FIBROBLAST GROWTH FACTOR 7: IS IT EFFICIENT IN DETECTING SEMINAL STAINS AFTER LONG TERM STORAGE?

Iman F. Gaballah; Khaled A. Bayoumi; Laila Rashed*; Ezz El Din Mostafa Abd El Wahed Shalaby

Forensic Medicine and Clinical Toxicology Department - Faculty of Medicine - Cairo University.

*Biochemistry Department - Faculty of Medicine - Cairo University.

Corresponding author: Iman Fawzy Gaballah: imangabo@kasralainy.edu.eg

ABSTRACT

Introduction: proper detection and analysis of the biological evidence is a crucial matter for crime scene reconstruction and the most promising method for tissue determination is the analysis of differential DNA methylation patterns according to cell or tissue type. These patterns are chemically and biologically more stable thus representing a more reliable identification tool. Identification of semen and sperms are particularly important, especially in sexual assaults, where sperms are the source of DNA in the human ejaculate. In practice, sperms isolated from sexual assault evidence lose most of their distinctive sub-cellular organelles upon which morphological identification depends. **AIM OF THE Research:** to identify stability of the FGF7 marker in stored seminal specimens. **MATERIAL and METHODS:** 15 seminal specimens stored for over 10 years were used along with 5 fresh samples for FGF7 assessment. **Results and Conclusion:** DNA methylation proved to be a stable marker for both fresh semen and stored semen samples.

KEYWORDS: Epigenetic; DNA methylation; differential DNA methylation; FGF7; forensic detection of sperms; bisulphite conversion.

INTRODUCTION

1. DNA profiling and Epigenetics

At crime scenes, biological material such as blood, saliva and semen help forensic doctor's link suspects to a specific crime with DNA extraction being the first step providing a profile of the suspect. This DNA profile does not give any information about the source of the cells of DNA in question. Therefore, many techniques were studied such as mRNA, micro RNA, immune-based assays and DNA methylation **(Zubakov et al., 2010; Lee et al., 2011; Harbison and Fleming, 2016)**.

Epigenetic markers, being more stable than mRNA, were studied. Although the DNA sequence (genotype) is identical in each cell, its epigenetic profile (phenotype) is not, leading to the result that each cell has its own unique epigenome. Epigenetic modification is a naturally occurring process needed for a good functioning genome and its transcriptional regulation. These markers are also associated with genomic imprinting, cell memory and X-inactivation. Epigenetic changes are controlled by DNA methylation, histone modification and non-coding RNA-associated gene silencing **(Paliwal et al., 2010; madi et al., 2012)**.

The fate of each cell is controlled by epigenetic profiles, as they transform, by activation and suppression of particular

sets of genes, from simple fertilized eggs to more functionally and metabolically specialized cells. These genes are responsible for the functional and morphological features of different cells (**Friedl et al., 2012; Madi et al., 2012)**. According to**Sabeeha and Hasnain (2019),** these changes are characterized by:

- Being spontaneous is induced by environmental factors (nutrition, aging) or as a consequence of mutations.
- Changes may last through cell divisions until the cell's death or it may continue through several generations even if it does not cause changes in the underlying DNA sequence of the organism.
- They change the way genes behave, but not the genetic code sequence of DNA; it is the associated chromatin protein that becomes modified leading to gene activation or silencing thus enabling differentiated cells to only express genes necessary for their activity.
- If gene inactivation occurs in a sperm or egg cell resulting in fertilization, then these epigenetic changes can be transferred to the following generation, otherwise these changes may be preserved through cell division and only occur in an organism's lifetime. However, changes may be the result of DNA damage, which occurs about 60,000times/day/cell. Damages are repaired, but at the site of this repair, epigenetic changes can remain.

2. DNA methylation

This is a functional change to the genome but does not change nucleotide sequence where it only alters gene expression. It is the addition of a methyl group to the 5^{th}position of the cytosine ring in CpG (cytosine-phosphate-guanine) dinucleotide, a reaction catalyzed by DNA methyl transferase. It inhibits gene expression aiming at specific locations of the CpG dinucleotide clusters (CpG islands). These islands are more than 500bp in length and are associated with promoter regions in the genome. They generally contain a greater number of CpG sites than expected. De novo methylation occurs in germ cells or early embryo stages leading to uniqueness of methylation patterns in different tissues. These patterns are chemically and biologically more stable representing a better identification tool than RNA or proteins **(Forat et al., 2016; Richards et al., 2019)**.

