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Menstrual cycle phase at the time of rape does not affect recovery of semen or amplification of STR profiles of a suspect in vaginal swabs

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ABSTRACT

The effect of women menstrual cycle on the forensic analysis of rapes was studied in a random group of 170 victims aged among 10 and 51 years. Participants were grouped according to the day of the menstrual cycle in which they were at the moment of the assault. From each participant, samples of vaginal fluid were taken and analyzed for sperm cells, p30 protein, total human DNA and human male DNA. Moreover, amplification of suspect's autosomal STR and Y-STR was attempted. Suspects' autosomal STR profiles were obtained from 92 of the 101 samples in which spermatozoa were found; and Y-STR haplotype was obtained in 1 of the 9 samples where autosomal STR profiles of a male were not obtained. On the other hand, Y-STR haplotypes were obtained in 2 of the 21 samples negative for sperm cells but positive for p30 protein. Y-STR haplotypes were also obtained in 11 of the 48 samples negative for sperm cells and p30 protein. It was found that groups of participants did not differ on the recovery of sperm cells from the vaginal swabs, quantification of suspect's DNA or amplification of their STR profiles. It is concluded that the menstrual cycle phase at the moment of the sexual assault does not affect the main outcomes of the forensic investigation of rapes.

1. Introduction

During sexual intercourse, millions of sperm cells are deposited into the anterior vagina, near the external os of the cervix, and less than 1% might be retained in the female reproductive tract [1]. What occurs with the spermatozoa that do not enter in the cervix has not been extensively studied in humans [2], but it has been estimated that most of them are expulsed from the vagina through flowback [1], while the rest is still detectable in the vaginal fluid several days after sexual intercourse [3,4].

Sperm cells are exclusive of semen. Therefore, their detection in cervico-vaginal fluid is the most convincing way to demonstrate sexual intercourse. Thus, semen is the first evidence sought in rape cases [5]. The coincidence between the DNA of spermatozoa found in vaginal swabs from a victim of rape and the DNA of an alleged perpetrator is not conclusive by itself to demonstrate sexual assault. However, it is an important tool for criminal justice systems [6,7].

The routine analyses of evidence associated with sexual crimes include microscopic examination for spermatozoa (i.e. sperm cytology or spermatoscopy), determination of p30 protein (i.e. prostate-specific antigen), and prostatic acid phosphatase activity [8]. Despite the analytical suitability of these assays for forensic purposes [9], it is not possible to demonstrate, in many cases, the presence of semen in vaginal swabs from rape victims [10].

Obviating the probability of false complaints, negative cases can result as a consequence of: (1) the reaction of the victim after rape, such as bathing before sampling, or waiting a long time after the sexual intercourse to make the accusation and to go through the sampling process; (2) the characteristics of the perpetrator, such as oligospermic, azoospermic or vasectomized offenders; or (3) the characteristics of the sexual intercourse, such as when the attacker use a condom or does not ejaculate [6,10].

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The phase of the menstrual cycle in which victims were at the moment of the assault has been considered as an additional factor which could affect the probability of finding semen in vaginal swabs used as evidence in rape investigation [11,12]. It has been assumed that the increase in the outflow during menstruation might accelerate the rate at which spermatozoa and seminal proteins are removed from the vagina, decreasing the probability to find semen during forensic analysis, when compared to samples from victims assaulted in a menstrual cycle phase other than menstruation. However, after a detailed review of the literature, we did not find any report in which this issue was systematically studied.

To determine how menstrual cycle affects the forensic analysis of rapes, we grouped victims of rape according to the menstrual cycle phase at the moment of the assault, and compared these groups in terms of: (1) recovery of sperm cells and p30 protein in samples of vaginal fluid of participants, (2) the amount of total human DNA and human male DNA in swab extracts, and (3) the amplification of suspect's genetic profiles.

2. Materials and methods

2.1. Study group

This study was approved by the Scientific Ethical Committee of the Universidad de Costa Rica. All the participants or their relatives authorized the use of their samples and testimonies in the forensic investigation. Participants in this study were randomly selected from rape victims who were assaulted in Costa Rica between March 2012 and February 2015. All participants reported the aggression to the prosecution offices, where they were transferred to the Forensic Sciences Center for medical-legal evaluation and evidence collection. We excluded from the study: (1) male, children, menopausal and pregnant women, (2) women who showed themselves for physical examination more than 48 h after the assault; (3) women who did not report the date of their last menstrual period; (4) women having irregular menstrual cycles or reporting their last menstruation more than 35 days ago; (5) women who bathed before the collection of vaginal swabs; and (6) women who were not certain if the attacker use condom. There were no concerns about age, religion, ethnic group, nationality, or geographical origin of the victim, nor for the veracity of the accusation. Participants were grouped according to the day of the menstrual cycle in which they were at the moment of the assault. It was assumed that: (1) group 0–5 included participants who were menstruating; (2) group 6–11 included participants who were in the first week of the proliferative phase; (3) group 12–17 included participants who were in the period around ovulation; and (4) groups 18–23, 24–29 and 30–35 included participants who were in the antepenultimate, penultimate and ultimate week of the secretory phase, respectively. Vaginal swabs were collected from each participant and analyzed for sperm cells, p30 protein, total human DNA and human male DNA. Additionally, amplification of suspect's autosomal STR and Y-STR was attempted.

