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Evaluation of vaginal mRNA markers in women from different age groups: A GeFI collaborative study

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ABSTRACT

Forensic mRNA profiling assays normally include a set of vaginal-specific markers. Although it is known that vagina undergoes characteristic age-related morphological and physiological changes over a lifetime, few studies have evaluated the efficacy of proposed forensic vaginal mRNA markers in women from different age groups.

In this collaborative study involving ten GeFI (Italian working group of ISFG) laboratories, a 19-plex mRNA profiling assay including three vaginal-specific markers (CYP2B7P1, MUC4, MYOZ1) was tested in a collection of vaginal swabs obtained from female volunteer donors in their reproductive years (n = 84) and postmenopausa (n = 55).

Differential expression of vaginal markers in the two age categories was assessed by means of: a) overall success rate of mRNA profiling (vaginal mucosa "observed" in the tested sample according to scoring protocol) b) average peak height ratios between vaginal-specific markers within mRNA profiling replicates.

Other factors potentially influencing mRNA profiling outcomes, like time interval between vaginal swab collection and analysis, concurrence of menstrual cycle and recent sexual activity at the time of sampling were also investigated.

1. Introduction

In the past few years mRNA profiling has emerged as a powerful tool for the identification of body fluids of forensic interest, including vaginal mucosa. Vaginal mucosa is a highly dynamic tissue, undergoing several morphological and physiological modifications over a lifetime in connection with hormonal modulation and ageing. The main purpose of this study was therefore to evaluate the efficacy of a set of proposed forensic vaginal mRNA markers (CYP2B7P1, MUC4, MYOZ1) in women from different age groups.

2. Materials and methods

Vaginal swabs were obtained through self-sampling from adult consenting women in their reproductive years (R) (n = 84) and in postmenopausa (PM) (n = 55). Donors were asked to fill an anonymous survey including information on menstrual cycle phase and sexual activity in the 10 days before sampling. Subsets of swabs (n = 13–14), designed as to minimize differences in the ratio between R and PM samples and mean time since swab collection (7.3 months ± 0.4 SD), were distributed to participating laboratories. RNA extraction, cDNA synthesis, mRNA profiling using a 19-plex mRNA assay [1] and scoring of results [2] was done according to protocols previously adopted in a preparatory GeFI collaborative exercise [3]. For capillary electrophoresis, each participant used the available instrumentation (310, 3130, 3500 and SeqStudio genetic analyzers), and the settings (analytical threshold, etc.) defined through internal laboratory validation.

3. Results

Nine out of ten laboratories returned complete results, corresponding to a final sample set of 75 swabs from R donors and 50 swabs from PM donors. mRNA profiling success rate (vaginal mucosa "observed" according to scoring guidelines) across laboratories was on average 86% (± 8% SE) for R samples, and 83% (± 5% SE) for PM samples. The observed difference in mRNA profiling success rate between the two age categories was not significant ($p > 0.05$, Mann–Withney rank sum test).

There was also no significant correlation ($p > 0.05$, Spearman rank test) between mRNA profiling success rate and time interval between

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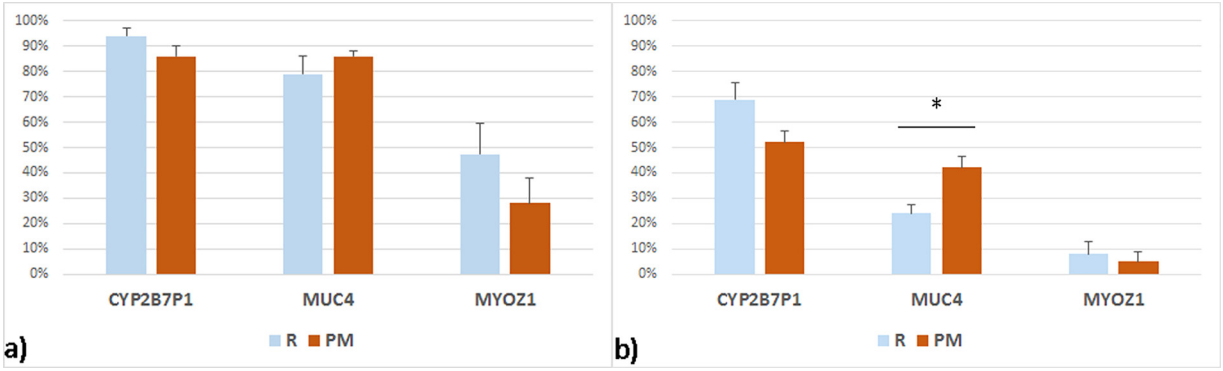