2.1 Differential DNA methylation

According to cell type, chromosome segments (tissue-specific Differentially Methylated Regions (tDMRs)) show different methylation profiles. Thus, methylation status at a certain CpG site helps identify cell type. Methylation patterns at tDMR sites are stable and specific rendering them excellent identification markers.

2.2 DNA methylation analysis (Bisulphate modification)

Since DNA polymerase cannot differentiate between both methylated and unmethylatedcytosines, therefore direct DNA sequencing cannot determine methylation patterns. Bisulphate conversion is the best method for determining a DNA methylation profile of unique regions, which is a reaction, based on selective de-amination of cytosine with sodium bisulphate. It is a reaction specific to unmethylatedcytosines but not 5-methylcytosine nucleotides. This reaction leads to 2uncomplementary DNA strands ending up in analyzing single strands **(Lee et al., 2011; Richards et al., 2019)**.

Bisulphate modification leads to the conversion of unmethylatedcytosines into uracils followed by being amplified as thiamines; they also lead to the conversion of methylated cytosines into cytosines. Methylated and unmethylatedcytosines are detected using DNA sequencing. This method can be coupled with methylation-specific PCR using specific primers that

anneal to sites where bisulphate converted DNA exists. Application of bisulphite DNA conversion on genome DNA can provide detailed methylation information of every CpG island. The only drawback is the need of large DNA quantities because DNA loss and degradation occurs during the process of bisulphate conversion. This problem has been overcome and conversion occurs nowadays with samples as little as 1ngm of DNA. This conversion is a step prior coupling with other methods for successful methylation determination **(Lee et al., 2011; Xu et al., 2012; Hernandez et al., 2019)**.

Methylation analysis begins with bisulphate modification where it differentiates methylated from un-methylated cytosine bases at CpG sites. It converts un-methylated CYTOSINE to URACIL and amplification of modified DNA converts URACIL → THYMINE.

Methylated Cs →Ts

Un-methylated Cs → Us

According to **Tanaka and Okamoto (2007),** post-PCR detection of Cs and Ts indicates methylation pattern and according to their relative numbers at a specific site, the methylation pattern can be detected as follows:

- If 10 out of 10 nucleotides at this position is 'C', then the DNA is 100% methylated at this position.
- If 3 out of 10 nucleotides at this position is 'C', then the DNA is 30% methylated at this position.

3. Body fluid identification

Differential DNA methylation, mRNA and miRNA are used as tools for body fluid identification. Enzymatic and immunological tests are usually used for the identification of tissue-specific proteins since they require the presence and activity of specific proteins. Degraded proteins lead to the degradation of the biological material, which in turn leads to false negative results **(Frumkin et al., 2011)**.

Protein-based tests determine the presence or absence of certain tissue proteins only, but composition of mixed samples and connection to short tandem repeats (STRs) obtained from these samples is not addressed. Another drawback is cross reactivity to non-specific targets which are not always human-specific. Weak positive signals are detected when extracted samples are of low amount making them hard to interpret. Body fluid stains are usually invisible, in minute amounts or in mixtures leading to difficult identification. DNA methylation-based assay is a method that will not consume additional samples and it can solve cases from which only DNA was retained **(Grskovic et al., 2013; Gomaa and Salehi, 2017)**.

In sexual assault cases, location of seminal stains and semen identification (presumptive by detection of acid phosphatase and confirmatory by detection of prostate specific antigen) is crucial followed by Staining and identification of spermatozoa. DNA typing is necessary and in spite of its specificity, it might be difficult in cases where evidence is in low amount or degraded (of low quality) and it might suffer low specificity if the sample is contaminated**(Allery et al., 2001)**.

4. Fibroblast Growth Factor (FGF7)

This gene encodes a protein that is a member of the FGF family, which possesses mitogenic and cell survival activities. A previous research, done by the same author together with some of the colleagues **(Elmahdy et al., 2016),**under the title "Molecular Assessment of DNA

methylation Profiling for Body Fluid Identification in Medical Forensic Application" was done at the Department of Forensic Medicine and Clinical Toxicology, KasrAlainy school of Medicine, Cairo University which compared DNA methylation between different body fluids (saliva, semen, blood) where FGF7 in semen showed relatively greater methylation values. According to other researchers, FGF7 showed low methylation levels in epithelial tissue, blood and saliva rendering them indistinguishable. On the other hand, semen showed higher methylation values across the CpG sites with a conclusion that this marker is more appropriate for detection of semen**(Madi et al., 2012; Zinkle and Mohammadi, 2019)**.

