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A Comparison Study of Cryopreservation of Seminal Fluid between Flinders Technology Associates (FTA-card) and Swab for Multiplex Short Tandem Repeats (STR) Analysis

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Abstract

Background Whatman™ FTA (Flinders Technology Associates) is a form of card soaked in chemicals that denatures proteins while protecting DNA and ensuring the safe handling of dried body fluid spots (blood, semen, and saliva) and buccal cells. To this day, these cards are still infrequently used in forensic science. Therefore, reference samples including biological material may be collected on FTA cards for genetic analysis that have been widely used for DNA preservation and analysis, particularly in forensic sciences and genetic studies. **The goal** of the study was to evaluate the effects of cryopreservation on DNA quantitative and short tandem repeats (STR) profiling by comparing the yield and quality of DNA extracted before and after cryopreservation using FTA card and swabs in the forensic analysis. The mean total DNA concentration and the purity of seminal fluid from FTA-cards and swabs before and after sperm cryopreservation were measured by using nano-drop spectrophotometer (93.688 ± 12.5) (90.94 ± 13.9) ng/ µl; (221.07 ± 20.97)(135.47 ± 19.6) ng/ µl respectively. Also a highly significant difference (p<0.001) was detected when the results of concentration DNA on FTA card were compared with dilution using wash media before and after sperm cryopreservation. Whereas significant difference (p<0.05) was observed between DNA concentration on FTA card with and without dilution after sperm cryopreservation. STR loci were present in the majority of data, particularly in FTA-card and swab samples where results explained that 100% of STR profiles were present in all 15 loci, both before and after sperm cryopreservation. In contrast to the conventional method of storing and analyzing STR profiling, it can be concluded that seminal fluid samples taken by FTA card and swabs can be taken and stored for a time by cryopreservation, leads to maintaining DNA integration in the field of criminal research.

Keywords: Sperm cryopreservation, Flinders Technology Associates FTA cards, DNA extraction, Short tandem repeats (STR) analysis

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دراسة مقارنة للسائل المنوي المحفوظ بالتبريد باستخدام بطاقة FTA والمسحات للكشف عن التتابعات الوراثية STR

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الخلاصة:

بطاقات واتمان (FTA) هو شكل من أشكال البطاقات المنقوعة في المواد الكيميائية والتي بدورها تغير من طبيعة البروتينات مع حماية الحمض النووي الريبوزي المنقوص الاوكسجين (DNA) للخلايا حيث يكون التعامل مع بقع سوائل الجسم الجافة (الدم والسائل المنوي واللعاب) بالاضافة للخلايا الطلائية امنأ. في علم الطب الشرعي لاتزال هذه البطاقات مستخدمة بصورة متكررة لكن بشكل نادر حتى يومنا هذا. لذلك يمكن جمع عينات مرجعية بما في ذلك المواد البيولوجية على بطاقات (FTA Flinders Technology Associates) للتحليلات الجينية، والتي تم استخدامها على نطاق واسع لحفظ الحمض النووي وتحليله، وخاصة في علوم الطب الشرعي والدراسات الوراثية .الهدف من هذه الدراسة هو تقييم تأثير الحفظ بالتبريد (النيتروجين السائل) على تحديد الحمض النووي الكمي والتكرار الترادفي القصير (STR) من خلال مقارنة إنتاج ونوعية الحمض النووي المستخرج قبل وبعد الحفظ بالتبريد و تحديد التنميط الوراثي من خلال التتابعات الوراثية (STR) لعينات السائل المنوي باستخدام بطاقة FTA والمسحات في تحليل الطب الشرعي. تم قياس متوسط تركيز الحمض النووي الكلي ونقاوته لعينات السائل المنوي من بطاقات FTA والمسحات قبل وبعد تجميد الحيوانات المنوية باستخدام مقياس الطيف الضوئي [(93.688 ± 12.5) (90.94 ± 13.9) نانوغرام / ميكرولتر] ؛ (135.47 ± 221.07) (20.97 ± 19.6)نانوغرام / ميكرولتر على التوالي .اظهرت النتائج وجود فرق معنوي ذو دلالة إحصائية (p <0.001) عند مقارنة نتائج تركيز الحمض النووي على بطاقة FTA مع التخفيف باستخدام الوسائط او الميديا قبل وبعد حفظ الحيوانات المنوية.بالتبريد. يوجد فرق معنوي (p <0.05) بين تركيز الحمض النووي على بطاقة FTA مع وبدون التخفيف بعد التجميد او حفظ الحيوانات المنوية بالتبريد. أوضحت النتائج أن 100% من ملفات مواقع التتابعات الوراثية STR كانت موجودة في جميع المواقع الخمسة عشر قبل وبعد حفظ الحيوانات المنوية بالتبريد في كلا عينات بطاقة FTA والمسحات. على عكس الطريقة التقليدية لتخزين وتحليل التنميط الوراثي STR أستنتج هذه الدراسة أن عينات السائل المنوي المأخوذة بواسطة بطاقة FTA والمسحات يمكن أخذها وتخزينها لفترة من الوقت عن طريق الحفظ بالتبريد مما يؤدي إلى الحفاظ على تكامل الحمض النووي المستخدم في مجال البحث الجنائي.

