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Efficacy of in vitro embryo transfer in lactating dairy cows using fresh or vitrified embryos produced in a novel embryo culture medium[1]

J. Block,*† L. Bonilla,* and P. J. Hansen*[2,3]
*Department of Animal Sciences and D.H. Barron Reproductive and Perinatal Biology Research Program, University of Florida, Gainesville 32611
†Ovatech LLC, Gainesville FL 32605

ABSTRACT

Objectives were to determine whether pregnancy success could be improved in lactating cows with timed embryo transfer when embryos were produced in vitro using a medium designed to enhance embryo development and survival after cryopreservation. In experiment 1, embryos (n = 569 to 922) were cultured in either modified synthetic oviduct fluid or a serum-free medium, Block-Bonilla-Hansen-7 (BBH7). Development to the blastocyst stage was recorded at d 7, and selected blastocysts (n = 79 to 114) were vitrified using open pulled straws. Culture of embryos in BBH7 increased development to the blastocyst stage (41.9 ± 2.0 vs. 14.7 ± 2.0%) and advanced blastocyst stages (expanded, hatching, hatched; 31.1 ± 1.3 vs. 6.4 ± 1.3%) at d 7 and resulted in higher hatching rates at 24 h postwarming compared with embryos cultured in modified synthetic oviduct fluid (59.0 ± 0.5 vs. 26.7 ± 0.5%). In experiment 2, embryos were produced using X-sorted semen and cultured in BBH7. At d 7 after insemination, embryos were transferred fresh or following vitrification. Lactating Holstein cows were either subjected to timed artificial insemination (TAI) on the day of presumptive ovulation or used as embryo recipients 7 d later. Embryo recipients received an embryo if a corpus luteum was present. The percentage of cows pregnant at d 32, 46, and 76 of gestation was higher among cows that received fresh embryos compared with TAI cows or cows that received vitrified embryos. At d 76, for example, the proportion and percentage pregnant was 47/150 (31.3%) for cows subjected to TAI, 48/95 (50.5%) for cows receiving fresh embryos, and 39/141 (27.7%) for cows receiving a vitrified embryo. No difference was observed in the percentage of cows pregnant among TAI cows and those that received vitrified embryos. There was a service or transfer number × treatment interaction because differences in pregnancy rate between embryo transfer recipients and cows bred by TAI were greater for cows with more than 3 services or transfers. Pregnancy success in lactating cows can be improved by transferring fresh embryos produced in BBH7 compared with TAI. Moreover, no decline in fertility was observed when cryopreserved embryos were transferred compared with TAI. Embryo transfer is particularly efficacious for infertile cows that have previously experienced several failed breeding attempts.
Key words: cryopreservation, embryo transfer, fertility

INTRODUCTION

Embryo transfer can be an important tool for genetic improvement and fertility enhancement in dairy cattle and other animals (Hansen and Block, 2004). Its use as an assisted-reproduction technology is based on embryo transfer bypassing pregnancy failure caused by defects in ovulation, fertilization, embryonic development, and AI technique. Improvements in pregnancy rate using embryo transfer were demonstrated for lactating cows subjected to heat stress (Al-Katanani et al., 2002; Block et al., 2003; Rodriques et al., 2004). In each of these studies, fertility in inseminated cows was low. When heat stress is not a contributing factor to pregnancy failure, pregnancy rates between embryo-transfer recipients and inseminated cows do not differ (Rodriques et al., 2004; Sartori et al., 2006). In another study carried out throughout the year, pregnancy rates after embryo transfer were higher than for AI, and high body temperature caused a reduction in pregnancy rate in both groups (Demetrio et al., 2007).

The failure of embryo transfer to consistently improve fertility in lactating cows when fertility in inseminated cows is not very low could reflect reduced competence of the embryo used for transfer to establish and maintain pregnancy. This problem is exacerbated when the embryo is derived in vitro because an embryo produced by that process has altered molecular and cellular properties that reduce its capacity for surviv-

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[2]Corresponding author: pjhansen@ufl.edu
[3]BBH7 will be made available to researchers wishing to replicate the results herein.

ing cryopreservation and establishing pregnancy after transfer into recipients. The period of embryo culture is an important one for producing embryos with high competence for survival because transfer of in vitro-produced embryos into the sheep oviduct has been shown to enhance development, gene expression, and cryosurvival (Rizos et al., 2008), and the addition of specific molecules, such as colony-stimulating factor 2 and IGF-1 improved posttransfer survival of in vitro-produced embryos (Block et al., 2007; Loureiro et al., 2009).

