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Efficacy of embryo transfer in lactating dairy cows during summer using fresh or vitrified embryos produced in vitro with sex-sorted semen

B. M. Stewart,* J. Block,†§[1] P. Morelli,* A. E. Navarette,‡ M. Amstalden,‡ L. Bonilla,§[1] P. J. Hansen,§[1] and T. R. Bilby*‡[2]
*Texas AgriLife Research and Extension, Texas A&M System, Stephenville 76401
†OvaTech LLC, Gainesville, FL 32605
‡Department of Animal Sciences, Texas A&M University, College Station 77843
§Department of Animal Sciences, University of Florida, Gainesville 32611-0910

ABSTRACT

The objective was to determine whether transfer of fresh or vitrified embryos produced in vitro with sex-sorted semen improves pregnancy and calving rates during summer in lactating dairy cows compared with artificial insemination (AI). Lactating dairy cows (n = 722) were enrolled during summer months at 2 commercial dairies in Central Texas and randomly assigned to 1 of 3 treatments: AI with conventional semen (n = 227), embryo transfer-vitrified (ET-V; n = 279) or embryo transfer-fresh (ET-F; n = 216). Embryos were produced in vitro using sex-sorted semen and with Block-Bonilla-Hansen-7 culture medium. For vitrification, grade 1 expanded blastocysts were harvested on d 7 after fertilization and vitrified using the open-pulled straw method. Fresh embryos were grade 1 blastocysts and expanded blastocysts harvested on d 7 after fertilization. Cows were submitted to the Ovsynch56 protocol: d −10 GnRH, d −3 $PGF_{2\alpha}$, d −1 GnRH and d 0 timed AI; or Select Synch protocol: d −9 GnRH, d −2 $PGF_{2\alpha}$, and AI following detected estrus (day of AI = d 0). On d 7, all cows were examined for presence of a corpus luteum (CL). A vitrified or fresh embryo was transferred to cows with CL in ET-V and ET-F groups. Cows were considered synchronized if progesterone was <1 ng/mL on d 0 and a CL was present on d 7. At d 40 ± 7 of gestation, the percentage of cows pregnant was greater for the ET-F compared with the ET-V and AI groups among all cows (42.1 vs. 29.3 and 18.3%, respectively) and synchronized cows (45.5 vs. 31.6 and 24.8%, respectively). Also, the percentage of cows pregnant was greater for the ET-V than the AI group among all cows and tended to be greater among synchronized cows. At d 97 ± 7 of gestation, the percentage of cows pregnant among all cows was greater for ET-F and ET-V groups than for the AI group (36.4 and 25.7 vs. 17.0%, respectively) and the percentage for the ET-F group was greater than for the ET-V group. Among synchronized cows, the percentage of cows pregnant was significantly increased for the ET-F group than for ET-V and AI groups (39.4 vs. 27.8 and 23.1%, respectively) and no difference was found between ET-V and AI groups. No effect of treatment on embryo loss was observed. The percentage of cows with live births was significantly increased for the ET-F than for ET-V and AI groups among all cows (27.5 vs. 17.1 and 14.6%, respectively) and synchronized cows (29.9 vs. 18.5 and 20.0%, respectively). The percentage of cows giving birth to a live heifer was significantly increased for the ET-F and ET-V groups compared with the AI group among all cows (79.1 and 72.5 vs. 50.0%, respectively) and synchronized cows (79.1 and 72.5 vs. 50.0%, respectively). No difference existed between ET-F and ET-V groups for percent live heifer births but both were greater than for the AI group. The transfer of fresh embryos produced in vitro using sex-sorted semen to lactating dairy cows during summer can effectively increase the percentage of cows that establish pregnancy and also the percentage of cows that give birth to a live heifer compared with percentages from AI with conventional semen.

Key words: embryo transfer, sexed semen, heat stress, dairy cattle

INTRODUCTION

The effects of heat stress on reproductive function in lactating dairy cows are well known (Hansen, 1997; Wolfenson et al., 2000; Hansen, 2007). Heat stress is not confined to cows in warm climates because hyperthermia can occur in lactating cows at temperatures as low as 25 to 28°C (Berman et al., 1985; Sartori et al.,

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[2]Corresponding author: trbilby@ag.tamu.edu

2002) and decreased reproductive function during the summer has been reported in regions with temperate climates (Udomprasert and Williamson, 1987; Sartori et al., 2002; Ambrose et al., 2006). Moreover, the effects of heat stress on fertility are likely to increase (Hansen, 2007), given that increased milk production makes it more difficult to regulate body temperature during heat stress (Berman et al., 1985; Berman, 2005) and also exacerbates the effects of heat stress on fertility (Umphrey et al., 2001). Thus, strategies that mitigate the negative effects of heat stress on reproductive function are likely to become essential for continued improvement in reproductive efficiency of dairy cows.