1. Introduction

Biological fluids (blood, sperm, and buccal cells) can be collected on FTA cards for forensic genetic investigations of the reference samples. To reduce or eliminate any risks to technical employees when processing samples, FTA cards are impregnated with chaotropic compounds that block infectious bacteria and reduce the possible biohazards of the sample. [1]. This procedure allows biological material to be stored on FTA cards for an extended period at room temperature. The need for refrigerators and freezers is eliminated, significantly lowering storage costs. It also makes it simpler to ship samples that may utilize regular mail [2, 3]. FTA® paper, which contains chemicals for cell lysis and protein denaturation, is often used for long-term storage of biological materials such as forensic casework. This material also protects nucleic acids from oxidative and UV degradation, allowing long-term storage of biological samples [4]. Cell membranes and organelles are disrupted, and nucleic acids become entangled in matrix fibers when crude biological samples such as whole blood, cells or plant or animal tissues, are immediately placed on a FTA card. The nucleic acids are immobilized and stabilized, allowing them to be processed immediately, transported, or stored at room

temperature for longer periods of time [5, 6]. Whatman FTA cards are a convenient solution for gathering, preserving, transporting, and isolating nucleic acid samples for analysis at room temperature. After removing a little disk from the FTA card, the STR typing of biological material (such as blood or buccal cells) contained on the card is performed (for example, by an automated puncher) [2]. Cryopreservation of spermatozoa (also known as sperm banking or sperm freezing) is a growing method that allows fertility preservation for men and animals that require such operations. Multiple procedures/protocols exist to cryopreserve sperm and spermatozoa in liquid nitrogen [7]. A cryoprotectant's primary function is to minimize cytoplasmic hyperosmolarity when frozen and to prevent intracellular and extracellular ice development. Following its accidental discovery as a cryoprotectant in the 1940s, glycerol has been widely used to protect spermatozoa against cryoinjury [7]. Swim-up techniques for sperm separation may cause sperm DNA damage as used in the IVF program. This technique was used in this study to investigate if it impacts the results of the STR profile when used for semen cryopreservation for forensic cases [8]. In the human genome, short tandem repeats (STR) repetitions are exceedingly polymorphic and prevalent which is a type of DNA polymorphism that involves the repetition of small DNA sequences. STRs are sometimes referred to as "junk DNA" since they are introns and do not contain protein-coding sequences. Given that the number of times a DNA sequence for a certain STR is replicated differs between individuals. STRs are typically useful for all forensic genetic analytical procedures used today, including genealogical studies, in addition medical research, and ethnographic studies [9,10]. In the 1990s the STRs were first used in forensic casework. STR usage has since then become the gold standard application in forensic laboratories around the world [11]. STRs are frequently amplified using PCR thath can produce millions of copies of DNA from a modest beginning amount of material. The length and quantity of repeat units present at each locus can be determined by analyzing the amplified STRs using techniques like capillary electrophoresis. An individual's DNA profile can be generated by examining several STR loci, allowing comparison to DNA samples from different sources. [12]. STR commercial assays that can correspond to up to fifteen different sites [13, 14]. The isolation of a tiny disk of the FTA card (e.g., by an automated puncher) is used to perform STR typing of biological material (e.g., blood, buccal cells) stored on FTA cards [15, 16]. STR marker amplification is then done on the extracted DNA or immediately on the FTA punch [17]. This study aimed to establish and validate a method to allow microsatellite analysis of DNA profiles obtained from cryopreservation (frozen-thawed) human sperm cells by using two types of collection FTA-papers and swab when considered as reference samples for forensic cases.

