Theriogenology 79 (2013) 551–557

Contents lists available at SciVerse ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Seminal plasma effects on sex-sorting bovine sperm

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ARTICLE INFO

Article history:
Received 23 February 2012
Received in revised form 19 October 2012
Accepted 29 October 2012

Keywords:
Sex-sorting
Sex-sorted semen
Sperm
Seminal plasma
Bull
Cattle

ABSTRACT

The objective was to determine which characteristics of bovine ejaculates affected efficacy of sex sorting bovine sperm by flow cytometry. The effects of first versus second ejaculates, seminal plasma content, addition of BSA, and seminal plasma from different bulls during staining were all studied, as was the effect of 8-hour storage with and without seminal plasma. Semen collected by artificial vagina was centrifuged at 1000 × *g* for 15 minutes to separate sperm from seminal plasma; seminal plasma was clarified by 10 minutes of additional centrifugation at 2000 × *g*. Sperm were rediluted to 160 × 10^6 sperm per mL with: Tyrode's medium plus albumin, lactate, and pyruvate (TALP) containing 0%, 5%, 10%, or 20% homologous seminal plasma, TALP containing 10% heterologous seminal plasma, or TALP containing 0.3% (control), 0.6%, or 1.2% BSA. After incubation with Hoechst 33342 for 45 minutes, an equal volume of TALP containing red food dye was added, and sperm were analyzed by flow cytometry/cell sorting to determine percent of live-oriented sperm, X sort rate, percent of membrane-impaired sperm, and split (degree of separation between X- and Y-bearing sperm populations). The percent of live-oriented sperm was higher for sperm incubated with 0% seminal plasma (64%) than for sperm incubated with 5%, 10%, or 20% seminal plasma (60%, 59%, and 58%, respectively; $P < 0.05$). The X sort rate was higher for sperm incubated with 0% seminal plasma than sperm with 20% seminal plasma (4.26 vs. 3.61 × 10^3 sperm per second). When seminal plasma was exchanged between bull ejaculates, only one bull had seminal plasma that was detrimental to sperm, resulting in 31% membrane-impaired sperm compared with a range of 16% to 19% for seminal plasmas from other bulls ($P < 0.05$). The addition of BSA did not affect sort efficiency at the concentrations studied. Sperm from six bulls stored for 8 hours without seminal plasma had more membrane-impaired sperm (which were discarded) during sorting (28%) than with seminal plasma (19%; $P < 0.01$), but higher postthaw motility postsorting (63%) than with seminal plasma (52%; $P < 0.05$). In conclusion, the presence of seminal plasma during staining and sorting decreased sort rates and percent of live-oriented sperm, and storing sperm without seminal plasma increased postthaw motility.

1. Introduction

The ability to choose the sex of offspring at conception has long been desired, but the technology to do so has only been available for 20 years and for less than 10 years commercially. Unfortunately, separation of X- and Y-bearing bovine sperm by flow cytometry is still an inefficient process, in which only approximately one-third of the sperm that pass through the flow cytometer are collected as live, sexed sperm, with an accuracy of >90% [1]. To improve efficiency, and therefore decrease the cost of the sex sorting process, each step of the process needs to be re-evaluated. In addition, current industry dogma is that ejaculates containing less 1 × 10^9 sperm per mL sort poorly. Because sperm are diluted to

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http://dx.doi.org/10.1016/j.theriogenology.2012.10.024

160×10^6/mL to optimize staining with Hoechst 33342 (H33342; Molecular Probes, Eugene, OR, USA) for routine sex sorting, seminal plasma is more diluted when initial sperm concentration of the ejaculate is high than when it is low. Thus, we hypothesized that seminal plasma might contribute to reduced sorting efficiency when initial sperm concentrations are low.

Seminal plasma is the fluid added to sperm during epididymal transport and ejaculation and is produced by the epididymis and accessory sex glands. During normal mating, sperm spend a relatively short interval in the seminal plasma originating from the accessory sex glands, but when processing sperm for cryopreservation or sex sorting, sperm might spend hours (up to 1 day) in diluted seminal plasma.

