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SEMEN QUALITY ASSESSMENT AND SEXED SEMEN

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Introduction

Sexing mammalian sperm, especially in cattle, has been the topic for several recent reviews (Johnson, 2000; Maxwell et al., 2004; Johnson and Seidel, 1999; Johnson and Welch, 1999; Garner, 2001; Seidel and Garner, 2002; Garner and Seidel, 2003; Seidel, 2003). This brief review examines the process of collecting semen and the seminal parameters necessary before sperm to be efficiently sexed. The objectives are (1) to outline the steps necessary in selection of bulls that produce semen suitable for flow cytometric sexing, and (2) to examine the limitations relative to seminal parameters necessary to make semen sexing relatively efficient.

Bull Screening

The semen from most, but not all bulls, can be efficiently processed for production of sexed semen. It is somewhat more difficult to sort sperm from beef bulls than it is from dairy bulls. This is because the process by which most dairy bulls have been selected for AI usually eliminates those males whose sperm are sensitive to in vitro manipulation and, thus, do not cryopreserve well. This selection pressure has not been exerted, at least in such a magnitude, on beef bulls because not nearly as large a proportion of beef cattle are artificial inseminated.

Bull Preparation

Bulls must be prepared properly prior to collection of semen for sexing sperm using flow sorting. The frequency of ejaculation is important because most bulls need to be collected several times prior to using their semen for sexing. This is done to rid the epididymides of aged, degenerating sperm. After a cleaning out procedure has been completed, collection of two ejaculates, two to three times a week, appears to be optimal for most bulls once they are flushed of unsuitable sperm.

Time Restriction from Semen Collection to Processing

Once semen has been collected and it has been determined to be suitable for sex sorting, there is a limited time interval before freshly collected semen becomes unsuitable for successful viable sexing. The probability of successful sexing sperm decreases progressively after the semen is collected. This time interval does vary somewhat with each bull or semen batch, but a limit of about 18 hr between semen collection and the final cryopreservation process of the sexed sperm should be adhered to if the sexing process is to be reasonably efficient.

Measurement of Sperm DNA Content

Rapid, high resolution measurement of sperm DNA content was first achieved by analyzing fluorescently stained, demembranated sperm nuclei using a flow cytometer (Otto et al., 1979; Pinkel et al., 1982; Garner et al., 1983). Precise DNA measurement in this early work required removal of the surrounding membranes to facilitate repeatable DNA staining. This process, however, severely damaged the sperm. It wasn't until the membrane permeant DNA-binding dye, Hoechst 33342 was utilized that precise measurement of DNA became possible with living sperm (Johnson et al., 1987)). With the added precision of DNA measurement provided with Hoechst 33342 stained living sperm, an effective means of separating viable gametes with either the X- or Y-chromosome was developed. An illustration of the staining and sperm sex sorting process is shown in Fig. 1. Currently sperm are separated with an accuracy of 85-95% (Seidel et al. 1999; Welch and Johnson, 1999; Johnson, 2000; Maxwell et al., 2004). Flow cytometric sex-sorting of sperm according to their DNA content is patented (Johnson, 1991) and has been sub-licensed for non-human mammals to XY, Inc., through Colorado State University.