2. Material and Method

2.1 Samples Collection

This study involved seminal fluid samples from 23 healthy human volunteers, ages ranging between 22-44 years. Each sample contained 2ml of seminal fluid which was collected in the Kamal Al-Samaria IVF Center, Ministry of Health in Baghdad-Iraq. The samples were classified as groups before and after semen samples underwent cryopreservation (Figure 1).).

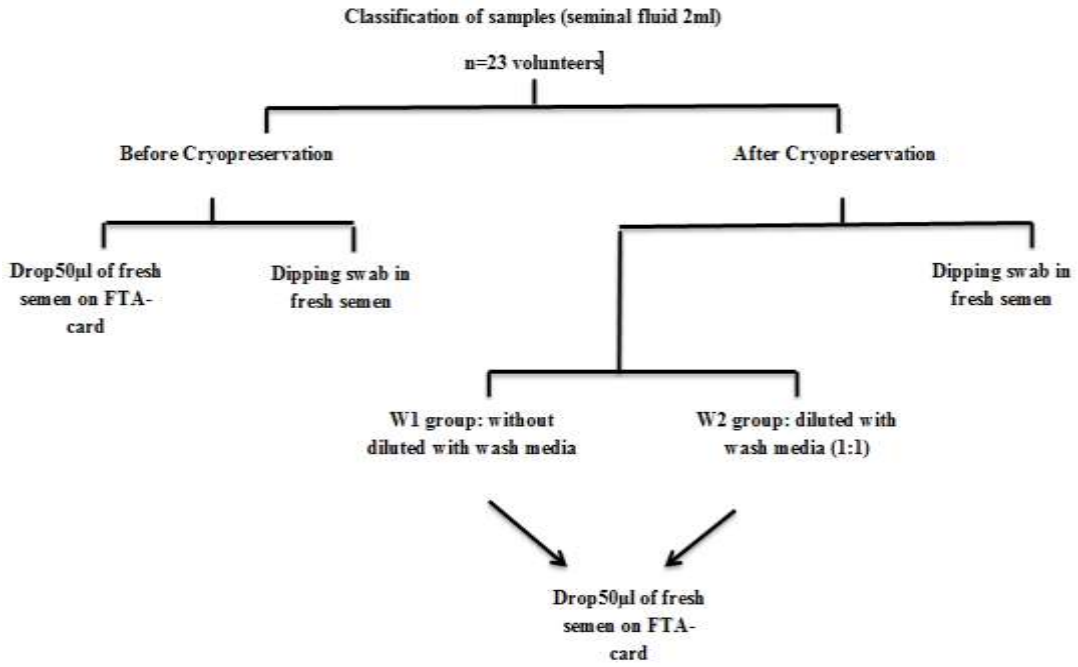

Figure 1: Classification of study groups before and after cryopreservation

2.2 Samples Preparation

2.2.1 Seminal fluid preparation before and after cryopreservation:

FTA cards*:* 50 µl of fresh semen from a healthy individual was spotted on FTA cards. The cards were allowed to dry and were stored for 3 days. Three 1 mm 2 punches were cut into a previously sterilized 1.5 mL tube and prepared for extraction. ***Buccal swab:*** a smear was taken by a buccal swab by dipping in a sample containing 1 ml of semen. The same semen samples underwent cryopreservation and were distributed in the same way as mentioned above.

Sperm cryopreservation: Sperm Freezing Medium (ORIGIO a/s, Denmark) was used for human spermatozoa cryopreservation. According to the firm, the protocol entailed the following: After liquefaction, the total volume of the ejaculate was determined and necessary semen analysis was performed. Prior to diluting the semen 1:1 (v/v) with the Sperm Freezing Medium, it was ensured that both the semen sample and the Sperm Freezing Medium are at room temperature. Drop by drop, the medium was applied to the sperm, and the solution was thoroughly mixed after each addition. Later the diluted semen was loaded into straws or cryo-tubes and sealed it according to the manufacturer's recommendations. It is very important to leave some air space in the lower part of the straw for sealing as well as to allow expansion of the solution during freezing. Straws were suspended horizontally for 30 minutes, just above the liquid nitrogen's surface. Finally, the straws or cryo-tubes were placed in a liquid nitrogen tank and stored at -196℃. The frozen samples were thawed after 24 hours. The sperm thawing protocol involved the following according to the company (ORIGIO a/s, Denmark); immediately after thawing, sperms were prepared by the swim-up procedure (in this step after semen cryopreservation; the study group was divided into two groups; **W1 Group:** without dilution that did not undergo swim-up procedure); **W2 Group:** with dilution that underwent swim-up procedure).

