Animal Reproduction Science 178 (2017) 61–66

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Comparison of fertility of liquid or frozen semen when varying the interval from CIDR removal to insemination☆

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ARTICLE INFO

Article history:
Received 31 August 2016
Accepted 23 January 2017
Available online 24 January 2017

Keywords:
Beef cattle
Liquid semen
Frozen semen
Fertility

ABSTRACT

Cryopreservation allows for long-term storage of semen; however, it leads to damage of sperm that may result in complete loss of viability or changes that possibly decrease sperm functionality. Liquid semen is not exposed to these stressors and may result in a longer lifespan in the female reproductive tract, thus increasing the range in timing of insemination without affecting fertility. The objective of this study was to compare fertility of liquid and frozen semen when varying the interval from CIDR removal to AI using the 7-day CO-Synch + CIDR protocol for synchronization of time of estrus. Within age group, crossbred cows ($n = 389$) were randomly assigned to insemination at 36 or 60 h after CIDR removal with either liquid or frozen semen (36L, 60L, 36F, and 60F) from one of two Angus bulls. Cows were monitored for estrous activity from CIDR removal until 60 h thereafter. Cows that failed to exhibit estrus received GnRH (100 μg, i.m.), and a blood sample was collected for analysis of estradiol concentration. There was no difference in pregnancy rates when liquid or frozen semen (53% and 52%) was used, but cows inseminated at 60 h had a greater ($P < 0.01$) pregnancy rate than those inseminated at 36 h (72% and 31%). There was no time of AI by semen type interaction ($P = 0.57$). Estrus was detected in 63%, 61%, 56%, and 62% of 36F, 36L, 60F, and 60L, respectively (only 5% and 1% of 36F and 36L were detected in estrus before insemination). Overall cows that exhibited estrus had a greater pregnancy rate compared with cows that did not ($P < 0.01$; 79% compared with 24%). Among cows that did not exhibit estrus, those inseminated with liquid semen tended to have greater pregnancy rates than those inseminated with frozen semen ($P = 0.06$). Cows that became pregnant had greater ($P < 0.01$) concentrations of estradiol at 60 h than those that did not (10.7 ± 0.55 compared with 7.9 ± 0.26 pg/mL). In summary, there was no difference in pregnancy success between liquid and frozen semen. However, cows that exhibited estrus and were inseminated at 60 h after CIDR removal had greater pregnancy success compared to cows that did not exhibit estrus.

☆ Summary Sentence: There was no difference in pregnancy success between liquid and frozen semen; however, among cows that did not exhibit estrus, those inseminated with liquid semen tended to have greater pregnancy rates than those inseminated with frozen semen.

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http://dx.doi.org/10.1016/j.anireprosci.2017.01.010
0378-4320/

1. Introduction

Cryopreservation allows for the long-term storage and international dispersal of gametes from genetically superior animals (Vishwanath, 2003); thus this ability has allowed for transformation of the AI industry from small, regional bull studs to the majority of domestic and international frozen semen sales coming from a fewer number of larger AI organizations. Despite the benefits of estrous synchronization and AI in beef cattle (Seidel, 1995), increased labor and greater animal handling has led to a slow incorporation of AI in the beef industry with only 8% of cow-calf operations in the U.S.A. utilizing AI (McBride and Mathews, 2011) despite conception rates to a single timed- AI ranging from 50% to 70% (Lamb et al., 2010).

Insemination at the correct time is essential for pregnancy success, and depends on the viable lifespan of gametes, the time it takes for viable sperm to reach the site of fertilization, and the timing of ovulation relative to insemination (Nebel et al., 2000). A significant reduction in the motility of frozen-thawed sperm is characteristic of cryopreserved bull semen (Watson, 1995), which can be, but not always, compensated for by increasing the number of sperm per insemination (Saacke, 1983, 2008). Other changes to sperm as a result of the freezing and thawing process cannot be compensated for, such as the damage and removal of surface proteins (Lasso et al., 1994), alterations to lipid composition within the plasma membrane (Buhr et al., 1994), and premature capacitation (Cormier and Bailey, 2003). It has been hypothesized that these prematurely capacitated sperm have a shorter life-span and only have a limited amount of time to come in contact with an oocyte before these cells are no longer capable of fertilization (Watson, 1995).

