On the Examination of Seminal Stains by Exposure to Ultrasonics

1. Determination of Spermatozoa from Seminal Stains

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INTRODUCTION

The presence of semen is proved by various methods and the best proof may be due to demonstration of spermatozoa morphologically. Even prolonged in spection of preparation does not always reveal spermatozoa in spite of their undoubted presence in the material investigated. Spermatozoa adhere rather tenaciously to cloth fibers, and this characteristic is thought to be the chief reason for the relative failture of many methods. The authors attempted to free spermatozoa from seminal stains by the exposure to ultrasonic, to collect them without destruction and to observe microscopically on the staining smear.

MATERIALS AND METHODS

Healthy human semen gathered by masturbation was diluted with saline solution or dried material beating on gauzes or preserved as seminal stains for long time (longest 14 years).

As an ultrasonic generator, multiple wave generator (T–A–4014 Type) made by the Kaijo Denki Co. was used and exposed frequency was 8 kinds from 20 kc to 2 Mc, exposed strong was 40 W and exposed time was 10, 20, 30 and 60 minutes.

The spermatozoa was collected in a test tube, adding saline solution or 0.1% antiformine and exposed to ultrasonics. Removing fibers and adding a drop of Löffler methylene blue solution (10 times the dilution) the solution was left standing for a few minutes and centrifuged for 3 minuites at 1500 rpm and the precipitate was observed microscopically.

RESULTS

At first we did test to see whether exposure to ultrasonics injured spermatozoa in fresh semen or not. The table 1 shows detective rate of spermatozoa by 8 kinds of wave cycles of ultrasonics, from 20kc to 2000kc, for 10, 20, 30 and 60 minutes at 37°C. The exposure to ultrasonics at over 400kc for 10-60 minutes did not injure the spermatozoa morphologically, but under 200kc caused separation of the spermatozoa tail. The lower the ultrasonics became, the more this injury became increased. This result was common in neat and any dilution of semen.

Table 1.

Effect of Ultrasonics on the Morphological Structure of Spermatozoa

temperature		37°C			
time (min)		10	20	30	60
frequency (kc)	20	−	±	+	++
	50	−	±	+	++
	100	−	−	±	±
	200	−	−	±	±
	400	−	−	−	−
	600	−	−	−	−
	1200	−	−	−	−
	2000	−	−	−	−
control (unexposure)		−			

− : no destructive spermatozoa
± : a few destructive spermatozoa
+ : moderate destructive spermatozoa
++ : many destructive spermatozoa

Next, the most suitable frequency and exposed time to free the maximum number of spermatozoa from seminal stains was investigated. The spermatozoa was counted by a blood mélangeur and a Thoma-Zeiss blood counter. As showing on the table 2 the largest number of free spermatozoa from seminal stains preserved for 3 months and a year obtained at 400kc for 20-30 minutes and next at 600kc. The frequency of ultrasonics at 1200kc and 2000kc, and exposed time for 10 minutes decreased in number and exposed time for over 60 minutes caused the separation of the tail.

Spermatozoa obtained from seminal stains preserved for 3 or 6 years caused generally the separation of tails and the number of complete spermatozoa decreased, but the best condition of exposure was at 400kc or 600kc for 20-30 minutes. On the seminal stains preserved for 14 years, exposed frequency at 400kc or 600kc and exposed time for 20 minutes was most suitable.

Table 2.
Experiment of the Separation of Spermatozoa from Dried Seminal Stains

preservation time		within 3 months				1 year				3 years				6 years				14 years			
exposed time (min)		10	20	30	60	10	20	30	60	10	20	30	60	10	20	30	60	10	20	30	60
frequency (kc)	400	++++	++++	++++(++)		++++	++++	++++(++)		(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(+)	(++)	(+)	(+)
	600	++++	++++	++++(++)		++++	++++	++++(++)		(++)	(++)	(++)	(++)	(+)	(++)	(++)	(++)	(+)	(++)	(+)	(+)
	1200	++++	++++	++++(++)		++++	++++	++++(++)		(++)	(++)	(++)	(++)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
	2000	++++	++++	++++(++)		++++	++++	++++(++)		(+)	(++)	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(+)	(+)	(+)

( ) : spermatozoa separated tail

DISCUSSION

Since Baecci's[1] investigation, various staining method has been used for the proof of semen, but most of methods stained the material or teased fibers from the seminal stain and observed microscopically and direct observation of spermatozoa separated from the meterials was few. In 1960 Ellis[2] observed the filter papers filtrating the separated spermatozoa from the seminal stains by means of agitation on a magnetic stirrer microscopically. Yasoshima[4] (1962) modified this method and recommended as a easy method. Marcinkowski[3] (1966) reported the separation of the spermatozoa from the material by the vibration of an apparatus fitted in domestic laundering devices. The authors separated the spermatozoa from the material by the exposure to ultrasonics.

On the resistance and morphological changes of the spermatozoa by the exposure to ultrasonics, morphological destruction is not caused by the exposure at over 400kc for 10-60 minutes, but the exposure at under 200kc for half an hour occurs the separation of tail. This change may be due to strong vibration, one of the physical activity of the ultrasonics. On the separation of spermatozoa from the preserved seminal stains, the exposure at 400kc for 20-30 minutes is most suitable and next 600kc. The decrease of the number of spermatozoa at 1200kc or 2000kc and for 10 minutes is observed and the exposure for over 60 minutes causes the separation of tail because of overtime exposure. The separation of spermatozoa may be due to not only the vibration, but also direct or secondary washing effect by cavitation. At that time, using 0.1% antiformine increases the number of spermatozoa in solution and dye affinity by the proteolytic effect and aids the morphological recovery of the spermatozoa by its swelling effect. From the oldest seminal stains which was preserved for 14 years, the spermatozoa was demonstrated, but twice amount of semen is necessary in unexposed material, when compared with exposed case.

CONCLUSION

In the determination of the sprmatozoa from seminal stains, the exposure to ultrasonics can more easily separate the spermatozoa from the material and more clearly prove the semen, when compared with usual methods.

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