2.2.2. Genomic DNA Extraction from Semen

*DNA Extraction Protocol****:*** Organic or Phenol-chloroform method: DNA extraction with modification (the lysis step taken 24 h) according to Souvik and Goodwin [18, 19]. *DNA quantification and quality assessment:* The concentration and purity of extracted DNA was

determined using Nanodrop spectrophotometer (Thermo Fisher / USA). By electrophoresis on a 0.8% agarose gel and detection with Red- safe staining dye, the integrity of genomic DNA was evaluated.

*Real-Time PCR Amplification***:** Twenty-three sperm samples were examined using a multiplex Real-time PCR assay prior to cryopreservation. The 7500 fast system SDS software v2.0.5 (Applied Biosystems, Foster City, CA) was used to evaluate the data after the amplification reactions, using the commercial kit (Quntifiler human DNA quantification Kit) and the specific gene detection TaqMan method. In order to amplify SRY (FAMTM-labeled probe), RPPH1 (VIC®-labeled probe), and an Internal Positive Control-IPC (NEDTM-labeled probe), a multiplexed TaqMan ® was constructed.

Capillary Electrophoresis and Data Analysis for STRs: The Applied Biosystems AmpFlSTR® IdentifilerTM kit was used to genotype fifteen autosomal STR markers or loci on the X and Y chromosomes which simultaneously amplifies the loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433. VWA, TPOX, D18S51, D5S818, FGA) as well as the amelogenin locus by 3130 XL Genetic Sixteen capillary array system (Applied Biosystems, Foster City, CA, USA) was used following the genetic analyzer manufacturer'srprotocol [20].

3. Analytical Statistics: SPSS for Windows, version 21 (SPSS Inc. Chicago, IL, USA) was used to statistically analyze the data. The data was used to determine the standard error of the mean (SEM), after performing ANOVA testing, additional comparisons were made using Tukey's and Bonferroni post hoc tests.

4. Results: Certain sperm function parameters used in this study are shown in Table 1.

Table 1: Certain sperm functions parameters of the study group.

Age (Years)	Sperm Count (Million/Ejaculated)	Progressive Motility (%)	Non Progressive Motility (%)	Immotile (%)	Morphologically Abnormal Sperm (%)
37.16 ± 1.3	42.51 ± 3.56	10.3 ± 2.13	15.3 ± 3.48	74.4 ±5.61	70.31 ± 1.73

Data were expressed as mean ± SE.

The total DNA from twenty-three semen samples on the FTA card and swabs were successfully extracted before and after cryopreservation. The concentration of total DNA ng/µl (mean ± SE) was measured using Nano-drop spectrophotometers in the study group according to the organic method to determine the most effective concentration and integrated DNA that was obtained from FTA cards and buccal swabs. Upon comparing FTA card and buccal swab before and after sperm cryopreservation, the total DNA concentration (ng/ µl) was found to be significantly different (p<0.05) (Table 2).

Table 2: Comparison results of DNA concentration comparison between FTA card and buccal swab before and after sperm cryopreservation.

Sample Collection	DNA Concentration (ng/µl) (Mean + SE)		Purity (%) (Mean + SE)
FTA Card (n= 23)	Before	93.688 ± 12.5	1.6 ± 0.02
	After	90.94 ± 13.9	1.69 ± 0.01
	P-value	0.88	0.07
Buccal Swab (n=23)	Before	221.07 ± 20.97	1.57 ± 0.03
	After	135.47 ± 19.6	1.6 ± 0.02
	***P*-value**	**0.005***	0.115

Data were expressed as mean ± SE; Statistical analysis were performed by ANOVA; Significant differences * ($p \leq 0.05$).

