J. Dairy Sci. 97:229–239
http://dx.doi.org/10.3168/jds.2013-6943

Consequences of transfer of an in vitro-produced embryo for the dam and resultant calf[1]

L. Bonilla,*[2] J. Block,*‡ A. C. Denicol,* and P. J. Hansen*[3]
*Department of Animal Sciences, D. H. Barron Reproductive and Perinatal Biology Research Program, and Genetics Institute, University of Florida, Gainesville 32611
‡Ovatech LLC, Gainesville, FL 32608

ABSTRACT

No reports exist on consequences of in vitro production (IVP) of embryos for the postnatal development of the calf or on postparturient function of the dam of the calf. Three hypotheses were evaluated: calves born as a result of transfer of an IVP embryo have reduced neonatal survival and altered postnatal growth, fertility, and milk yield compared with artificial insemination (AI) calves; cows giving birth to IVP calves have lower milk yield and fertility and higher incidence of postparturient disease than cows giving birth to AI calves; and the medium used for IVP affects the incidence of developmental abnormalities. In the first experiment, calves were produced by AI using conventional semen or by embryo transfer (ET) using a fresh or vitrified embryo produced in vitro with X-sorted semen. Gestation length was longer for cows receiving a vitrified embryo than for cows receiving a fresh embryo or AI. The percentage of dams experiencing calving difficulty was higher for ET than AI. We observed a tendency for incidence of retained placenta to be higher for ET than AI but found no significant effect of treatment on incidence of prolapse or metritis, pregnancy rate at first service, services per conception, or any measured characteristic of milk production in the subsequent lactation. Among Holstein heifers produced by AI or ET, treatment had no effect on birth weight but the variance tended to be greater in the ET groups. More Holstein heifer calves tended to be born dead, died, or were euthanized within the first 20 d of life for the ET groups than for AI. Similarly, the proportion of Holstein heifer calves that either died or were culled for poor health after 20 d of age was greater for the ET groups than for AI. We observed no effect of ET compared with AI on age at first service or on the percentage of heifers pregnant at first service, calf growth, or milk yield or composition in the first 120 d in milk of the first lactation. In a second experiment, embryos were produced using 1 of 2 culture media: synthetic oviductal fluid–bovine embryo 1 (SOF-BE1) or Block-Bonilla-Hansen 7 (BBH7). We detected no difference between cows receiving an SOF-BE1 or BBH7 embryo in gestation length, the percentage of cows in which parturition was induced, or the percentage of cows that experienced calving difficulty, retained placenta, prolapse, or metritis. Among Holstein heifers, birth weight was higher for BBH7 calves than for SOF-BE1 calves. Treatment had no significant effect on calf death. Results indicate that calves born as a result of IVP-ET are more likely to experience alterations in birth weight and increased death in early life but that there were few consequences to the dam of carrying a fetus derived by IVP-ET.

Key words: embryo transfer, in vitro fertilization, calf mortality, reproduction

INTRODUCTION

In vitro production of embryos (**IVP**) is a technology that can be used to increase genetic selection (Moghaddaszadeh-Ahrabi et al., 2012; Weigel et al., 2012), utilize sex-sorted semen (Xu et al., 2006; Rasmussen et al., 2013), and improve fertility during heat stress (Block et al., 2010; Stewart et al., 2011; Vasconcelos et al., 2011) and in repeat-breeder females (Son et al., 2007; Block et al., 2010; Canu et al., 2010). The opportunities created by IVP have not been fully realized, however, because the IVP embryo is aberrant in certain respects compared with embryos produced in vivo. Molecular differences in the function of embryonic blastomeres, as illustrated by alterations in DNA methylation (Niemann et al., 2010), the transcriptome (Driver et al., 2012), and lipidome (Sudano et al., 2012), result in an embryo with reduced capacity for establishment of pregnancy after transfer to recipient females (Farin et al., 1999; Pontes et al., 2009; Siqueira et al., 2009). In vitro production has longer-term conse-

Received April 19, 2013.
Accepted September 10, 2013.
[1]Conflict of interest: LB, JB, and PJH have an ownership position in Cooley Biotech LLC, Gainesville, Florida, the licensee of BBH7 medium.
[2]Current address: Minitube of America, International Center for Biotechnology, Mt. Horeb, WI 53572.
[3]Corresponding author: hansen@animal.ufl.edu

quences, too, with reports of higher pregnancy losses in the late embryonic and fetal period (Agca et al., 1998), abnormal placental development (van Wagtendonk-de Leeuw et al., 1998; Bertolini et al., 2004; Miles et al., 2004, 2005), heavier fetal (Bertolini et al., 2004; Miles et al., 2004) and birth weights (Behboodi et al., 1995; Kruip and Den Haas, 1997; van Wagtendonk-de Leeuw et al., 1998; Numabe et al., 2000; Breukelman et al., 2005), and increased rates of dystocia (Behboodi et al., 1995; van Wagtendonk-de Leeuw et al., 1998), congenital abnormalities (Wagtendonkde Leeuw et al., 1998), stillbirths, and neonatal mortality (Kruip and Den Haas, 1997; van Wagtendonk-de Leeuw et al., 1998; Numabe et al., 2000). Also, the sex ratio at birth can be skewed toward the male (van Wagtendonk-de Leeuw et al., 1998; Block et al., 2003). Increased frequency of developmental defects in IVP-derived offspring has not always been observed (Jiang et al., 2011; Rasmussen et al., 2013). Discrepancies in effects of IVP on the incidence of developmental defects could be due to differences in the culture systems used to produce embryos in vitro (Fischer-Brown et al., 2005) or be an artifact of small sample size.