2.2. Spermatoscopy

Cotton swabs were used for the collection of vaginal fluid samples. Fiber fragments of the swabs were cut and placed in 1.5 mL microcentrifuge tubes. Then, 300 μL of sterile deionized water were added. After vortexing, samples were incubated under constant stirring for 6 h at room temperature. The fiber fragments were placed into a spin basket filter unit and centrifuged 6 min at 18,407 x *g*. Three microliters of the pellet were placed in a glass slide. The dry material was colored by the Christmas tree staining and microscopically examined (at 1000x magnification) to evaluate the presence of sperm cells.

2.3. Detection of seminal p30 protein

Seminal p30 protein was assessed only in samples negative for sperm cells, by using a commercial immunochromatographic kit for semen identification (ABAcard p30 Test; Abacus Diagnostics Inc.) according to the manufacturer's instructions.

2.4. Differential lysis

Differential lysis was performed following a procedure based on the method described by Gill and co-workers [13]. In cases where sperm cells were found, cellular bottoms obtained in the samples extracted from swabs were suspended in 100 μL of deionized sterile water. Then, 5 μL of 20 mg/mL proteinase K (Catalogue # MC5005; Promega) were added. The mixtures were incubated at 37 °C for 1 h, and then centrifuged 6 min at 18,407 x g. The supernatants containing DNA mostly from female epithelial cells (i.e. the epithelial fractions) were placed in a second microcentrifuge tube and then, 50 μL of 20% Chelex were added. The pellets (i.e. the spermatic fractions) were washed three times with 500 μL of Tris buffer (10 mM Tris, 10 mM EDTA, 50 mM NaCl, 2% SDS (w/v), pH 7.5–8.0) and one time with 500 μL of deionized sterile water. After centrifugation, the spermatic fractions were mixed with 50 μL of 5% Chelex, 8 μL of 20 mg/mL proteinase K and 7 μL of 1.0 M dithiothreitol (DTT). After vortexing, the mixtures were incubated at 56 °C for 1 h. Then both, the epithelial and spermatic fractions were boiled for 15 min and centrifuged for 3 min at 18,407 x g. Extracts were stored at 2–8 °C until DNA quantification.

In cases where sperm cells were not found, cellular bottoms obtained in samples extracted from swabs were added with 50 μL of 5% Chelex, 8 μL of 20 mg/mL proteinase K, and 7 μL of 1.0 M DTT. After vortexing, the mixtures were incubated at 56 °C for 1 h, boiled for 15 min, centrifuged for 3 min at 18,407 x g and stored at 2–8 °C until DNA quantification.

2.5. Amplification of suspect's STR profiles

Total human DNA and human male DNA were quantified in all samples using a DNA quantification kit (Quantifiler Duo DNA; Applied Biosystems) in a real time PCR equipment (7500 Fast Real Time PCR System; Applied Biosystems), according to the manufacturer's instructions. DNA was amplified with two multiplex STR systems (PowerPlex 16 HS System and/or PowerPlex Y23 System; Promega), using a thermal cycler (Veriti; Applied Biosystems), according to the manufacturer's instructions. The PowerPlex 16 HS System was used only in samples positive for sperm cells. The PowerPlex Y23 System was used exclusively in samples in which concentration of human male DNA was higher than 0.01 ng/μL. The amplified products were mixed with formamide and ILS, incubated at 95 °C for 3 min, cooled in ice for 3 min, and analyzed in a genetic analyzer (3500 Genetic Analyzer; Applied Biosystems) using the Genemapper ID-X software v 1.2 (Life Technologics).

2.6. Statistical analyses

Statistical analyses were performed using IBM SPSS® v 22.0 (SPSS Inc., Chicago, IL, USA) software. The categorical Pearson's chi-square test was used to evaluate differences between groups of participants regarding the following ratios: samples positive for sperm cells/total number of samples, autosomal STR profiles/total number of tested samples, Y-STR profiles/total number of tested samples and samples positive for p30 protein/total number of