Figure 2 demonstrates agarose gel electrophoresis of total DNA isolation from both FTA-card and buccal swab samples after seminal fluid cryopreservation.

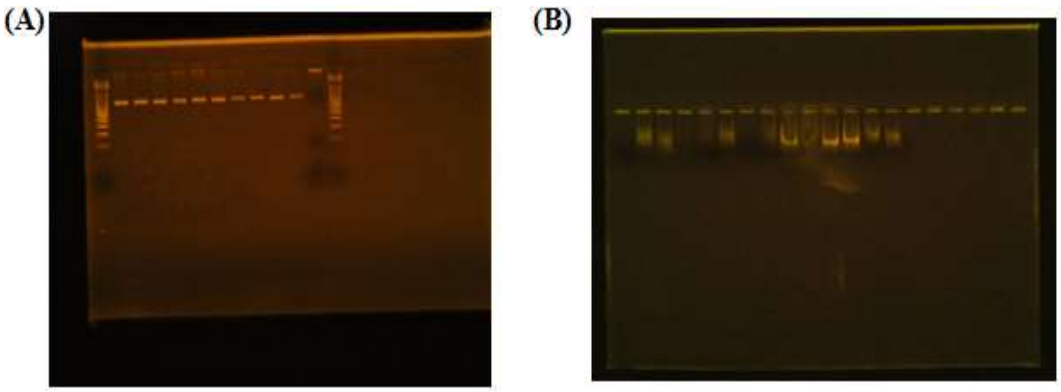

Figure 2: Electrophoresis of extracted DNA from seminal fluid samples, (A) FTA card samples after seminal fluid cryopreservation; (B) Buccal swab samples after seminal fluid cryopreservation; on 1% agarose gel electrophoresis stained with Red-Safe; and then electrophoresed at 5 volts per cm for 30-45 minutes.

Table 3 shows the highly significant difference ($p<0.001$) that was recorded upon comparing the results of concentration DNA on FTA card with dilution using wash media before and after sperm cryopreservation. Whereas significant difference ($p<0.05$) was observed between DNA concentration on FTA card with and without dilution after sperm cryopreservation. However, a non-significant difference appeared in DNA purity when compared between study groups with and without diluted samples on FTA cards. The purpose of this study was to acknowledge the effects of semen cryopreservation on the yield and quality of DNA extracted on FTA-card and using this protocol for forensic sciences.

Table 3: Results of DNA concentration on FTA card with and without dilution before and after sperm cryopreservation

Sample Collection	DNA Concentration (ng/µl) (Mean + SE)		Purity (%) (Mean + SE)
FTA Card (With dilution W2) (n= 23)	Before	93.688 ± 12.5	1.6 ± 0.02
	After	51.59 ± 6.29	1.62 ± 0.01
	P-value	0.004**	0.09
FTA Card (With & without dilution) (n=23)	After (W1)	90.94 ± 13.9	1.69 ± 0.01
	After (W2)	51.59 ± 6.29	1.62 ± 0.01
	P-value	0.013*	0.08

Data was expressed as mean ± SE; Statistical analyses were performed by (ANOVA); Significant differences * (P≤0.05) & highly Significant differences ** (p≤0.01); W1= without dilution (*underwent swim-up procedure*) sample; W2= with dilution (*underwent swim-up procedure*) sample.

Fifteen autosomal STR loci and amelogenin were discovered in the results of complete DNA profiles acquired from semen samples that were typed using the AmpFlSTR® Identifiler kit (Table 4).

Table 4: Results of full DNA profiles obtained from semen sample from both FTA-card & swabs by 15 autosomal STR loci and amelogenin were identified).

