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Effect of transporting donor or recipient does and their embryos on the outcome of fresh embryo transfer in Boer goats

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ABSTRACT

Between-farm embryo transfer of livestock animals can potentially increase the spread of quality genetic material. However, the transporting of donor or recipient animals or their embryos has become a practical problem. The objective of this study was to compare the effect of transporting donor and recipient does and their embryos between various farms on inter-farm fresh embryo transfer in Boer goats. Results indicate the transportation of donor does within 4 h before embryo collection not to have a significant effect on embryo recovery number, embryo survival rate and the subsequent pregnancy in recipient does. Also, the transportation of embryos at 36.5–38 °C within 2 h before embryo transfer did not significantly affect the embryo survival rate and subsequent pregnancy rate, but the transportation of embryos at 20 °C resulted in a significant ($P < 0.05$) lower survival rate (41.7%) and pregnancy rate (42.0%). The transportation of recipient does resulted in a significantly lower pregnancy rate (42.0%) and embryo survival rate (32.1%) than the transportation of donor does and embryos. Results suggest the transportation of donor does to be the best method for embryo transfer programs on the farm. Alternatively, the supply of fresh embryos kept at body temperature (36.5 °C) was also preferred for short or long distances between farms.

1. Introduction

Embryo transfer (ET) is an advanced, but well established, animal breeding technology. This technique involves embryo collection from a donor female and transfer to recipient females, which serve as surrogate mothers for the remainder of pregnancy. Embryo transfer techniques have been applied to nearly all species of domestic animals and many species of wildlife and exotic animals, including humans and non-human primates. In the goat, the superovulation of donor does and the pregnancy in recipient does are the main factors affecting the efficiency of this embryo transfer technology (Armstrong et al., 1983; Cognié et al., 2003; Gonzalez-Bulnes et al., 2004; Thibiera and Guérinb, 2000). The pregnancy and the offspring numbers derived from embryo transfer directly reflect the MOET breeding program efficiency.

Cryopreservation of gametes and embryos also facilitate the long-distance transportation and increases the international exchange of genetic material. However, such transportation may cause chemical and physical damage to embryos and lead to a decrease in embryo viability and pregnancy rates of recipient does (Kasai, 1994; Hasler, 2001; Papadopoulos et al., 2002). Furthermore, the cryopreservation of embryos

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requires complex procedures, special skills and expensive equipment.

In contrast to cryopreservation, fresh goat embryo transfer generally results in higher pregnancy rates. China has many small goat farms in the rural areas and embryo transfer is often carried out between these different farms. This inter-farm embryo transportation has become a major problem for embryo transfer on the small rural farms. Some reports indicate that fresh cattle and sheep embryos may survive at 0 °C for a few hours or transport over a long distance at normal temperatures before transfer to recipient females (Leibo and Winninger, 1986; Yang et al., 1991; Takahashi et al., 1996; Vajta et al., 1997). However, embryo survival rates and pregnancy rates have recorded a significant decrease. Based on the Chinese rural farming practice, two strategies may be considered to avoid the disadvantages of fresh embryo transportation. The general first choice is to transport the donor does from the nucleus farm to the recipient farm for embryo transfer. Another option is to the transport recipient does to the donor doe farms. However, this transportation effect on embryo transfer efficiency remains unclear. The objective of this study was to compare the effect of transporting donor, recipient does or their embryos between the different farms on the outcome of an inter-farm fresh embryo transfer program in Boer goats.

2. Materials and methods

2.1. Preparation of donor and recipient does

Purebred multiparous, mature Boer goat does ($n = 205$) were used as embryo donors and mature Guanzhong dairy goats ($n = 1790$) used as recipients in this study. The experimental donor and recipient goats were obtained from four farms in China of which two farms were in the Shaanxi Province (the KeYuan Farm and the Boer Goat Center) and two farms in the Shandong Province (the Well-bred Livestock Breeding Center and the Dongying Chaoda Boer Goat Farm). The embryo donor does were 3–5 years of age and their offspring had been weaned more than 60 days previously. Similarly the recipient does were 2–5 years of age and certified not pregnant. All goats were kept outdoors with access to indoor facilities, fed a concentrate and hay diet, and supplied water ad lib. The experiments were conducted during the natural breeding season (September to November-autumn) for the period 2002–2004.

