The Effects of Exposure to Ambient Temperature on Sperm Cells Stored in Straws

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Story in Brief

Several artificial inseminations in the past two years have converted partially or completely to the use of either ¼ cc. or ½ cc. straws as their method of storing semen. This particular method of storage has been used in Europe for a few years and the movement of semen of exotic bulls into this country in straws has stimulated our artificial insemination industry to attempt to use this particular method of semen storing.

Little is known concerning how straws should best be stored and thawed. It is known that improper handling of ampuls can significantly decrease the percent live cells that are present in an ampul of stored semen. The time element concerning how long an ampul of semen can be safely exposed before returning it to the nitrogen tank has been well established. Such parameters have not been established for handling straws. It was the intent of this research to establish the safe handling procedures necessary for satisfactory use of semen stored in straws.

Semen was collected from four bulls on two different occasions and stored in ¼ cc. straws. These straws were exposed to room temperature for 10-second intervals and then returned to the nitrogen tank. Straws were exposed for a maximum of ten 10-second exposures and then thawed and evaluated for the speed of movement of the cells and the percent of the cells that were showing progressive mobility.

There was little doubt that even one 10-second exposure had a significant effect ($P<.01$) in lowering the percent live cells in the stored straws. Approximately 15 percent of the live cells were lost after exposing the straws for one 10-second interval and then returning the straw to the tank. After the third 10-second exposure, the percent live cells had decreased to less than half of the original percentage. This indicates that the sperm cells stored in straws cannot be exposed to ambient temperature and returned to the nitrogen tank as has been typical of cells in ampuls. Poor fertility can obviously result from careless handling of semen stored in ¼ cc. straws.

Introduction

Since about 1956, the artificial insemination industry has utilized the glass ampul as the preferred method of freezing, storing and delivering semen to the artificial insemination customer. This particular system has worked well and satisfactory fertility has been typical throughout the industry from the use of the ampul. For the past several years, several European countries have converted to extensive use of the straw as the preferred method of semen packaging.

Several reports (MacPherson, et al, 1966; Carpenter, 1971; MacPherson and Penner, 1972; Day, 1972; Bean, 1972) utilizing organizational technicians have indicated some small advantage for straws in conception rate, usually one to two percent. Other advantages have been cited, namely, greater quantities of semen can be stored in the same space occupied previously by ampuls and better post-freeze recoveries are typical with semen stored in straws.

A big percentage of the artificial inseminations in this country are done by personnel other than technicians hired directly by the artificial insemination stud. There is much greater opportunity for mistakes to be made by people who will not be typically as well trained or controlled as would be a technician hired by the bull stud. No controlled research has been published in which straws were purposely exposed to the typical mistakes that may be made in the field. This experiment was conducted to measure the influence of routine mistakes that could be made on the farm in using straws.

Previous research with semen stored in ampuls indicate that an ampul can be exposed safely to room temperature for 10 to 12 seconds and returned to the nitrogen tank without significant loss of live sperm cells. Exposure to room temperature for one minute and then returning to the tank has resulted in significant loss of live sperm cells stored in the ampul. This research is an attempt to define the effect of length of exposure to room temperature on cells stored in ¼ cc. French straws.

Procedure

Semen was collected from four dairy bulls on two different occasions and stored in ¼ cc. French straws for use in this study. All bulls were housed and handled similarly and were on routine maintenance rations. Semen was collected and initially evaluated for the percent live cells and the rate of movement of the cells. Each ejaculate was then diluted with egg yoke-citrate-glycerol extender to yield 20 million live cells per straw prior to freezing. The straws were filled, plugged and then placed in 5°C water for approximately three hours. The straws were then placed

on a freezing rack and placed in a MVE Model CBF-21 vapor freezing unit at a point above the nitrogen where the vapor temperature was −130°C. The cells were left at this level for seven minutes and then placed in the liquid nitrogen for transfer into the storage unit. Three straws on each ejaculate of each bull were then thawed in a 35°C water bath and evaluated for the percent live cells and the rate of movement of the cells.