Samples NO. (n=23)	STR Locus (allele1/allele 2)															
	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	Alleles (%)
	A1/A2	A1/A2	A1/A2	A1/A2	A1/A2	A1/A2	A1/A2	A1/A2	A1/A2	A1/A2	A1/A2	A1/A2	A1/A2	A1/A2	A1/A2	
Sample 1	13/14	28/31	11/12	10/11	14/15	7/9	8/8	10/12	16/20	13/13	16/16	8/8	14/16	9/13	22/24	100
Sample 2	13/14	28/28	11/11	11/12	15/15	7/9	8/8	10/10	16/20	13/13	16/6	8/8	14/16	9/9	22/22	100
Sample 3	13/15	28/31	11/12	10/11	15/15	7/9	8/8	10/12	16/25	13/13	15/18	8/8	14/16	9/13	22/24	100
Sample 4	15/15	28/28	11/11	13/13	14/15	6/6	12/12	8/12	19/19	13/15	14/18	8/11	15/15	12/12	21/21	100
Sample 5	10/15	30/31	10/10	11/12	15/17	9/9.3	8/10	11/13	9/25	14/15	14/14	8/9	13/16	11/3	12/22	100
Sample 6	14/15	30/32.2	10/11	10/12	15/17	8/8	12/14	11/12	21/21	15/15	16/17	8/10	17/19	11/13	20/21	100
Sample 7	14/14	29/29	10/10	11/12	17/18	6/9	8/10	10/12	17/23	14/15.2	15/15	8/11	17/17	10/11	23/23	100
Sample 8	10/13	20/31.2	9/9	11/12	16/16	8/9.3	9/12	10/13	19/23	12/14	14/17	9/11	13/15	13/13	22/23	100
Sample 9	13/13	30/30	10/11	10/12	14/15	8/9.3	11/11	11/12	19/23	14/15	17/19	8/8	15/19	11/11	20/21	100

Sample 10	13/15	32.2/322	10/11	13/13	15/18	8/9.3	11/12	8/9	22/26	13/14	16/17	8/8	13/15	12/12	19/19	100
Sample 11	11/14	29/322	11/12	10/13	16/17	6/9	8/13	13/13	23/24	14/14	18/19	8/9	12/17	13/14	24/24	100
Sample 12	13/14	29/322	9/10	11/11	15/17	8/10	8/13	10/11	15/17	14.2/152	14/18	8/9	14/16	9/11	20/20	100
Sample 13	13/15	28/32.2	8/10	11/12	16/17	7/9.3	8/11	10/11	17/25	14/15.2	17/17	9/11	16/17	11/11	24/27	100
Sample 14	12/13	29/31.2	10/11	5/12	14/18	6/6	8/11	11/11	20/23	12/16.2	16/17	8/10	13/19	12/13	23/25	100
Sample 15	12/15	30/30	11/12	10/11	17/17	9.3/9.3	13/14	11/12	17/20	12/13	16/18	8/11	16/18	12/13	21/23	100
Sample 16	11/14	29/30	8/11	11/11	17/18	7/9.3	9/12	9/11	23/25	12/14	14/29	8/12	14/16	13/13	24/24	100
Sample 17	11/15	30/32.2	11/12	10/11	15/18	9/9	11/12	9/10	23/24	13/18.2	16/16	8/11	13/15	11/13	21/29	100
Sample 18	13/15	29/322	7/12	8/13	16/16	6/9.3	12/12	11/13	17/17	14/14	17/19	8/9	12/13	12/13	23/25	100
Sample 19	13/15	28/29	11/11	11/11	17/17	9/9.3	12/12	11/13	16/19	13/14	14/17	8/8	12/18	12/13	25/25	100
Sample 20	14/15	30/30	11/12	11/12	14/16	6/11	11/12	11/12	20/26	13/14	14/18	10/11	12/16	12/13	21/25	100
Sample 21	13/14	29/30	10/10	10/12	16/17	9/9.3	11/11	10/11	20/26	13/15.2	17/18	8/9	14/15	10/12	22/24	100
Sample 22	10/12	27/29	10/11	11/11	18/18	9/9	12/12	9/11	25/25	12/12	16/17	8/11	12/18	11/11	24/24	100
Sample 23	13/14	32.2/322	8/11	10/13	16/16	6/6	8/13	9/11	17/17	14/15	16/16	8/11	15/15	11/13	24/25	100
Mean Percentage	STR Profiles % from FTA-card															**100 %**
	STR Profiles % from swabs															**100 %**

15 STR loci of AmpF1STR1 Identifier-TMPCR Amplification Kit (L1=D851179, L2= D21S11, L3=D7S820, L4=CSF1PO, L5=D3S1358, L6=THO1, L7=D13S317, L8=D16S539, L9=D2S1338, L10=D19S433, L11= VwA, L12=Tpox, L13=D18S51, L14= D5818, L15=FGA), Allele 1 (A1), Allele 2 (A2). Alelles % = *The Alleles percentage of 15 STR loci*; Black Bold (results of sperm samples before &after cryopreservation collected by FTA-card& swabs).

