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Brief Communication

METHOD FOR THE INACTIVATION OF VIRUS ON SEMEN STRAWS

A number of infectious agents including some viruses may be transmitted through semen. In methods for the processing and storage of frozen semen where liquid nitrogen has free access to the semen such as in the pellet method the possibility exists of infectious agents being transferred from one dose of semen to the other. The assumption that transmission may take place has been supported by *Lorrmann* (1968) who demonstrated that sperm could be transferred from one pellet to another. In other freezing procedures such as the straw method the processing is so that the vials (straws) may be contaminated on their outside surface from the semen for which they are used or otherwise, and the possibility exists, therefore, of contamination of the liquid nitrogen in the storage container with viruses and other infectious agents.

The present investigation was undertaken with the aim of finding a method for treatment of the filled and sealed straws which could be assumed to inactivate viruses on their outside surface.

A pH of $\geqq$ 10 provides an effective and with pH increasing inactivation rate for viruses. In Danish rules and regulations for the control of notifiable virus diseases, the use of 0.2 % NaOH is included for virus inactivation purposes. It was therefore decided to investigate whether the filled and sealed semen straws in their further processing could pass a bath with 0.2 % NaOH solution without detrimental effects to the straws and semen.

Physical properties. Straws, medium (*Cassou* 1966) were machine-printed (Graul) using Schwarz no. 03. The straws were closed according to *Cassou* (1950). They were then submerged in a bath containing 0.2 % NaOH in distilled H_2O at 4°C (pH 12.8) for 5 hrs. After this treatment no effect was noticed on the physical qualities of the straws, seals and printed texts, and no liquid was noticed inside the straws.

pH. Straws were filled with water (pH 7.0) and sealed. Some of the straws were submerged in the aforementioned bath, and others were placed in a bath containing tap water at 4°C. After 5 hrs. pH of the contents of the straws was measured. In 5 trials pH was found to be 6.7—6.8 in both groups.

Motility. Straws were filled with semen processed from the same ejaculate. The straws were sealed and placed in a water bath at 4°C. After a few minutes half of them were transferred to the aforementioned bath with NaOH solution and the other half to an ordinary water bath where they remained for 10 min., whereafter both groups were transferred to new ordinary water baths and left there during the remaining equilibration period. Microscopic determination of motility rates by two persons working independantly took place 1) before filling of the straws, 2) 1 hr. after submerging in the final water baths, 3) 24 hrs. after freezing. Seven ejaculates from 7 bulls were included. The two groups did in no case differ significantly in respect of motility rate.

Fertility. Twelve ejaculates from 6 bulls were diluted according to standard procedure and split in two. One half of the diluted semen was filled in straws labelled B and the other half in straws labelled C. The two groups of straws were kept separate and carried through the same processing procedures as described above, so that B-straws passed through water baths and C-straws through water baths and the NaOH bath. They were then frozen according to standard procedure and stored for 5—20 days. Eighteen technicians used the semen for insemination. Each

Table 1. Breeding efficiency (60—90 day non-return percentage) for experimental (C) and control groups (B).

	C		B		
Bull	number of ins.	N.-R.%	number of ins.	N.-R.%	Difference C-B
Aalborg Bill	195	68.2	207	67.6	0.6
Aalborg Bos	258	66.7	286	60.1	6.6
Aalborg Ask	381	58.3	335	66.0	—7.7*
Aalborg Horn	302	71.9	266	69.9	2.0
Aalborg Bast	126	62.7	134	61.9	0.8
Aalborg Foch	193	61.1	177	63.3	—2.2
	1455	64.7	1405	65.1	—0.4

*: $P < 0.05$

technician was equipped with the same number of B- and C-straws for each ejaculate. The use of B- and C-straws was randomly distributed in respect of time. The breeding efficiency was expressed by 60—90 day non-return percentages based on 1., 2. and 3.-inseminations. The results are given in Table 1. It will be seen that the totals of the C- and B-groups did not differ significantly. The true difference has been calculated to be between —3.9 and +3.1 at the 5 % level. One bull (Aalborg Ask) shows a difference which is significant at the 5 % level, but one such difference in a material of 6 differences in total is not unlikely to occur from incidental causes.

The results indicate that the passage through 0.2% NaOH for 10 min. can be included in the processing of straw semen without reducing its fertilizing capacity.

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