

Original article

# Circadian Rhythm Effects on the Extraction and Identification of Semen on Semen-Stained Fabrics Using Seminal Fluid Biochemistry

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## ABSTRACT

The rate of rape and other sexually induced crimes is rapidly on the increase worldwide with weak empirical confirmational tools, and expensive financial implications. This is further compounded by poor proof of the time of the committal of the crime and its attendant effect on the parameter of interest. This called for a cheap and accurate approach involving the use of biochemical parameters indicative of seminal fluids on fabrics and other ancillary materials found in a crime scene. This study was therefore designed to validate a suitable seminal fluid biochemical approach to detecting seminal stained fabrics and the attendant circadian rhythm effects. Seminal fluid biochemical parameters used for the study include prostate-specific antigen, gamma-glutamyl transferase, acid phosphatase, magnesium, zinc, and inorganic phosphorus. These parameters were analyzed using WHO-approved methods after the extraction phase from dried stained fabrics. Forty (40) participants were recruited for the study divided into two groups; nocturnal and diurnal. The mathematical and empirical analysis data were analyzed using One-way ANOVA (Post Hoc) on an SPSS 25 version platform. The result showed a significant increase ( $p < 0.05$ ) in seminal PSA, GGT, and ACP in the semen-stained fabrics when compared to distilled water-stained fabrics. In a similar vein, there was a significant increase in seminal ACP and PSA concentrations in the nocturnal group when compared to the diurnal, whereas seminal GGT decreased. In conclusion, this study has shown that semen-stained fabric can be detected for the presence of seminal fluids using seminal PSA, ACP, and GGT. Also, the effect of circadian rhythm should be considered during result interpretation.

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## INTRODUCTION

Semen is a gray, opalescent, and turbid fluid that is secreted by male gonads, and released during ejaculation [1]. It is a blend of cells, sperm, and a combination of inorganic and organic substances [2]. Average male ejaculate approximately 3.5 ml of seminal fluid. Every milliliter can generate around 10-50 million sperm cells [2]. These figures may differ

with the age of the male and are adversely affected by medical conditions, genetic history, diet, smoking habits, and the use of illegal drugs [1].

In this context, seminal biochemistry involves the organic and inorganic molecules found in semen excluding sperm and other cellular components. Seminal fluid is made up of a lot of biochemical parameters inclusive of enzymes, hormones, lipids, proteins, and sugar. These molecules play vital roles in the sustenance of sperm and fertility in general. Some are in abundance in the fluid and others in trace quantity. This study employed those in abundance in the seminal fluid such as zinc, magnesium, inorganic phosphate, gamma-glutamyl transferase (GGT), acid phosphatase (ACP), and prostate-specific antigen (PSA). These seminal biochemicals are found in seminal fluids in higher concentrations compared to blood and are easily measured for clinical or research purposes.

Seminal fluid parameters are also of importance to the practice of forensic science. Forensic science encompasses the application of scientific knowledge and its techniques for jurisprudence and criminal justice. This includes evidence recognition, collection, processing, and interpretation for legal purposes [3]. Physical evidence is of great importance to the courts of law. Indeed, such evidence can be more valuable than an eyewitness account in particular cases [4-5]. One of the physical types of evidence commonly encountered and recovered from crime scenes is human body fluid like semen.

The detection of seminal fluid on fabric is a pointer to the occurrence of sex, masturbation, or maybe a spontaneous emission. This could form a fulcrum upon which investigations involving the occurrence of rape or other sex-induced crimes are carried out. Detecting and validating the presence of seminal fluid on fabric or clothing is quite difficult and complex. There are various methods which include alternate light source (ALS), sperm microscopy, rapid stain identification series (RSID), and DNA. These investigations could be preliminary or confirmatory, however, some are quite expensive, and beyond the reach of underdeveloped and developing nations due to the high, and complex technological involvements. In a similar vein, the methods enumerated above could be inconsistent and at variance with the investigation at hand due to some external, and internal factors.

