetected (0)	Undetected (0)	Strong (3)	Undetected (0)	Undetected (0)

Table 3- *Observation table for Christmas tree staining*

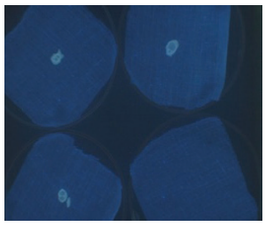
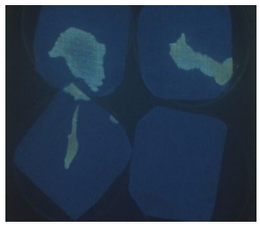
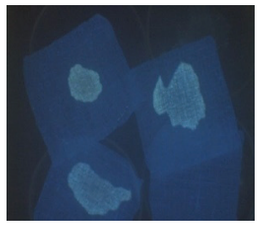
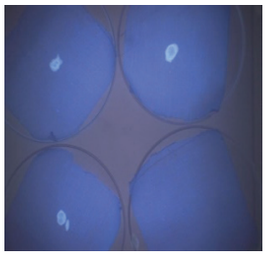
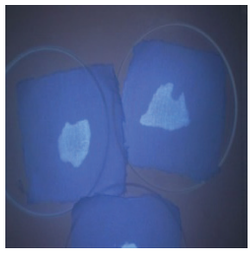
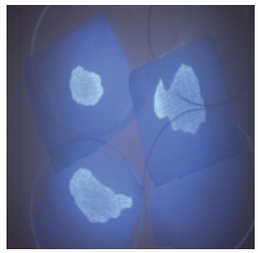
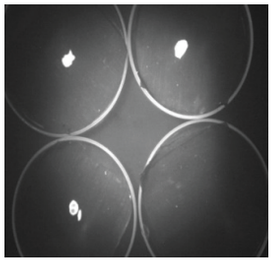
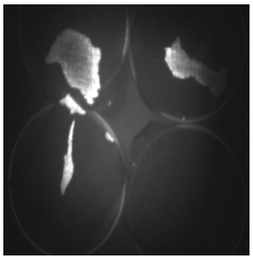
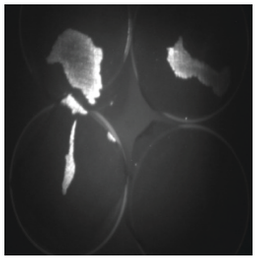
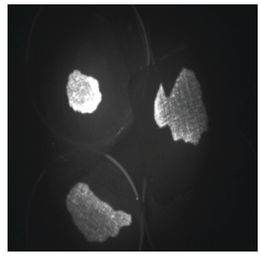
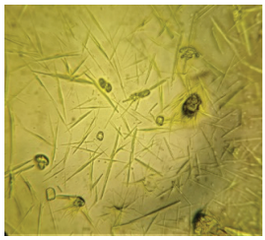
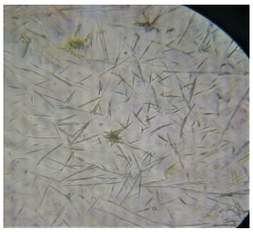
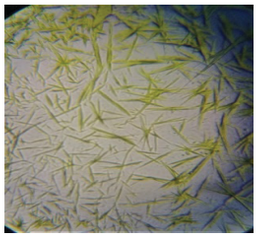
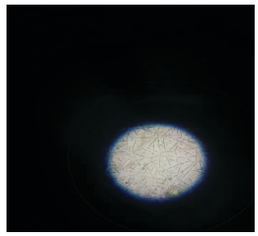
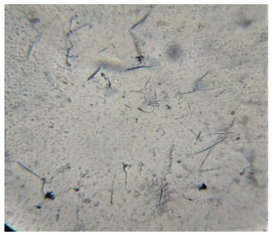
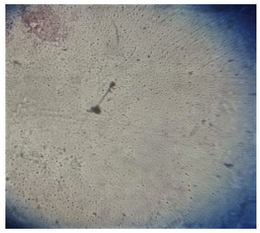
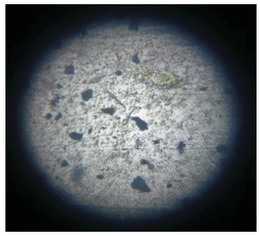
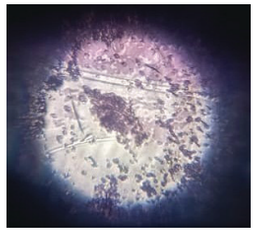
TIME INTERVAL (hrs)	FABRIC TYPE					
	COTTON		NYLON		DENIM	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
24 (1 day)	+3	+3	+2	+2	+3	+2
72 (3 days)	+2	+2	+1	+1	+1	+1
120 (5 days)	+1	+1	+1	+1	+1	+1
168 (7 days)	+1	+1	+1	+1	+1	+1

