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Research article

Single sperm cell isolation by micromanipulation for human identification in sexual assault

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Abstract

This study proposes a new alternative solution in preferential extraction methods or microdissection to isolate and analyse single sperm cells in case of sexual assault. After the transfer of swabs in liquid culture medium, perpetrator's spermatozoas can be physically separated from victim's epithelial cells by using classical techniques of micromanipulation as ICSI (IntraCytoplasmic Spermatozoa Injection), usually applied for IVF (In vitro Fertilization).

Keywords: Sperm cell isolation; Preferential extraction; Laser microdissection

1. Introduction

In case of tardive sample collection or low number of spermatozoids, the genotype determination can be very difficult because of an important feminine contamination. Moreover, profile interpretation is very complex in case of sexual aggression implying several perpetrators.

This study proposes a new alternative solution in preferential extraction method or in microdissection to isolate and analyse one or several sperm cells in case of sexual assault [1–5].

We tested here if a simple micromanipulation with fine micropipette allows to isolate spermatozoids from a liquid medium according to the same technique used for ICSI (IntraCytoplasmic Spermatozoa Injection) in IVF (In vitro Fertilization).

2. Material and method

2.1. Sample collection

Ten vaginal swabs were performed, with patient consent, 6–8 h after a sexual relation for a medical post-coital exploration of infertility. Reference samples were obtained from saliva or hair collection and the results of spermogramm were controlled to verify the spermatozoa concentration. Swab was transferred in 300 μl of buffer TE.

2.2. Spermatozoa isolation

One drop of sample diluted in TE buffer is prepared in a Petri dish and covered with paraffin oil. Spermatozoids were isolated under inverted microscope with a 20 μ of diameter micropipette controlled by a Narishige micromanipulator (Fig. 1). Isolated gametes were transferred in a 3 μl of PBS prepared in a 0.2 ml microtube.

2.3. Cell lysis and whole genomic amplification

To obtain sufficient quantity of DNA for STR amplification, all single cell samples were preamplified by Multiple Displacement Amplification (MDA) with Repli-G Mini-Kit® (Qiagen) after a cell lysis in DTT according to manufacturer's manual. One microliter of a 100-fold diluted MDA product was used as template for STR analysis. For control profiles, 100 spermatozoids were isolated and only extracted on column QIAAmp DNA Microkit just after cell lysis without MDA.

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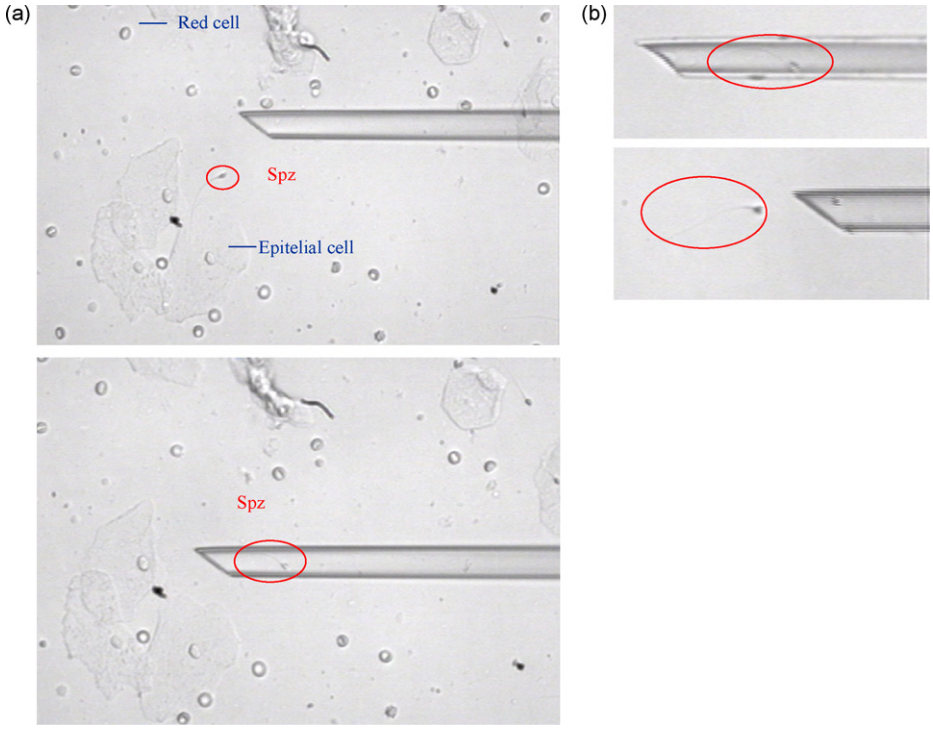

