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The Effect of Freezing and Thawing On Sperm Cell Dimensions

M. E. Wells, T. L. Bright, and P. J. Hefley

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

laboratory procedures. Sperm head dimensions were determined prior to dilution, immediately after freezing and at 1½ hours and 3 hours incubation at 100°F. Head length and width varied widely among bulls while acrosome thickness was basically similar among bulls. Freezing had no effect on head length but did significantly decrease head width. Incubation caused an increase in both head length and width. Acrosome thickness was most affected by freezing with very little effect attributable to incubation.

Introduction

The size of sperm cells has been the object of research interest for several years. It is known that the membrane system of the sperm cell is semi-permeable in nature and the dimensions of a cell can be influenced by the tonicity of the medium surrounding it. Thus, one of the characteristics of a suitable semen extender is that it should not contribute to excessive swelling or shrinking of the cell which could lead to destruction of the cell. The integrity of a cell can also be disrupted by drastic treatments. Since sperm cells undergo drastic ranges of temperature in the course of dilution, processing, freezing, thawing and ultimately insemination, we were interested in how some of these affected sperm cell dimensions.

One of the current concepts of sex control suggests that one can separate male-causing from female-causing sperm cells due to size differences in the cells. Very little has been published which defines the variation in size of cells that is encountered in an ejaculate or among bulls. One of the objectives of our research was to determine how sperm cell size varied within an ejaculate and among bulls.

Experimental Procedure

Single ejaculates from each of 11 yearling Holstein bulls were used for this study. The bulls were housed and managed similarly. The bulls were ejaculated via the artificial vagina with ejaculates then being processed according to standard procedures for storage of sperm cells in glass ampules. Ampules from each ejaculate were frozen and stored in liquid nitrogen. Two ampules of each ejaculate were thawed in ice water and one incubated for 1½ hours and the other for 3 hours at 100°F. This approximates cow body temperature and such treatment would perhaps give an indication of how well sperm cells can withstand temperature stress.

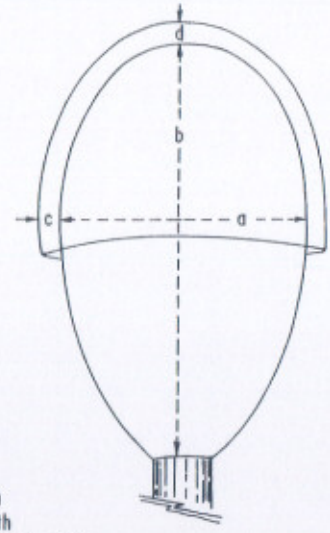
Stained sperm cell smears were prepared on the initial ejaculates

(prior to dilution), immediately post-freeze, after 1½ hours of incubation and after 3 hours of incubation at 100°F. Twenty-five cells were measured on each preparation to characterize cell dimensions. The features measured are presented in Figure 1. The head length and width and acrosome thickness at the side and apex of the cells were utilized to determine initial cell size characteristics as well as cell changes due to freezing and incubation.

Sperm cell dimensions were measured with a micrometer eyepiece that was inserted into one of the ocular tubes of a binocular microscope. All measurements were made with oil immersion lens (100x) of the microscope. The same technician did the measurements throughout the study.

Results and Discussion

The dimensions of sperm cells in the initial ejaculates, prior to any treatment, are presented in Tables 1 and 2 and part of Table 3. Data from 3 of the bulls are presented so that variation encountered in this



a = Head Width
b = Head Length
c = Acrosome Width, Side
d = Acrosome Thickness, Apex

Figure 1. Sperm cell head schematic drawing showing cell dimension measured.

study can be described. Table 3 indicates that the average size of cells pre-freeze was 8.43 microns long, 4.50 microns wide with acrosome dimensions of .33 microns on the side and .60 microns at the apex. Table 1 indicates the standard deviations in individual bulls for these same measurements while Table 2 indicates the range encountered in each individual bull. The head length and width measurements agree quite well with other studies. The acrosome measurements are among the first attempts to determine the size of the acrosome and should aid in efforts to understand cell components and how they are affected by routine procedures.

