J. Dairy Sci. 96:4447–4454
http://dx.doi.org/10.3168/jds.2012-6484

Reducing sperm concentration is critical to limiting the oxidative stress challenge in liquid bull semen

C. Murphy,* A. G. Fahey,† A. Shafat,‡ and S. Fair*[1]
*Department of Life Sciences, Faculty of Science and Engineering, University of Limerick, Limerick, Ireland
†School of Agriculture and Food Science, University College Dublin, Dublin 4, Ireland
‡Department of Physiology, National University of Ireland, Galway, Ireland

ABSTRACT

Because of the short breeding season, the use of liquid bull semen is a viable option in seasonal grass-based dairy systems such as Ireland. Currently in Ireland, liquid bull semen contains approximately 5 million sperm per insemination dose and is used within 2.5 d of collection. The hypothesis of this study was that reducing the sperm number per insemination dose would enable bull sperm to be stored for longer. Semen was collected at a commercial AI center and diluted to 1 (T1), 2 (T2), 3 (T3), 4 (T4), and 5 (T5) million sperm per 0.25-mL dose in caprogen diluent. On d 0.25 (6 h postcollection), 1, 2, 3, 4, and 5 postcollection, viability, oxidative stress, and mitochondrial activity were assessed using flow cytometry and the fluorescent probes propidium iodide, CM-H_2DCFDA, and rhodamine 123, respectively. On the same days, glucose consumption, total antioxidant capacity, and progressive linear motility were assessed. We observed an effect of day and treatment on sperm cell viability, with the highest percentage live found in T1 and the lowest in T5 on all days. Oxidative stress in live sperm increased with duration of storage and was affected by treatment, being highest in T5 and lowest in T1 on all days (d 5: 56.4 ± 2.76% and 28.8 ± 1.22%, respectively; mean ± SEM). Both the total antioxidant capacity and percentage of live sperm positive for rhodamine 123 were unaffected by treatment. The concentration of glucose in caprogen declined with time and was lowest in T5 and highest in T1 on d 5. In conclusion, higher concentrations of sperm have detrimental effects on sperm cell viability and increase oxidative stress but have no effect on the mitochondrial activity of sperm.

Key words: sperm, dairy bull, oxidative stress, reactive oxygen species

INTRODUCTION

With the abolition of milk quotas within the European Union now imminent (March 31, 2015), the Irish dairy industry has set a target of a 50% increase in dairy output by the year 2020 (Department of Agriculture, 2010). The underlying basis for this is that Ireland (like New Zealand) has a seasonal grass-based milk production system, which has a significant cost advantage in the form of low-cost milk production. This production system is highly dependent on excellent reproductive performance with compact calving to coincide with the start of the grass growing season (Dillon et al., 1995). Although milk production per cow has increased since the 1970s (Foote, 1996), reproductive performance has dramatically declined (Walsh et al., 2011). To combat this decline in fertility, Ireland, through the Irish Cattle Breeding Federation (ICBF), has implemented an Economic Breeding Index (Berry et al., 2005). This is a profit index that enables farmers to select the most elite and profitable bulls to breed replacement heifers, which will have an increased ability to produce more milk solids per cow per year and an increased ability to become pregnant. Therefore, the intensive use of bulls with a high Economic Breeding Index is the best way to optimize profitability by reversing the fertility problems in the Irish national dairy herd while facilitating increases in milk production (McCarthy et al., 2007).

In 2009, Ireland began to select dairy bulls for use in AI programs using genomic selection so as to reduce the generation interval with greater reliability and increase the rate of genetic gain (Taş et al., 2007; Berry and Kearney, 2011). Potential AI bulls are now being identified within weeks of birth and are entering into AI programs at approximately 10 mo of age, when demand for their semen far exceeds supply. Although these young bulls (<12 mo of age) have undergone puberty, they yield a low volume of semen, which can normally only be collected once per week (Helbig et al., 2007; R. Monaghan, National Cattle Breeding Centre, Enfield, Co. Meath, Ireland; personal communication) compared with 3 times per week for a mature bull. Liquid semen provides an opportunity to maximize the

Received December 14, 2012.
Accepted March 13, 2013.
[1]Corresponding author: sean.fair@ul.ie

number of insemination doses from these elite bulls; however, the industry must devise a protocol to optimize its use.