MATERIAL & METHODS

- Initial tests used 5 semen samples (freshly obtained) to determine the marker specificity. Fifteen semen samples stored for 10 years in fabric and at room temperature in a clean dry place were alsotested to determine if the pattern of methylation remained constant during long-term storage.

DNA Extraction:

It was extracted utilizing nucleic acid extraction kit (NucleoSpin®) as follows:

- **Cells lysis:** 350µl buffer RA1 + 3.5µl of β-mercaptoethanol+ 200µl of each sample and vortex vigorously to perform lyaste.
- **Lysate filtration:** to reduce viscosity and clear lysate filtrate, NucleoSpin®Filter was added to collection tube (2ml) and centrifuged at 11,000 rpm for 1 min.
- **Adjustment of DNA binding conditions:** NucleoSpin®Filterwas discarded and 350µl of 70% ethanol + lysate and mixed by up and down pipetting (5 times).
- **DNA binding:** NucleoSpin® DNA column was placed in collection tube loaded with lysate and centrifuged for 30sec at 11,000rpm.
- **Washing and drying silica membrane:** first wash was done by adding 600µl buffer RA3 to DNA column and centrifuged. The flow-through was discarded. A second wash was done by adding 250µl buffer RA3 to Column and centrifuged for 2 min until complete dryness.
- **DNA elution: into a nuclease free collection tube, 1.5ml of** column was placed; DNA was then eluted in 60µl DNase-free H_2O and centrifuged for 1 min.

Primers:

Sequence of usedoligonucleotide primers for DNA methylation analysis are shown in the following table.

Table (1): Primers used for analysis of DNA methylation.

Gene symbol	Primer sequence (5' → 3')	Gene Bank accession number
FGF7	F:GGGTTTATATGTATTATTTGGTGGT R:CATTATATACTCCTCAAAACACACAC	AC087742.9
B-actin	F:TGGTGATGGAGGAGGTTTAGTAAGT R:ACCACCACCCAACACACAATAACAAACACA	AP013035.1

Bisulfite DNA Conversion:

Methylation status was determined using sodium bisulfite. Bisulfite modification of extracted DNA was done using EpiTect Bisulfite Kit as follows:

- DNA was thawed and for dissolving the bisulphate mix, 800µl RNAse-free water

was added to each aliquot, then vortexed until completely dissolved (up to 5 min).

- Bisulfite reactions were prepared in 200µl PCR tubes. The following was added
  a. 1-2µg DNA solution
  b. RNAse-free water to 20µl total (DNA + water must be 20µl)
  c. 85µl dissolved bisulfite mix
  d. 35µl DNA Protect Buffer
- Storage of the tubes occurred at room temperature.
- As an indication of sufficient mixing and correct pH, DNA buffer turns from green to blue after the addition of DNA-bisulfite mix.
- Tubes are then placed into thermal cycler and the program was started.

Quantitative real time PCR assessment:
DNA undergoing bisulphate modification was used as a template for fluorescence-qPCR**(Brait et al., 2012)**.Reaction mix samples were then applied in real time PCR.

Table (2): qPCR reaction mix components.

Reagent	Volume	Final concentration
2x SensiFAST™ SYBR®Hi-ROX Mix	**10µl**	**1x**
10 µM forward primer	**0.8µl**	**400nm**
10 µM reverse primer	**0.8µl**	**400nm**
H_2O	**Up to 16µl**	
Modified DNA template	**2µl**	
20µl Final volume		

Methylation Analysis:

Samples were measured as triplicates on each PCR run using the universal methylated human DNA standard (Zymo Research) and semen DNA as sample. Quantitative methylation was performed to detect and quantify the studied methylated genetic marker in human methylated recent and old semen samples. This technique allows accurate quantitative assessment of DNA methylation. Success of the PCR amplification was assessed with 1.5% agarose gel electrophoresis. The relative quantitation (RQ) of methylation is quantified according to the calculation of delta-delta Ct (ΔΔCt).

The RQ of each gene was calculated by taking $2^{-\Delta\Delta Ct}$ as follows:

ΔΔCt =
[(Ct target-Ct reference) of sample] – [(Ct target-Ct reference) of control]

Where:

- Ct target sample: Ct value of required gene in tested sample.
- Ct reference sample: Ct value of gene of reference in tested sample.
- Ct target control: Ct value of required gene in control DNA.
- Ct reference control: Ct value of gene of reference in control DNA.

