Confirmation of Dog Semen Fluid in an Abuse Scenario by Morphometrics and mtDNA

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Abstract

Some types of violence as an abuse in minors are sometimes difficult to prove and to be related from the crime scene. Cases where non-human bodily fluid is encountered in the routine examination are rare. We describe the case of a minor-victim denouncing a presumptive sexual abuse from male adults with the implication of a domestic animal in the analysed evidences. The dog sperm-head microstructure and morphometrics, for the first time in a forensic context, were described and the following species genetic confirmation as Canis lupus familiaris was by mtDNA analysis. Animal relating evidences in a sexual assault scenario were discussed.

Keywords

Non-Human Semen Fluid; Dog Spermatozoa; Morphometrics; Canis Lupus Familiaris; Mitochondrial DNA

Introduction

Bodily fluid identification plays a significant role in the crime description or confirmation of the abuse suspicion [1, 2]. In particular, case history descriptions with abuses implicating animals, more commonly dogs, in a sexual crime scene are scarce in literature [3]. There is a lack of a screening technique for the presence of dog semen confirmation in routine samples [3, 4]. Some reports of canine assaults were alleged where some physical violence was described and no canine sperm cells were finally confirmed [3-7]. Previous attempts to locate and identify dog semen were: one positive dog semen case whose species was not finally concluded [3] and, secondly, one case where dog hairs were reported [4]. In our present study, a juvenile denounced sexual abuse by male adults where routine human semen analysis at the laboratory revealed, unexpectedly, animal sperm. Species analysis was confirmed by mitochondrial DNA (mtDNA) and microscopic morphometrics are discussed.

Case History

A 17-year-old girl denounced two adults for reiterated sexual abuse. The girl had history of destructured family context with histrionic personality, limit-low IC and quetiapina, 200 mg/day, olanzapina 2.5 mg/day and lorazepam 1/day treatment. Some type of

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sexual harassment was probable and vaginal and anal penetrations were reported. She washed herself and brought her unwashed-clothes to the physician for analysis. Ecchymosed vaginal introit, imperforate hymen and anal fissures were encountered. Human semen analysis was firstly requested in the current forensic investigation and a posterior conversation revealed the presence of a dog in the scene.

Materials and Methods

For semen screening, acid phosphatase and alternate forensic light, Christmas-tree staining for spermatozoa microscopic visualization and PSA immunodiagnostics, when necessary, were employed. Both human and non-human sperm cells were microscopically visible with 40x and 100x-oil immersion magnifications. A dog semen control was visualized, in parallel, for our evidences’ comparison.

Genetic analysis of human and non-human semen stains was followed with Phenol-Chloroform DNA extraction and Amicon Ultra-30 ultrafiltration (Millipore). The presence of human male DNA identification was confirmed by AmpF/STRYfiler (Applied Biosystems). Whereas, for dog genetic characterization, mtDNA amplification was carried out after [8] following quantification by microchip (Agilent Bio 2100) for mtDNA analysis.

Results

Semen was found in the form of scarce human and abundant non-human sperm in clothes (Table 1) No semen was found in genital, vaginal and rectal swabs. Canine spermatozoa were visualized in the inner thigh zone of pants, the posterior zone of panties (knickers); sleeves, central-front and posterior zones of the jacket, as well as in both socks. Similarly, human sperm was visualized in the inner-thigh zone of pants and in the central-front area of the jacket. PSA was only positive in the pants. The sperm head morphology description with the Christmas-tree stain was as follows: rose head with concave proximal end, the acrosome extends to one third of the total and gets a deep rose dye while degrades towards the distal end (Figure 1).

Besides, the dog sperm head gave a three-distinct-bands pattern: A) the acrosomal cap, B) the red and colourless bands region, and C) the post-acrosomal zone. Spermatozoa tails stained in light green colour and were easily lysed. Average dog spermatozoa heads were 5 um and 3.7 um for major and minor parameters, respectively. Scarce DNA traits from human semen were present in all the clothes analysed, except for socks, and did not match with reference suspects. There was human DNA scarcity in the samples (eg. male DNA 0.08 ng quantification in panty) and no match conclusion finally arrived. However, by mtDNA analysis confirmation, the animal species revealed Canis lupus familiaris as present in the evidences, including intimate zones from the clothes.

