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The effect of ascorbic acid during biopsy and cryopreservation on viability of bovine embryos produced *in vivo*

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Abstract

Multiple ovulation embryo transfer (MOET) is used to make more rapid progress in animal breeding schemes. On dairy farms, where female calves are more desired, embryo sex diagnosis is often performed before embryo transfer. Fresh transfers have been favored after biopsy due to cumulative drop in pregnancy rates following cryopreservation. The aim of this study was to explore whether exposure to ascorbic acid (AC) during biopsy and freezing increases the viability of biopsied embryos after cryopreservation. Data on presumptive pregnancy and calving rates of biopsied and cryopreserved/overnight-cultured embryos were gathered. Results showed differences in presumptive pregnancy rates between the groups: 45% for both biopsied-cryopreserved groups (control and AC), 51% for biopsied-overnight-cultured embryos and 80% for intact-fresh embryos. Differences between the groups were also apparent in calving rates: 22% for biopsied-cryopreserved control embryos, 31% for biopsied-cryopreserved AC-embryos, 23% for biopsied-overnight-cultured embryos and 63% for intact-fresh embryos. It is concluded that manipulated embryos are associated with lower presumptive pregnancy and calving rates compared with intact-fresh embryos. The highest calving rates for groups of manipulated embryos were achieved in the AC-group. Therefore, addition of AC can be recommended if biopsy is combined with freezing before transfer.
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1. Introduction

Multiple ovulation and embryo transfer (MOET) allows farmers to make more rapid progress in herd improvement and breeding schemes. Since the first embryo transfers (ET) in the 1970s, the ET business has evolved into an international system where 500,000 fresh or frozen embryos are traded each year [1,2]. In 2008, 101,149 embryos were transferred in Europe, 2761 of which were in Finland [3].

Female calves are more desired than males on dairy farms, and embryo biopsy is often carried out for sex determination before transfer. Biopsy can, however, reduce embryo viability dramatically when combined

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with cryopreservation. Freezing biopsied embryos reduces pregnancy rates significantly [4,5]. To avoid having to carry out a biopsy, sex-sorted semen can be used for embryo production. However, not all bulls produce semen suitable for sexing, and embryo flushing results can be impaired when sex-sorted semen is used, especially for cows [6,7].

When embryonic cells are exposed to stress factors, such as biopsy, freezing and thawing, as well as light or pH fluctuations, mitochondria start producing reactive oxygen species (ROS), leading to lipid peroxidation, disruption of cell membranes, and in the worst case, cell death [8]. Ascorbic acid (AC) is an antioxidant and a potent scavenger of oxygen radicals, which protects cells against the deleterious effects of ROS. Lane et al [9] reported that addition of 0.1 mM of AC to the cryopreservation solution of mouse embryos benefited the subsequent embryo development. It reduced the level of hydrogen peroxide in embryos after thawing and enhanced the inner cell mass development in blastocyst stage embryos.

Here we present data from a field MOET study, the "AATE project, Analysed embryos increase productivity of dairy herds in the Northern Savo", that was organized by local and national breeding authorities in the Northern Savo area of Finland during 2005–2007. The main goals of the project were to obtain information on the pregnancy rates of biopsied and cryopreserved embryos of top breeding dairy cattle and to explore whether ascorbic acid exposure during biopsy and freezing could increase the viability of biopsied and cryopreserved embryos after thawing [10].

2. Materials and methods

2.1. Experimental design

Embryo flushings were conducted on Finnish dairy farms located in the eastern part of the country. Animals included in the study were Ayrshire and Holstein-Friesian dairy heifers and cows. Flushed embryos were transported to the laboratory of the University of Eastern Finland (Kuopio) for subsequent analysis, unless embryo yield was less than four, in which case embryos were transferred fresh and intact to recipients on farms without manipulation.

A total of 49 animals (26 heifers and 23 cows) were flushed on day 7 after inseminations. In the laboratory, biopsy for sex determination were done on transferable embryos (quality code 1–3) followed by either freezing (quality code 1), or culture overnight (quality code 2–3). After completing the sex diagnosis, female embryos were transferred to recipients (quality code 1 embryos as frozen-thawed, and quality code 2–3 embryos as fresh on day 8).

The data for animals and pregnancies used in this study were gathered from the Finnish database, which was updated by veterinarians and ET-technicians. Since the study was conducted on dairy farms rather than in research facilities, pregnancy rates were likely subject to overestimation. After the transfer, the recipient was classed as non-pregnant if the animal returned to clearly visible heat within two weeks post-transfer. All the other recipients were classed as presumptively pregnant, even though the pregnancy was confirmed only later (week 6 onwards), either using ultrasound or rectal examination during regular on-farm calls by veterinarians or ET-technicians. If an animal was classed non-pregnant later than 53 d after transfer, it was considered a lost pregnancy, which included recipients incorrectly counted as pregnant (silent first two heats) or those which aborted. The exact incidences of abortions cannot, therefore, be assessed from these data, but generally, at the farm level in Finland, the abortion rate is lower than 5%. Animals delivering a full term calf were classed as calved.

2.2. Superovulation treatment and embryo flushings

The superovulation program was started 9–12 d (cows) or 10–11 d (heifers) after natural heat. Donors were superovulated with eight decreasing doses of follicle stimulating hormone (FHS) (Folltropin-V, Bioniche, Ontario, Canada or Pluset, Laboratorios Calier S.A., Barcelona, Spain) during four days. In total, 18 ml and 12 ml of FSH was administrated to cows and heifers, respectively. To induce ovulations, the animals were treated with 2 ml of prostaglandin (Genestran, Forte Healthcare Ltd., Ireland) in the evening of day three after initiation of FSH treatment. Donors showing standing heat were inseminated two to three times 12 h apart with frozen-thawed semen of top breeding bulls, starting in the morning of day six after initiation of FSH treatment [11]. Embryo flushings were conducted using standard procedures.

2.3. Embryo sexing, freezing and storage in culture

Embryos were recovered non-surgically on day 7 after insemination, and transported in straws (max 10 embryos in a straw) to the laboratory in Emcare Holding Solution (ICPbio, Auckland, New Zeeland) at room temperature (RT). In the laboratory, embryos were classified according to IETS recommendations [12]. Qual-

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