Increasing conception rates to a single timed-AI may offer an economic incentive sufficient to overcome the perceived inconveniences associated with the use of the AI technology in beef cattle. One approach to increase conception rates is to develop semen extension and delivery techniques that will increase sperm lifespan relative to the current technique, specifically addressing animals that do not express estrus following imposed procedures for synchronization of estrus. Previous studies (Geary and Whittier, 1998) have indicated this population of animals may benefit from delayed timing of insemination due to a long interval (approximately 30 h) from injection of GnRH to ovulation. Sperm cells of liquid semen are not exposed to stress associated with cryopreservation and may have a longer viable lifespan in the female reproductive tract compared with sperm of frozen semen. Therefore, the objective of this study was to compare fertility using liquid or frozen semen when varying the interval from CIDR removal to insemination using the 7-day CO-Synch + CIDR ovulation synchronization protocol to test the hypothesis that use of liquid semen would result in a greater conception rate compared with use of frozen-thawed semen when the interval from AI to ovulation increased.

2. Materials and methods

2.1. Experimental design

Postpartum Angus-crossbred cows ($n = 389$; 2–13 yr of age and 30–100 d post-partum) from the South Dakota State University Antelope and Cottonwood research stations were estrous synchronized using the 7-d CO-Synch + controlled intravaginal progesterone insert (CIDR) protocol, which consisted of the insertion of an Eazi-Breed CIDR insert (1.38 g progesterone; Zoetis, Florham Park, NJ) and administration of GnRH (100 μg, i.m.; Factrel, Zoetis) on d -7, CIDR removal and administration of $PGF_{2\alpha}$ (25 mg, i.m.; Lutalyse, Zoetis) on d 0, and fixed-time AI after CIDR insert removal. Within age group, cows were randomly assigned to insemination at 36 or 60 h after CIDR removal with either liquid or frozen semen (36L, 60L, 36F, and 60F). Cows were monitored for estrous activity from CIDR removal to 60 h after CIDR removal. Cows that failed to exhibit estrus prior to 60 h after CIDR removal received an injection of GnRH (100 μg) at this time and a blood sample was collected for analysis of estradiol concentration.

2.2. Semen processing and quality control

A single ejaculate was collected from each of two Angus bulls and each ejaculate was divided into aliquots to prepare the liquid and frozen semen doses. Frozen semen was processed per standard operating procedures of Select Sires, Inc. in a proprietary whole-milk-glycerol extender, frozen, and stored in liquid nitrogen until the time of use. Liquid semen was processed in an egg-yolk-TRIS-glycerol extender (pH = 6.6; Foote, 1970). Liquid semen was stored at 5 °C until the time of AI (2 or 3 d post-collection). Liquid and frozen semen was packaged in 0.5-mL straws (IMV Technologies, L'Aigle, France) at an average rate of 24 million sperm per straw.

Semen (liquid and frozen) was evaluated using flow cytometry (Cell Lab Quanta SC MPL; Beckman Coulter Inc., Brea, CA, USA) and computer-assisted sperm analysis (CASA; IVOS II; Hamilton Thorne, Beverly, MA, USA) after warming to 37 °C in a water bath on d 2 and 3 post-semen collection. For each flow cytometric and CASA evaluation, sperm contained in two straws were analyzed and the mean reported. Plasma membrane viability was assessed using propidium iodide (PI). Briefly, extended semen (2 μL) was diluted in TC buffer (198 μL; 0.14 M TRIS, 0.14 M Citric Acid, 10% BSA w/v, pH 7) and PI (12 μM) and incubated for 5 min at 37 °C without exposure to light. Emission spectra were collected using a 670-nm long-pass filter. All sperm were distinguished from debris by gating based on side scatter and electronic volume. Data are expressed as percent viable sperm, (total sperm – PI positive sperm)/(total sperm). DNA stability was evaluated using a modified procedure of the Sperm Chromatin Structure Assay (SCSA; Evenson et al., 1980; Ballachey et al., 1988). Semen (20 μL) was diluted in TNE buffer (180 μL; 0.01 M TRIS-HCl, 0.15 M NaCl, and 1 mM disodium EDTA, pH 7.4), followed by addition of an acid-detergent solution (400 μL; 0.1% Triton X-100, 0.08 N HCl, and 0.15 M NaCl, pH 1.2), and incubation at room temperature for 30 s. This was followed by addition