The Effect on Length of Equilibration Time on Sperm Cells Stored in Straws

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Story in Brief

Glycerol is widely used as a critical component in several extenders utilized in freezing sperm cells. Extended semen is typically exposed to glycerol (to "equilibrate") for 6 to 18 hours at 40°F. before freezing to —320°F. With increasing quantities of semen being processed in straws, it is important to identify methods of straw processing which result in greatest yield of useful cells post-freeze. This pilot study was conducted to determine if exposing sperm cells to glycerol for varying lengths of time had any effect on percent live cells and cell quality post-freeze when ½ cc. French straws were utilized as the dosing vehicle.

Ejaculates from mature bulls were processed using standard procedures which require the addition of glycerol to the extender. Each ejaculate was split with the two fractions then exposed for either 4 or 18 hours to glycerol prior to freezing.

Examination of ejaculates after freezing revealed significant differences between the treatments imposed. The longer equilibrium time resulted in significantly higher percentages of live cells and cells showing normal morphology. Aging of the acrosome was reduced greatly with the longer equilibration time.

Introduction

Storage of bull sperm cells in the deep-frozen state (—320°F.) has been practiced for several years. The early search for critical extender components revealed that glycerol was needed in the commonly used extenders to assure recovery of live cells in needed numbers post-freeze (Polge and Rowson, 1952, Mixner and Wiggins, 1957).

For several years, 7 to 10 percent glycerol has been added routinely to extenders. Early research indicated that glycerol should be added to the cooled, diluted semen with the mixture then being held for 6 to 18 hours at 40°F. before freezing the cells. This equilibration, or reaction time, was thought to be necessary for cells to acquire the protective properties of glycerol. Later research has shown that reasonable recovery of live cells post-freeze is possible with much shorter equilibration times (Bernsdtson and Foote, 1969). Consequently, some artificial insemination organizations have altered their ampule processing procedures to utilize the shorter equilibration time. Several of these organizations are now attempting to utilize the straw dosing system as their primary vehicle for semen dissemination.

Recent research attention has been devoted to freezing rates and thawing rates that would result in consistent quality of the product post-freeze (Almquist and Wiggin, 1973a, 1973b). Results have indicated that procedures for processing ampules cannot be applied successfully to straw processing. Consequently, several aspects of processing semen in straws need to be investigated. Information on the effect of equilibration time on post-freeze quality of cells stored in straws has not been reported.

The objective of this pilot study was to evaluate the effect of length of exposure time to glycerol on post-freeze quality of semen stored in ½ cc. French straws.

Materials and Methods

One ejaculate was collected from each of 3 mature dairy bulls for use in this pilot study. All bulls were housed and handled similarly and were on routine maintenance rations with no treatment other than weekly semen collection being imposed. Following collection, semen was evaluated microscopically for percentage of progressively motile cells and normal cells. Stained slides of each ejaculate were prepared to determine the morphological condition of the acrosome prior to dilution and freezing. Each ejaculate was diluted in an extender consisting of 74 percent of a 2.9 percent sodium citrate solution, 20 percent fresh egg yolk and 6 percentglycerol. Each ½ cc. straw was filled with 20 million live cells, sealed and placed in a water bath at 40°F. One half of the straws from each ejaculate was held for 4 hours at 40°F. and then frozen while the remaining straws were held for 18 hours at 40°F. and then frozen. Straws were frozen by placing them in an MVE Model CBF21 vapor freezing unit at the point where vapor temperature was —180°C. The straws were maintained at this level for 8 minutes then lowered into the liquid nitrogen for transfer to the storage unit.

Eight straws were thawed at 90-95° F and evaluated on each bull in each treatment for rate of movement and percent progressively motile cells. Morphological examination of the cell population in each straw was conducted to determine percent normal cells and the extent of acrosomal aging noted on normal and abnormal cell types (Wells and Awa, 1970). The following summarizes the measurements (as percentages) that were made on each initial ejaculate and the 8 straws per bull in each treatment after freezing.

1. Progressively motile cells
2. Aged acrosomes
3. Normal morphology
4. Normal cells with non-aged acrosome
5. Normal cells with aged acrosome
6. Abnormal cells with non-aged acrosome
7. Abnormal cells with aged acrosome

Results and Discussion

Results from the motility and morphology analysis are presented in Table 1.