Furthermore, there are also challenges and difficulties encountered in tracing or retrieving seminal fluid from clothing, the effect of the time of emissions, cases of azoospermia or aspermia, and the admissibility of evidence in the court of law [6-7]. Time of emission is also crucial in the determination of seminal fluid biochemical parameters due to the peak and other variations associated with circadian rhythms. This could affect the affirmation of semen-stained fabrics. This study is therefore designed to address the above by evolving parameters, and methods that are cheap, reproducible, accurate, and admissible in the court of law. Also, the effect of time of emission on the identification of semen-stained fabrics using seminal fluid biochemistry will be interrogated.

## **METHODS**

### ***Study area/sample size***

The study was conducted at the Federal University Otuoke in Otuoke town. Otuoke is a town in Ogbia Local Government Area of Bayelsa state, Southern Nigeria. The biochemical investigations were carried out at the Eni-yimini Laboratories (eL) LTD, Yenezue-Gene Epie, Yenagoa, Bayelsa State.

The study employed the G-Power software for the generation of the sample size. A total of forty (40) students consented and were consequently recruited into the study. The study was divided into two groups; nocturnal (midnight) and diurnal (noon) emissions of equal sample size.

### ***Ethical approval/selection criteria***

The ethical clearance and experimental protocol were approved and granted by the Department of Biochemistry, Federal University Otuoke, Bayelsa State. Individual consents were also extracted from the subjects before the study with an elaborate education of the thematic basis of the study.

All the students recruited were healthy and fit as established by the university physician after thorough physical examinations and medical laboratory evaluations. Students with a history of seminal disorders or who have been operated upon for reproductive ailments were excluded from the study. The research utilized mainly students from the Department of Biochemistry between the age range of 20 to 30 years old.

### ***Collection of samples/ Sample Preparation***

Sterile sample containers were given to forty male volunteers a day for the study. The subjects were asked to produce the semen into the containers using masturbation. The containers were then transported to the laboratory for the seminal dissemination of the various fabrics used for the study. Semen-stained fabrics were kept dry before the preparation for the extraction phase and then analytical.

The area of the fabrics stained with semen was marked with water-insoluble inks and then excised carefully (Figure 1). The excised portions were subsequently soaked in 5 mL of distilled water for 30 mins. The resultant mixtures were spun for 10 mins at 2000 rpm, the supernatant was decanted, then the residue was used for the biochemical analysis.



Figure 1. Pictorial representations of semen-stained cloth

### Laboratory analysis

Agappe reagents (UK) were used for the estimation of seminal inorganic phosphate, magnesium, and zinc concentrations. Also, Biosystem Kits (UK) was used for the estimation of the seminal acid phosphatase (ACP) and gamma-glutamyl transferase activities. In a similar vein, an Accubind ELISA kit (USA) was used for the estimation of seminal total prostate-specific antigen (tPSA) concentration using a microplate.

### Statistical analyses

Data were analyzed with the Statistical Package for Social Sciences (SPSS) program (SPSS Inc., Chicago, IL, USA; Version 18-21) and Microsoft Excel. One-way ANOVA (Post Hoc) was used in comparing the means of the various seminal biochemical parameters used for the study.

## RESULTS

The result section of this study elaborated on the comparisons of biochemical parameters found in abundance in seminal fluids in the various groups. These presentations were captured in tables, whereas the graphical patterns of these parameters were presented in figures.

Table 1 parameters (zinc, magnesium, inorganic phosphate) were not significant ( $p < 0.05$ ) when compared within and between the groups.

Table 1. Mean concentrations of some studied electrolyte concentrations of the various groups

Parameters	Distilled water	Semen-stained A	Semen-stained B	F-test	P-values
Mg (mmol/L)	0.4995±0.0082	0.3143±0.0997	0.3848±0.2208	1.786	0.222
Zn (mmol/L)	221.0250±1.6329	134.4120± 97.4589	249.7815± 51.3376	3.566	0.072
P (mmol/L)	0.0525±0.0050	0.7738±0.8279	2.4467±2.0525	2.565	0.180

Semen-Stained A- Nocturnal emission; Semen-Stained B- Diurnal emission. Mg- Magnesium, Zn- Zinc, P- Inorganic Phosphorus.  $P < 0.05$  - Significant