These observations represented the degree of recovery of the cells post freeze with no treatment imposed. The effect of repeated exposure to room temperature was evaluated using from one to ten 10-second exposures to room temperature. After each individual exposure, the three straws in each ejaculate for each bull were returned to the nitrogen tank for at least one minute before thawing and evaluating for the rate of motility and the percent of live cells.

Treated straws were also thawed in 35° C water for one minute and then placed on a warm microscope slide for evaluation purposes.

Results and Discussion

Table 1 shows the average quality of the ejaculates used in this study for both pre-freeze and post-freeze motile cells. The average pre-freeze quality was 85 percent live cells. The average quality post-freeze with no treatment imposed was 55 percent live cells. This gives an average post-freeze recovery rate of 65 percent. Table 1 also presents the average recovery rate that has been experienced in ampul studies with these particular bulls although ampuls were not stored from these ejaculates.

The average recovery rate that we have experienced with these bulls before with semen stored in ampuls was 45 percent. It has been our experience as well as others, that semen stored in straws typically will have a better recovery rate than will semen stored in ampuls. This is one

Table 1. Comparison of Percent Motile Cells Pre-Freeze and Post-Freeze

Method of Storage	Pre-Freeze	Post-Freeze	Recovery Rate
		%	
Straw	85	55	65
Ampul	85	38	45

of the advantages that straw storage has over ampul storage in that better utilization of the bulls can be obtained with straws.

Figure 1 presents the average effect of multiple exposure of straws to room temperature. Successive exposure of straws to roow temperature results in a drastic decrease in the percent live cells. It is significant to note that the first few exposures take a heavy toll of the percent live cells in the straws. Figure 1 illustrates that with just one 10-second exposure an average of 25 percent of the live cells in the straw was lost. This alone could result in a significant lowering of fertility of

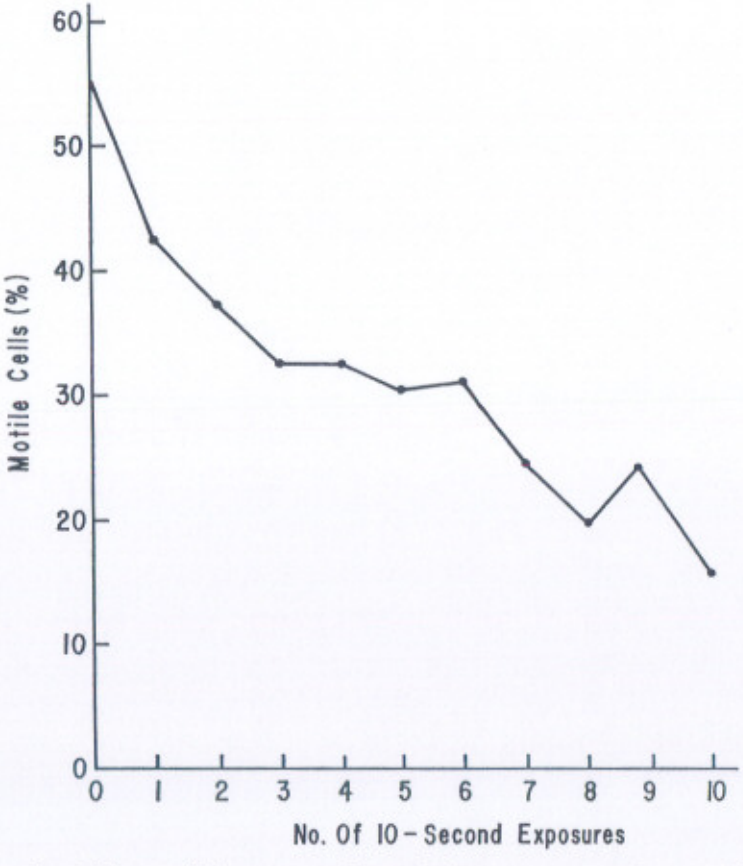

Figure 1. Effects of Exposure to Room Temperature on Percent Motile Cells in Straws.*