There are no reports on consequences of IVP for the postnatal development of the calf or on postparturient function of the dam of the calf. It is reasonable to hypothesize that milk yield of cows that calved as a result of transfer of an IVP embryo would be altered because the fetus can affect subsequent milk yield (Cue and Hayes, 1985; Moya et al., 1989; Guilbault et al., 1990). Large birth weights and associated dystocia could also compromise the subsequent health of the dam because of the relationship between dystocia and subsequent reproductive function and milk yield (Zaborski et al., 2009).

Three hypotheses were evaluated in the current study. The first was that animals born as a result of IVP have reduced neonatal survival and altered postnatal growth, fertility, and milk yield compared with animals born as a result of AI. The second was that cows giving birth to calves produced as a result of IVP would have lower milk yield and fertility and higher incidence of postparturient disease than cows giving birth to calves born as a result of AI. Finally, we hypothesized that the culture medium used to produce embryos in vitro would affect the incidence of developmental abnormalities. The media assessed were synthetic oviductal fluid–bovine embryo 1 (**SOF-BE1**), a serum-free medium that includes BSA as the only protein source (Fields et al., 2011), and Block-Bonilla-Hansen 7 (**BBH7**; Cooley Biotech LLC, Gainesville, FL), a proprietary medium reported to increase the percentage of embryos that develop to the blastocyst stage and improve embryo survival after cryopreservation (Block et al., 2010).

MATERIALS AND METHODS

Experiment 1: Characteristics of Offspring and Dams in an IVP Program Compared with an AI Program

This study examined the characteristics of calves produced as a result of embryo transfer (**ET**) using a fresh IVP embryo (**ET-F**), ET using a vitrified IVP embryo (**ET-V**), or AI. In addition, health traits, reproductive function, and milk yield of their dams was determined. The experiment was conducted at the University of Florida Dairy Unit (Hague, FL; 29.77904 N, 82.48001 W). Data from 426 cows and their calves were analyzed. Data for pregnancy diagnoses at d 32 and 76 of gestation for 386 of the cows have been reported previously (Block et al., 2010). Details of reproductive management and embryo production for all 426 cows are provided in Block et al. (2010). For the ET groups, embryos were produced in vitro using BBH7 culture medium (Block et al., 2010). Data on cows were analyzed from d 76 of pregnancy through 210 d of the subsequent lactation, whereas data on calves were analyzed from birth until 120 d of the first lactation.

Embryos were produced using 2 bulls who together had an average PTA for various traits as follows: $209 lifetime net merit (range 167 to 251), 12 kg of milk yield (−93 to 117), 3.9 yr of productive life (3.8 to 4), 7.2% sire calving ease (6.0–8.4%), 6.8% daughter calving ease (6.0 to 7.6%), 8.7% sire stillbirth (8.6 to 8.8%), 7.7% daughter stillbirth (6.6 to 8.8%), and 1.6% daughter pregnancy rate (0.8 to 2.3%). For AI, a total of 8 bulls were used, with average PTA as follows: $257 lifetime net merit (range 15 to 447), 352 kg of milk yield (−147 to 651), 2.0 yr of productive life (−1.3 to 3.5), 6.3% sire calving ease (5.2 to 7.6%), 6.7% daughter calving ease (6.0 to 8.6%), 7.5% sire stillbirth (6.1 to 10.8%), 7.0% daughter stillbirth (6.6 to 10.8%), and 0.8% daughter pregnancy rate (−1.3 to 3.7%).

Dams. Holstein cows that were pregnant as a result of ET or AI were housed in sand-bedded freestall barns equipped with fans and sprinklers that were programmed to run when dry bulb temperatures exceeded 21.0°C. Cows were milked and fed a TMR twice daily. Cows were dried off 46 d before expected calving by cessation of milking and intramammary infusion of each quarter with antibiotic (Quartermaster, Pfizer Animal Health, Kalamazoo, MI). Parturition occurred spontaneously or, for animals that did not calve by 280 d of gestation, by induction via i.m. injection of 20 mg of dexamethasone (Aspen Veterinary Resources, Liberty, MO) and 25 mg of $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health). Occurrence of one or more calving difficulties (needed assistance, slight problem, rear presentation, considerable force, extremely difficult and died) was re-