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In Vitro Changes in Human Spermatozoa Exposed to Gastric Juice: Laboratory Findings as a Support for Forensic Practice

P. Hooft and H. van de Voorde

Laboratory of Forensic Medicine, University of Leuven, Vital Decosterstraat 102, B-3000 Leuven, Belgium

Summary. Samples of complete human semen were incubated in gastric juice for different time periods at 37°C, and by simulating the post-mortem temperature decrease of the human body. The changes in the spermatozoa were similar in both experiments. Short incubation specimens were examined directly with interference contrast microscopy and showed an almost immediate immobilization of spermatozoa when they were brought in contact with gastric juice. Specimens with longer incubation periods were stained with alcalic fuchsine and examined by immersion microscopy. There was a morphologically stable plateau for the heads of the spermatozoa for up to 6 h of incubation. The tails disappeared progressively in the first 45 min. After more than 6 h of incubation a progressive swelling and lysis of the heads was observed. Spermatozoa could be recognized for up to 7 days of incubation.

Key word: Spermatozoa, in contact with gastric juice

Zusammenfassung. Menschliche Spermaproben wurden bei 37°C für unterschiedliche Zeitspannen mit Magensaft versetzt und die Änderungen von Motilität und Morphologie der Spermien untersucht. In einer zweiten Versuchsanordnung wurde zusätzlich die postmortale Temperatursenkung simuliert. In beiden Versuchsanordnungen waren die Spermienbefunde identisch. Die Spermien wurden nahezu unmittelbar nach Kontakt mit dem Magensaft immobilisiert. Bei den längeren Inkubationszeiten verschwanden alle Spermienschwänze in den ersten 45 min, die Morphologie der Spermienköpfe änderte sich nicht während der ersten 6 h, danach kam es schnell zu einer Lysis. Dennoch konnten bis zum 7. Inkubationstag morphologisch normale Spermien beobachtet werden.

Schlüsselwort: Spermatozoen, Verhalten im Magensaft

Offprint requests to: P. Hooft

Introduction

In forensic literature the detection period for spermatozoa in the oral cavity and the rectum after sexual assaults has well been described in several reports [1, 2, 4, 7, 8]. In 1982, Willot and Allard [7], using oral and rectal swabs, were able to detect spermatozoa in the mouth for up to 9 h after the facts, and in the rectum for up to 65 h. Willot and Crosse [8] in 1986 estimated the proportion of fellatio cases among sexual assaults to be 20% and found spermatozoa in saliva for up to 16 h after the offence.

There is, however, a remarkable shortage of literature on the detection of spermatozoa in the stomach after sexual assaults with oral intercourse. This paper is intended to estimate the possibilities for and the time period of in vitro detection of spermatozoa in gastric juice. Although lab conditions may in large differ from real casework, such experiments can be a support for forensic practice. As it is the result of a swallow reflex induced by the projection of semen into the pharynx at the time of ejaculation, the finding of spermatozoa in the stomach can be seen as a proof of life at the time of the offence.

Material and Methods

One drop of complete human semen (pH 7.3) was suspended in two drops of gastric juice (pH 2.4) on a microscopic glass and was examined directly using interference contrast microscopy (Leitz Dialux microscope) to evaluate short-term mobility changes of the spermatozoa.

Next, 0.1-ml samples of semen were incubated in 5 ml gastric juice. In a first experiment, series of 18 samples were incubated at 37°C, together with control series of 0.1 ml semen in 5 ml physiologic saline. The samples were incubated for 15-min periods during the first hour (samples 1–4), for 30-min periods during the second hour (samples 5–6), and for 1-h periods during the next 4 h (samples 7–10). Samples 11 and 12 were incubated for 12 and 24 h, respectively. The remaining samples were incubated for day periods for the next 6 days (samples 13–18). At the right time, 5 ml of a phosphate buffer pH 7 (Merck) were added for neutralization of the acidity. The samples were then centrifuged at 4000 rpm for 10 min (Hettich rotor). The sediment was spread out on a microscopic slide and dried at the air for 20 min. After fixation in a flame, the specimens were stained with alcalic fuchsine for 20 min (standard solution: 1 g basic Fuchsin (Gurr Ltd.), 10 ml ethanol, 5 ml phenol, 95 ml distilled water; work solution: 3 ml standard solution, 3 ml ethanol, 4 ml distilled water) and were carefully rinsed with distilled water. The stained specimens were air-dried and mounted with inert Depex mounting medium (Searle). They were examined microscopically at a magnification of × 1,250 using immersion oil (Merck Cederoil).