Fig. 1. a) Average percentage of marker replicate peaks above analytical threshold (error bars indicate SE). b) Average relative contribution of each marker to total peak height of vaginal markers in single replicates (error bars indicate SE). Asterisk indicates significance at $p < 0.05$.

swab collection and RNA extraction, which ranged from 6 to 20 months with a median of 11 months.

Differential contribution of the three vaginal markers to mRNA profiling results in the two age groups is summarized in Fig. 1.

In Fig. 1a, the percentage of peaks above analytical threshold observed in mRNA profiling replicates (4 for each swab) is shown for each vaginal marker. The average relative contribution of single vaginal markers to total peak height (measured in rfu) observed for vaginal markers within each mRNA profiling replicate is shown in Fig. 1b. In this case, data from one participating laboratory that reported several off-scale peaks for CYP2B7P1 and MUC4 were not considered in calculations. A significant increase in average relative contribution to total peak height of vaginal markers was observed for MUC4 in PM samples, compared to R samples ($p < 0.05$, *t* test).

Percentages of tested swabs in which body fluids other than vaginal mucosa were "observed" by mRNA profiling were the following: blood (20%); menstrual secretions (21%); semen, not considering in scoring calculations marker PRM1 specific for spermatozoa (21%); semen, considering PRM1 in scoring calculations (5%); saliva (5%); nasal mucosa (4%); skin (51%). The observation of semen (with and without spermatozoa, depending on the presence/absence of PRM1 in at least half of the PCR replicates), blood and menstrual secretions was then considered in connection with available information on menstrual cycle phase ($n = 95$) and sexual activity in the 10 days before sampling ($n = 124$). In donors who did not report sexual activity in the last 10 days, semen with and without spermatozoa was "observed" in 1% and 6% of cases, respectively. Semen with and without spermatozoa was "not observed" by mRNA profiling in a large number of donors who reported sexual activity in the 10 days before sampling: 37% and 30% of cases, respectively. However, it must be underlined that the anonymous survey did not differentiate between protected and unprotected sexual activity. Menstrual secretion and blood were "observed" in donors outside menstrual cycle in 12% and 9% of cases, respectively. On the contrary, menstrual secretion and blood during menstrual cycle were "not observed" in 7% and 6% of the tested samples, respectively.

4. Discussion

Of the three vaginal markers included in the tested multiplex, MUC4 was originally selected for forensic mRNA profiling through a combination of literature and database searches [4] and it is known to be expressed both in R and PM women [5]. On the contrary, CYP2B7P1 and MYOZ1 were originally identified as vaginal markers through transcriptome profiling (RNA-Seq) of vaginal swab samples obtained from two donors (26 and 30 yr old, respectively) [6]. The function of CYP2B7P1 and MYOZ1 in vagina remains unknown and their expression in women from different age groups has not been previously investigated. The results obtained confirm that, in general, MYOZ1 is a less sensitive vaginal marker compared to MUC4 and CYP2B7P1, both in R and PM donors [3]. Overall, the three combined vaginal markers included in the 19-plex mRNA profiling assay appear highly efficient in identifying vaginal mucosa irrespective of the donors age category, even in non-recent samples (6–20 months). A relative reduction of amplification signal for CYP2B7P1 and MYOZ1, corresponding to a significant relative increase of MUC4 was observed in vaginal swabs from PM donors.

Declaration of Competing Interest

None.

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None.

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