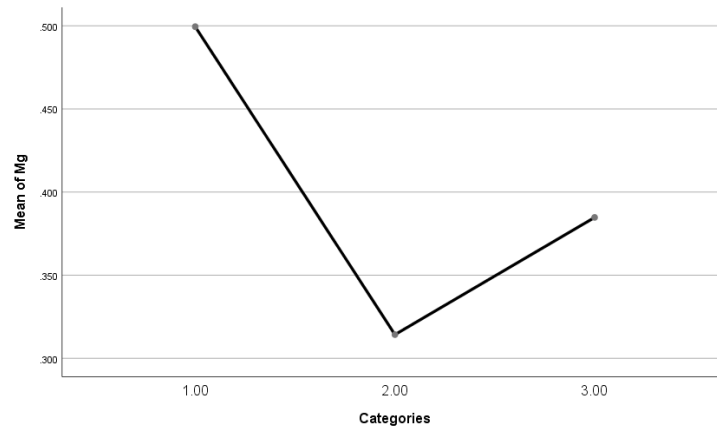
Table 2 shows a significant increase ( $p < 0.05$ ) in GGT activity in semen stain B when compared to semen stain A and distilled water. In a similar vein, there was a significant increase in ACP and PSA activities in group A when compared to that of B and distilled water. A comparison between semen-stained A and B, revealed a significant increase in concentrations of PSA and ACP in A when compared to B, whereas GGT was on the contrary.

Table 2: Multiple comparisons of seminal enzymes and antigen between the studied groups.

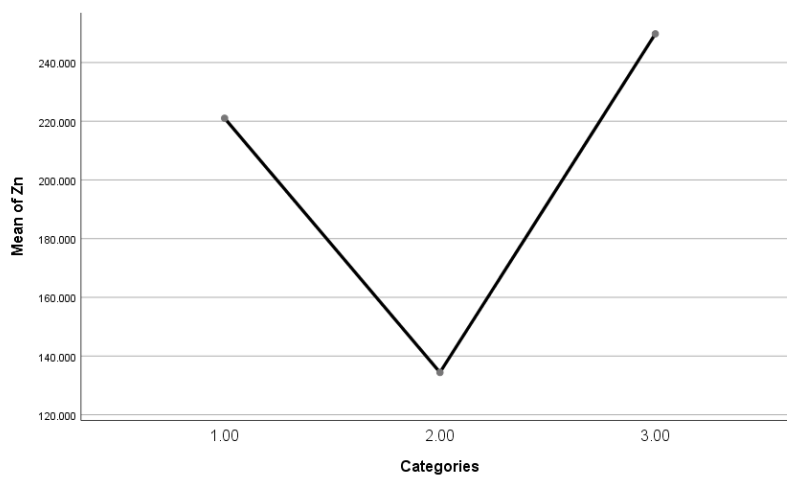
Parameter	Distilled water	Semen-stained A	Semen-stained B	F test	P value
GGT(U/L)	0.000 ± 0.000	0.0046 ± 0.0037 <sup>a</sup>	0.0700±0.0506 <sup>a,b</sup>	6.913	0.015
ACP(U/L)	0.4490±0.0083	1.6232±0.7087 <sup>a</sup>	1.3120±0.4266 <sup>a</sup>	6.489	0.018
PSA (ng/ml)	3.2695±0.0082	287.4410±0.0000 <sup>a</sup>	76.7655±21.5591 <sup>a,b</sup>	561.705	0.000

Semen-Stained A- Nocturnal emission; Semen-Stained B- Diurnal emission. GGT- Gamma-glutamyl transferase; ACP- Acid phosphatase; PSA- Prostate specific antigen,  $P < 0.05$  - Significant.

