DIFFERENTIAL EXTRACTION OF DNA TYPES IN SEMEN MIXTURES FROM MULTIPLE MALE CONTRIBUTORS

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requirements for the award of the degree of
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To my beloved parents and husband

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ABSTRACT

The analysis of samples obtained from sexual assault cases usually involve the separation of mixtures comprising cells from male and female contributors in order to produce the single profile of suspect and victim. However, specific research on the separation of mixed-male samples without the interference from female cells is lacking. This study is necessary since certain cases of sexual assault involve male victim (sodomy cases) or multiple male suspects (gang-rape cases) and their effects to the pattern of separation should not be neglected. Differential extraction (DE) is the most commonly used method to separate the mixed sample as, theoretically, it is able to produce complete separation of mixtures from male and female individual. However, incomplete separation following DE is possible since the separation is affected by various factors. In this research, the pattern of sperm cells separation in male mixtures contributed by multiple numbers of sperm contributors with different proportion of major and minor contributors was studied. This research utilized semen specimens obtained from volunteering donors which were used to prepare four designed mixtures from multiple numbers of contributors in varying proportion of major and minor contributors in order to determine the effects of both factors to the pattern of cells separation by DE method. It was found that the sperm cells from mixtures contributed by as low as two individuals were successfully separated into both sperm cells (SC) and non-sperm cells (NSC) fraction. This verified that the male DNA profiles obtained from the NSC fraction may originate from the sperm cells and not the epithelial cells of male contributors. Mixture samples contributed by maximum of four males with various proportions of major and minor contributors revealed that the sperm cells from all contributors were separated into both SC and NSC fraction. However the ability to identify the major and minor contributors in both fractions were found to be dependent on different major and minor proportion of the original sample.

ABSTRAK

Analisis sampel yang diperolehi daripada kes-kes serangan seksual biasanya melibatkan pemisahan campuran yang terdiri daripada sel-sel daripada penyumbang lelaki dan perempuan bagi menghasilkan profil tunggal suspek dan mangsa. Walau bagaimanapun, penyelidikan spesifik mengenai pemisahan sampel campuran lelaki tanpa gangguan dari sel-sel wanita adalah kurang. Kajian ini adalah didapati perlu kerana sebahagian kes serangan seksual melibatkan mangsa lelaki (kes liwat) atau berbilang suspek lelaki (kes rogol berkumpulan) dan kesannya kepada corak pengasingan tidak harus diabaikan. Pengekstrakan Pembezaan (DE) adalah kaedah yang paling biasa digunakan untuk memisahkan sampel campuran kerana secara teorinya, ia dapat menghasilkan pemisahan lengkap campuran daripada individu lelaki dan perempuan. Walau bagaimanapun, pemisahan tidak lengkap selepas DE boleh berlaku memandangkan pemisahan tersebut dipengaruhi oleh pelbagai faktor. Dalam kajian ini, corak pemisahan sel sperma dalam campuran lelaki yang disumbangkan oleh beberapa penyumbang sperma dengan perkadaran penyumbang major dan minor yang berbeza telah dikaji. Kajian ini menggunakan spesimen air mani yang diperolehi daripada penderma sukarela yang digunakan untuk menyediakan empat campuran yang direka daripada berbilang nombor penyumbang dalam pelbagai perkadaran penyumbang major dan minor bagi menentukan kesan dua faktor tersebut kepada corak pemisahan sel menggunakan DE. Kajian mendapati bahawa sel-sel sperma daripada campuran yang disumbangkan oleh serendah dua individu telah berjaya dipisahkan kepada kedua-dua pecahan sel sperma (SC) dan sel bukan sperma (NSC). Sampel campuran yang disumbangkan oleh maksimum empat lelaki dengan pelbagai nisbah penyumbang major dan minor menunjukkan bahawa sel-sel sperma daripada semua penyumbang telah dipisahkan kepada kedua-dua pecahan SC dan NSC. Walau bagaimanapun, keupayaan untuk mengenal pasti penyumbang major dan minor dalam kedua-dua pecahan didapati bergantung kepada perbezaan bahagian major dan minor daripada sampel asal.

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LIST OF ABBREVIATIONS AND SYMBOLS

ATP	-	Adenosine triphosphate
CCD	-	Charge-coupled device
CODIS	-	Combined DNA Index System
DE	-	Differential extraction
DNA	-	Deoxyribonucleic acid
DTT	-	Dithiothreitol
EDTA	-	Ethylenediaminetetraacetic acid
EXT	-	Extraction
FBI	-	Federal Bureau of Investigation
Hi-Di	-	Highly deionized
HSA	-	Hospital Sultanah Aminah
ID	-	Identification
ISFG	-	International Society of Forensic Genetics
JKM	-	Jabatan Kimia Malaysia
LCM	-	Laser capture microdissection
NA	-	Non-applicable
NSC	-	Non-sperm cells
PCR	-	Polymerase chain reaction
PHs	-	Peak heights
POP	-	Performance Optimized Polymer
Pro- K	-	Proteinase K
PSA	-	Prostate- specific antigen
RFLP	-	Restriction fragment length polymorphism
RFU	-	Relative fluorescence units
rpm	-	Revolutions per minute
RSID	-	Rapid stain identification