Discussion

Dog Semen Confirmation and Target Evidences

The present study showed for the first time in a forensic perspective the importance of identifying non-human bodily semen fluid, firstly by morphometry and secondly, by mtDNA analysis. In our case, semen was found in the form of scarce human and abundant non-human spermatozoa heads from the clothes of the victim. Firstly, suspects were not matched from the scarce human semen, and secondly, it is also possible that the dog was around the scene but we cannot confirm whether that was relevant in the abuse scenario.

We here described the dog spermatozoa head.
morphic and showed an up-to-date review of the presence of non-human species to relate a crime scenario. Variability ranges in visual estimation of dog sperm morphology were in line as previously reported [9, 10] the dog spermatozoa heads from the present study were slightly shorter than the reported average major lengths (5 um versus 5.7 um) although within the range for the minor parameter (3.7 versus 3.5-4.4 um) [10]. According to experts, the real morphometric spermatozoa dimensions are rarely described in dogs and a larger number of individuals should be measured for major accuracy in identification [10]. Besides, different results for the morphometric parameters have been obtained when the same population was analysed at a different magnification level (40x versus 60x) [10]. After cryopreservation or dehydration, and probably after a long time interval, like in our present case, significantly lower morphometric dimensions were obtained for the evaluated sperm samples [10]. Semen samples dilutions to approximately 50 x 100 spermatozoa/ml and an objective lens magnification higher than 40x, and analysing at least 100 spermatozoa, were some technical settings previously proposed to obtain reliable sperm morphometries in a veterinary context of the species [10].

No assault of a dog, as such, was alleged in our present case, however, the examination of the clothes smears from intimate zones and underwear, evidenced dog sperm cells that posterior helped to relate the scenario. In literature, there has been a lack of a screening method for dog semen confirmation in the scene [3, 4], however, we hereby could confirm the methodology as satisfactory with the Christmas-Tree staining method (see pattern in Figure 1). In literature, there is one single case describing dog spermatozoa morphology but no conclusion finally arrived to the species because the morphology slightly differed from a dog semen control [3]. In that case, the fresh dog sperm control exhibited the staining pattern in the three described sections, however, evidence sperm cells were damaged, the pattern slightly differed and no genetics were applied [3]. Prior DNA identification, the assessment of sperm morphology is determinant but it may be influenced by factors such as the fixation and staining technique, sperm handling procedures, the quality of the microscope and, possibly most important, the evaluator’s skills [10,11]. Schudel D [3] found that other than microscopy, there was no screening test reported in the literature for canine semen, or for the identification assessment of stains. This may be particularly problematic and so a molecular diagnosis comes necessary for the species conclusion (present study). Furthermore, animal spermatozoa stained with less intensity than human sperm cells (present study); more particularly, bull and pig sperm morphology is result comparable to dog spermatozoa, with a concave proximal head end, but with longer spermatozoa heads (8–10 um) [12]. Thus, the examiner must be able to identify target stains and if obvious targets are not present, the analyser must use his/her experience, the case history information and a proper visual examination to select areas for further analysis [3]. The latter is important as the sperm visualization in selected (intimate) areas of the clothes (for example) might implicate the determination of the accidental/non-accidental nature of the encounter.

**Animal Assault/Induced Harassment in the Scenario**

Fatal dog bites and animal violent acts occur every year, however, fatal dog cases are rare and affect children and old people [6]. This is contrary to our case where it seemed that the animal was in the scene. Accidental assaults are different from induced assaults or types of harassment and, reviewing the literature, a strategy for prevention should come up at least for awareness and more income in the forensic investigation [6, 7]. Dog semen presence in our case may be more unlikely from a result of coincidence than by an induced procedure. The sperm-rich fraction of the dog ejaculate is emitted after courting and mounting [13] and so the high spermatozoa presence in our evidences was more unlikely if the latter was not previously induced. One published case of provoked anal penetration of a child by a dog was previously described; however, the proof to sexual abuse was hardly solved out [5]. Although sexual abuse is a concern for many researchers, therapists, and advocates, for the past three decades, some fundamental issues remain unresolved [2]. Sexual abuse in children and adolescents, as well as, sexual harassment involving animals should be substantially investigated for preventing strategies and more determinant conclusions arriving to court [14].

**Confirmation**

Both human and non-human semen was encountered in the victims’ clothes. This study includes the confirmation procedure for differentiating human from dog sperm in an abuse scenario.
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Conflict of interest
The authors declare that they have no conflict of interest.

References