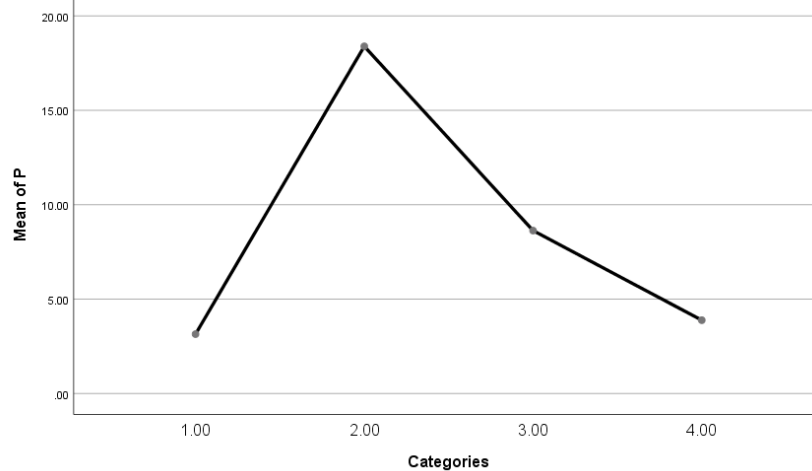
Figures 2-7 are the presentation of the comparative patterns of the various seminal parameters measured across the study groups.



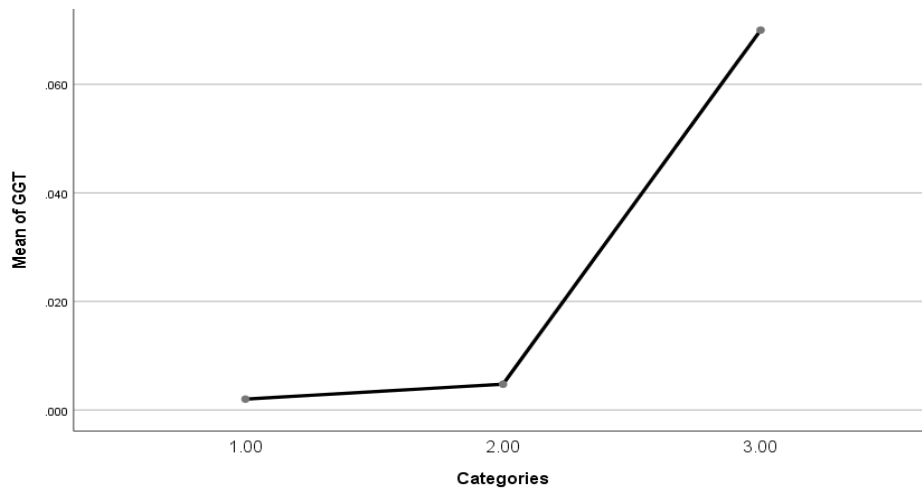
**Figure 2. Mean concentrations of Magnesium across the study groups.**  
1- Distilled water; 2- Semen-Stained A; 3- Semen-Stained B.



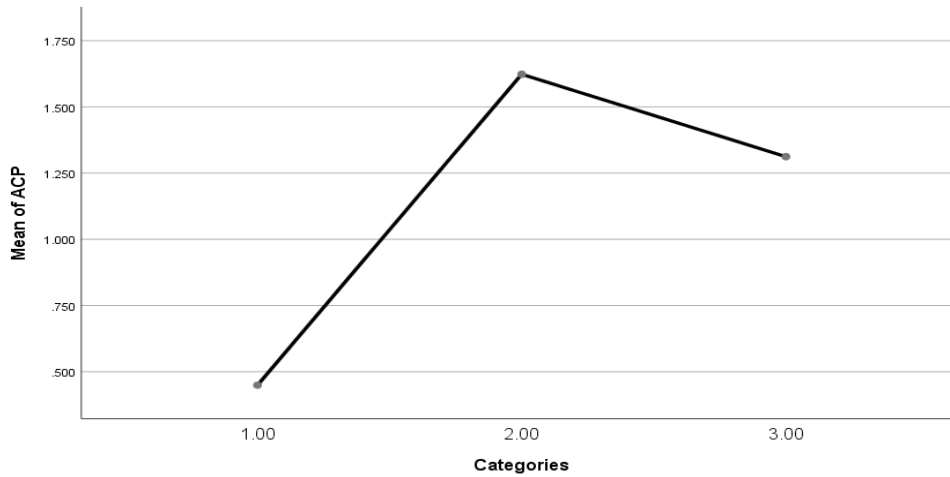
**Figure 3. Mean concentrations of Zinc across the study groups**



