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Transfer of sexed caprine blastocysts freshly collected or derived from cultured morulae

M. El-Gayar[1], W. Holtz*

Institute of Animal Husbandry and Genetics, University of Goettingen, Albrecht-Thaer-Weg 3, 37075 Goettingen, Germany

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Abstract

This paper describes a simplified method for biopsying goat embryos for sex determination, followed by the transfer of these embryos to recipient does. A 90° angle holding pipet is described, which permits holding the embryo in position and, with minimal effort, open the zona pellucida and excise 5–10 trophoblast cells with the aid of a microsurgical blade. Twenty-four embryos were biopsied, 12 of which were freshly collected (day 7) and 12 of which were cultured in vitro from collected morulae (day 6). In the course of the polymerase chain reaction (PCR) a 447 (male) and 445 bp (female) fragment of the DNA, derived from the trophoblast cells, was amplified. The primers used were specific for the highly conserved mammalian sequences of the ZFX and ZFY genes. Individual biopsied embryos were transferred to 24 synchronized recipient does. The pregnancy rate following transfer was 67% (8/12) in recipients receiving freshly collected blastocysts while only 8% (1/12) in the recipients receiving blastocysts cultured in vitro from morulae ($P < 0.01$). Four of the kids born were males and four females. The sex assigned to the embryos was correct in 83% of the cases.

Keywords: Goat; Sex determination; Embryo transfer; Micromanipulation; PCR

1. Introduction

Knowledge of the sex of embryos to be transferred would be of great benefit to animal producers. The established means of sexing embryos in several farm animal species is the application of the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) to a small number of biopsied cells. The method has been used for embryo sexing in cattle (Pollevick et al., 1992; Bredbacka et al., 1995), pigs (Fajfar-Whetstone et al., 1993), horses (Peippo et al., 1995), sheep (Schmoll et al., 1995; Bernardi and Delouis, 1996) and goats (Leoni et al., 1996). Among the possible methods of biopsying mammalian embryos (microblade, micro-pipette or glass needle), the blade-biopsy is the simplest and most effective to perform. However, occasionally the embryo is re-

* Corresponding author. Tel.: +49 551 395605; fax: +49 551 395587.
E-mail address: wholtz@gwdg.de (W. Holtz).
[1] Present address: Department of Animal Production, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt.

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leased from the zona pellucida in the process (Shea, 1999).

There is little information on sex determination in caprine preimplantation embryos. Also, transfer of sexed caprine embryos has not been reported in the literature. Aasen and Medrano (1990) first demonstrated that DNA samples isolated from goat blood could be sexed using the polymerase chain reaction (PCR). Using universal primers they amplified 447 or 445 bp fragments from male or female genomic DNA, corresponding to the ZFY or ZFX genes. The restriction fragment length polymorphism (RFLP) yielded a specific banding pattern with two bands in females and three in males. Similar observations were reported by Pomp et al. (1995). A simple method, using PCR, has been standardized for accurate sex determination in goats (Rao and Totey, 1992). A pair of bovine Y-chromosome-specific primers was applied to genomic DNA isolated from blood samples of adult male and female goats. The primers recognized and amplified the Y-chromosome-specific sequences of male goats. The assay was found to be accurate, reliable and rapid. Leoni et al. (1996) were the first to describe a method for sex determination in goat embryos, using PCR and RFLP analysis. They amplified a DNA fragment derived from four to eight cells that had been biopsied from embryos as described by Aasen and Medrano (1990). The objective of the present study was to establish an efficient way of biopsying embryos, using a specially designed holding pipet, determine the sex of the biopsied cells by subjecting them to PCR and RFLP, and transfer the sexed embryos to recipients to examine their viability.

2. Materials and methods

2.1. Collection of embryos

Parous Boer goat does (age: 21–103 months, average body weight: 48–80 kg), were induced to ovulate during the breeding season using 1.5 mg Norgestomet implants (Crestar®, Intervet, Boxmeer, Netherlands) for 10 days (Holtz and Sohnrey, 1992). Upon implant removal, 2 $PGF_{2\alpha}$ injections (5 mg Dinoprost in 1 ml Dinolytic®, Pharmacia and Upjohn, Erlangen, Germany) were administered at 12 h intervals. In order to induce multiple ovulations, donors were treated with 16 Armour Units (AU) of highly purified pFSH supplemented with 40% LH, as described by Nowshari et al. (1995). Briefly, beginning 48 h before implant removal, s.c. injections of 4, 4, 2, 2, 2 and 2 AU were administered at 12 h intervals. Does were tested for standing estrus with an aproned buck twice daily and were hand mated repeatedly until no longer in estrus. Embryos were collected 7 days after the last mating, applying the transcervical approach described by Holtz et al. (2000) and Suyadi et al. (2000). $PGF_{2\alpha}$ (1 ml Dinolytic®) was administered 20 h before flushing. Both uterine horns were flushed by 10 consecutive infusions of 20 ml of flushing medium (Dulbecco's PBS containing 100 IU/ml penicillin and 100 μg/ml streptomycin, supplemented with 2% heat-inactivated goat serum). Twenty-four embryos were collected, 12 of which were expanded blastocysts, with blastomeres of uniform shape and color and intact zona pellucida. These were biopsied within 1 h following collection. Another 12 embryos in the morula stage from 7 different donors were cultured in 0.1 ml drops of M16 medium (Hogan et al., 1986) for 24 h and were biopsied at the expanded blastocyst stage.

2.2. Fabrication of 90° holding pipets

Using a microforge (Research Instruments, Cornwall, England), a pipet of o.d. 1.0 mm and i.d. 0.58 mm was heated at one point by the glass bead-covered filament until it softened and bent to an angle of about 15–20°. The process was repeated four to six times moving the pipet progressively further away from the tip, until the pipet was curved to an angle of 90° (Fig. 1).

2.3. Sexing of embryos

Biopsies were carried out with a pair of micromanipulators (Leitz, Wetzlar, Germany), mounted on both sides of an inverted microscope (IM 35, Zeiss, Goettingen, Germany). One embryo at a time was picked out from the M16 medium and rinsed five times in M2 flushing medium devoid of BSA (Hogan et al., 1986), before being transferred to a drop of M2 medium on a level glass plate placed on the warming stage of an inverted microscope. The embryo was fixed with the bent holding pipette and mounted on the microinstrument holder of the left micromanipulator. It was then centered in the microscopic field at a magnification