STR systems detect DNA at several different locations on the DNA strand at each of these locations (loci); the alleles (A1, A2) of 15 STR loci appeared in most results, especially in FTA-card samples appeared 100% of STR profiles in all 15 loci before and after sperm cryopreservation. Whereas results of STR profiles in swabs samples, results appeared 100 % before and after sperm cryopreservation in all 15 loci (Table 4). Figure 2 explains the DNA profile electropherogram derived from semen FTA-card sample amplified with 15 loci of STR.

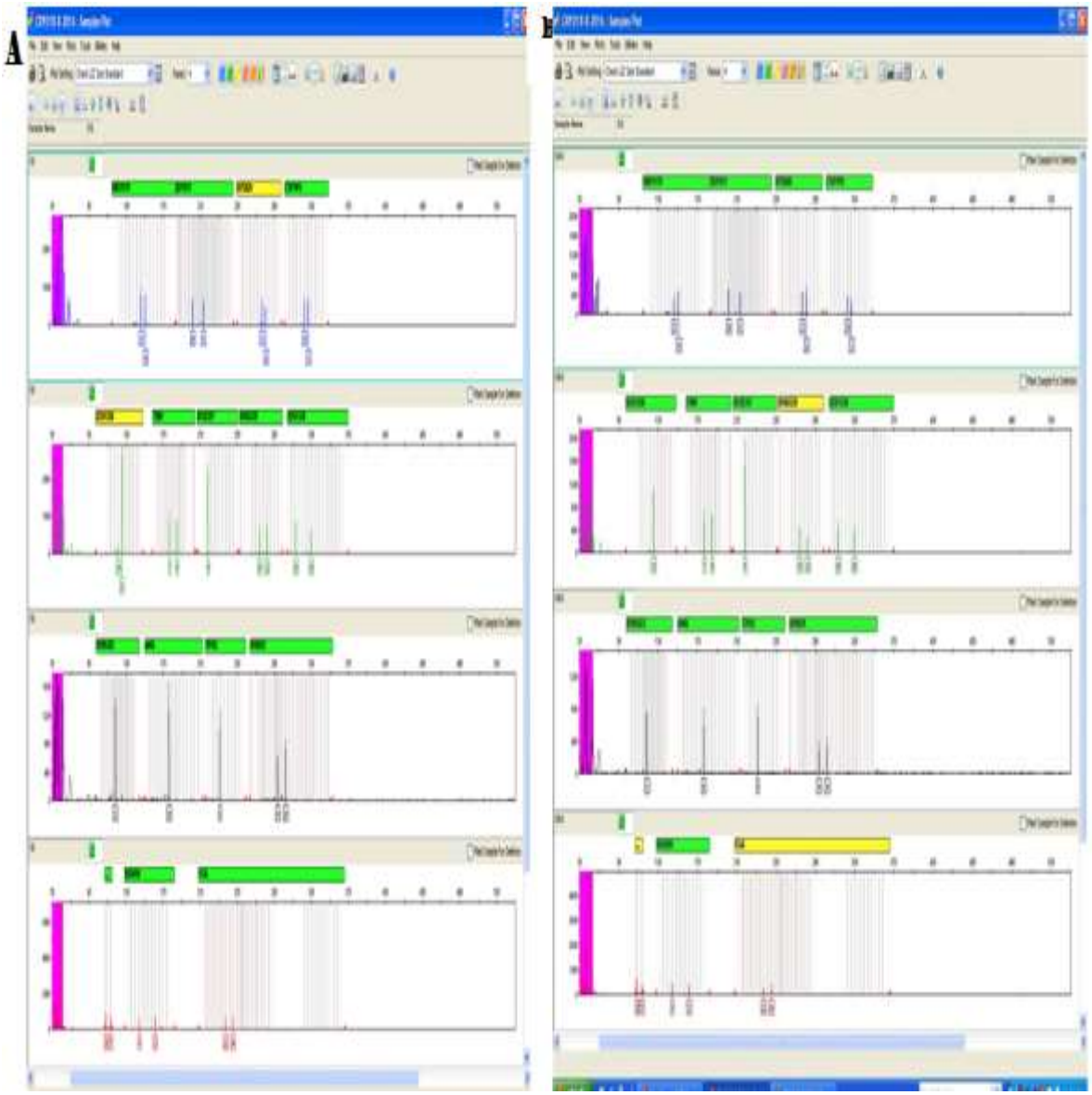

Figure 2: DNA profile electropherogram derived from semen FTA-card sample amplified with 15 loci of STR. (A) STR profile for FTA-card samples before seminal fluid cryopreservation; (B) STR profile for FTA-card samples after seminal fluid cryopreservation; The amplification was obviously successful. Image acquired directly from the 3130 XL Genetic Analyzer device.