2.2. Superovulation and insemination of donor does

Controlled internal drug release (CIDR, Hamilton, New Zealand) devices containing 0.3 g medroxyprogesterone were placed in the vagina of the donor does on day 0 and the CIDR's were again replaced on day 12. The full period of progestagen treatment (16 days) including a total of 200 mg FSH (Folltropin-V 400 mg/20 ml, Vetrepharm, Canada) which was administered intramuscularly twice daily (12 h intervals), in decreasing doses to induce superovulation. After 7 FSH treatments (total of 4 days), a single intramuscular injection of PGF2α (Shanghai Institute of Planned Parenthood Research, China; 0.2 mg/dose) was administered to each donor doe. Estrus was detected with the aid of adult bucks, beginning 12 h after CIDR removal. Goats were mated to bucks twice daily at 6:00 and 18:00 until the demonstration of estrus terminated. A single, 50 μg intramuscular injection of LRH-A3 (luteinizing hormone releasing hormone A3) (First Hormone Manufacturing Company, Ningbo, China; 25 μg/dose) was given to each donor at the onset of mating. An additional intravaginal sponge containing 45 mg fluorogestone acetate (FGA, Key Laboratory of Animal Reproductive Endocrinology & Embryo Biotechnology, Ministry of Agriculture, Northwest A&F University, China) was placed in the vagina of each donor doe at 60–72 h after the end of mating until embryo collection (6.5–7 days after estrus).

2.3. Synchronization of estrus in recipient does

In order to synchronize estrus, CIDR's were inserted into the vagina of the recipient does for 14 days with 300 IU PMSG (Pregnecol, Bioniche, Australia; 20,000 IU/dose) injected at CIDR withdrawal to each ewe. Forty-eight hours after CIDR removal, the recipient ewes received a single 0.1 mg injection of PGF2α. Estrous detection was performed using bucks from 12 h after CIDR withdrawal and the onset and end of the induced estrous period recorded.

2.4. Recovery and assessment of embryos

Embryo recovery was performed 6.5–7 days after the onset of estrous behavior. All donor does were fasted for 24 h prior to surgery and the number of corpora lutea (CLs) were determined by laparoscopy just before laparotomy. Embryos recovery was performed only on goats with more than 4 CLs. Donor does were anesthetized using a single intravenous injection of 0.5–0.6 ml Sumianxin (Changchun Veterinary University, China; 1.5 ml/dose), with embryo recovery performed by flushing the two uterine horns with a PBS solution, supplemented with 3% BSA.

Embryos were evaluated morphologically under a stereomicroscope (60× magnification). The morula or blastocyst stage embryos at flushing were graded on a scale—from grade 1, excellent; grade 2, good/fair; grade 3, poor and grade 4, degenerated (Lindner and Wright, 1993; Rubianes et al., 1995). Grade 1–3 embryos were regarded as transferable. Transferable embryos were then washed 2–3 times with PBS + 3% BSA and stored in a TCM-199 holding medium (Sigma, St. Louis, MO, USA), supplemented with 20% FBS and 100 μM β-Mercaptoethanol (Sigma, St. Louis, MO, USA).

2.5. Embryo transfer to recipient does

Recipient does were fasted for 24 h prior to transfer and transported to recipient farms 2–4 h prior to transfer. Embryo transfer was performed under general anesthesia, using 0.3–0.5 ml Sumianxin. One or two embryos were transferred to each recipient, depending on the CL developmental status of the recipient. A recipient with 2 CLs or one well-developed CL would receive two embryos.

2.6. Experimental design

2.6.1. Experiment 1: effect of transporting donor does for embryo transfer

A total of 52 potential donor does were transported to the recipient farm in trucks, 6.5–7 days after the onset of overt estrus, but only 49 does were used for embryo collection. In order to determine the effect of the transportation times of the donors on embryo collection, the donor does were delivered at 0 h (control), 1 h, 2–3 h and 4 h before the flushing procedure, respectively.

2.6.2. Experiment 2: effect of transporting embryos for embryo transfer

A total of 1504 fresh embryos were used in the transportation trial. This trial consisted of two parts: (i) to determine the effect of transportation temperature on embryo survival. Fresh embryos were stored in straws at 20 °C, 36.5 °C, or 38.5 °C in portable incubators (INC 30, Australia) and transported 2 h before implantation to the recipient does and (ii) to determine the effect of transportation time on embryo survival, all fresh embryos were stored in straws in portable incubators at 36.5 °C. Embryos were then transported at 0.5 h, 1 h or 2 h before embryo transfer (0 h transport, control). The holding medium during embryo transportation was TCM-199, supplemented with 20% FBS and 100 μM β-Mercaptoethanol.

2.6.3. Experiment 3: effect of transporting recipient does for embryo transfer

A total of 400 recipient does were divided into 3 groups and transported to recipient farmers 0.5 h, 1 h or 2 h before transfer, respectively. After embryo transfer, the recipient does were kept on these farms for 2–3 days and transported back to the original farm.

2.7. Statistical analysis

The mean numbers of recovered embryos and transferable embryos were indicated by the mean ± SD. The difference between treatments were analyzed using Duncan's multiple range test ANOVA procedure,