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First live offspring born in superovulated sika deer (*Cervus nippon*) after embryo vitrification

L. Wang[a,b,1], G.B. Zhou[c,1], W.Q. Shi[b], J.M. Shi[a], X.Z. Tian[a], C. Gao[a], L. Zhang[a], S.E. Zhu[a], T.T. Zhang[d], S.M. Zeng[a], G.S. Liu[a,*]

[a] *Key Laboratory of Animal Genetics and Breeding of the Ministry of Agriculture, National Engineering Laboratory for Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, China*

[b] *Animal Husbandry and Veterinary Station of Beijing, Beijing, China*

[c] *Institute of Animal Genetics and Breeding, College of Animal Science and Technology, Sichuan Agricultural University (Chengdu Campus), Wenjiang, China*

[d] *School of Applied Sciences, Bournemouth University, Poole, UK*

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Abstract

The rapid growth in sika deer (*Cervus nippon*) farming and interest in their conservation is an impetus for development of embryo transfer (ET) procedures. However, a paucity of research has prevented widespread application of ET in this species. The objective of the present study was to establish a multiple ovulation and ET procedure with both fresh and vitrified embryos in sika deer. Multiparous weaned hinds (N = 18) were used as embryo donors during the reproductive season of 2008 at a local breeding farm in China. Estrus was synchronized in donors and recipients (N = 38) by inserting a controlled internal drug release for 12 days (insertion = Day 0). Superovulation was induced with a total of 320 mg of NIH-FSH-P1 (Folltropin-V; Bioniche, Belleville, ON, Canada) given as 40 mg im every 12 h from the afternoon of Day 9 to the morning of Day 13. After estrus was detected, donors were artificially inseminated using a transcervical technique. The embryo recovery rate was 76.8% (63/82), including 1.6% (1/63), 77.8% (49/63), and 1.6% (1/63) blastocysts, morula, and eight-cell embryos, respectively. After transfer of fresh and vitrified embryos, pregnancy rates were 85.7% and 61.6% and birth rates were 64.3% and 53.9% ($P > 0.05$). In conclusion, we developed a satisfactory multiple ovulation and ET procedure in farmed sika deer using vitrified embryos.

Keywords: Sika deer; Superovulation; Estrous detection; Vitrification; Embryo transfer

1. Introduction

Sika deer (*Cervus nippon*) inhabit much of east Asia. They are short-day seasonal breeders whose reproductive activity in China spans from September to November. In Asia, sika deer are of great value in the antler industry because of their large size and top-ranked antler quality. However, as a result of high commercial interest, sika deer have been overhunted the past few decades. Therefore, the sika deer population has been reduced, with a slow increase, because they are monotocous. Therefore, it is important to develop effective reproductive technologies, for example, embryo transfer (ET), to effectively increase the population of sika deer.

Since publication of reports on surgical ET in white-tailed deer [1] and red deer [2], this technique has also been successfully used in fallow deer [3–5] and red deer [6]. Multiple ovulation and ET (MOET) programs for red

[1] These two authors contributed equally to the work.

* Corresponding author. Tel.: +86 10 62732735; fax: +86 10 62732735.
E-mail address: gshliu@cau.edu.cn (G.-S. Liu).

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deer (*Cervus elaphus*) have been developed and used commercially over the past decade, particularly in New Zealand [7,8]. However, there have apparently been no successful MOET procedures developed for ET of sika deer. Because of their limited distribution and small herd size, ET research in sika deer has been very limited.

Embryo cryopreservation is widely used for facilitating the storage, transport, and dissemination of valuable genetic resources. The transfer of cryopreserved red deer embryos has previously been reported using slow freezing methods with glycerol as the main cryoprotectant [9–11]. However, glycerol requires multistep dilution after thawing, and embryos are passaged through decreasing concentrations of cryoprotectant as sucrose is included to prevent osmotic shock [12]. This relatively time-consuming technique could be replaced by other cryopreservation methods (mainly used in cattle) that do not require either serial dilutions after thawing [13,14] or expensive freezing machines [15–17]. Vitrification could also be an appropriate alternative for red deer embryo cryopreservation [8]. However, apparently no reports to date have described embryo vitrification in sika deer.

In this study, our objectives in sika deer included: (1) in vivo embryo production from superovulated females via AI; (2) vitrification of embryos using the straw method; and (3) surgical ET of vitrified-warmed embryos into recipients.

2. Materials and methods

2.1. Animals

Donors (N = 18) and recipients (N = 38) were chosen during the reproductive season of 2008 from farmed sika deer females (aged 3 to 5 y) at a farm located in Huairou District, Beijing, China. The adult male deer used for semen collection had the highest yield of antlers on the farm. Adult male deer with strong sexual desire and docile temperament were chosen for estrous detection. All experimental protocols regarding handling of sika deer were in accordance with the requirements of the Institutional Animal Care and Use Committee at China Agricultural University.

2.2. Estrous synchronization and superovulation

Estrous synchronization treatment both in recipients (N = 38) and donors (N = 18) consisted of a 12-day placement of an ovine controlled internal drug release (CIDR) (InterAg, Hamilton, New Zealand) containing 100 mg of progesterone. The date of CIDR insertion was designated Day 0 (initial day of treatment). At the time of CIDR removal (Day 12), 300 IU of pregnant mare serum gonadotropin (PMSG; Folligon; Intervet International, Boxmeer, Holland) was given to each recipient hind.

Superovulation was conducted with a total dose of 320 mg of NIH-FSH-P1 (Folltropin-V, Bioniche, Belleville, ON, Canada). Intramuscular injections were given every 12 h (40 mg at each injection), beginning on the afternoon of Day 9. The CIDRs were removed from donors on Day 12, concurrent with the seventh injection of FSH.

2.3. Estrous detection

Estrous was detected using a teasing method [8,18] which is a standard in the deer industry.

Twelve h after CIDR removal, an adult buck of proven fertility was introduced to the donor group for 30 min to 1 h, every 2 to 4 h. During this time, the buck's penis was covered by a 60 × 40-cm soft cloth, which effectively prevented intromission. Estrus was recorded when the female stood still for mounting by the male, a process known as mounting behavior. Estrus was similarly evaluated in recipient hinds.

2.4. AI

First, the frozen-thawed semen (sperm motility ≥ 0.3) was loaded in 0.25-mL plastic straws, each of which contained $> 1 \times 10^8$ sperm. Donor insemination was done (transcervical technique) 8 to 12 h after estrus was detected, with insemination repeated 8 to 12 h later.

2.5. Embryo recovery

Embryos were surgically collected from donors 6 to 7 days after the first insemination. The donors, which had been starved for 48 h, were anesthetized (100 mg of xylazine hydrochloride im; Lumianbao; Qingdao Hanhe Animal and Plant Pharmaceutical, Co, Ltd., China) and a midline ventral laparotomy performed (4-cm incision). Uterine horns and ovaries were exteriorized, and corpora lutea (CL) were counted.

A cannula attached to a syringe was inserted into the lumen near the uterotubal junction, and the uterine horn was flushed with 30 to 40 mL of a warm solution of PBS + 0.3% BSA (Fraktion, 735078; Roche Diagnostics, GmbH, Mannheim, Germany). From the opposite uterine horn, the medium flowed out through the other cannula into a Petri dish (90 mm in diameter; Corning, Inc., Corning, NY, USA). The flushing procedure was then repeated on the contralateral uterine horn. The reproductive tract was next washed with 0.9% sodium chloride solution (to minimize adhesions) and then re-