5. Discussion

The primary goal of this study was to compare the separation, extraction capabilities, quantitation and STR data from cryopreservation (frozen-thawed) human seminal fluid using two different collecting techniques, FTA paper and swabs that use organic extraction. Historically, forensic DNA laboratories have used organic extraction and manual solid-phase extraction as common DNA extraction techniques [21]. Semen samples were mainly chosen in this study as they could be transported and stored for up to 20 years at room temperature when using FTA-card technology [22]. Cryopreserved sperm may theoretically be stored indefinitely because all metabolic responses and biological processes are halted [23]. Semen being so heterogeneous, different species, individuals within the same species, and even ejaculates from the same person can all be successfully cryopreserved in different ways. (Table 1) [24]. The current investigation allowed for a comparison between the yield of total DNA extraction from

FTA-card samples to the yield obtained after the identical FTA-card samples were cryopreserved. Sperm in seminal plasma enhances DNA integrity as it contains abundant antioxidants. Sperm in unprepared seminal fluid appeared to be more resilient to freezing damage than sperm in prepared seminal fluid. Further improvement can be made by preparing sperm and freezing it after adding seminal plasma [25, 26].

A forensic laboratory receives various exhibits such as clothing, cotton swabs, smears on a glass slide, liquid and dry sperm, sperm combined with other bodily fluids, and other case-related items, to be examined for the presence of seminal stains [27]. No biological evidence is degradation resistant. Since meticulous biological sample collection and storage can therefore yield information that is important in forensic analysis. There are few acceptable pieces of evidence for semen identification [28]. Using swabs to collect semen in this study served two purposes: first, to determine the amount and quality of DNA that could be obtained under the circumstances similar to those in which semen samples are taken in criminal cases, particularly rape cases; second, to determine whether collecting and preserving the semen by cryopreservation using the aforementioned technique had any impact on the DNA's quality.

An individual will have two alleles (heterozygous) as DNA is packed into pairs of chromosomes. The fragment size (allele) at a specific locus on one chromosome is distinct from the fragment size (allele) at the same locus on the other chromosome.. An individual will only have one allele at that site if the sizes of the identified fragments are the same on both chromosomes (homozygous) [29]. In forensic research, short tandem repeats (STRs) are amplified and typed to identify individuals and establish relationships between them. STRs markers are length-variant, highly variable repeating DNA sequences that are present in non-coding areas of the genome. They are hence perfect markers for DNA profiling indicators [30, 31].The smaller size of STR alleles on chromosomes also makes it simpler to distinguish them from other chromosomal regions, preventing the selection of closely related loci. Closely related loci do not exhibit the population's expected random distribution pattern, making statistical analysis challenging. The data is more reliable and predictable since STR alleles also have lower mutation rates [32, 31].

6. Conclusion

It can be concluded that FTA cards have evolved into a practical and effective tool for the preservation and extraction of seminal fluid DNA in forensic disciplines. With the help of these cards, it is simple and inexpensive to stop DNA degradation and inhibitors from degrading semen sample DNA. The cost of all samples collected on both FTA-card and swabs shows that the whole process becomes less expensive. Time is another factor that needs to be considered as there is a need for a method that can analyze several samples quickly in forensic laboratories. The FTA-card and swab method is one such procedure. When both these approaches were compared in the current study the findings demonstrated that FTA is a practical and sensitive technique for collecting, preserving, and retrieving samples and genetic sequences in the forensics field. Through access to hereditary STR profiles from both swabs and FTA card before and after seminal cryopreservation, this investigation was able to conclude that seminal fluid samples when taken using FTA card and swabs can be taken and stored for a longer time, leading to maintain DNA integration in forensic research.

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