The average pre-freeze characteristics indicate that these samples were of acceptable quality prior to initiation of treatment. A comparison of percent progressively motile cells shows that there was a significant reduction due to either treatment. It has long been accepted that there will be a significant percentage of cells killed during freezing. Therefore, dilution rates are calculated to yield the desired number of live cells post-freeze. As Table 1 shows, the 18 hour equilibration time gave signi-

Table 1. Mean Effect of Treatment on Sperm Cell Characteristics

Characteristic	Prefreeze	4 Hr. Exposure	18 Hr. Exposure
		(percent)	
Progressive Motility**	83.0	35.3[1]	42.3[1]
Normal Cells*	88.3	85.2	88.1
Normals with non-aged Acrosome*	81.1	48.7	64.4
Normals with aged Acrosome*	7.1	36.6	23.7
Abnormals with non-aged Acrosome	7.0	6.1	5.2
Abnormals with aged Acrosome	4.3	8.6	6.7
Total aged Acrosomes**	10.8	45.2	30.4

[1] Each treatment mean is the average of 3 bulls, 8 straws per bull.
*Treatment means were significantly different at the .05 level.
**Treeatment means were significantly different at the .01 level.

ficantly better ($P<.05$) post-freeze recovery. Apparently, the longer holding time enabled a higher percentage of the cells to withstand the freezing process.

A similar treatment effect is seen in the percent normal cells. The difference between pre-freeze and post-freeze percentages was not as great as motility differences. The 18 hour equilibration period was again significantly superior ($P<.05$) to the shorter holding period.

The next group of measurements attempted to categorize the changes occurring in acrosome state caused by treatment. In general, freezing caused a larger decrease in the percentages of normal cells showing no aging of the acrosome while the changes on the abnormal cells were of much smaller magnitude. This agrees with other studies and indicates that the normal sperm cells in a population are most likely to be damaged during the freezing process. As Table 1 shows, the total percentage of aged acrosomes post-freeze in an ejaculate is principally due to the large increase in the percentage of aged normal cells.

Table 2 shows the mean effect of treatment on the ejaculate characteristics of each bull. There were significant differences among bulls in response to treatment for several of the measured characteristics. The pattern of response was similar in that the longer equilibration time resulted in a more desirable post-freeze condition. However, the magnitude of response was quite different among bulls. This is best illustrated by comparing progressive motility ratings for the bulls' ejaculates for each treatment. The 4 hour exposure was much harsher for bull C than for other bulls. This agrees well with industry observations that semen of some bulls freezes better than that of others.

It is generally accepted that in order for a population of cells to be fertile, a reasonable percentage of the cells must be normal with no apparent damage or aging of the acrosome (Saacke and White, 1968). Research on boar sperm cells by Graham et al. (1971) and Pursel (1971) verified that the integrity of the acrosome must be preserved on some percentage of the cells before fertility can be apparent. The precise percentage of number of intact cells necessary is the object of continuing research.

Our research herein reported strongly suggests that significantly better post-freeze percentages of live cells and normal cells with no aging of the acrosome are achieved with longer exposure to glycerolated extender prior to freezing. The optimum length of time may well be between the two time intervals employed in this study. Research is continuing to better define optimum treatments.

Table 2. Mean effect of Treatment on Bull ejaculate Characteristics[1]

Ejaculate Characteristic	Prefreeze			4 Hr. Exposure			18 Hr. Exposure		
	Bull			Bull			Bull		
	A	B	C	A	B	C	A	B	C
%									
Progressive motility	85.0	80.0	85.0	52.0	39.0	15.0	50.0	40.0	37.0
Normal cells	81.0	94.0	90.0	82.2	85.0	88.5	84.3	88.8	91.1
Normal cells with non-aged acrosomes	72.0	87.0	84.5	49.8	51.1	45.1	59.1	68.1	65.8
Normal cells with aged acrosomes	9.0	7.0	5.5	32.5	33.8	43.3	25.1	20.6	25.3
Abnormal cells with non-aged acrosomes	11.0	4.5	6.5	7.0	6.8	4.4	5.8	5.7	4.1
Abnormal cells with aged acrosomes	8.0	1.5	3.5	10.6	8.1	7.1	9.8	5.5	4.8
Total aged acrosomes	17.0	8.5	9.0	43.1	42.0	50.4	35.0	26.1	30.0

[1] Each mean is the average of 8 observations per bull.

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The Effect of Freezing and Thawing On Sperm Cell Dimensions

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Story in Brief

Although the dimensions of sperm cells of several species have been published, the effects of dilution, freezing and thawing on sperm dimensions have not been thoroughly studied. Single ejaculates were secured from each of 11 yearling Holstein bulls and processed with routine