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Embryo loss in cattle between Days 7 and 16 of pregnancy

D.K. Berg, J. van Leeuwen, S. Beaumont, M. Berg, P.L. Pfeffer *

AgResearch, Ruakura Campus, Hamilton, New Zealand

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Abstract

Embryo loss between embryonic Days 7 and 16 (Day 0 = day of IVF) in nonlactating cattle, *Bos taurus*, was analyzed using transfer of 2449 (in groups of 3 to 30) in vitro–produced (IVP) blastocysts. In 152 transfers, pregnancy losses attributable solely to recipient failings amounted to between 6% (beef heifers) and 16% (parous dairy cows), of which 3% were caused by uterine infections. Neither season, year, nor the age of the embryos on retrieval affected pregnancy rates. The latter observation indicated that the reason that a recipient failed to retain embryos was already present at the time of transfer. Notably, the proportion of embryos recovered decreased ($P = 0.03$) as more embryos were transferred, particularly at later stages (Day 14, $P < 0.01$). The average length of embryos decreased by approximately 5% for every additional embryo transferred ($P < 0.0001$). These effects may be linked to embryonic migration. Embryo mortality inherent to the embryo during the second week of pregnancy was 24%. Additionally, 9% of Day 14 embryos were of inferior quality, as they did not contain an epiblast. Combining embryo and recipient causes but excluding infection effects, embryonic loss of IVP embryos during the second week of pregnancy amounted to 26% (heifers) or 34% (parous dairy cows). The length of embryos doubled every day between Days 9 and 16, with a 4.4-fold range in sizes representing two thirds of the variation in length. Embryos retrieved from heifers were twice the size of those incubated in parous cows ($P < 0.0001$), indicating faster embryonic development/trophoblast proliferation in heifers. Whereas season did not affect embryo recoveries, length was lower (50%) in winter (winter–autumn, $P < 0.05$; winter–spring, $P < 0.001$). Lastly, transuterine migration in cattle, when transferring multiple embryos, commenced at Day 14 (4%) and had occurred in all recipients by Day 16 (38% of embryos found contralaterally).

Keywords: Bovine embryo; Embryonic mortality; Recipient; Preimplantation; Transuterine migration

1. Introduction

Embryonic mortality in cattle is a major source of economic loss for livestock producers. This problem is compounded for New Zealand's pasture-fed dairy cattle that need to conceive within a short seasonal window to be aligned with pasture growth and the following breeding season. In general, fertilization rates are accepted to be close to 90%, whereas calving rates lie somewhere between 40% and 55% and are on the decline in dairy cows [1]. This implies an embryonic and fetal mortality rate (excluding fertilization failure) of between 40% and 56% for moderate- and high-producing cows. Most of these losses occur during the embryonic period [2], defined as conception to the end of differentiation and encompassing approximately the first 45 d of gestation [3]. Within this period, embryonic death appears to be highest within the first 3 wk of gestation [2,4–6], some reports attributing greatest losses to the first week [7], particularly for high-producing dairy cattle [8], whereas others have pinpointed the second week as the most sensitive period [9,10]. From an evolutionary view, it is

* Corresponding author. Tel.: +64 7 838 5002; fax: +64 7 838 5628.
E-mail address: peter.pfeffer@agresearch.co.nz (P.L. Pfeffer).

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advantageous for species to impose the greatest selection pressure for embryo viability at early gestational stages, as it minimizes the reproductive cost for the mother. From a developmental view, early losses are expected, as errors during early events tend to have more severe consequences than those involving later developmental programs. The first week of development requires the critical events of correct embryonic genome activation and the establishment of the first lineage decision leading to trophectoderm and inner cell mass. During the second week of bovine development, the hypoblast lineage is set up, gastrulation is orchestrated, and embryonic-maternal signaling necessary for pregnancy recognition has been initiated. These critical themes continue, leading to an attached embryo with three body layers and rudimentary organs, as well as extraembryonic membranes, by the end of the third week.

Embryonic death during the critical second and third week of embryogenesis was investigated in some detail, retrieving embryos at multiple time points within this interval on an unprecedented large scale. Multiple transfers of in vitro–produced (IVP) embryos allowed, for the first time, a direct assessment of the relative importance of recipients and embryos to early pregnancy failures. Effects of season, recipient type (heifer vs. parous cows), and number of embryos transferred on pregnancy, embryo viability, size, and uterine migration were examined. Confounding, lactation-associated effects (including the lactational hormonal environment, postpartum interval, negative energy balance) on the uterine environment were excluded by using only dry animals, thus allowing the characterization and quantification of inherent embryonic mortality and setting the baseline for studies aimed at specifically dairy-farming associated stressors.

2. Materials and methods

The degree of embryo loss and factors influencing such losses between Days 7 and 16 of cattle, *Bos taurus*, pregnancies were determined. To allow noncompromised comparisons between heifer and cow recipients, lactational effects were excluded by using only dry cows. Fertilization and oocyte quality effects were minimized by using abattoir-derived oocytes, the same bull's semen for all fertilizations, and selecting only Grades 1 and 2 Day-7 blastocysts for transfer into recipients. A total of 2449 blastocysts were transferred into 106 cows and 90 heifers. Animal procedures were conducted under the approval of the Ruakura Animal Ethics Committee (R.A.E.C. 11183).

2.1. Generation of IVP embryos

For the generation of IVP embryos, ovaries were sourced from local abattoirs, being predominately from Friesian, Jersey, or Friesian-Jersey cross dairy cows. Oocytes were aspirated, matured, and fertilized using standard cattle techniques [11]. Day 0 refers to the day on which in vitro fertilization (IVF) was performed. Frozen-thawed spermatozoa from one Friesian bull was used throughout the entire data set. Zygotes were cultured in vitro for 7 d in biphasic SOF, synthetic oviduct fluid, medium supplemented with 10 μmol 2,4-dinitrophenol from Days 5 to 7 [11].

2.2. Embryo transfer

Seven days after IVF, groups of Grades 1 and 2 blastocysts (early to expanded) were selected for transfer by morphologic evaluation [12]. Grading and selection of blastocysts were completed by the same experienced embryologist throughout the entire data collection period, thereby reducing variability. The number of blastocysts transferred per recipient ranged from 3 to 30. For determination of recipient-only effects, at least eight blastocysts/animal were transferred. Blastocysts were washed twice in Emcare Hold (ICPbio, Auckland, New Zealand) and loaded into 0.25-mL plastic straws for transfer. Blastocysts were transferred transcervically with a standard 0.25-mL embryo transfer instrument into the uterine horn ipsilateral to the corpus luteum (CL) of estrus-synchronized animals 7 d after standing estrus.

2.3. Recipients

All recipients were maintained under pastoral farming conditions. Two types of recipients were used, depending upon availability. Parous nonlactating dairy cows, 3 to 5 yr of age, were used throughout the year. Additionally, during summer and autumn, crossbred beef heifers (15 to 18 mo of age, ~450 kg) were used. Recipients (usually 20% more than required) were synchronized using a single intravaginal progesterone-releasing device for 10 to 12 d (CIDR-B; InterAg, Hamilton, New Zealand). At device insertion, 1 mg estradiol benzoate (CIDIROL; InterAg) was administered intramuscularly. Four days before device removal, these cattle were given 500 μg cloprostenol (Estroplan; Parnell Laboratories, Auckland, New Zealand). Cattle were checked for signs of estrus three times daily, and only those observed in standing estrus (Day 0) and that possessed a palpable