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Intrauterine inoculation of seronegative heifers with bovine viral diarrhea virus concurrent with transfer of in vivo–derived bovine embryos

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Abstract

Bovine viral diarrhea virus (BVDV) has been shown to be associated with single transferable in vivo–derived bovine embryos despite washing and trypsin treatment. Hence, the primary objective was to evaluate the potential of BVDV to be transmitted via the intrauterine route at the time of embryo transfer. In vivo–derived bovine embryos (n = 10) were nonsurgically collected from a single Bos tarus donor cow negative for BVDV. After collection and washing, embryos were placed into transfer media containing BVDV (SD-1; Type 1a). Each of the 10 embryos was individually loaded into an 0.25-mL straw, which was then nonsurgically transferred into the uterus of 1 of the 10 seronegative recipients on Day 0. The total quantity of virus transferred into the uterus of each of the 10 Bos tarus recipients was 878 cell culture infective doses to the 50% end point ($CCID_{50}$)/mL. Additionally, control heifers received 1.5×10^6 $CCID_{50}$ BVDV/.5 mL without an embryo (positive) or heat-inactivated BVDV (negative). The positive control heifer and all 10 recipients of virus-exposed embryos exhibited viremia by Day 6 and seroconverted by Day 15 after transfer. The negative control heifer did not exhibit a viremia or seroconvert. At 30 d after embryo transfer, 6 of 10 heifers in the treatment group were pregnant; however, 30 d later, only one was still pregnant. This fetus was nonviable and was positive for BVDV. In conclusion, the quantity of BVDV associated with bovine embryos after in vitro exposure can result in viremia and seroconversion of seronegative recipients after transfer into the uterus during diestrus.

Keywords: Bovine viral diarrhea virus; BVDV; Cattle; Embryo transfer; In vivo–derived embryos

1. Introduction

Assisted reproductive techniques such as artificial insemination (AI) and embryo transfer (ET) of in vivo–derived and in vitro–produced Bos tarus embryos are widely used to improve genetics throughout the world. The International Embryo Transfer Society (IETS) reported that approximately 577,900 and 245,260 in vivo–derived and in vitro–produced (respectively) bovine embryos were transferred worldwide in 2007, and ET continues to increase in other species, including swine, goats, sheep, horses, and wildlife [1]. Also, approximately 1.0×10^8 straws of semen are used each

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year [2]. These techniques have been thought to substantially reduce and/or eliminate the potential for transmission of a variety of pathogens [3–12]. However, for some pathogens, specifically bovine viral diarrhea virus (BVDV), which can be transmitted via contaminated semen [13–28], resulting infections can culminate in increased early embryonic death, abortion, and birth of persistently infected offspring [14–18,22,23,28]. In addition, results from studies concerning in vivo–derived and in vitro–produced embryos have pointed to the possibility of BVDV being transmitted via ET [14,22,24,29–41]. Current research by Gard et al. [42] reported that 27% of in vivo–derived and 42% of in vitro–produced embryos had embryo-associated virus after artificial exposure to a high-affinity strain of BVDV (SD-1; Type 1a) after washing procedures in accordance with the IETS manual (without trypsin) [43]. Additionally, embryo-associated BVDV was previously determined to be infectious in an in vitro culture system and in an in vivo model through intravenous inoculation of embryos into virus negative and seronegative recipients [30,31]. However, it is not yet known if embryo-associated BVDV is infectious after intrauterine inoculation. Therefore, the objective of this study was to determine if BVDV, in quantities associated with embryos [42], when placed intrauterine with an embryo, could result in an infection in a susceptible recipient.

2. Materials and methods

2.1. Experimental design

For this study, 10 Code 1 (excellent to good embryos as graded according to the IETS manual [44]), in vivo–derived embryos were collected from a seronegative and BVDV-negative donor cow. Ten seronegative and BVDV-negative Bos tarus, heifers each were implanted with a single embryo and 878 cell culture infective doses to the 50% end point ($CCID_{50}$) BVDV/mL. Two recipients served as the positive and negative controls and were implanted with 1.5×10^6 $CCID_{50}$ BVDV/straw and 1.5×10^6 $CCID_{50}$ heat-inactivated virus/straw, respectively. Serum and whole blood samples were collected from all recipients on Days 0, 3, 4, 6, 7, 8, 9, 10, 12, 15, and 30 after intrauterine inoculation and analyzed for virus and serum neutralizing antibodies on Days 0, 15, and 30. Recipients were assessed for pregnancy using transrectal ultrasonography on Days 30 and 60. One conceptus was recovered via colpotomy on Day 60, and the fetus and fetal membranes were assessed for BVDV via immunohistochemistry, virus isolation, and PCR.

2.2. Bovine viral diarrhea virus exposure

Stocks of SD-1 (Type 1a) BVDV were propagated in Madin Darby bovine kidney (MDBK) cells that were cultured in Minimum Essential Medium with Earle's salts (MEM). Virus was harvested from the cells by a single freeze-thaw method, aliquoted, and stored (–80 °C) until needed. The viral titer of the stock virus was quantified by applying the method of Reed and Muench [45]. On the day of the trial, aliquots of stock virus were thawed and diluted in MEM to achieve two concentrations of virus: 6.0×10^6 and 3.5×10^3 $CCID_{50}$/mL. Embryos were placed in MEM containing 3.5×10^3 $CCID_{50}$ BVDV/mL in 35-mm Petri dishes and loaded into 0.25-mL straws. Straws were taken to the North Auburn Beef Unit and maintained in a warm environment, approximately 38.5 °C, until transfer. The titer of each concentration of virus was determined again at the end of the exposure period.

2.2.1. Heat inactivation of virus

Virus at a concentration of 6.0×10^6 $CCID_{50}$/mL was heated at 85 °C for 10 min to inactivate infectious virus, as done previously [23]. The negative control heifer received 1.5×10^6 $CCID_{50}$ heat-inactivated BVDV/straw. The remainder of the heat-inactivated virus was tested via virus isolation to ensure no infectious virus was present.

2.3. In vivo embryo collection, embryo washing, and transfer of embryos

2.3.1. Synchronization of estrus, superovulation, and breeding

Estrus was synchronized in a seronegative and viral-negative Angus crossbred donor cow with 25 mg prostaglandin $F_{2\alpha}$ (Lutalyse; Pfizer, Kalamazoo, MI, USA). Superovulation was initiated 9 d after estrus by administration of decreasing doses of follicle-stimulating hormone (FSH; Folltropin-V; Bioniche Animal Health, Belleville, ON, Canada), as described previously [30,31]. Approximately 24 h after the superovulation protocol, the cow was bred by a BVDV-free Angus bull. All cows, heifers, and bulls used in this study were tested 30 d prior to the beginning of the study and on Day 0 using virus isolation and serum neutralization assays to ensure freedom from BVDV and anti-BVDV antibodies, respectively. Additionally, all animals were housed away from other animals, and all contact with other animals was suspended for the length of the study.

Twelve Angus crossbred recipient heifers underwent estrus synchronization by insertion of a controlled intravaginal drug release (CIDR; 1.38 g progesterone;