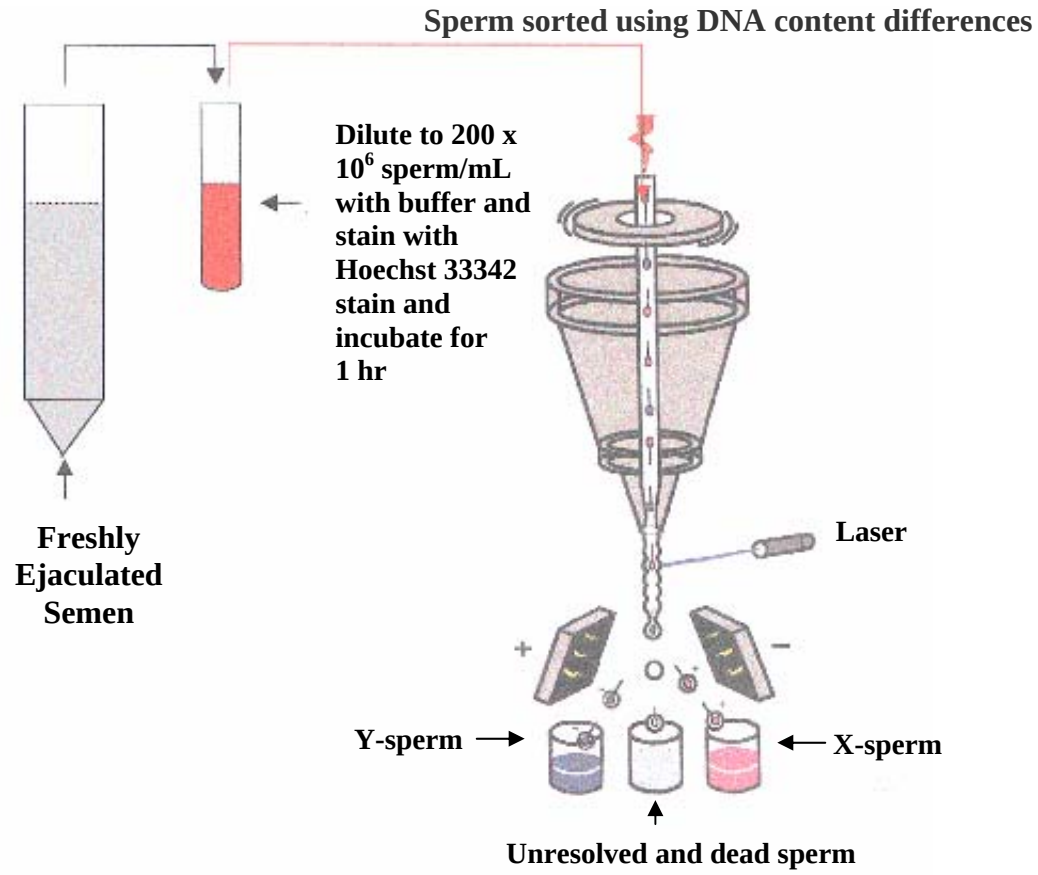

Figure 1. Semen processing, staining and flow sorting with an SX MoFlo® whereby sperm are sexed into Y- and X- populations at 85-95% purity.

Removal of Dead Sperm

The efficiency of sperm sorting was enhanced by identifying dead or damaged sperm and gating them out so that only intact, living sperm are actually sorted (Johnson et al., 1994). Membrane damaged or dead sperm are identified in Hoechst 33342-stained samples by their uptake of a membrane impermeant food dye, FD&C #40 (Warner Jenkinson, St. Louis, USA). Compromised gametes are thereby identified so that they can be gated out and disposed of as waste along with those sperm not measured properly (Johnson and Welch, 1999; Schenk et al., 1999).

Survival and Cryopreservation of Sexed Sperm

The sorting process greatly dilutes sperm so that re-concentration by centrifugation is necessary to prepare the sexed sperm before packaging in 0.25 mL straws. Sexed sperm have been cryopreserved almost identical to conventional cryopreservation of unsexed sperm with the exception that the number of sperm/dose is greatly reduced. Due to the fewer sperm/dose with sexed sperm their survival becomes progressively more important not only because of the fewer sperm/dose but also because considerable time and money has been invested in sorting these gametes. A 0.25 mL straw of sexed sperm contains only about 10% of the sperm that is normally present in unsexed straws. Some gains in the proportion of sperm that can survive cryopreservation have been achieved by development of an enhanced cryopreservation process known as the Multi-Thermal Gradient freezing system. Cryopreservation in a vacuum, as used by this system, enables more of the sorted sperm to survive cryopreservation and thawing (Arav et al., 2002). Although the sorting process eliminates dead and damaged sperm, those cells that do survive tend to degenerate faster than unsorted sperm (Rath et al., 2003). Any gain in the efficiency either of sorting sperm or increases in cryopreservation survival or storage reduces the time required to sort each dose of sexed sperm (Centurion et al., 2003). Cryopreserving sperm overcomes the distribution problem because sexed bovine sperm can be stored indefinitely and transported for use almost anywhere in the world.

Each of the procedures by which sperm are sex-sorted, processed, cryopreserved and stored need to be carefully carried out so that higher proportions of sexed sperm can remain functional for insemination. The susceptibilities of sperm of individual bulls to staining, laser exposure, high dilution, elevated pressure and resistance to the several changes in media composition that occur during the sexing process differ slightly (Maxwell et al., 2004; Garner, 2005). Comparative examination of the integrity of the DNA (Sperm Chromatin Structure Assay, SCSA, Ballachey et al., 1987) of bovine sperm during various steps in the sex-sorting process indicated that mechanical injury, not exposure to Hoechst 33342 and/or 150 mW of laser illumination, inflected the greatest damage (Garner, 2001). This finding was verified and expanded with sperm viability tests using SYBR-14 and PI (Garner and Suh, 2002). Reducing the fluidic pressure during sorting from 50 to near 40 psi increased the survivability of sorted gametes while the resolution of the X - and Y-chromosome bearing sperm populations was maintained (Suh and Schenk, 2002).

