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Embryo production after in vitro fertilization with frozen-thawed, sex-sorted, re-frozen-thawed bull sperm

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Abstract

The objective of this study was to determine the in vitro fertilizing capacity of bull sperm derived from fresh or frozen samples and subjected to sex sorting and re-cryopreservation. Four sperm types were assessed for their ability to fertilize and sustain early embryo development in vitro. Semen from three Bos taurus bulls of different breeds (Jersey, Holstein and Simmental) was collected and either sorted immediately and then frozen (SF) or frozen for later sorting. Frozen sperm destined for sorting were thawed, sex-sorted, and re-frozen (FSF) or thawed, sex-sorted (FS), and used immediately for in vitro fertilization (IVF). Frozen-thawed nonsorted semen from the same ejaculate was used as a control. Oocytes from donor cows were aspirated via ovum pick-up and matured in vitro prior to IVF and culture. On average, 19.0 ± 1.7 (mean ± SEM) oocytes were aspirated per donor cow, of which 74.4 ± 2.2% were selected for maturation. The proportion of cleaved embryos (Day 3) did not differ between sperm groups (P = 0.91). Likewise, IVF with FSF sperm resulted in similar Day 7 blastocyst rates (as a percentage of total oocytes) as those of control, SF, and FS sperm (FSF, 34.5 ± 4.7; control, 32.2 ± 4.6; SF, 35.9 ± 4.8; and FS, 26.9 ± 4.1%; P = 0.23). These encouraging results show that frozen-thawed sex-sorted sperm may be re-frozen and used for in vitro embryo production with similar blastocyst production as that of nonsorted frozen-thawed and sex-sorted frozen-thawed sperm.

Keywords: Cryopreservation; Embryo production; IVF; Sex preselection; Sperm sorting

1. Introduction

Sperm sorting remains the only effective and reproducible method of sex selection, and sexed sperm is commercially available to many dairy farmers for artificial insemination (AI). A limiting aspect of the technology is the necessity for donor bulls to be located within a short distance of the sorting facility. As there are fewer than 20 commercial facilities worldwide, sperm from many animals are currently unable to be sorted, and the interest in sorting from frozen-thawed samples is growing from both commercial entities and producers. In sheep, in vitro embryo production using frozen, sorted, and re-frozen (FSF) sperm has been highly successful, with blastocyst rates after in vitro fertilization (IVF) with FSF ram sperm higher than that with nonsorted control sperm [1]. Also, after laparoscopic AI of ewes, FSF sperm was equally fertile compared with fertility of nonsorted and sorted frozen-thawed sperm [2]. In cattle, recent research has allowed frozen-thawed bull sperm to be sex-sorted with high

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resolution and re-cryopreserved while retaining motility and high acrosome integrity [3], and after artificial insemination of FSF sperm, one heifer calf was born [4]. In that study and others [5], the in vivo fertility of frozen-thawed, sex-sorted, re-frozen-thawed bull sperm was greatly reduced after AI compared with that of nonsorted controls, possibly due to rapid decline in motility and decreased velocity compared with non-sorted sperm. Thus, FSF sperm may be better utilized in an in vitro fertilization and embryo transfer program where sperm transport within the female reproductive tract is not necessary. However, the fertility of FSF bull sperm has not been assessed in vitro after IVF. When a sperm sorting facility is in close proximity to an in vitro embryo production laboratory, frozen-thawed sperm have been sex-sorted for immediate IVF, without sperm undergoing the additional stress of a second freeze-thaw cycle. Puglisi et al. [6] reported the birth of two calves after IVF of abattoir-sourced oocytes and transfer of embryos produced with frozen-thawed sex-sorted (FS) sperm, and Malcolm et al. [7] reported IVF with FS sperm resulted in comparable blastocyst formation with that of sex-sorted frozen-thawed (SF) and control sperm. The current study aimed to sort frozen-thawed sperm at high rates after incubation with low concentrations of Hoechst 33342 and compare the in vitro fertilizing capacity of (1) frozen-thawed, sex-sorted, re-frozen-thawed sperm; (2) frozen-thawed, sex-sorted sperm; and (3) sex-sorted, frozen-thawed sperm.

2. Materials and methods

2.1. Experimental design

Cumulus-oocyte complexes (COCs) were aspirated from the ovaries of sexually mature, unsynchronized Bos indicus (Brahman) cows every 2 wk (n = 7 replicates), matured in vitro, and fertilized with either nonsorted frozen-thawed (control), SF, FS, or FSF sperm from three bulls of different breeds (Jersey (n = 3 ejaculates), Holstein (n = 2 ejaculates), and Simmental (n = 2 ejaculates)). Presumptive zygotes were cultured in vitro and cleavage rates and blastocyst production recorded to ascertain the developmental competence of embryos produced by each sperm type.

2.2. Semen collection, preparation, and sorting

Unless otherwise stated, all media components were purchased from Sigma-Aldrich (St. Louis, MO, USA). Semen was collected by artificial vagina from sexually mature Holstein, Jersey, and Simmental bulls. Semen was checked for motility and morphology, and only ejaculates having greater than 65% motility and less than 30% morphologic abnormalities were used. The proportion of semen designated for immediate freezing was diluted in a Tris-based freezing diluent with 20% egg yolk, cooled for 1.5 h and then diluted 1:1 with the same diluent containing 12% glycerol to give a final composition of 177 mM Tris, 58.3 mM citric acid, 55.5 mM fructose, 20% egg yolk, and 6% glycerol. Sperm were loaded into 0.5-mL straws (IMV, L'Aigle, France) at a concentration of 30×10^6 motile sperm/straw and frozen in a programmable freezer (Beltron Instruments, Bryan, TX, USA) at a rate of –3 °C/min to –4 °C, –73 °C/min until –15 °C, and –21.4 °C/min until –140 °C, at which point straws were plunged into liquid nitrogen for storage.

The remainder of the ejaculate was diluted to 150×10^6 sperm/mL with Tris medium [4] supplemented with 49 to 65 mM Hoechst 33342 (Invitrogen Molecular Probes, Eugene, OR, USA) and incubated for 50 min at 34 °C. After staining, samples were diluted 1:1 with Tris medium supplemented with 4% egg yolk and 0.0015% food dye (FD&C #40; Warner Jenkinson Company Inc., St. Louis, MO, USA) and filtered through a 50-μm filter (GCAT, Fort Collins, CO, USA) to remove any debris or agglutinated cells prior to sorting.

Frozen sperm destined for FS and FSF groups were thawed (10 straws per gradient) and centrifuged through a PureSperm gradient using methods previously described [4]. Briefly, thawed sperm were placed over a 45:90% PureSperm gradient (Nidacon International AB, Mölndal, Sweden) and centrifuged ($600 \times g$, 20 min). The sperm pellet was diluted with Tris medium (1:4 sperm pellet:diluent) and washed by further centrifugation at $300 \times g$ for 11 min, then stained and prepared for sorting as described above for fresh sperm.

A high-speed cell sorter (MoFlo SX; Beckman Coulter, Inc., Fullerton, CA, USA) was operated at 40 psi with a diode pumped solid-state pulse laser (Vanguard 350 HMD-355; Spectra Physics, Mountain View, CA, USA) at 125 mW, with bovine sheath fluid (CHATA Biosystems Inc., Fort Collins, CO, USA). Gates were set to attain ≥90% purity, and X-chromosome–bearing sperm were sorted into Tris I medium [8].

2.3. Post-sort processing of sperm

Both SF and FSF sperm were concentrated by centrifugation at $750 \times g$ for 6 min. The resultant pellet