Liquid semen accounts for 5% of the total AI usage within the Irish dairy industry, but this can increase to 25% during the peak dairy breeding season (Al Naib et al., 2011b). Given that 60% of all dairy inseminations in Ireland occur in a 6-wk period in April and May, the extensive use of fresh semen during the peak breeding season is a viable option, whereby the number of insemination doses per ejaculate could be increased up to 10-fold compared with frozen semen (Verberckmoes et al., 2005). Currently in Ireland, the norm is to include 20 million sperm in each frozen semen dose (Al Naib et al., 2011a), whereas 5 million sperm are included in each liquid dose, which, combined with its lower processing costs, makes liquid semen an attractive option. However, liquid semen is not used beyond 3 d of collection because of concerns over declining fertility with the use of aged semen (Vishwanath and Shannon, 2000).

The hypothesis of this study was that storing liquid semen at lower sperm concentrations than usual (5 million sperm per dose) would reduce levels of reactive oxygen species (**ROS**), preserving the antioxidants in the extender and metabolic activity of the sperm, and thereby extending the sperm lifespan. In addition, lowering the sperm number per dose would maximize the use of both young and mature elite sires during the peak dairy breeding season. The objective of this study was to examine the effect of reducing the sperm number per insemination dose, for up to 6 d postcollection, on a range of in vitro parameters; namely, progressive linear motility (**PLM**), viability, mitochondrial activity, oxidative stress, total antioxidant capacity (**TAC**), and glucose consumption.

MATERIALS AND METHODS

Experimental Design

Semen was collected from Holstein, British Friesian, Belgian Blue, and Hereford bulls, at the National Cattle Breeding Centre, and the effect of reducing the number of sperm per insemination dose was examined on a range of in vitro parameters. The experiment included 6 collections, with semen collected from 3 to 4 bulls in each collection (collection = replicate). Different bulls were used for most but not all collections, and 12 bulls in total were used over the 6 collections. The minimum quality criteria for semen used on the study were ejaculates with >500 million sperm per mL and with greater than 75% progressively motile sperm. The sperm concentration of each ejaculate was assessed using a photometer (IMV Technologies, L'Aigle, France) and was initially diluted in 20% egg yolk caprogen diluent (1:1), followed by a further dilution in 5% egg yolk caprogen to give final concentrations (treatments) of 1 (**T1**), 2 (**T2**), 3 (**T3**), 4 (**T4**) and 5 (**T5**) million sperm per insemination dose, packaged into straws, as one straw represents one insemination dose (0.25-mL straws; IMV Technologies). Before dilution, the caprogen medium was purged in fresh food-grade nitrogen gas (BOC Gases Ireland, Dublin, Ireland) to dispel oxygen from the medium and create an anaerobic environment to limit the metabolic activity of sperm during liquid storage. Semen from each bull used in a given replicate was separated in a split sample design and equally represented in each treatment. Samples were transported to the laboratory at the University of Limerick (Limerick, Ireland) and stored at ambient temperature (18–20°C) for up to 6 d postcollection. Four straws from each bull at each concentration were pooled and analyzed for viability, PLM, oxidative stress, and mitochondrial activity, at 6 h postcollection (referred to as d 0.25) and on d 1, 2, 3, 4, and 5. In addition, on each of the assessment days, the contents of 4 straws from each treatment were centrifuged at 10,000 × *g* at 4°C for 10 min, and the supernatant was removed and frozen at −20°C for later batch analysis of glucose concentration and TAC.

PLM

Progressive linear motility was assessed using a phase contrast microscope (BH-2; Olympus, Center Valley, PA) at a magnification of 400×. A droplet of semen (10 μL) was placed on a prewarmed slide, covered with a prewarmed coverslip, and assessed by counting 50 motile sperm for each treatment on each assessment day. A sperm cell was deemed to display PLM if it moved in a linear fashion; PLM was expressed as the percentage of live and motile sperm that displayed forward progressive linear motion.

Assessment of Oxidative Stress and Mitochondrial Activity and Viability

Diluted semen (350 μL) from each treatment was washed twice in PBS (5 mL) at 800 × *g* at 32°C for 10 min. To detect general oxidative stress, the fluorescent probe 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA; Molecular Probes, Eugene, OR) was added to 367 μL of the washed sample (1.4×10^6 sperm/mL) to give a final concentration of 125 μ*M* and incubated in the dark at 35°C for 30 min. Following the incubation period, propidium iodide (**PI**; Invitrogen, Grand Island, NY) was added to give a final concentration of 50 μ*M* and