The Effect of Freezing Rate On Sperm Cell Characteristics

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Story in Brief

Bull sperm cells can be stored successfully for several years in liquid nitrogen. Although the freezing process does kill a relatively large number of live cells, this problem is compensated for by starting with an increased number of cells prior to freezing. Research through the past years has defined the optimum rate of freezing bull cells with the major criterion for gauging success being the survival pattern post-freeze. Apparently, the typically used freezing rates also have given reasonable acrosome condition post-freeze.

On some bulls and in boars and rams, it is most difficult to successfully freeze their cells. Sperm cells can apparently survive the freezing process but fertility is not acceptable. It is known that the freezing process does affect the acrosome and it was of interest to explore the effect of freezing rate on the acrosome. Bull sperm cells were frozen at five different rates, ranging from very slow to very fast. Ampules were thawed and examined for percent live cells and aged acrosomes.

Under the conditions used in these trials the moderate rate of freeze (5.5° F/minute) gave the best survival post-freeze with acceptable condition of the acrosome.

Introduction

The backbone of our modern-day artificial insemination industry is the capability to freeze and store sperm cells for an extended period of time. Bull cells and stallion cells can now be frozen and stored successfully as judged by fertility and livability studies. However, on some bulls' cells and cells from boars and rams, good post-freeze livability can be achieved, however, fertility is not acceptable.

Recent studies on boar sperm suggest that when acrosomal integrity is preserved, satisfactory fertility is achieved even when post-freeze motility is low. The above facts suggest that criteria other than post-freeze motility are involved in maintenance of fertility. In view of this, several facets of sperm cell preservation procedures are being re-examined to determine how the acrosome is affected. This study was undertaken to evaluate the effect of freezing on morphological integrity of the acrosome and sperm cell livability.

Materials and Methods

Four dairy bulls, 4 years old, housed and managed similarly, were used in this study. Semen was collected from these bulls once weekly for 5 consecutive weeks. Initial ejaculate characteristics measured were as follows:

a) Volume
b) Sperm cell concentration
c) Percent live sperm cells
d) Percent aged acrosome

All ejaculates were diluted to obtain approximately 30 million live sperm per ampule prior to freezing. The diluter used was a standard egg yolk-sodium citrate-glycerol mixture. Freezing was accomplished 5-6 hours after dilution by placing the canes of semen in a wire rack and lowering them into a thick-walled styrofoam box containing 3 inches of liquid nitrogen.

In this split-ejaculate study, ampules from each bull were frozen at the following rates:

a) slow, .9° F/min from 41° F to —22° F, then 5.5° F/min to —55° F;
b) moderate, 5.5° F/min from 41° F to —55° F;
c) intermediate, 7.5° F/min from 41° F to —22° F, then 30° F/min to —55° F;
d) rapid, 17° F/min from 41° to —55° F;
e) accelerated, 35° F/min from 41° F to —55° F.

The ampules were all frozen from —55° to —125° F at 35° F/min and then transferred to a liquid nitrogen tank. The ampules were thawed 24 hours later and were evaluated for acrosomal state and percent live cells.

Results and Discussion

Effects of freezing rate on percent live cells.

All rates of freeze drastically reduced the percentage of live cells from the initial evaluations (Figure 1). The slow rate was apparently the most harmful as there was only an average of 17 percent live cells post-freeze. The moderate rate gave the highest percent live, 29 percent, with the faster freezing rates resulting in fewer live cells per ampule. These results are in good agreement with several studies that indicate that sperm cells should be frozen at a rate of 5-8° F per minute from +41° F to —4° F.