Block-Bonilla-Hansen 7 (**BBH7**) is a defined, serum-free culture medium that was developed to increase the yield and cryotolerance of blastocysts produced in vitro. For the present experiment, it was hypothesized that BBH7 would increase embryo competence for development and cryosurvival, and that the resultant embryos would be of sufficient quality that transfer of either a fresh or vitrified embryo would increase pregnancy success of lactating cows. Furthermore, it was hypothesized that benefits of embryo transfer would be greater for cows that had been bred more than 3 times previously (i.e., repeat breeder cows) because defects in a subpopulation of these cows associated with failure of ovulation, fertilization, and early development would be bypassed.

MATERIALS AND METHODS

Materials

All materials were purchased from Sigma Aldrich (St. Louis, MO) or Fisher Scientific (Fairlawn, NJ) unless specified otherwise. HEPES-Tyrode's lactate and in vitro fertilization (**IVF**)-Tyrode's lactate were purchased from Caisson Laboratories Inc. (Logan, UT) or Millipore (Billerica, MA). These media were used to prepare HEPES-Tyrode's albumin lactate pyruvate (**TALP**) and IVF-TALP, as described previously (Parrish et al., 1986). Oocyte collection medium consisted of tissue culture medium-199 with Hank's salts without phenol red and supplemented with 2% (vol/vol) bovine steer serum (Pel-Freez, Rogers, AR), 2 U/mL of heparin, 100 U/mL of penicillin-G, 0.1 mg/mL of streptomycin, and 1 m*M* glutamine. Oocyte maturation medium was TC-199 with Earle's salts (Invitrogen, Carlsbad, CA) supplemented with 10% (vol/vol) bovine steer serum, 2 μg of estradiol 17-β/mL, 20 μg of bovine FSH/mL (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 Healthcare (Chalfont St. Giles, UK). Modified synthetic oviductal fluid was purchased from Millipore (Billerica, MA). The formula was as described by Takahashi and First (1992) except that phenol red and BSA were omitted. The synthetic oviductal fluid was modified (**mSOF**) before use to contain 1.0 m*M* alanyl-glutamine, 5.3 m*M* sodium lactate, 0.5 m*M* trisodium citrate, 2.77 m*M* myo-inositol, 0.5 m*M* fructose, 20 μL of essential AA/mL (Eagle's basal medium), and 10 μL of nonessential AA/mL (minimum essential medium). The medium BBH7 is a proprietary, serum-free culture medium developed by the University of Florida and licensed to Cooley Biotech LLC (Gainesville, FL). Gonadotropin-releasing hormone was Cystorelin from Merial (Duluth, GA), and $PGF_{2\alpha}$ was Lutalyse from Pfizer (New York, NY). Lidocaine was obtained from Pro Labs (St. Joseph, MO). Open pulled straws were from Mintube (Verona, WI). Fetal bovine serum was purchased from Atlanta Biologicals (Lawrenceville, GA).

Experiment 1: Effect of Culture Medium on Blastocyst Development, Survival Following Vitrification, Cell Number and Differentiation, and Lipid Content

In Vitro Embryo Production. All procedures related to in vitro embryo production were as described previously (Soto et al., 2003) unless otherwise noted. Immature cumulus oocyte complexes (**COC**) were collected from abattoir-derived ovaries (Central Packing, Center Hill, FL). Harvested COC were matured for 21 to 24 h in a humidified atmosphere of 5% (vol/vol) CO_2 in air. Following maturation, COC were washed once in HEPES-TALP and then coincubated with a pool of sperm from 3 different bulls. To eliminate bull effects, a different combination of 3 bulls was used for each replicate. Spermatozoa and COC were coincubated for 8 h. Following fertilization, presumptive zygotes were cultured in groups of approximately 30 in 50-μL microdrops of either mSOF or BBH7. Culture drops were overlaid with mineral oil and placed at 38.5 °C in a humidified atmosphere of 5% CO_2, 5% O_2, and 90% (vol/vol) N_2 for 7 d. The proportion of oocytes that cleaved was recorded on d 3 after insemination. The proportion of oocytes that developed to the blastocyst stage (i.e., all blastocysts including those that were nonexpanded, expanded, hatching, and hatched) and advanced blastocyst stage (expanded, hatching, and hatched blastocysts) were recorded on d 7 after insemination. Embryos were produced in 12 replicates and used as described below.

Vitrification and Postwarming Survival. For 8 replicates, selected embryos were vitrified and warmed using the open pulled straw method as previously described (Vajta et al., 1998) with minor modifications. All steps were carried out with media kept on a warmed