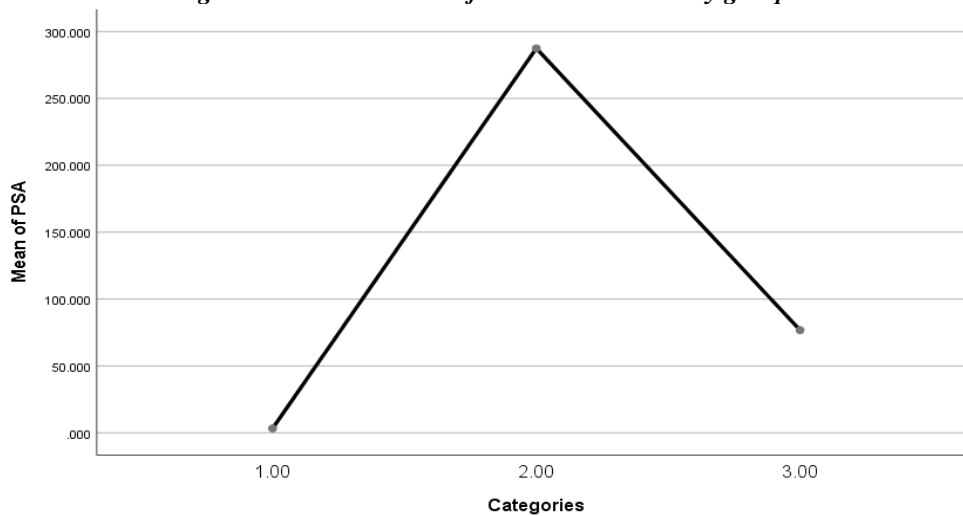
**Figure 4. Mean concentrations of Inorganic Phosphorus across the study groups**



**Figure 5. Mean activities of GGT across the study groups of PSA**



**Figure 6. Mean activities of ACP across the study groups**



**Figure 7. Mean concentrations of PSA across the study groups**



## DISCUSSION

The study revealed a non-significant difference in the studied electrolyte concentrations across the groups (Table 1; Figure 2-4). Irrespective of the non-significant posture, the presence of the study electrolytes was detected in the study matrices. The significant presence of the electrolytes in the matrices creates a major difficulty in its utilization for semen-stained fabric identification. This has demonstrated that the concentrations of magnesium, zinc, and inorganic phosphate cannot be used to detect rape cases and other sexually induced crimes. In a similar vein, the nocturnal and diurnal effects on seminal electrolytes analyzed were not significantly different, and hence cannot be factored in when considering the time effect.

Supporting the above discourse, zinc is naturally present in water, the average concentration is 0.6 – 5.0 ppb, and that of magnesium is between 40mg/L – 50mg/L, whereas the amount of inorganic phosphate in water is mostly within 1mg/L [8]. As posited by WHO on the concentrations of the studied electrolytes in water, coupled with that of detergent and the fabrics itself. The utilization of electrolytes in forensics is quite remote considering the combinational propensity. The need for seminal electrolyte tagging could be the only solution to the conundrum.

Furthermore, the statistical results showed that seminal activities of GGT, ACP, and PSA were significantly increased in the semen stains (A & B) when compared with the control (distilled water) (Table 2; Figure 5-7). The result of this study is suggestive of the suitability of PSA, ACP, and GGT in the detection of seminal fluids on fabrics both at nocturnal and diurnal. Seminal PSA, GGT, and ACP are enzymes and antigens produced by the testis and secreted in semen. Their presence is indicative of seminal fluids, though are also indicative of some other intracellular and extracellular fluids with considerably lower concentrations. This finding follows a handful of other studies [9-15].

