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Large scale *in vivo* risk assessment of bovine viral diarrhea virus (BVDV) transmission through transfer of bovine embryos produced via somatic cell nuclear transfer (SCNT)

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Abstract

The objective was to use the bovine viral diarrhea virus (BVDV) as a model to assess the risk of infectious disease transmission in the system of *in vitro* embryo production and transfer via somatic cell nuclear transfer (SCNT) technology. The risks of BVDV transmission in the SCNT embryo production were previously evaluated [1]. In that *in vitro* study, following standard operating procedures (SOP), including pre-nuclear transfer donor cell testing, oocyte decontamination and virus-free cell and embryo culture conditions, SCNT embryos produced were free of detectable viral RNA. The current study focused on the evaluation of the potential risk of disease transmission from SCNT embryos to recipients, and the risk of producing persistently infected animals via SCNT embryo transfer. Blood samples were collected from 553 recipients of SCNT embryos and 438 cloned calves and tested for the presence of BVDV viral RNA via a sensitive real time PCR method. All samples tested were negative. These results, in conjunction with the previous *in vitro* study, confirmed that the established SCNT embryo production and transfer system is safe and presents no detectable risk of disease transmission.

Keywords: BVDV transmission; Risk Assessment; Somatic Cell Nuclear Transfer (SCNT); Embryo; Recipient

1. Introduction

Bovine embryo transfer technology has been widely used around the globe to transfer and exchange genetics between farms, countries, and continents. Comprehensive studies have been conducted on the biosafety of conventional bovine embryo production and transfer. A standard operating procedure (SOP) for embryo production and transfer has been published by the International Embryo Transfer Society (IETS) to ensure the safety of this technology [2]. Large amounts of research data demonstrated that when the IETS guidelines were followed, the application of embryo transfer technology did not increase disease transmission, but can, on the contrary, be used as a means to prevent disease transmission and improve the health status of a given herd [3–5]. Commercial embryo production and transfer via the somatic cell nuclear transfer (SCNT) technology is relatively new. Since oocytes used for SCNT embryo production are predominantly from abattoirs and the nuclear transfer procedure produces small openings on the zona pellucida (ZP), concerns have been raised regarding the biosafety of this technology [5–7]. There-

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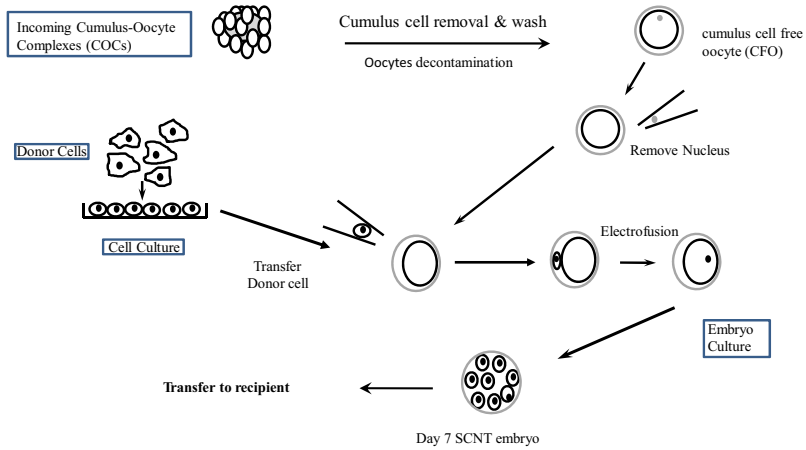

Fig.1. Schematic diagram of the SCNT embryo production system. The boxes indicated possible entry points for BVDV contamination.

fore, a comprehensive evaluation of the risk of infectious disease transmission via the SCNT technology is necessary.

Bovine viral diarrhea virus (BVDV) is prevalent worldwide, causing substantial economic losses for the cattle industry. It has been found in semen samples of infected bulls [8,9] and in ovarian follicular fluid, oviductal cells, and embryos from infected heifers [10,11]. Due to its prevalent nature and its association with the reproductive tract, BVDV is an ideal model to study the risk of disease transmission associated with SCNT embryo production and transfer. In a previous study, we carefully evaluated all risk factors present in an *in vitro* SCNT embryo production system [1]. Our study led to the establishment of a standard operating procedure for SCNT embryo production, including pre-nuclear transfer somatic donor cell testing, stringent oocyte washing, and virus-free cell and embryo culture conditions, to insure virus free SCNT embryo production [1]. The limitation of the previous study was that tests performed on SCNT embryos were collected by proportional sampling. Although the sample size was large enough to predict that all embryos produced in the system were virus free, it was not absolute. Moreover, BVDV viral molecules have been previously detected in oocytes of persistently infected (PI) animals [12,13]. There is a potential risk of BVDV positive oocytes from the PI animals being used to produce infected SCNT embryos. These infected embryos may be transferred into recipients, resulting in the production of PI calves, with disease transmission occurring from embryos to recipients. The objective of the current study was to evaluate the risk of disease transmission through SCNT embryo production and transfer in a large embryo transfer recipient herd. In addition to the previous *in vitro* study [1], this study would provide a conclusive risk assessment of disease transmission through SCNT embryo production and transfer.

2. Materials and methods

2.1. Experimental design

The process of *in vitro* SCNT embryo production and transfer is summarized (Fig. 1). Several potential pathogen entry points were marked by rectangle boxes. “Could SCNT embryo transfer produce PI animals of BVDV infection?” “Is there a risk of disease transmission from the SCNT embryo to its recipient?” These were the two key questions that the current study aimed to address. To address the first question, blood samples from 438 new born cloned calves were collected and tested for the presence of the BVDV viral molecule via a sensitive real-time PCR test [14]. To address the second question, more than 553 recipients receiving SCNT embryos were tested for BVDV viral RNA on the day of embryo transfer and again 40 d after embryo transfer.

2.2. Embryo production via SCNT

The SCNT embryos were produced as described previously, with minor modifications [15]. To avoid BVDV contamination, all somatic donor cell lines were cultured in medium containing gamma irradiated fetal bovine serum (FBS). Each donor cell line was tested for the presence of BVDV viral RNA prior to using it for nuclear transfer. The *in vitro* matured oocytes obtained from the commercial oocyte suppliers (Bomed Inc. Madison, WI, USA, and Trans Ova Genetics, Sioux Center, IA, USA) were processed and prepared for