RESULTS

In this research, FGF7 (semen specific epigenetic marker) was selected to differentiate between recent (5 samples) and 10year old semen samples (15 samples).

Table (3): mean and standard deviation compared between recent and old semen samples.

Epigenetic marker	Mean ±SD	Recent samples	10 year old samples	P-value
FGF7	Mean	28.6	25.44	0.9647
	±SD	±6.42	±2.7	

Table (3) showed no statistically significant difference between both recent and 10year old semen samples where the p value = 0.9647 (statistical significance occurs at $p<0.05$).

In addition, Receiver Operating Characteristic Curve (ROC curve) analysis was performed and area under the curve (AUC) values was evaluated. FGF7 showed good sensitivity and specificity which can serve as a good molecular marker for semen discrimination at crime scenes.

Table (4): showing sensitivity and specificity of FGF7 in semen identification.

AUC	p-value	95% confidence interval		Cut-off value	Sensitivity %	Specificity %
		Lower bound	Upper bound			
0.839	<0.001	0.774	0.901	0.7961	82.2	74.1

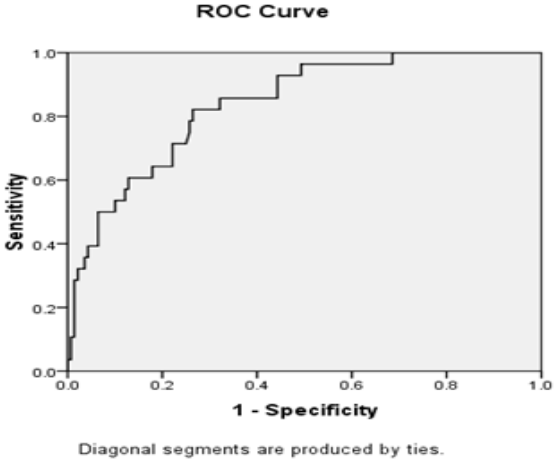

Fig. (1): ROC curve for detection of semen using FGF7.

DISCUSSION

DNA methylation is an investigative lead that solves crimes in forensic genetics. Identifying body fluids that utilize tissue-specific DNA methylation can contribute to solving crimes by predicting the activity that led to evidence material deposition **(Lee et al., 2016)**. Tissue of origin is the main difference in methylation profiles from different samples, regardless of its origin, whether the same or different individuals **(Ziller et al., 2013; Jiang et al., 2015)**.

In the present study, the potential of DNA methylation for semen was examined. FGF7 was selected, and DNA methylation profile was produced by bisulfite treatment using pooled seminal DNA. Several studies applied DNA methylation in body fluid identification but each study processes its own methodology and its own DNA methylation markers that differentiate it from other studies. Results proved that FGF7 methylation values were the same when comparing recent and 10 year old semen samples.

As for the specificity of FGF7 to semen, **Madi and his colleagues (2012)** examined a few genomic loci using bisulfite modification and pyrosequencing. Different biological samples were collected and methylation patterns at ZC3H12D and FGF7 loci succeeded in differentiating sperms from other biological samples.

Using pyrosequencing, **Fu and others (2015)** studied venous blood, saliva, semen, menstrual blood and vaginal fluid using 5 tDMRs for BIK, CYTH4, GAS2L1, MDFI, and OSM genes. Semen was successfully differentiated from other

body fluids using CYTH4-G-6 (6^{th}CpG site of the gene CYTH4). Sources of these body fluids were identified in 78% samples with good sensitivity and specificity. As for age and gender, no differences were found regarding the assays.

Since the time when Illumina's Human Methylation450 (450K) Bead Chip array provided methylation profiles at more than 450,000 CpG sites using genomic DNA quantities as little as 0.5 ug, researchers reported CpG markers that showed differential DNA methylation patterns in different types of body fluids based on the array results **(Lee et al., 2016)**.

Park and other researchers (2014) investigated novel DNA methylation markers (cg23521140 and cg17610929) for detection of semen to be utilized in forensic science and were evaluated using pyro sequencing that showed high sensitivity and specificity for identification of the target body fluid. **Lee and his colleagues (2015)** also identified cg17610929, cg26763284 and cg17621389 markers for semen using similar approach. Both studies suggested that cg17610929 was a semen-specific marker.

However, the comparison between recent and old male semen samples denoted that FGF7 showed no significant difference between both groups.

CONCLUSION

- FGF7 marker displays a specific methylation pattern in semen samples.
- In comparison to recently collected samples, samples collected 10 years before the date of the study showed the same methylation pattern.
- The process of bisulphate modification, PCR amplification and pyro-sequencing is highly feasible offering rapid results after short time (as short as 2 days).