to age, the number of disintegrated sperms seen i.e. broken heads and tails, increases. Table-3 shows the observations made during the microscopic examination of the extracted fluid using the Christmas tree staining method. In the initial period of 24 hours, the stain consisted of intact spermatozoa with long tails. However, as the time duration increases, the number of intact sperms seen decreases and the number of heads and tails in disintegrated form get detected to a greater extent. It was also seen that the disintegration of spermatozoa was seen to be increasing in the order of denim greater than nylon, and the latter greater than cotton. The number of spermatozoa seen was also recorded to be usually greater in cotton fabric than the remaining two. This might be occuring due to efficient extraction of the seminal fluid and spermatozoa from cotton fabric as compared to both nylon and denim.

4. Result

From the observations it could be devised that the fluorescence of the stain was more easily detectable on the fabrics as the time interval was increased. Also, it was found to be more detectable

Table 4- *Observations for cotton fabric for different tests**

Hours 24	Hours 72	Hours 120	Hours 168
 Figure 1	 Figure 6	 Figure 11	 Figure 16
 Figure 2	 Figure 7	 Figure 12	 Figure 17
 Figure 3	 Figure 8	 Figure 13	 Figure 18
 Figure 4	 Figure 9	 Figure 14	 Figure 19
 Figure 5	 Figure 10	 Figure 15	 Figure 20

*** Description:** Figure 1, *Figure 6, Figure 11, Figure 16: Cotton fabric as viewed under UV (365 nm; Figure 2, Figure 7, Figure 12, Figure 17: Cotton fabric as viewed under Transmitted UV (365 nm); Figure 3, Figure 8, Figure 13, Figure 18: Cotton fabric as viewed under Fluorescent light (400- 535nm); Figure 4, Figure 9, Figure 14, Figure 19: Barberio test: Cotton fabric; Figure 5: Many sperms seen in cotton; Figure 10: Sperm seen in cotton; Figure 15: One sperm seen at the centre in cotton; Figure 20: Sperm heads seen in cotton.*