Fig. 1. Isolation of one spermatozoa. (a) Selection of one spermatozoa in liquid medium. (b) Transfer into the PCR microtube containing 3 μl of PBS buffer.

2.4. STR amplification and genotyping

Dilutions of preamplification products were subjected to multiplex STR typing by using the AmpFlSTR Identifiler Kit according to usual protocols without any modifications of conditions (28 cycles). PCR products were separated by capillary electrophoresis with 310 AB Prism Genetic Analyser and results were analyzed by Genescan and Genotyper softwares. One to five selected spermatozoids were amplified for each patient.

3. Results and discussion

A profile could be obtained for all the 30 amplified spermatozoas with a mean of 8.3 alleles/spermatozoa (Fig. 2). None female allele could be observed among all the 30 obtained profiles. The global rate of amplification success for all expected alleles was 56% with a large dispersion of signal intensity between 28 and 7230 rfu.

This method seems very efficient to avoid any risk of feminine contamination since no female allele was observed

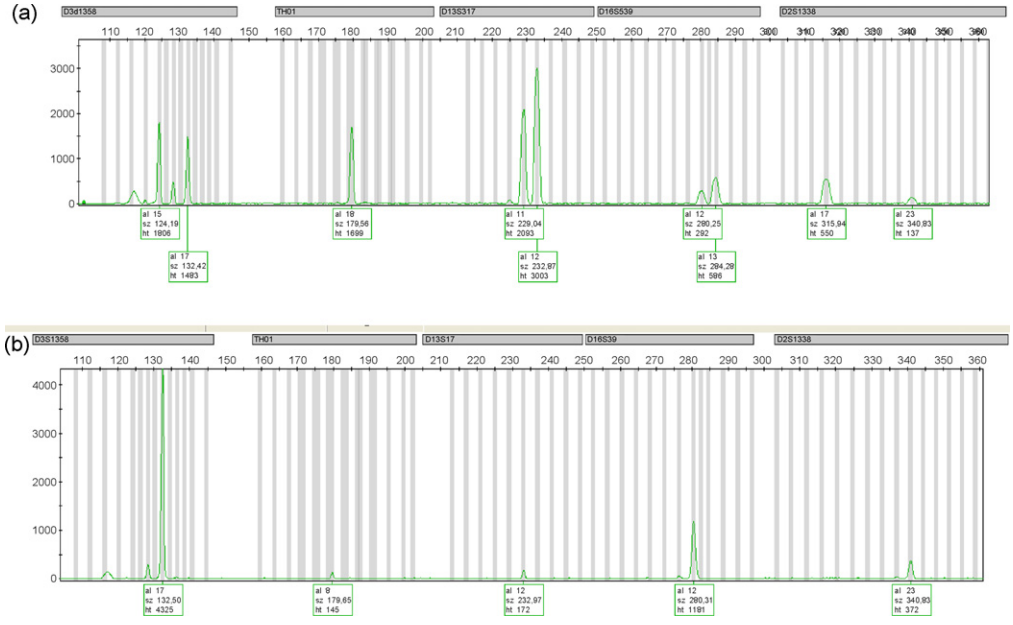

Fig. 2. Example of STR profiles. (a) Control profile on 100 spermatozoas. (b) Profile of one isolated spermatozoa.

among all the 30 obtained profiles. On the other hand, if a haploid male profile could be obtained for all the 30 amplified spermatozoa, none of them was integral with the presence of 1 allele for each of the 16 tested STR. However, as several spermatozoas can be isolated for specific amplification, a complete genotype can be always determined.

This approach has to be now tested on dried swabs and on other samples as anal swabs or textile fibres but we can already conclude to the practicability of this technique for liquid sample collections.

Conflict of interest

None.

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