Seminal plasma is composed of the proteins, sugars, and ions necessary for maintaining sperm viability, inhibiting sperm capacitation, protecting sperm membranes, and initiating sperm motility [2]. However, the presence of seminal plasma has been described as being beneficial, or, in some studies, detrimental, to bovine sperm survival during storage, cryopreservation, or sex sorting [3–6]. The effect of holding sperm in seminal plasma for extended periods has not been studied thoroughly in the context of sex sorting bovine sperm, although some benefit of added seminal plasma during sex sorting of ovine and porcine sperm has been demonstrated [7]. Therefore, the main null hypothesis of these experiments is that seminal plasma has no effect on efficacy of the sex sorting process.

2. Materials and methods

2.1. Semen collection and initial analysis

First ejaculates were collected by artificial vagina from dairy and beef bulls collected at least once in the week before the study, and housed at Sexing Technologies, Inc. (Navasota, TX, USA). Second ejaculates were also collected an average of 1 hour after first ejaculates in experiment I. For all experiments, raw semen was processed by determining the initial sperm concentration (Nucleocounter SP-100; ChemoMetec, Allerod, Denmark), subjective percent progressively motile sperm, percent morphologically normal sperm, and pH. Antibiotics were then added, as recommended by Certified Semen Services (National Association of Animal Breeders, Columbia, MO, USA). All ejaculates were evaluated by the same person, and only ejaculates containing at least 60% motile sperm and 70% morphologically normal sperm were used. The semen was centrifuged at $1000 \times g$ for 15 minutes, and seminal plasma was removed by aspiration. Less than 1% of sperm were discarded with the seminal plasma. The seminal plasma was clarified by an additional 10 minutes of centrifugation at $2000 \times g$, with sperm pelleted in this step discarded. The sperm pellets were resuspended in staining Tyrode's medium plus albumin, lactate, and pyruvate (TALP [8]) and/or seminal plasma to appropriate concentrations. In experiment II, a semen sample was removed before centrifugation as a control.

2.2. Experimental designs

In experiment I, sperm were diluted to 160×10^6 sperm per mL with staining TALP [8] and 0%, 5%, 10%, or 20% seminal plasma from either the same ejaculate or from the other (first or second) ejaculate from the same bull. In experiment II, frozen seminal plasma collected during experiment I was thawed in a 34.5 °C water bath and stored at 4 °C during the experiment. For experiment II, all treatments were diluted to 160×10^6 sperm per mL by adding staining TALP to: control semen (not centrifuged), centrifuged sperm resuspended in TALP with no seminal plasma, centrifuged sperm resuspended in TALP with 10% seminal plasma from the same ejaculate, and centrifuged sperm resuspended in TALP with 10% seminal plasma from bulls used during experiment I (Table 1). Seminal plasma was chosen from bulls used in experiment I based on whether the ejaculate sort efficiency was good or poor in relation to other ejaculates in the study. Poor was defined as having no split (degree of separation between the X- and Y-bearing sperm populations), and good had a split of 30% or greater. In experiment III, centrifuged sperm without seminal plasma were diluted to 160×10^6 sperm per mL using staining TALP; for staining, sperm were exposed to 0% or 10% seminal plasma and 0.3% (control), 0.6%, and 1.2% bovine serum albumin (Fraction V) for 1 hour before staining.

In experiment III, seminal plasma was removed and sperm were reconstituted with staining TALP to 1.4×10^9 sperm per mL , and then stored in 1.5 mL Eppendorf tubes at 16 °C without seminal plasma. Sperm samples were taken at 0 and 4 hours of storage for staining and sorting.

In experiment IV, sperm from six bulls was centrifuged as in previous experiments and seminal plasma was added back to original sperm concentrations (0.75 to 2.21×10^9 sperm per mL) for half of the sperm; for the other half, staining TALP was added to produce 1.4×10^9 sperm per mL. Sperm were stored in 15 mL conical tubes at 16 °C for 8 hours until staining for sorting.

2.3. Staining

For all experiments, H33342 was added at a final concentration of 65 μM, and sperm were incubated for 45 minutes in a 34.5 °C water bath to facilitate H33342 movement into sperm cells. The pH of the sperm samples was measured before and after incubation. After staining,

Table 1
Experiment II design, indicating source and concentration of seminal plasma used in each treatment.

Treatment	Seminal plasma (%)	Seminal plasma origin
Control	Control	Sperm bull
Own	10	Sperm bull
None	0	NA
Good1	10	Good1
Good2	10	Good2
Good3	10	Good3
Poor1	10	Poor1
Poor2	10	Poor2
Poor3	10	Poor3