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Summary

Frozen semen samples from Holstein bulls were measured by the sperm chromatin structure assay (SCSA), a new procedure utilizing flow cytometry for the evaluation of sperm quality. Fertility ratings of the bulls were known based on their use in artificial insemination matings. Values obtained by the SCSA were highly correlated ($r = -.58$, $P<.01$) with bull fertility ratings. Results of this research indicate the SCSA may be a valuable technique for measurement of sperm cell quality and detection of suboptimal fertility in bulls.

(Key Words: Bulls, Artificial Insemination, Flow Cytometry, Fertility, Semen Quality.)

Introduction

An important problem in the livestock industry is accurate determination of sire fertility. Tests presently used to estimate semen quality include measurement of motility and morphological abnormalities of sperm. However, these tests have not always proven useful for prediction of fertility, and there is a definite need for improved methods to determine semen quality and ultimately sire fertility. Flow cytometry is a technique which provides a new method for evaluating sperm cells.

A flow cytometer is an instrument designed for the measurement of cells in liquid suspension flowing past a measuring point. The cells may be labeled with a fluorescent dye which binds to the component of interest (e.g., DNA) and then passed single file in a fluid stream through a laser beam. The fluorescent signals emitted from the cells are detected by photomultiplier tubes and processed by a computer interfaced to the flow cytometer.

A method has been developed called the Sperm Chromatin Structure Assay (SCSA) which uses flow cytometry to measure the structure of chromatin (DNA and associated proteins found in the nucleus of the cell) in sperm cells. The SCSA involves treating cells briefly with either acid or heat to partially denature the DNA, i.e., to convert the more susceptible regions of the double-stranded (ds) DNA to single-stranded (ss) DNA. The sperm are then stained with acridine orange (AO) dye and measured by flow cytometry. When the AO is bound to double-stranded DNA, it fluoresces green when excited by the laser beam and, when bound to single-stranded DNA, it fluoresces red. Measurement of green vs red fluorescence in an AO-stained cell indicates the relative content of double- vs single-stranded DNA and thus provides a measure of the structural stability of the cell. To quantify the degree of denaturation in a sample, alpha-t (α_t), defined as the ratio of red to total (red and green) fluorescence, is used.

Higher α_t values are associated with increased susceptibility of the sperm to denaturation.

Previous work suggested that subfertile individuals had sperm samples with higher α_t values when measured by the SCSA, indicating defective or altered chromatin structure of the sperm could be a factor in decreased sire fertility. During the past 2 years, a study has been conducted to verify these early results. The major objective of this research was to determine the relationship between sire fertility and flow cytometric measurements on sperm chromatin structure.

Experimental Procedure

Frozen semen samples from 49 mature Holstein bulls representing a cross-section of regular production sires were supplied by Eastern Artificial Insemination Cooperative, Ithaca, NY. Based on post-thaw motility, all samples were suitable for use in artificial insemination. Fertility ratings had been previously computed for these bulls based on nonreturn rates from a larger number of matings. Higher fertility ratings were associated with increased sire fertility.

Sperm samples were stored in liquid nitrogen until measurement. At that time, samples were thawed, diluted with physiological saline and immediately measured by the SCSA method. Flow cytometry measurements were made using a Cytofluorograph II and 2150 computer system from Ortho Diagnostic Systems, Inc. (Westwood, MA). For each sample, the levels of green and red fluorescence were recorded on 5000 cells and the distribution of α_t values was computed. The standard deviation (a measure of the variation among cells) of the distribution of α_t values (SD α_t) was used to describe the extent of denaturation of a sample. The correlation coefficient was computed to quantify the association between SD α_t and FR.

Results and Discussion

An example of the data collected for each sample is shown in figure 1. In the cytograms (A and C), green fluorescence (ds DNA) is on the vertical axis and red fluorescence is on the horizontal axis (ss DNA), and each dot in the cytogram represents a single cell. The alpha-t (α_t) frequency histograms (B and D) corresponding to the cytograms show the distribution of α_t values for the samples. The first sample (A, B) is from a bull with a high fertility rating and the second (C, D) from a bull with a low fertility rating. Note the greater proportion of cells with increased red fluorescence in C, indicating a greater susceptibility to denaturation, and the associated shift in α_t values seen in D. The SD α_t values were 35.9 and 80.0 for the first and second samples, respectively.