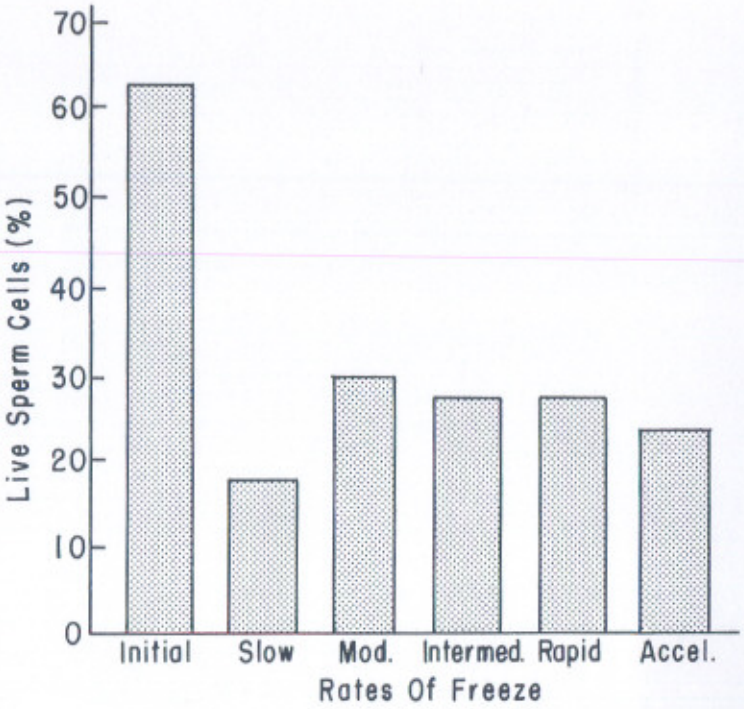

Figure 1. The effect of rate of freeze on percent live sperm cells. (20 observations per mean).

The data also indicated that bull differences and semen quality differences unique to a given collection day may influence how a sample of cells will freeze. This agrees well with our experience that some bulls' cells are occasionally difficult to freeze.

Effects of freezing rates on acrosome status.

Figure 2 presents the average percentage of aged acrosomes for all rates of freeze. All rates of freeze essentially doubled the degree of aging noted in the average initial ejaculate (21 percent). The slow, intermediate and accelerated rates appear to cause aging to a greater extent than the moderate and rapid rates. However, more ejaculates from a greater number of bulls are needed to verify this as fact. There were significant differences among bulls and dates of semen collection in response to the rates of freeze. This reinforces the facts as we already know them, namely, we can expect bulls to differ in response to treatment of

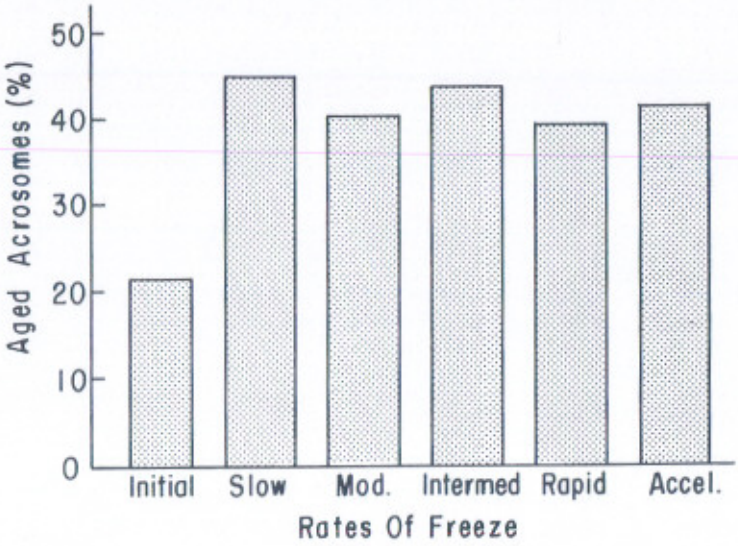

Figure 2. The effect of rate of freeze on percent aged acrosomes. (20 observations per mean).

their cells and it is not unusual to have large differences among the ejaculates of a bull in response to rates of freezing.

There were highly significant interactions among bulls, rates and dates of collection. Simply stated, this means that (1) semen from different bulls responded differently to the various rates of freeze, (2) the semen from different bulls responded differently on different dates, and (3) different rates of freeze had different effects on different dates.

This complexity of factors tells us that it is most difficult to make clear-cut projections as to how freezing will affect the acrosome of bull sperm cells. It is imperative that further research be conducted to clarify each factor's relative contribution to the maintenance of acrosomal integrity through the freezing process.