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Risk of transmission of *Mycobacterium avium* ssp. paratuberculosis by embryo transfer of in vivo and in vitro fertilized bovine embryos

A. Bielanski *, J. Algire, G.C.B. Randall, O. Surujballi

Canadian Food Inspection Agency, Animal Diseases Research Institute, Germplasm Centre of Expertise, Ottawa Laboratory Fallowfield, Ottawa, Ont., Canada K2H 8P9

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Abstract

Over a 5-year interval, experiments were conducted to determine if *Mycobacterium avium* ssp. paratuberculosis (Map) is associated with in vivo and in vitro fertilized (IVF) embryos and whether it can be transmitted by embryo transfer. The present studies included: collection of embryos from five asymptomatic, naturally infected donors and transfer to uninfected recipients; collection of oocytes from two naturally infected donors with overt clinical signs; exposure of in vivo and IVF embryos to Map and transfer to uninfected recipients; and the inoculation (transfer) of ''clean'' IVF embryos to the uterine lumen of infected cows. The presence of Map was confirmed in the uterine horns of all asymptomatic, infected donors. None of the tested embryos, which were not used for embryo transfer, or unfertilized ova (two per batch), were positive for Map, as determined by culture ($n = 19$) or by PCR ($n = 13$). However, all in vivo fertilized embryos exposed to Map in vitro (and subsequently sequentially washed) tested positive for Map, by both culture (12 batches) and PCR (15 batches), whereas IVF embryos treated in the same manner tested positive on culture (51%, 18/35 batches) and by PCR (28%, 20/71 batches). Transferring both in vivo embryos and IVF embryos potentially contaminated with Map into 28 recipients resulted in 13 pregnancies and eight calves born without evidence of disease transmission to either the recipients or the offspring over the following 5-year period. In samples collected from one of the clinically infected animals, two of seven (28%) cumulus oocyte complexes (COC) and follicular fluid tested positive by PCR and 10/10 cumulus oocyte complexes on culture for Map. From the second clinically infected cow, three of five batches of IVF embryos ($n = 20$) were positive on PCR and two of four batches containing unfertilized oocytes and embryos were positive on culture. Only 10% of embryos reached the morula and blastocyst stage 10 days after fertilization. In conclusion, Map is unlikely to be transmitted by embryo transfer when the embryos have been washed as recommended by the International Embryo Transfer Society.
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Keywords: *Mycobacterium paratuberculosis*; *Mycobacterium avium*; IVF; Embryos; Embryo transfer

1. Introduction

Paratuberculosis (Johne's disease) is a chronic infectious disease of cattle and other domestic and wild ruminants caused by *Mycobacterium avium* ssp. paratuberculosis (Map) and is widespread in countries with a temperate climate. The disease is characterized by a persistent progressive diarrhea, weight loss and eventual death. In addition to economic losses, a recent increase of interest in the eradication of this disease in some countries has been prompted by the potential involvement of Map as a causative microorganism in Crohn's disease, a debilitating chronic enteritis in

* Corresponding author. Tel.: +1 613 228 6698; fax: +1 613 228 6669.
E-mail address: bielanskia@inspection.gc.ca (A. Bielanski).

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humans. Generally, infection with Map occurs via ingestion of the organism, but in utero transmission to fetuses during the later stages of pregnancy has been reported [1]. The microorganism has been isolated from semen and the endometrium of pregnant and non-pregnant, clinically and nonclinically infected cattle [2]. Despite substantial information regarding Map, there are very limited data regarding the association of Map with germplasm and potential for transmission [3,4]. The objective of the present study was to determine if *Mycobacterium avium* ssp. paratuberculosis is associated with in vivo and in vitro fertilized (IVF) embryos and whether it can be transmitted by embryo transfer.

2. Materials and methods

Two series of experiments were conducted to determine the association of Map with embryos and the ability of the organism to be transmitted (to recipients and offspring) by embryo transfer. In the first experiment (in vivo), embryos and oocytes were collected from heifers naturally infected with Map and transferred to Map-free recipients or used for IVF; in the second experiment (in vitro), embryos were produced by IVF and exposed in vitro to Map prior to embryo transfer, testing for the presence of Map, or both.

2.1. Animals

2.1.1. Embryo and oocyte donors

Seven Holstein cows (from 4 to 8 years of age) were purchased (from a farm with a history of herd Map infection) to serve as oocyte/embryo donors. Fecal samples from all seven cows were positive for Map on culture and on polymerase chain reaction (PCR) test for Map. Five had no clinical symptoms of Johne's disease, whereas the other two had profuse diarrhea and over a period of a few weeks, became emaciated and cachexic. These two cows were euthanized and samples of cumulus oocyte complexes (COC), uterine flushes and follicular fluids were collected post-mortem. Furthermore, IVF embryos were produced from one of these cows.

All infected donors were housed in separate cubicles in an isolation facility. It was mandatory for personnel to have a shower before entering the cubicle and after leaving it.

2.1.2. Embryo transfer recipients

Embryo transfer recipients (28 Holstein heifers from 1.5 to 2 years of age) were purchased from a farm with no herd history of Map; all were negative on ELISA, fecal culture and PCR. These heifers were housed in remote, pathogen-free facilities, with a specially designated animal care staff. After embryo transfer, recipients were housed in an isolation unit until calving. During the next 4 years, animals were kept at an isolated farm facility where they were periodically tested for the presence of Map.

2.2. In vivo experiments

2.2.1. Production of embryos and embryo transfer

Five subclinically infected Holstein donors were used for embryo production. These animals were superovulated with a total dose of 400 mg of FSH-P (Folltropin; Bioniche Animal Health, Belleville, Ont., Canada) over 4 days, using a decreasing dose regimen. Seven days after insemination, embryos were collected non-surgically by washing each uterine horn with 500 mL of phosphate buffered saline (PBS), supplemented with 2% estrous cow serum. To prevent accidental contamination of the embryos by feces, the closed system for uterine washing between the flushing cathether and embryo filter was used. Embryos were collected by filtration of wash fluids through a 75 μm filter membrane in an Emcon (Immuno System Inc., Spring Valley, WI, USA) system, and then retrieved by washing the filter membrane with 25 mL PBS (supplemented with 2% estrous cow serum).

The superovulation and nonsurgical embryo collection procedures were repeated on some donor animals over a period of 2 years. After washing, embryos were selected and transferred non-surgically into synchronized Map-free recipients on Days 7 or 8 of the estrus cycle (Day 0 = estrus). Embryos recovered from donors which were not used for embryo transfer and uterine sediments retained on the embryo collection filters (uterine cells and mucus) were tested for Map.

2.2.2. Oocyte and embryos production from clinically infected donors

Cumulus oocyte complexes were collected post-mortem from two cows with clinical signs of Johne's disease (see Section 2.1.1); the COC from one of these cows were used for IVF. Embryos were generated using the same protocol as described below.

2.3. In vitro experiments

2.3.1. Production of IVF embryos

The IVF embryos produced were used for: (a) in vitro exposure to Map and embryo transfer; (b)

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