RECOMMENDATIONS

Further studies should be done to identify:

- Different species.
- Body fluids in contaminated and/or mixed samples.
- Body fluids present in minute amounts.

REFERENCES

Brait, M.; Maldonado, L.; Begum, S.; Loyo, M.; Wehle, D.; Tavora, F.F.; Looijenga, L.H.; Kowalski, J.; Zhang, Z.; Rosenbaum, E.; Halachmi, S.; Netto, G.J. and Hoque, M.O. (2012):DNA methylation profiles delineate epigenetic heterogeneity in seminoma and non-seminoma. *Br. J. Cancer*, **106**: 414-423.

Elmahdy, R.M.A.; Shokri, D.A.; Gaballah, I.F. and Abdel-Fattah, D.S. (2016): Molecular Assessment of DNA methylation Profiling for Body Fluid Identification in Medical Forensic Application" was done at the Department of Forensic Medicine and Clinical Toxicology – KasrAlainy School of Medicine – Cairo university. Unpublished Doctorate (MD) Thesis.

Forat, S.; Huettel, B.; Reinhardt, R.; Fimmers, R.; Haidl, G.; Denschlag, D. and Olek, K. (2016): Methylation Markers for the Identification of Body Fluids and Tissues from Forensic Trace Evidence. *PloS one*, **11(2)**: e0147973.

Friedl, A.A.; Mazurek, B. and Seiler, D.M. (2012): Radiation induced alterations in histone modification patterns and their potential impact on short-term radiation effects. Front.Oncol. **2**:117.

Frumkin, D.; Wasserstrom, A.; Budowle, B. and Davidson, A. (2011): DNA methylation-based forensic tissue identification. *Forensic Science International: Genetics,* **5(5)**: 517-524.

Fu, X.D.; Wu, J.; Wang, J.; Huang, Y.; Hou, Y.P. and Yan, J. (2015): Identification of body fluid using tissue-specific DNA methylation markers. *Forensic Science International: Genetics Supplement Series,* **5**: e151-e153

Gomaa, R. and Salehi, J. (2017): DNA methylation as a biomarker for body fluid identification. Research gate

Gršković, B.; Zrnec, D.; Vicković, S.; Popović, M. and Mršić, G. (2013): DNA methylation:the future of crime scene investigation? *Molecular Biology Reports,* **40(7)**: 4349-4360.

Harbison, S. and Fleming, R. (2016): Forensic body fluid identification: State of the art. Research and Reports in Forensic and Medical Sciences, **6**:11-23.

Hernandez, H.G.; Lopez-Cepeda, M.L.; Contreras-Garcia, G.A.; Vargas-Porras, C. and Arboleda, H (2019): Bisulphite conversion DNA visualiser for designing DNA methylation primers. Trends in Bioinformatics, **12**:1-6.

Jiang, R.; Jones, M.J.; Chen, E.; Neumann, S.M.; Fraser, H.B.; Miller, G.E. and Kobor, M.S. (2015): Discordance of DNA methylation variance between two accessible human tissues. *Scientific Reports,* **5**.

Lee, H.Y.; An, J.H.; Jung, S.E.; Oh, Y.N.; Lee, E.Y.; Choi, A. and Shin, K.J. (2015): Genome-wide methylation profiling and a multiplex construction for the identification of body fluids using epigenetic markers. *Forensic Science International: Genetics,* **17**: 17-24.

Lee, H.Y.; Jung, S.E.; Oh, Y.N.; Choi, A.; Yang, W.I. and Shin, K.J. (2015): Epigenetic age signatures in the forensically relevant body fluid of semen: a preliminary study. *Forensic Science International: Genetics,* **19**: 28-34.

Lee, H.Y.; Lee, S.D. and Shin, K.J. (2016): Forensic DNA methylation profiling to obtain investigative leads from evidence material. BMB reports.

Lee, H.Y.; Park, M.J.; Choi, A.; An, J.H.; Yang, M.I. and Shin, K.J. (2011): Potential forensic application of DNA methylation profiling to body fluid identification. International Journal of Legal Medicine, **126(1)**:55-62.

Madi, T.; Balamurugan, K.; Bombardi, R.; Duncan, G. and McCord, B. (2012): The determination of tissue-specific DNA methylation patterns in forensic bio fluids using bisulphate modification and pyrosequencing. Electrophoresis, **33**:1736-1745.