SC	-	Sperm cells
SD	-	Standard deviation
SDS	-	Sodium dodecyl sulfate
STR	-	Short tandem repeats
SWGDAM	-	Scientific Working Group on DNA Analysis Methods
TE	-	Tris-EDTA
VNTR	-	Variable number of tandem repeat
Y-STR	-	Y- chromosom STR analysis
kV	-	Kilovolts
M	-	Molar
min	-	Minute
mL	-	Mililiter
µL	-	Microliter
ng	-	Nanogram
°C	-	Degree celsius
s	-	Second

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

DNA analysis of biological samples is important for human identification in forensics. While samples that have a single contributor and contain sufficient amounts of DNA are easy to interpret, the analysis of mixed samples can cause problems. The evidences from sexual assault case, such as vaginal swabs and stained clothing, most often contain nucleated cells from the male contributor (predominant sperms) and the female victim (epithelial cells) (Patrinos & Ansorge, 2010). Elucidation of the individual contributors' DNA profiles can, at times, be complicated in these mixtures. However, sperm cells can be separated from other cells/non-sperm cells using a type of procedure called differential extraction, a variation of the organic extraction procedure (Yoshida *et al*., 1995; Rudin & Inman, 2002; Kochl *et al.*, 2005; Montesino *et al*., 2007). The separation of mixed DNA using this technique will finally produce the sperm cells (SC) and non-sperm cells (NSC) fraction which are expected to give the DNA profiles respectively for the male perpetrator and female victim.

It is the aim of forensic analyst to completely separate the DNA of perpetrator and victim in sexual assault cases using differential extraction method, however, the success of this type of analysis may vary due to several factors. These include whether the sperm is intact, the number of contributors present, the relative contributions of the donors and time elapsed between the aggression and collection

of evidence (Vuichard *et al.*, 2011). Due to these factors, the separation of mixed DNA in a sample may not be completed, leaving the SC and NSC fraction containing mixture of cells from both contributors, either in one or in both fractions.

Sexual assault need not necessarily involve only one suspect and one victim. Cases like "gang-rape" involving multiple suspects and/or multiple victims are considered more serious than sexual assault involving one suspect (Alderden, 2008), thus the analysis of DNA from such cases would produce results which are complicated to interpret. Since the separation of cells by differential extraction is reported to be affected by the number of contributors to the sample (Vuichard *et al.*, 2011), the pattern of cell separation in the mixture samples from gang-rape case is expected to deviate from the normal separation pattern.

Another issue aroused in sexual assault sample analysis involving multiple suspects is regarding the origin of cells recovered from the separated cell fractions. In the case where incomplete separation occurs and the DNA analysis reveals male profile to be developed in the NSC fraction, the origin of the male profiles developed is normally questioned in court as to whether it is due to the sperm cells or epithelial cells of male contributors present in the NSC fraction. With the absence of publication on the separation pattern of male mixtures contributed by multiple male individuals, the explanation on the issue is hardly explained in court. Due to that, it is found essential to assess the separation of mixed-male samples by differential extraction in order to study the pattern of separation within mixtures contributed by multiple male contributors. In this research study, the effect of the number of contributors to the mixture and relative contributions of the donors on the pattern of separation will be established.

1.2 Objectives of Study

The objectives of this research are:

i. To employ differential extraction method on simulated male mixture samples.
ii. To determine the effect of different proportions of major and minor male contributors in the mixture sample on the sperm cells separation pattern.
iii. To determine the pattern of sperm cells separation (into sperm and non-sperm cells fraction) in mixed-male contributed by multiple number of sperm contributors.

1.3 Scope of Study

The scope of this study involved the analysis of simulated male mixture samples which were prepared from semen specimens from five male individuals for which consent from each donor was obtained prior to sample collection. Different types of male mixtures were prepared using different number of sperm contributors and controlled proportion of the major and minor contributors to the cell mixture. The simulated male mixtures were then extracted using differential extraction method to determine the pattern of sperm cells separation into sperm and non-sperm cells fractions.

1.4 Significance of Study

The results of this study will be particularly useful for the forensic DNA analysts who are directly involved in the analysis of sexual assault samples mainly in cases involving multiple male suspects and/or victims. From this study, the sperm cells separation from different male mixtures was assessed to determine any specific pattern developed in order to gain better understanding on how the identified factors affect the sperm cells separation in the real sexual assault mixture samples. The depth of understanding in this pattern of separation system should be able to assist

the forensic analyst in the interpretation of complex mixture profiles during data analysis and to strengthen their testimony in court.

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