One of the most effective strategies for improving fertility in lactating dairy cows exposed to heat stress is embryo transfer (**ET**; Ambrose et al., 1999; Al-Katanani et al., 2002; Demetrio et al., 2007). Despite the effectiveness of ET during the summer, use of this approach commercially has been limited. The high costs of embryo production by superovulation and transvaginal oocyte aspiration can be overcome through the use of abattoir-derived oocytes in conjunction with in vitro embryo production (**IVP**). However, decreased survival following cryopreservation (Al-Katanani et al., 2002; Lazzari et al., 2002; Rizos et al., 2002) and skewed male gender ratio (van Wagtendonk-de Leeuw et al., 1998; Hasler, 2000; Block and Hansen, 2007), limits the widespread application of IVP embryos in the commercial dairy industry.

Suboptimal embryo culture conditions are one reason for the decreased survival of bovine IVP embryos following cryopreservation (Lazzari et al., 2002; Rizos et al., 2002). Recently, it was reported that culture of embryos in a novel serum-free culture medium, Block-Bonilla-Hansen-7 (**BBH7**), could improve survival following cryopreservation compared with embryos cultured in synthetic oviductal fluid (Block et al., 2010).

The use of sex-sorted semen for embryo production represents one strategy to overcome the male biased gender ratio typically observed for embryos produced in vitro. Xu and colleagues (2006) reported that pregnancy rates achieved in non-heat-stressed recipients following transfer of IVP embryos produced with sex-sorted semen were similar to those obtained with IVP embryos produced with non-sorted semen. To date, no study has evaluated the effectiveness of using IVP embryos produced with sex-sorted semen during the summer with heat-stressed, lactating recipients.

The objective was to determine whether transfer of vitrified and fresh embryos produced using sexed semen and following culture in BBH7 medium would improve pregnancy rates in lactating dairy cows during the summer compared with AI.

MATERIALS AND METHODS

Materials

All materials were purchased from Sigma (St. Louis, MO) or Fisher Scientific (Fairlawn, NJ), unless specified otherwise. The media in vitro fertilization (**IVF**)-Tyrode's Lactate and Hepes-Tyrode's Lactate were purchased from Caisson Laboratories, Inc. (Logan, UT) and Millipore (Billerica, MA), respectively. These media were used to prepare IVF-Tyrode's Albumin Lactate Pyruvate (**TALP**) and Hepes-TALP as described previously (Parrish et al., 1986). The oocyte collection medium was Tissue Culture Medium-199 (**TCM-199**) with Hanks' salts without phenol red (Atlanta Biologicals, Norcross, GA) and supplemented with 2% (vol/vol) bovine steer serum (Pel-Freez, Rogers, AR), 2 U of heparin/mL, 100 U of penicillin-G/mL, 0.1 mg/mL of streptomycin (Chemicon, Temecula, CA), and 1 m*M* glutamine. The oocyte maturation medium was TCM-199 (Invitrogen, Carlsbad, CA) with Earle's salts supplemented with 10% (vol/vol) bovine steer serum, 2 μg of estradiol 17-β/mL, 20 μg/mL of bovine FSH (Folltropin-V; Bioniche, Belleville, ON, Canada), 22 μg of sodium pyruvate/mL, 50 μg of gentamicin sulfate/mL, and 1 m*M* glutamine. Percoll was from GE Health Care (Waukesha, WI). The medium BBH7 is a proprietary, serum-free culture medium developed by the University of Florida and licensed to Cooley Biotech (Gainesville, FL). For the present study, BBH7 was prepared at the University of Florida. Gonadotropin-releasing hormone (2 mL i.m.; Cystorelin; Merial, Duluth, GA) and $PGF_{2\alpha}$ (5 mL i.m.; Lutalyse; Pfizer, New York, NY) were used for synchronization of estrous and lidocaine (2% wt/vol; Sparhawk Laboratories, Inc., Lenexa, KS) was used as a local anesthetic before embryo transfer.

Animals

The experiment was conducted on 2 commercial dairies in North Central Texas between July and October 2009. Data were obtained from a nearby weather station in North Central Texas and recorded by the Weather Underground (http://www.wunderground.com). Values obtained from the weather station were used to calculate temperature-humidity index (**THI**), using the equation THI = (9/5 temperature °C) – (0.55 – 0.0055 × humidity) × (9/5 temperature °C – 26.8), described by (NRC, 1971; Dikmen and Hansen, 2009). The maximum daily temperature and average relative humidity from July 9 through October 18, 2009 (from 10 d before experiment was initiated until 10 d after the last ET) was used to calculate the THI, as shown in Figure 1.