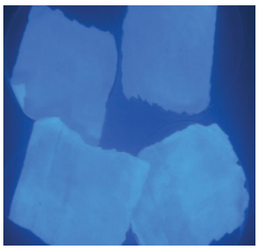
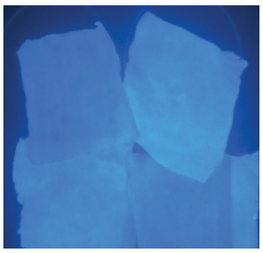
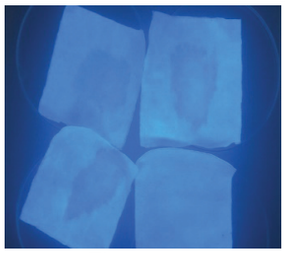
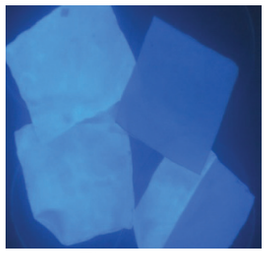
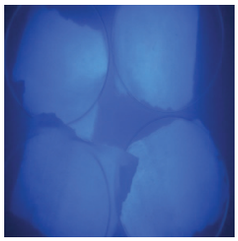
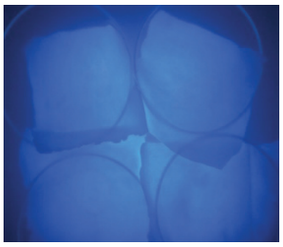
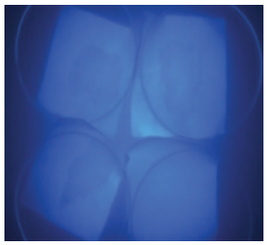
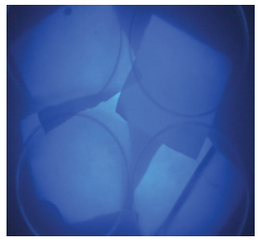
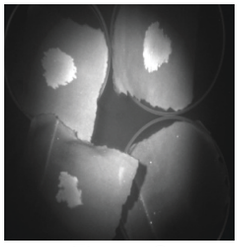
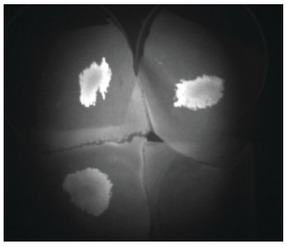
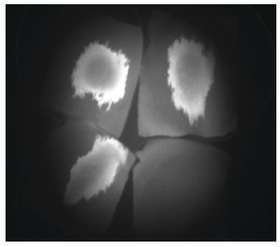
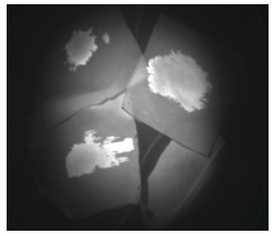
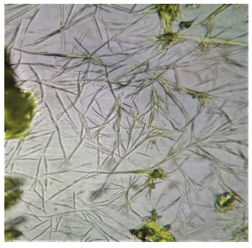
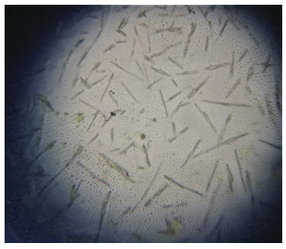
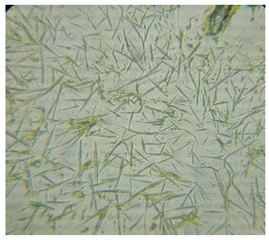
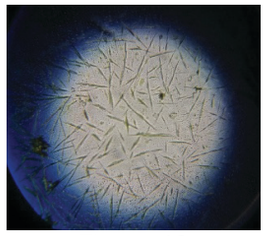
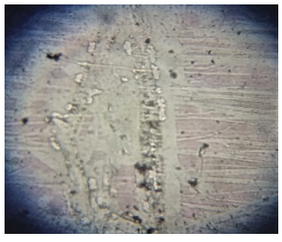
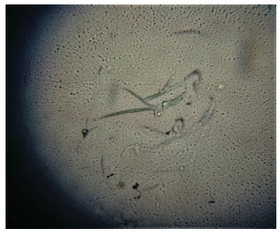
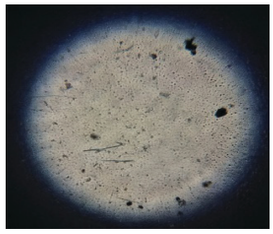
on cotton than the remaining two. Conversely, in Christmas tree staining, the sperm density was seen to have decreased as the time interval increased. It was also seen that the extraction of spermatozoa from the fabric was most efficiently carried out on the cotton fabric. In order to obtain the result statistically, the Pearson correlation test was applied.

It could be inferred from the analysis that the value of the coefficient of correlation for all the three fabrics namely cotton, nylon and denim for the fluorescence test was 0.89, 0.77 and 0.94 respectively. The positive value of the correlation coefficient indicated that there exists a positive correlation between the fluorescence detected on the fabric and the time interval after which it was tested. Thus, it suggests that as the time interval of the test increases, the fluorescence detection also becomes comparatively more efficient. Similarly, the value of the coefficient of correlation for cotton, nylon and denim for the microscopic examination of spermatozoa using the Christmas tree stain was found to be -0.94, -0.77 and -0.77 respectively. The negative value of the correlation coefficient indicated that there exists a negative correlation between the sperm density found in the extract and the time interval after which the test was conducted. This suggests that as the time interval increases, the sperm density of the stain decreases. This relationship is however not found to be linear. In case of Barberio test, no such relation was found. Thus, the increase or decrease in the time interval had no effect on the result of the Barberio test. Thus, from these results we can conclude that the null hypothesis could be rejected and the alternate hypothesis is accepted. This means that there is a correlation between the variables: fluorescence of the seminal stain, the sperm density and the ageing of the stain (time duration for which the stain was incubated). This correlation is found to be positive between fluorescence of the stain and the ageing of the stain whereas it is found to be negative for the sperm density of the stain and the ageing of the stain.