The effects of circadian rhythm on the studied biochemical were interrogated. This will aid in the understanding of the expected concentrations of the studied parameters as they affect the time of ejaculation. The study presented that there was a significant increase in ACP and PSA activities during nocturnal ejaculation when compared to diurnal, whereas GGT was on the contrary. Other parameters measured were not significant (Table 1-2, Figure 1-6). This depicts that ACP and PSA were more elevated at night, whereas GGT at the daytime. The variation could be due to circadian rhythm. Studies have implicated circadian rhythm in several biochemical parameter alterations and are now considered in drug administration, and also sampling. A study observed a diurnal fall in PSA concentration in the afternoon [16]. Growth hormones, insulin, leptin, ghrelin, adiponectin, and melatonin have been seen to fall in the daytime, whereas they peak at night [17-21]. These findings are in line with the results of this study, hence circadian rhythm should be considered in forensic sampling especially cases involving hormones, enzymes, and antigens that will be used for the identification of seminal fluid stains.

## CONCLUSION

The study revealed a significant increase in seminal PSA, GGT, and ACP in semen-stained fabrics, whereas the electrolytes studied were not significant. Furthermore, the effect of circadian rhythm was elucidated. Nocturnal ejaculation tends to have higher concentrations of seminal PSA and ACP compared to diurnal, whereas GGT was on the contrary. Based on the findings, seminal PSA, ACP, and GGT could be used to identify semen stain fabrics in rape and other sex-induced cases.

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## Conflicts of Interest

The authors declare no conflicts of interest.

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## تأثيرات إيقاع الساعة البيولوجية على استخلاص وتحديد السائل المنوي على الأقمشة الملطخة بالسائل المنوي باستخدام الكيمياء الحيوية للسائل المنوي

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### المستخلص

يتزايد معدل الاغتصاب وغيره من الجرائم ذات الدوافع الجنسية بسرعة في جميع أنحاء العالم مع ضعف أدوات التأكيد التجريبية، والآثار المالية الباهظة الثمن. ويتفاقم هذا الأمر بسبب ضعف الأدلة على وقت ارتكاب الجريمة وتأثيرها المصاحب على معيار الفائدة. وهذا يستدعي اتباع نهج رخيص ودقيق يتضمن استخدام المعايير البيوكيميائية التي تدل على وجود السوائل المنوية على الأقمشة والمواد المساعدة الأخرى الموجودة في مسرح الجريمة. لذلك تم تصميم هذه الدراسة للتحقق من صحة النهج البيوكيميائي المناسب للسائل المنوي للكشف عن الأقمشة الملطخة المنوية وتأثيرات إيقاع الساعة البيولوجية المصاحبة. تشمل المعلمات البيوكيميائية للسائل المنوي المستخدمة في الدراسة مستضد البروستاتا النوعي، ترانسفيراز جاما جلوتاميل، الفوسفاتيز الحمضي، المغنيسيوم، الزنك، والفوسفور غير العضوي. تم تحليل هذه المعلمات باستخدام الطرق المعتمدة من منظمة الصحة العالمية بعد مرحلة الاستخراج من الأقمشة الملونة المجففة. تم تجنيد أربعين (40) مشاركاً للدراسة مقسمين إلى مجموعتين؛ ليلية ونهارية. تم تحليل بيانات التحليل الرياضي والتجريبي باستخدام ANOVA أحادي الاتجاه (Post Hoc) على منصة الإصدار SPSS 25. أظهرت النتائج زيادة معنوية ( $p < 0.05$ ) في PSA و GGT و ACP في الأقمشة الملونة بالسائل المنوي مقارنة بالأقمشة الملونة بالماء المقطر. وعلى نفس المنوال، كانت هناك زيادة كبيرة في تركيزات ACP و PSA المنوية في المجموعة الليلية بالمقارنة مع المجموعة النهارية، في حين انخفض GGT المنوي. في الختام، أظهرت هذه الدراسة أنه يمكن الكشف عن النسيج الملون بالسائل المنوي لوجود السوائل المنوية باستخدام PSA، ACP، و GGT. أيضاً، ينبغي النظر في تأثير إيقاع الساعة البيولوجية أثناء تفسير النتائج.

**الكلمات الدالة:** السائل المنوي، الإنزيمات المنوية، PSA، إلكترونات، أقمشة مصبوغة بالسائل المنوي، الاغتصاب، الطب الشرعي.