* Each point is the average of 4 bulls, 2 ejaculates/bull, 3 straws per ejaculate.

the cells in the straws as a result of the decreased usable sperm cell population. As you continue to expose the straws at 10-second intervals, you continue to lose more live cells. At the end of the second exposure, over 30 percent of the live cells have been lost, and at the third exposure 40 percent of the live cells have been lost. It is apparent that with the thin dimension of the straw, rapid thawing can apparently occur in just one 10-second exposure and its subsequent refreezing then results in highly significant losses of live sperm cells. The practical interpretation of this is that we do not have the latitude for making mistakes with straws that we have with ampuls.

This information suggests that a straw should not be removed from the tank for any purpose except to thaw it enroute to ultimately being placed in the cow's reproductive system. This means that we must have adequate maps of our semen storage tanks so that one can readily locate the desired animal without resorting to pulling the straws out of storage to read the name or number and then return the straw to the tank. Mishandling of the straws in the manner described here will surely take away any advantage that might potentially be there for breeding our cattle with straws.

Figure 2 illustrates another deleterious effect that multiple exposures to room temperature can have. The relative strength of the cells was measured throughout this trial and is indicated by the rate of their motility. As can be seen in Figure 1, the cells in the straws started out with a very acceptable rate of motility. However, as the straws were exposed to increasing numbers of 10-second exposures, the strength of the cells apparently failed. Again, a practical interpretation of this means that the repeated exposures apparently weakened the cells. Although we did not collect fertility data in this study, other data would suggest that when cells are so weakened, fertility of the cells would be lessened.

The data presented indicates strongly that serious mistakes can be made as we attempt to utilize straws in implementing artificial insemination programs in our livestock herds. The great damage is apparently done by the stress incurred by the partial thawing and refreezing that occurs when straws are removed or exposed to room temperature and then returned to the nitrogen tank. In this process, we lose a large percentage of the live cells and undoubtedly have a significant effect on the potential fertility of the stored cells.

The conclusions in this report about exposing cells stored in straws to room temperature agree quite well with similar research conducted on cells stored in ampuls (Pickett, et al, 1961). Other research is presently in progress seeking to better define how semen stored in straws should be handled.

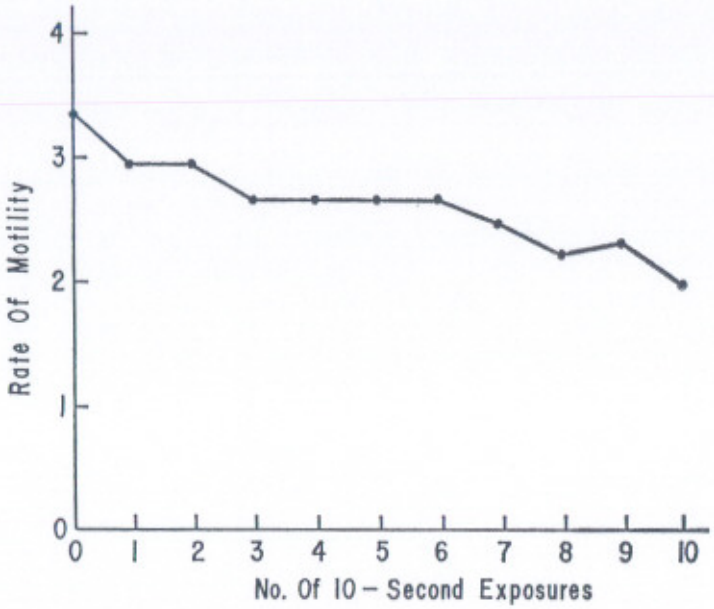

Figure 2. Effects of Exposure to Room Temperature on Rate of Movement of Cells in Straws.*

* Each point is the average of 4 bulls, 2 ejaculates/bull, 3 straws per ejaculate.

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