Conclusion And Discussion

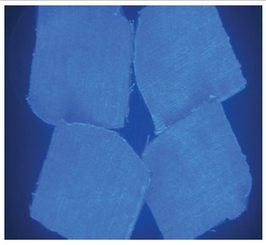
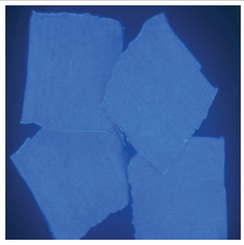
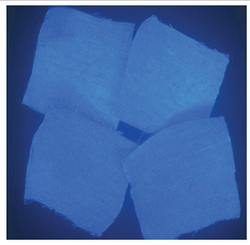
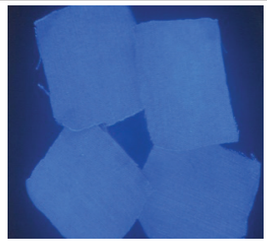
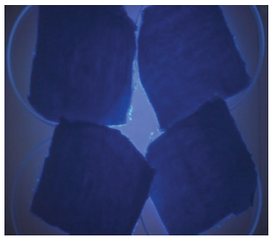
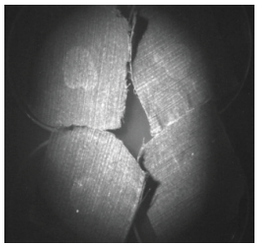
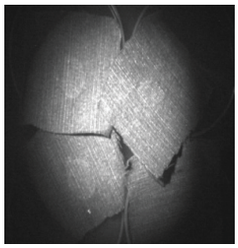
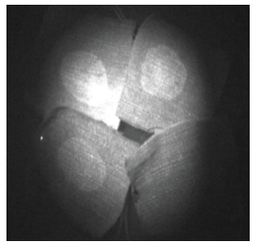
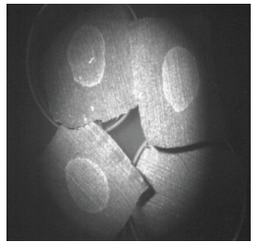
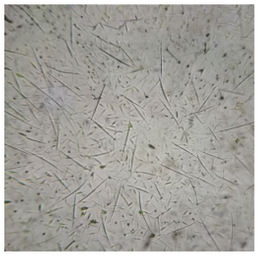
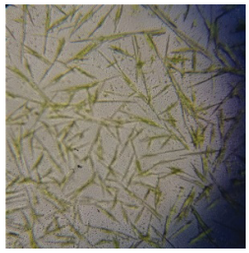
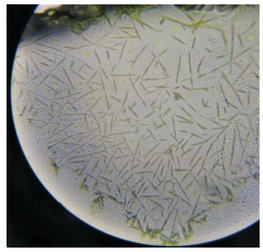
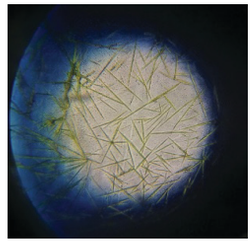
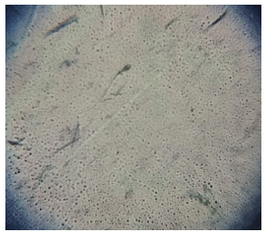
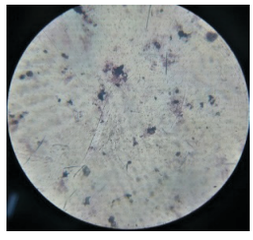
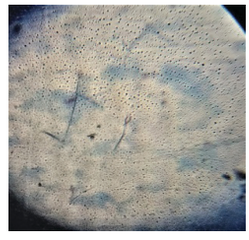
The study helped in understanding that the fluorescence of seminal stain on a fabric depends on the type of fabric onto which it was deposited, the amount of diffusion that has occurred on the fabric, the wavelength of light that has been used to visualise the stain and the time interval after which the stain was being visualised. It could be inferred from the study that the fluorescence was more easily detected on cotton and nylon in comparison to denim. The dyes used in the production of denim might act as interfering materials and thus lead to masking of the inherent fluorescence of the stain. As the time duration was increased, the stain diffused more onto the fabric and got dried and fixated on the fabric itself. This helped in increasing the fluorescence of the stain. However, deeper absorption into the bulk of the fabric might lead to deterioration in the fluorescence of the stain. The current study was subject to a maximum duration of one week. It was found that the stain could be more easily located when light of wavelength 400- 535 nm was made incident onto the stain. The other test conducted for the microscopic examination of seminal fluid was Barberio's test. This test led to positive results for all the samples irrespective of the type of fabric and the time duration. This increases the sensitivity and reliability of the test. The reliability of the test was checked by conducting the test for a negative control sample which led to a negative result. Thus, this test could be used to check for presence of semen in case of abnormalities such as oligospermia, aspermia or surgical procedures such as vasectomy. The next test conducted was a microscopic examination of spermatozoa in the seminal fluid. This test was used to check

