

CERVICAL MUCUS PENETRATION *IN VITRO* BY FRESH AND FROZEN-PRESERVED HUMAN SEMEN SPECIMENS

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A serious difficulty in the use of frozen-preserved human semen for donor insemination is our inability to predict, without rather extensive clinical trials, which specimens will be successful in producing conception and which will be generally infertile. Predictions cannot be made from the pre-freeze motility of the specimen (Behrman & Sawada, 1966) nor, in our experience, from the fertility of a donor's freshly ejaculated specimen.

A technique for determination of cervical mucus penetration by spermatozoa using capillary tubes (Kremer, 1965) has been thoroughly investigated and found to have several advantages (Kremer, 1968). This capillary tube test has been shown to be a reasonably reliable predictor of the fertility of fresh human semen (Fjällbrant, 1968). Specifically, a penetration by the leading spermatozoa in the capillary tube of more than 5 mm was found to be a necessary condition for fertility. Thus, it appears that this test may be a good screening device in the selection of frozen-preserved specimens for clinical use.

To make the test still more reliable and useful, some modifications were performed. The inner diameter of the capillary tube was decreased to 0.4 mm. This made it possible to fill more than forty tubes to a length of about 40 mm, using only one specimen of cervical mucus drawn from a donor at the expected time of ovulation. In this way, all the semen specimens could be tested with the same mucus sample.

Instead of keeping the mucus-filled capillary tubes at +4° C, they were stored deep-frozen at -70° C, since it had been shown that this procedure did not change the penetrability of the mucus. Thawing was performed at room temperature. The penetration distance was noted each hour for 3 hr.

As can be seen from Table 1, six fresh specimens, two frozen-thawed specimens and twenty-seven frozen-preserved specimens were compared by means of the penetration test. Modes of low temperature storage were compared: aliquots of the same specimens, which had aged almost 3 years in liquid nitrogen

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at -196°C and in a mechanical freezer at about -90°C , respectively, were studied. The influence of length of storage was investigated as well.

Frozen semen specimens were treated with egg yolk-glycerol preservative medium, and cooled to storage temperature according to standard practice (Behrman & Sawada, 1966). Specimens were thawed at room temperature, and count and motility were recorded.

A summary of results is given in Table 1. These indicate that: (1) fresh, untreated spermatozoa penetrate the full length of the capillary (usually by the 1st hr); (2) spermatozoa, which have been frozen and then thawed immediately, seem to have been only slightly damaged; but (3) spermatozoa, which have remained frozen for any length of time, are damaged to the extent that their

TABLE 1
SPERM PENETRATION (IN MM) OVER 3 HR, MEAN \pm S.E. OF MEAN

Fresh specimens		Frozen-thawed specimens		Frozen-preserved specimens		Specimens from the same donors stored 3 years at:							
						-90°C		-196°C		Specimens frozen-preserved in mechanical freezer for:			
No.	mm	No.	mm	No.	mm	No.	mm	No.	mm	No.	mm		
6	>30	2	25.2 ± 3.2	27	9.52 ± 1.78	7	4.14 ± 2.48	7	14.29 ± 2.26	16	8.44 ± 2.39	8	6.25 ± 2.93

penetration ability is severely reduced. The average penetration of these specimens is, however, greater than the 6 mm which is taken to be minimal if a specimen is to have any likelihood of being fertile; (4) it is quite clear, also, that storage in liquid nitrogen causes far less damage to spermatozoa than does storage in a mechanical freezer. This may be due, in part, to the fact that the latter does not maintain stable low temperatures, and specimens stored in them must undergo recrystallization. This is known to affect cell motility adversely (Ackerman, 1968); (5) two sets of specimens were compared with respect to length of storage; all had been preserved at -90°C . One set included those aged 1 to 12 months; the other, those aged from 13 to 35 months. A reduction of penetration ability with age was observed. This reduction is probably associated with the more extensive recrystallization which had occurred in the older specimens.

It is of interest to determine which of several factors seem best to predict the cervical mucus penetration of frozen-preserved spermatozoa. The relation of (1) post-thaw motility (%); (2) sperm density; and (3) days in storage to penetration was determined by multiple regression analysis. Among the partial correlation coefficients, only that for post-thaw motility (%) was significant: $b'_{Y_1, 23} = 0.678$, $F_{1, 33} = 33.70$, $P > 0.001$.

It is concluded that freeze-preservation of semen reduces the mucus-penetration ability of the spermatozoa. If liquid nitrogen is used for storage, this reduction is limited to a degree that might be compatible with fertility, even for storage periods approaching 3 years.

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