The correlation coefficient between bull fertility rating and SD α_t was -.58, measured on 49 bulls. This indicates that higher SD α_t values are associated with lower fertility ratings.

In consideration of several factors, this correlation is quite high and encouraging for the application of the SCSA as a test of sperm quality in the artificial insemination industry. First, only a single sample from each bull was measured by the SCSA, whereas fertility ratings were based on matings using semen

from a large number of collections. Secondly, when there is little variation among bulls, other studies have generally obtained low correlations between conventional semen quality tests and sire fertility. Although the bulls measured in this study had a relatively narrow range of fertility (all were acceptable for routine use in artificial insemination), the fertility ratings were nevertheless significantly correlated with the SCSA results. The association between fertility and SD α_t may be even stronger if a group of bulls with greater variation in fertility were measured.

To test the accuracy of the technique, repeat measurements were made on the samples. The results of the two measurements agreed very well, demonstrating that error due to the instrumentation is minimal. Furthermore, for some bulls, samples were obtained from collections taken over a several year time period and the α_t values of several different collections from a bull were, in most cases, similar. Thus, a single collection of a bull should be representative of further collections from that bull.

The SCSA can also be applied to sperm from other species. To date, stallion, ram, human, mouse and turkey samples have all been measured and studies on these species are presently underway. Whether or not a correlation exists between chromatin structure and fertility in species other than cattle has not yet been determined. An important question regarding the value of the SCSA in fertility prediction will be whether or not the correlation between the SCSA and bull fertility measured in artificial insemination matings will also be obtained when fertility is measured under natural mating conditions. Future research will also investigate the relationships between the SCSA and traditional methods (e.g., motility, morphology) of evaluating sperm quality.

In conclusion, the SCSA is a new method which utilizes flow cytometry for measurement of chromatin structure in sperm cells. Studies on frozen sperm samples from Holstein bulls have shown a significant association between proportion of sperm with structurally abnormal chromatin and bull fertility ratings. The SCSA should be of value in the artificial insemination industry for detection of fertility problems in bulls and eventually may also be useful for evaluation of sires in other livestock species.

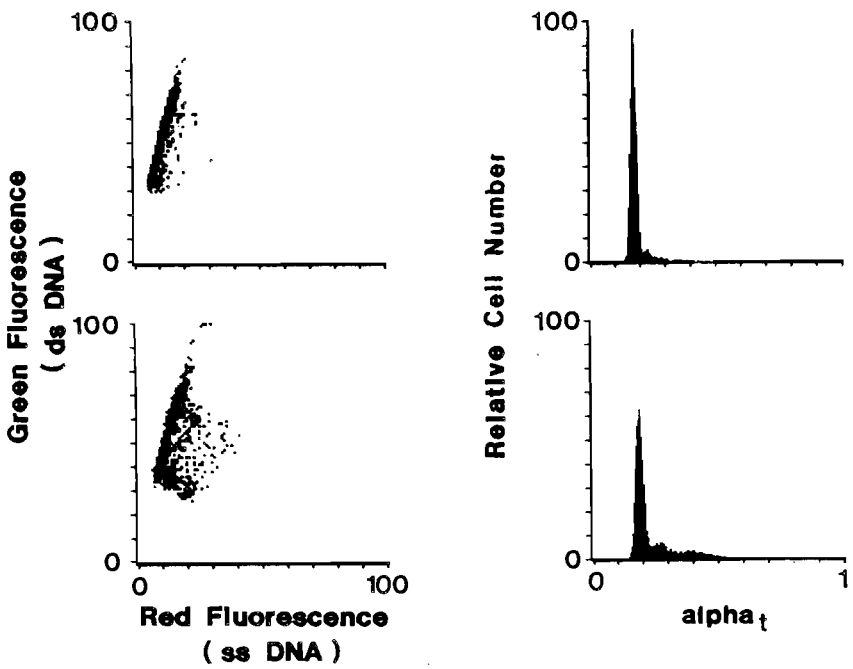

Figure 1. Green vs red fluorescence cytograms (A,C) and corresponding alpha-t frequency histograms (B,D) of sperm prepared by the SCSA method from two bulls.