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The Influence of Thaw Temperature On Bovine Spermatazoa Commercially Frozen In Straws

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Story in Brief

Bovine semen commercially frozen and packaged in .5 ml straws, was subjected to thaw temperatures of 32 F, 68 F, 95 F and 203 F for 2 min, 1 min, 20 sec and 7 sec, respectively. Following completion of thawing, the semen was incubated at 98.6 F for either 5 min, 1 hr, 3 hr or 5 hr. The effects of these treatments were evaluated by changes in the percentage of "live" cells and changes in cell structure during the incubation periods. Faster thaw rates increased the percentages of "live" cells and cells showing no alteration of the acrosome. Of the four thaw temperatures, the 95 F/20 sec provided more live cells of desirable type than the others.

Introduction

The artificial insemination industry currently utilizes several semen packaging methods in selling semen to the public. The most recent package consists of a plastic straw, or tube, which normally contains either .3 ml or .5 ml extended semen. The straw shape and size is substantially different from the conventional glass ampule. Several problems have become apparent as breeders have used recommended ampule thawing procedures for the straw. The major problems have been poor cell viability post-thaw and variable fertility.

The straw semen packaging system was introduced before research had defined optimum handling procedures. Various straw thawing techniques are common. These include thawing in the cow, thawing between the hands,

thawing in the shirt pocket, thawing in 32 F water and air thaw. These techniques obviously will result in a wide range in rate of thaw. Research has since determined that the geometric shape of the straw (surface to volume ratio) and straw freezing methods dictates particular thawing procedures. These procedures involve fast thawing rates which can be facilitated by thawing in 32 F water or 95 F water.

It appears that thawing rapidly results in improved motility and acrosome status. Thaw temperatures ranging from 40 F to 203 F have been utilized to determine optimum thawing procedures. However, the extremely rapid rates are exceptionally difficult to achieve in routine artificial insemination programs. Consequently, current recommendations by bull studs are a mixture of research findings and apparent achievable field practices.

The purpose of this study was to evaluate four thawing temperatures applied to semen frozen in plastic straws and determine the apparent usefulness of the four thaw temperatures used.

Materials and Methods

Semen frozen in .5 ml plastic straws was provided by a commercial AI Company for the study. Five different bulls were used, with eight straws provided per bull. Two straws for each bull were thawed at either 32 F, 68 F, 95 F or 203 F in a water bath for 2 min, 1 min, 20 sec and 7 sec, respectively. After thawing, straws were pooled in centrifuge tubes and placed in a constant temperature water bath at 98.6 F for incubation. At each of four incubation times, 5 min, 1 hr, 3 hr, and 5 hr, a small sample was withdrawn and evaluated for percent live cells and acrosome condition. To accomplish this, .25 ml semen was centrifuged for 2 min at 5,000 × G, washed and centrifuged for 2 successive times and re-suspended in .25 ml of 2.9 percent sodium citrate.

The evaluation for percent live cells utilized the nigrosin-eosin live-dead stain. A drop of the centrifuged semen was combined with a drop of stain for 3 min. Stained samples were smeared on glass slides, mounted and numerically coded for later evaluation. Coding was used to preclude bias or evaluator knowledge of the time the sample was taken from the incubation tube and thaw temperature used. Two hundred cells per smear were evaluated using a bright field microscope; red stained cells were considered dead while unstained cells were considered live.

The second evaluation utilized a stain that would differentiate the acrosome from the cell nucleus. A drop of the centrfuged semen was combined with a drop of the Wells-Awa acrosome stain for 3 min. Stained samples were smeared on glass slides, mounted and coded for later evaluation. Two hundred cell-counts were made on each slide to determine the percentages of acrosomes in the following categories: 1) normal cell with non-aged acrosome, 2) normal cell with aged acrosome, 3) total aged acrosome, 4) missing acrosome.