Paliwal, A.; Vaissiere, T. and Herceg, Z. (2010): Quantitation detection of DNA methylation states in minute amounts of DNA from body fluids Methods, science direct journal, **52**:242-247.

Park, J.L.; Kwon, O.H.; Kim, J.H.; Yoo, H.S.; Lee, H.C.; Woo, K.M. and Kim, Y.S. (2014): Identification of body fluid-specific DNA methylation markers for use in forensic science. *Forensic Science International: Genetics,* **13**: 147-153.

Richards, R.; Patel, J.; Stevenson, K. and Harbison, S. (2019): Assessment of DNA methylation markers for forensic application. Australian Journal of Forensic Sciences, **51(1)**:S99-S102.

Rodriguez, R.B.; Calacal, G.C.; Lande, R.P. and De Ungria, M.C. (2019): Integrating presumptive and confirmatory semen tests into DNA profiling of sexual assault evidence: A Philippine example. Egyptian Journal of Forensic Sciences, **9**:45.

Sabeeha and Hasnain, S.E. (2019): Forensic epigenetic analysis: The path ahead. Med.Princ.Pract. **28(4)**:301-308.

Schübeler, D. (2015): Function and information content of DNA methylation. *Nature*,**517(7534)**: 321-326

Tanaka, K. and Okamoto, A. (2007): Degradation of DNA by bisulfite treatment. *Bioorganic and Medicinal Chemistry Letters*, **17(7)**: 1912-1915.

Xu, H.; Zhao, Y.; Liu, Z.; Zhu, W.; Zhou, Y. and Zhao, Z. (2012): Bisulfite genomic sequencing of DNA from dried blood spot micro volume samples. *Forensic Science International: Genetics*, **6(3)**: 306-309.

Ziller, M.J.; Gu, H.; Müller, F.; Donaghey, J.; Tsai, L.T.Y.; Kohlbacher, O. and Gnirke, A. (2013): Charting a dynamic DNA methylation landscape of the human genome. *Nature*, **500(7463)**: 477-481.

Zinkle, A. and mohammadi, M. (2019): Structural Biology of the FGF7 Subfamily. Front.Genet. **10**:102.

Zubakov, D.; Boersma, A.W.M.; Choi, Y.; van Kuijk, P.F.; Wiermer, E.A.C. and Kayser, M. (2010): Micro RNA markers for forensic body fluid identification obtained from microarray screening and quantitative RT-PCR confirmation. Int.J. Legal Med., **124(3)**:217-226.

هل عامل نمو خلايا المنشأ الليفية٧ ذو كفاءة في تحديد البقعة المنوية بعد فترة طويلة من التخزين؟

ايمان فوزي جاب الله *خالد بيومي*ليلي راشد عزالدين مصطفى عبد الواحد شلبي***
قسم الطب الشرعي والسموم الإكلينيكية، كلية طب، جامعة القاهرة *
قسم الكيمياء الحيوية، كلية الطب، جامعة القاهرة**

إن تحليل الحمض النووي بمسرح الجريمة أداة لا غنى عنها في تحديد الجناة المشتبه بهم في الطب الشرعي. ان إضافة مجموعة مثيل للحامض النووي تعطيه ثباتا وحفظا من التكسير في المستقبل.

ان السائل المنوي له دور كبير في الطب الشرعي حيث انه يحتوي على الحامض النووي الذي يلعب دورا محوريا في تحديد الهوية خاصة في حالات الاعتداء الجنسي ولكن مع مرور الأيام يفقد السائل المنوي مكوناته وبذلك يكون دوره ضعيف في الأدلة الجنائية.

وتهدف دراستنا الي دراسة دور عامل نمو خلايا المنشأالليفية ٧ في تحديد البقعة المنوية بعد فترة طويلة من التخزين.

مواد وطرق البحث: وذلك عن طريق دراسة استطلاعية على خمسة عشرة عينة من البقع المنوية تم تخزينها لمدة عشرة أعواما مقارنة بخمسة عينات حديثة من السائل المنوي لتحديد عامل نمو خلايا المنشأالليفية ٧ بالعينات.

لقد ثبت من هذه الدراسة ان إضافة مجموعة مثيل للحامض النووي قد أضفى ثباتا على البقع المنوية فظهر بوضوح في البقع المنوية المخزنة وأمكن الاستدلال عليه في العينات القديمة بنفس الكفاءة التي توجدبالعينات الحديثة بما يشير الي امكانية استخدامه بكفاءة كدليل اكيد في إجراءات البحث الجنائي