Table 5- *Observations for nylon fabric for the different tests*.*

Hours 24	Hours 72	Hours 120	Hours 168
 Figure 1	 Figure 6	 Figure 11	 Figure 16
 Figure 2	 Figure 7	 Figure 12	 Figure 17
 Figure 3	 Figure 8	 Figure 13	 Figure 18
 Figure 4	 Figure 9	 Figure 14	 Figure 19
 Figure 5	 Figure 10	 Figure 15	 Figure 20

*** Description:** *Figure 1, Figure 6, Figure 11, Figure 16: Nylon as viewed under UV (365nm); Figure 2, Figure 7, Figure 12, Figure 17: Nylon as viewed under Transmitted UV (365nm); Figure 3, Figure 8, Figure 13, Figure 18: Nylon as viewed under Fluorescent light (400-535nm); Figure 4, Figure 9, Figure 14, Figure 19: Barberio test: Nylon fabric; Figure 5: Many intact sperms seen in one field in nylon, Figure 10: Sperms seen in nylon but lower than cotton; Figure 15: Few intact sperms seen in nylon, Figure 20: Sperm heads seen in nylon.*

Table 6- *Observations for denim fabric for the tests conducted**

Hours 24	Hours 72	Hours 120	Hours 168
 Figure 1	 Figure 6	 Figure 11	 Figure 16
 Figure 2	 Figure 7	 Figure 12	 Figure 17
 Figure 3	 Figure 8	 Figure 13	 Figure 18
 Figure 4	 Figure 9	 Figure 14	 Figure 19
 Figure 5	 Figure 10	 Figure 15	 Figure 20

*** Description:** *Figure 1, Figure 6, Figure 11, Figure 16: Denim as viewed under UV (365nm); Figure 2, Figure 7, Figure 12, Figure 17: Denim as viewed under Transmitted UV (365nm); Figure 3, Figure 8, Figure 13, Figure 18: Denim as viewed under Fluorescent light (400- 535nm); Figure 4, Figure 9, Figure 14, Figure 19: Barberio test: Denim fabric; Figure 5: Sperm seen at the centre in denim , Figure 10: Intact sperm seen at the centre in denim, Figure 15: Few sperm heads seen in denim, Figure 20: Two sperms seen at the centre in denim.*

for the presence of sperms in the given stain and the effect of increasing time interval on their identification. Accordingly, it was seen that as the time interval for incubation was increased, the sperm density as seen under a compound microscope gradually decreased. The reason behind this can be attributed to the drying of the stain which might make it difficult to extract the sperms from the fabric, thus leading to a lower sperm count in the sample. It could also be inferred from the results that the extraction of spermatozoa was most efficiently carried out from cotton fabric in comparison to the remaining two. Also, it could be concluded that as the standing time (here, incubation time) was increased, the probability of finding intact sperms decreased. The number of disintegrated sperms, sperm heads and tails were seen in a greater number as the time interval proceeded to one week. In certain slides, the length of the tail of the sperms was also seen to be smaller due to disintegration owing to time duration.

However, there could be certain limitations to the current study. For the purpose of analysis seminal fluid was collected separately during each of the four conditions (24 hours, 72 hours, 108 hours, 120 hours and 168 hours) from the same individual. It has been taken into consideration that the seminal fluid provided during each of the conditions would not be subject to much variation (as belonging to the same individual) and the variation occurring would be within permitted limits, not affecting the result of the tests.

The study could pave a path for future studies wherein other environmental factors such as humidity, bacterial contamination, exposure to rain, etc. could be taken into consideration. Further researches could also be carried out to understand the components of a spermatozoon that undergoes fastest degradation and the ones that could be detected for a longer period of time. Once this is known, different methods and techniques could be devised ensuring efficient extraction of spermatozoa from old seminal stains deposited on fabrics.

Acknowledgements

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