In a second experiment, series of six 0.1-ml semen samples in 5 ml gastric juice were incubated simulating the post-mortem temperature decrease of the human body. The samples were incubated at 37°C for 1 h (sample 1), then at 30°C for the next 6 h (samples 2–3), and further at room temperature (samples 4–6). They were treated as described above after 1, 3, 6, 9, 12, and 24 h. A control series of 0.1-ml semen samples in 5 ml physiologic saline was run in the same way, together with the experiment.

Results

On the addition of two drops of gastric juice to one drop of complete semen, the seminal pH dropped from 7.3 to 3.2. There was a rapid immobilization of the

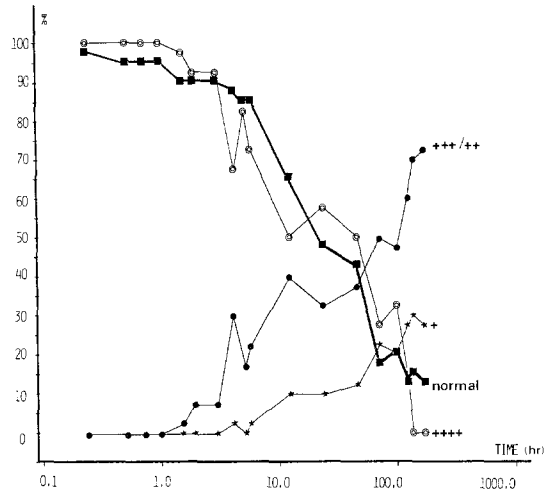

Fig. 1. In vitro changes in the morphology and the number of spermatozoa incubated in gastric juice at 37°C for different time periods (logarithmic hour scale). Percentage of morphologically normal sperm heads with normal fuchsine color pattern (■). Percentage of microscopic fields with many spermatozoa (++++), moderate numbers of easy to find spermatozoa (+++/++), and few spermatozoa (+)

spermatozoa, almost immediately on the moment of contact with gastric juice. The sperm tails all disappeared within 45 min. There was a rapid initial swelling and lysis of a small fraction of sperm heads, but the major proportion showed no morphological changes for up to 6 h of incubation. Samples 11, 12, and 13 (12–48 h of incubation) showed a progressive accumulation of swelling sperm heads and lysis. The control samples at this time still resembled the initial experimental specimens, with only swelling and lysis of a small fraction of tailed spermatozoa. Although massive destruction of spermatozoa became obvious in further specimens, well recognizable heads with an almost normal color gradient were found, even in the last sample that was incubated for 7 days (Fig. 1).

In the second experiment with decreasing incubation temperature, changes similar as those described above were found. The control samples kept on showing morphologically normal spermatozoa for this whole period.

Discussion

Human semen is composed of a small fraction of spermatozoa and a great amount of seminal plasma. It has an almost neutral pH (7.1 to 7.5) and consists of water for approximately 90% [6]. The seminal plasma acts as a nutritient medium and as a buffer. Sperm motility is very sensitive to environmental pH changes [3, 5]. The almost immediate immobilization observed in this experiment is the result of an acidity shock that exceeds the buffer capacity of the seminal plasma.

The most enemic acidity for spermatozoa in normal situations is found in the vagina around mid-cycle (pH 4) [3]. Although the acidity of gastric juice in large exceeds this value and thus is very hostile, there were only minor morphological changes observed in the sperm heads during the first hours of incubation. After 6 h of incubation the situation altered, when a progressive swelling and spermo-

lysis became obvious. This could be the result of a late breakdown of the sperm head membrane, leading to a passive diffusion with lysis of the sperm heads.

More important to forensic practice than the possible physiologic and biochemical explanations for these changes, is the fact that spermatozoa were clearly recognizable for up to 7 days of incubation in gastric juice. Since most of the forensic necropsies take place less than 48 h after death, it may be possible to detect spermatozoa in the stomach of victims of sexual assaults with oral intercourse, on condition that the stomach is empty. In cases where the stomach is filled with food, however, there may be little hope to detect sperm heads under the microscope. In these cases, a thorough examination of the esophagus might lead to the same results.

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