

Exploitation of embryos collected from Maedi-Visna seropositive ewes during eradication programs[☆]

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Abstract

It was demonstrated that Maedi-Visna virus is not transmitted through transfer of in vivo derived, either intact or micromanipulated sheep embryos, provided washing and trypsin treatment is applied immediately after collection. Thus, genetic material of highly producing Maedi-Visna seropositive sheep can be exploited or/and preserved prior to slaughter, (a) facilitating application of eradication schemes and accelerating flock replacement and (b) enabling preservation of genetic material (in the form of frozen embryos) from sheep of endangered breeds in cases when most or all available females are Maedi-Visna seropositive.

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1. Introduction

Maedi-Visna (ovine progressive pneumonia) is a “slow infection” caused by a lentivirus (Retroviridae). The major pathways of transmission are nasal discharge in cases of respiratory distress, colostrum and milk (Sihvonen et al., 2000; Straub, 2004). The financial impact of Maedi-Visna virus (MVV) is due to its broad distribution and adverse effects on animal productivity and on the trade of animals, semen and embryos (Ploumi et al., 1997; Gunn et al., 1998;

Scheer-Czechowski et al., 2000). Identification of infected sheep using serological tests (Dawson et al., 1982; Simard and Briscoe, 1990; Pepin et al., 1998; Zanoni et al., 1994), separation of seropositive from seronegative animals, colostrum deprivation and artificial rearing of the lambs, as well as slaughter of seropositive animals are essential for eradication of the disease from infected flocks (Houwens et al., 1984; Cutlip and Lehmkuhl, 1986; Sihvonen et al., 2000; Scheer-Czechowski et al., 2000). The necessity to slaughter females of high genetic value is an important hindrance for application of eradication programmes, especially in the case of endangered or rare sheep breeds when few female animals are available.

Genetic material from seropositive ewes either of high genetic value or of endangered breeds could be preserved before (or at) slaughter using superovulation

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and embryo transfer. To date the possibility of MVV transmission through embryo transfer techniques to the offspring or to the recipient ewes has not been investigated.

Although certain viruses (e.g. bluetongue virus, bovine herpesvirus-1, bovine herpesvirus-4) adhere to the surface of the zona pellucida (Gillespie et al., 1990; Stringfellow et al., 1990; Wrathall and Stumöller, 1998), experimental works with bovine leukaemia (Eaglesome et al., 1982; DiGiacomo et al., 1986), bovine immunodeficiency (Bielanski et al., 2001) and bovine vesicular stomatitis (Sutmoller and Wrathall, 1997) viruses, bluetongue virus in cattle (Bowen et al., 1983) and sheep (Hare et al., 1988), foot-and-mouth-disease virus in cattle (McVicar et al., 1986; Mebus and Singh, 1991; Sutmoller and Wrathall, 1997) and pigs (Singh et al., 1986), pseudorabies virus (James et al., 1983) and parvovirus (Gradil et al., 1994) in pigs and caprine arthritis-encephalitis virus (Lamara et al., 2002a) have demonstrated that, if the washing protocols recommended by the International Embryo Transfer Society (Stringfellow, 1998) for in vivo derived, intact embryos are followed, there is negligible risk of transmitting these viruses through embryo transfer techniques (Stringfellow and Givens, 2000).

The objective of this work was to investigate the possibility of transmission of MVV through the transfer of either intact or micromanipulated embryos collected from Maedi-Visna seropositive donors to seronegative recipients and/or to the offspring.

2. Materials and methods

2.1. Animals

In experiment I, 54 ewes (20 Chios-breed, 18 Pelagonia-breed and 16 Kozani-breed) were selected as embryo donors. The Chios-breed ewes were of particularly high genetic value (milk production, reproductive performance, phenotypic characteristics); actually, they were the best animals in the flock of the Animal Research Institute (Giannitsa, Greece). In experiment II, 123 Chios-breed ewes or Chios-with Friesian-breed cross ewes were used as zygote (experiment IIa, 98 ewes) or embryo (experiment IIb, 25 ewes) donors. In both experiments, all ewes used as zygote/embryo donors were Maedi-Visna seropositive.

One-hundred-and-sixty ewes of various breeds (25 in experiment I, 93 in experiment IIa and 42 in experiment IIb) were used as embryo recipients. All ewes used as recipients came from a flock of lambs that had been reared artificially during application of an animal health program in the Animal Research Institute (Vainas et al., 1999); all were found to be MVV seronegative in three successive tests, carried out in 6-monthly intervals.

2.2. Oestrus synchronization and superovulation

Fluorogestone acetate intravaginal sponges for 12 day were used for oestrus synchronization of ewes. One of the following three superovulation protocols was used in each case: 16 mg FSH-P in six decreasing 12 h interval doses, 17.6 mg o-FSH (OVAGEN) in eight equal 12 h interval doses or 200 mg p-FSH (FOLL-TROPIN) in six decreasing 12 h interval doses.

2.3. Fertilization

In the case of Chios-breed and Chios-cross donor ewes, intrauterine artificial insemination was performed through a laparoscope, 24 h after beginning of clinical oestrus. Pelagonia- and Kozani-breed donor ewes were mated to fertile, MVV seronegative, rams of the same breed, respectively.

2.4. Embryo collection

In experiment I, embryos were collected on day 6 (day 0 = oestrus onset) using a laparoscopic technique. In experiment IIa, zygotes and two-cell embryos were collected at 18–24 h after artificial insemination, whilst in experiment IIb, blastocysts were collected 5 day after artificial insemination, immediately after slaughter. Immediately after collection, zona pellucida intact zygotes and embryos were repeatedly washed in PBS and treated with trypsin, according to the recommendations of IETS (Stringfellow, 1998).

2.5. Embryo transfer

In all cases, zygotes/2-cell embryos or morulae/blastocysts were transferred to the oviducts or the uterine horns of recipient ewes, respectively, by means of a laparoscopic technique. Ultrasonography was used

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