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Use of laser microdissection greatly improves the recovery of DNA from sperm on microscope slides

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Abstract. Traditionally, sperm are isolated from vaginal cell mixtures by preferential extraction methods. However, this method is problematic when there are limited amounts (c. 250 pg). In particular, the analysis of sperm from microscope slides has proven difficult. Here, we describe the use of Laser Capture Microdissection (LM) for the isolation of spermatozoa from microscope slides containing sperm and vaginal cells.

Keywords: Laser Capture Microdissection; Preferential lysis

1. Introduction

Evidence in sexual assault investigations frequently comprises semen stains and intimate swabs from victims. One of the first steps in the investigative process is to identify the presence of spermatozoa by staining with haematoxylin and eosin (HE) followed by microscopic examination. If there is evidence of spermatozoa, this is followed by DNA profiling of the evidential material, e.g., swab. With the advent of techniques such as low copy number (LCN) [1,2], the sensitivity of DNA profiling has increased significantly. However, there are many cases that have been unsuccessfully profiled in the past, with the consequence that the only remaining evidence is the original slide. At the FSS, such slides are now routinely submitted for DNA profiling using a modified version of preferential lysis [3] and LCN analysis [1,2].

Use of the preferential lysis technique is universal. However, this method is frequently ineffective on slides with low numbers of sperm, because of unavoidable contamination of the profile by victim DNA. The inefficiency with which the sperm are liberated from the swab (following removal from the slide) may also reduce the effectiveness of preferential lysis.

Laser Capture Microdissection (LM) is a relatively new technique that can be used to isolate single cells from complex tissue samples mounted on microscope slides.

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The effectiveness of LM in processing old microscope slides containing sperm and vaginal cells was evaluated by comparing LM with preferential lysis.

2. Materials and methods

2.1. Slide preparation and Laser Capture Microdissection

Slides were prepared 6–30 months prior to LM processing. Slides were prepared and sperm were captured using LM as described by Elliott et al. [4].

2.2. Comparative study

Sixteen pairs of slides were used in the comparative study. Each pair consisted of slides prepared concurrently from a single post-coital vaginal swab cell harvest. Slide pairs were only included in the study if both contained similar numbers of sperm, vaginal cells and debris. From each pair of slides, one was processed using preferential lysis and organic extraction as described by Gill et al. [3] and the other using the LM technique.

The numbers of sperm present on each slide were first accurately counted by visual inspection of the entire slide. From each pair of slides, the one with the least sperm was processed using preferential lysis. The same number of sperm was then removed from the other slide using LM. Pairs of preferential lysis and LM samples were processed concurrently from the PCR stage onwards.

In addition to comparing the technique with preferential lysis, 77 slides were analysed using the LM protocol. Sperm numbers per slide ranged from 2 to 326.

2.3. Sperm lysis

Sperm lysis was carried out using Qiagen extraction chemistry modified for LM as described by Elliott et al. [4]. Following LCN PCR as described by Gill et al. [1] and Whitaker et al. [2], electrophoresis, GeneScan software analysis and Genotyper software analysis were carried out and AMPFlSTR® SGM Plus™ DNA profiles were generated as described by Cotton et al. [5].

2.4. Statistical analysis

2.4.1. Likelihood ratio (LR) calculation

Likelihood ratios were calculated for each DNA profile produced, as described by Evett et al. (1991) [6].

2.4.2. Regression analysis

To analyse the associations between three different variables—namely, time since intercourse (TSI), number of sperm recovered on a slide and the quality of a DNA profile, multiple regression analysis, robust regression and stepwise regression were employed to calculate regression coefficients and associated confidence intervals and test statistics [7]. All analyses were carried out using the MATLAB statistics toolbox, The MathWorks, 3 Apple Hill Drive, Natick, MA 01760-2098, USA.

3. Results and discussion

In a comparative study, where LM and preferential extraction were compared, LR analysis demonstrated the former to be markedly superior, with 15 out of the 16 samples producing better results with LM. The relative ineffective performance of preferential lysis may be explained by sperm trapped on the cotton swab, inadequate removal of female DNA or loss of sperm whilst separating the female supernatant. Male/female mixtures were relatively common. Surprisingly, during prolonged exposure to the vaginal environment, it has been demonstrated that female DNA from lysed cells can actually adhere to the sperm head [8]. Whilst female DNA can be removed from the sperm during preferential lysis, the single extraction step used in LM is a physical process and therefore cannot be used to selectively remove female DNA that is attached to the sperm. There are a number of factors likely to affect the quality of post-coital sperm and DNA within these sperm. In particular, Spadafora [8] and Francolini et al. [9] suggest complex interactions between sperm and exogenous female DNA that results in nucleases within the sperm cleaving the sperm DNA and eventually resulting in apoptosis. The longer the period of TSI, the greater the amount of apoptosis that may occur. Sperm that have undergone nuclear cleavage but not the subsequent stages of apoptosis, which involves destruction of the sperm head, may therefore appear morphologically intact or normal during microscopic examination even though the DNA contained is highly degraded. This interpretation is supported by statistical analysis that demonstrated an association between TSI and profile quality that was independent of the number of sperm analysed. Consequently, the presence of relatively high numbers of sperm does not assure good DNA profiles.

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