Although these four categories were determined, only the data on "percent normal non-aged acrosomes" will be utilized in this report. The remaining categories are to be used in a subsequent experiment. Non-aged acrosomes are tightly adherent to the anterior portion of the cell nucleus and have a smooth entire appearance. Deterioration of the acrosome is evidenced by ruffling, swelling or detachment and is presumed to occur either *post mortem* or immediately before cell death. (Hancock 1953, Saacke 1968).

A split plot statistical analysis of the data was performed on the "Percent Live Cells" and "Percent Normal Non-Aged Acrosomes".

Results and Discussion

There were significant effects observed in percent live cells due to the different thaw temperatures used in the study (Table 1). These effects were seen as changes in the number of cells that survived the freezing and thawing process. Fewer cells thawed at 32 F were live after 1 hr incubation than cells thawed at warmer temperatures. Incubation for five hrs increased the difference between 32 F thaw and the warmer thaw temperature. Cells thawed at any of the higher temperatures were more able to withstand incubation stress than those thawed at 32 F, suggesting that slow thawing causes damage to sperm cells. Thawing ampules in 32 F water is the accepted practice. Our data indicates that using this procedure for straws may result in somewhat reduced numbers of live cells post-thaw.

Table 2 compares the effects of thawing temperature and incubation periods on percentages of cells with normal non-aged acrosomes. After 1 hr of incubation, there were no significant differences among thaw temperatures. However, 95 F thawed cells had more normal non-aged acrosomes. Five hrs incubation produced varied changes with the cells thawed at 203 F having significantly higher percentages of non-aged acrosomes. It should be noted that populations of cells thawed at 68 F had the lowest percentage of cells with non-aged acrosomes at 1 hr incubation and had the greatest reduction in non-aged acrosomes after 5 hrs incubation. Thawing at 68 F would be approximately the rate achieved with air thaw and shirt pocket thaw procedures. Our data indicates that these procedures are inferior to either cold water or warm water thaw temperatures in maintenance of acrosome quality.

In order to further visualize thaw effects on straw packaged semen, averages over all bulls and incubation periods are shown in Table 3. The 95 F thaw resulted in the highest percent live cells and the highest percent normal non-aged acrosomes. Use of the 32 F thaw over the 95 F thaw would result in a 15.7 percent reduction in "live" cells and a 11.6 percent reduction of cells with normal non-aged acrosomes that would be available for insemination. It should also be noted that additional cell losses normally occur due to insemi-

Table 1. Mean percent "live cells" after 1 and 5 hrs of incubation*

	Thaw Temperatures			
	32 F	68 F	95 F	203 F
1 hr	57.8[a]	64.4[a]	64.7[a]	63.2[a]
5 hrs	45.6[b]	59.5[a]	60.8[a]	61.4[a]
% change	21.1	7.6	6.0	2.8

*Averages of five bulls.
[a,b]Means with different superscripts differ significantly (P<.05).

Table 2. Mean percent normal cells with non-aged acrosomes after incubation*

	Thaw Temperatures			
	32 F	68 F	95 F	203 F
1 hr	28.4[a]	25.8[a]	32.0[a]	27.4[a]
5 hrs	20.2[b]	13.0[b]	19.7[b]	23.3[a]
% change	29.0	50.0	38.0	15.0

*Averages of five bulls.
[a,b]Means with different superscripts differ significantly (P<.05).

Table 3. Percent "live" and percent normal non-aged acrosomes averaged over all incubation periods and bulls

	Thaw Temperatures			
	32 F	68 F	95 F	203 F
% "Live"	53.7	61.4	63.6	60.1
% Normal non-aged acrosomes	26.0	22.1	29.4	28.1

nation techniques and delays (from "thawed" to "inseminated"). In this case, use of the 95 F thaw would serve to provide an increased "safety factor" by the same percentages. A comparison of all thaw temperatures in Table 3 shows that thawing at 68 F was least effective in maintenance of acrosome condition. It can be concluded that use of the 95 F thaw provides more desirable cells for insemination and its use over a 32 F thaw will provide a substantial safety factor.

Literature Cited

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