In all bulls, there was obviously a wide range in the size of the sperm cells. One of the many current approaches to separate "X" and "Y" chromosome bearing (female causing and male causing, respectively) sperm cells assumes that there is a size and/or weight difference in these cells. The data in this study were pooled across bulls and examined for possible biomodality in distribution. No evidence or tendency for any of the measurements to separate into two types of cells was found. The reasons for such a wide range in cell size, what regulates cell size and the possible importance of cell size are under investigation.

The effects of freezing and incubation on sperm cells are summarized

Table 1. Mean Sperm Cell Dimensions Prior to Any Treatment[1]

Bull No.	Sperm Characteristic			
	Head length	Head width	Acrosome width (side)	Acrosome thickness (Apex)
433	8.50 ± .43	4.50 ± .38	0.29 ± .10	0.61 ± .21
439	8.02 ± .42	4.56 ± .33	0.26 ± .09	0.54 ± .15
440	8.40 ± .44	4.53 ± .27	0.28 ± .13	0.74 ± .27

[1] Each value represents mean ± standard deviation of 25 sperm cells.

Table 2. Range In Sperm Cell Dimensions Prior to Any Treatment[1]

Bull No.	Sperm Characteristic			
	Head length	Head width	Acrosome width (side)	Acrosome thickness (Apex)
433	7.80 — 9.45	3.40 — 5.10	.15 — .57	.30 —1.10
439	6.85 — 8.60	4.10 — 5.20	.15 — .45	.25 — .85
440	7.70 — 9.30	4.25 — 5.15	.10 — .67	.30 —1.40

[1] Each range is based on 25 cell measurements.

Table 3. Effect of Treatment On Sperm Cell Dimension[1]

Treatment	Head length	Head width	Acrosome width (side)	Acrosome thickness (Apex)
Pre-freeze	8.43	4.50	.33	.60
Post-freeze	8.42	4.16	.53	.73
Incubation 1½ hours	8.49	4.23	.54	.70
Incubation 3 hours	8.57	4.36	.51	.71

[1] All measurements are expressed in microns.

in Table 3. Head length was not affected by freezing but incubation caused a lengthening of the cell with the longer incubation time increasing the length of the cell. The analysis also revealed highly significant differences among bulls in length and width of sperm cells. Head width was significantly decreased ($P<.01$) by freezing as Table 3 illustrates. However, incubation caused an increase in cell size with the longer incubation period having the most drastic effect. It is not possible to clearly discern the reasons for the changes in head dimensions. The effect could be due to real changes in the cell membrane system caused by damage during freezing. It is known that a significant percentage of live cells are killed in the freezing process. The shift in mean dimensions from pre-freeze to post-freeze and the subsequent increase in cell size following incubation could well be due to changes in the dead cells. It was not possible to recognize dead from live cells in the preparations employed in this study.

Tables 1, 2, and 3 also present the data on acrosome dimensions measured in this study. Table 3 indicates that the average acrosome width (side) pre-freeze was .33 micron while the measurement at the apex was .60 micron. Table 1 shows the standard deviation of these measurements to be about .11 micron and .20 micron respectively. Table 2 reveals that there was considerable range within bull but quite similar ranges among bulls. Analysis revealed no significant differences among bulls for acrosome dimensions. Freezing had a significant effect ($P<.01$) on acrosome dimension (Table 3). The acrosome width increased over 60% after freezing. Incubation had no observable effect on width. The acrosome thickness at the apex of the cell also increased significantly due to freezing, with no further change being induced by incubation. This data verifies that freezing does effect the membrane system ot the sperm cell. However, it is not known what segment of the cell population is being affected or if the effect is across all ages or types of cells.

Improvement in semen processing techniques is the object of continuing research. Our study indicates that the physical dimension of sperm cells can be altered greatly by the freezing process. The definition of the nature and extent of these changes will be useful in pointing the avenues for continued improvement in protecting the fertility of stored sperm cells.