Sperm Insemination Dose Numbers

The current practice is to package 2 x 10^6 sexed sperm/0.25 mL straw. Considerable efforts determined that the minimal insemination dose for sexed sperm was 2 x 10^6 sperm/dose. At this insemination dose little room is left for packaging errors. Precise packaging of sperm/insemination dose is essential to minimize inadvertent production of straws containing less than the 2 x 10^6 sperm cryopreserved, sexed bovine sperm. An error of a million sperm/dose is of little consequence when packaging straws at 20 x 10^6/dose as is commonly used for unsexed semen doses. It is also important to minimize losses during sorting, centrifugation and packaging because nearly 60% of the total sperm in the sample are excluded or lost during sex sorting and processing (Garner and Seidel, 2003). The need for improvement in the sorting system is obvious. The use of sexed sperm requires strict management practices even when limited to use in heifers (Seidel, 2003). Proper handling of 0.25 mL straws is a necessity due to their temperature sensitivity.

Implementation of Sperm Sexing

Implementation of sperm sexing technology is driven by economics (Amann, 1999; Seidel, 2003). Sex-sorting of cattle sperm was brought to the point of commercialization by development of a more sophisticated orienting nozzle for the sperm sorter (Rens et al., 1996) and successful cryopreservation of the sorted gametes (Schenk et al., 1999). Sexing technology is highly sought after, but very few potential users have the capital necessary to implement and maintain such a program. It has been suggested that the yearly operating costs for the first year of a sex sorting facility would exceed US$ 2 million (Seidel, 2003). This is beyond sustainable cost range of most, if not all companies or cooperatives, currently processing and marketing bull semen. The first implementation of sperm sexing took nearly 20 years after the principle of measuring sperm DNA content for sexing purposes was established at the Lawrence Livermore National Laboratory (Pinkel et al., 1982; Garner et al., 1983). At least 50,000 calves have been born from sperm sorted for sex chromosome differences using flow cytometry. The majority of sexed calves have been produced in the United States, United Kingdom, and Argentina. Fewer sexed calves have been produced in other countries. Commercial applications of sperm sexing in cattle have resulted in the fertility of sex-sorted bull sperm at 2 x 10^6/dose remaining at 70 to 80% of unsexed sperm at normal doses of 10 to 20 x 10^6 sperm (Garner and Seidel, 2003).

Safety of Sperm Sexing

The safety of the sperm sexing process relative to potential genetic risks is of concern (Meistrich, 1996). Staining sperm with Hoechst 33342 and exposing the stained cells to UV-laser irradiation during flow sorting has been shown to increase the incidence of chromosome aberrations in hamster eggs microinjected with flow sorted sperm (Libbus et al., 1987). In a recent study with pigs, however, no genotoxic effects of sexing sperm were found (Parilla et al., 2004). Information on cellular toxicity, sperm staining efficiency, embryonic development of embryos resulting from Hoechst-stained sperm and the normalcy of offspring suggests that no marked difference exists from that seen with unstained, unsexed sperm for these parameters (Seidel and Garner, 2003; Tubman et al., 2004).

Summary

Reasonable fertility levels have been attained in a large number of field trials with sexed bull sperm (Morrell and Dresser, 1989; Seidel et al., 1999; Seidel and Garner, 2002; Seidel and Schenk, 2002). Enhancement of the sperm sorting system, especially the more efficient sorter nozzle, and successful cryopreservation of sexed sperm led to commercialization of bovine sperm sexing in the United Kingdom, Argentina and some parts of the United States. Under optimal conditions, the sperm sex sorter can produce 10 insemination doses of sexed bovine sperm at about 2 x 10^6 live sperm/dose for each sex/hr of actual sorting time. Production of sexed sperm, though, is less in actual production situations (Seidel and Garner, 2002). The flow sorting system is marginal because only one-third of the sperm passing through the system can be sorted and collected with the threshold set to achieve 90% accuracy (Seidel and Garner, 2002). Approximately 20% more of the sorted sperm are lost during the sperm concentration and packaging processes now in use (Seidel and Garner, 2002). Sorting efficiency can even be less with poor quality semen. However, flow sorting of X- and Y-chromosome bearing sperm has resulted in thousands of normally-appearing calves from numerous bulls (Seidel and Garner, 2002: Tubman et al., 2004).

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