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Superovulatory response and pregnancy after interspecies transfer of embryos in semi-wild Dulong (*Bos frontalis*) - short communication

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ABSTRACT

This study was conducted to examine the superovulatory response in semi-wild Dulong cows (*Bos frontalis*) and to evaluate whether their embryos can be maintained in pregnancy after interspecies transfer to cross-bred recipients (*Bos taurus*). Embryos were recovered from five Dulong and nine Red Angus (control) donors after superovulatory treatment. The average numbers of corpora lutea, collected ova and transferable embryos in Dulong donors were similar to those in Red Angus donors. The embryos from Dulong donors were transferred to three cross-bred recipients, two of which became pregnant. However, one embryo was lost between the 40th and 60th day of gestation. The second was lost between the 60th and 90th day of gestation. In Red Angus cows, 44.4% (4/9) of the recipients which received embryos became pregnant, and three delivered calves. We confirmed that Dulong cows respond to superovulatory treatment. Interspecies transfer of Dulong embryos into cross-bred recipients produced pregnancies, but not live offspring.

Key words: interspecies, embryo transfer, mithun, superovulation, wild animal

Introduction

The conservation of wild animals is a key to the maintenance of genetic variation (CHEN, 1995). Recently, exotic ruminants have tended to be overused for obtaining the effects of cross-breeds. Moreover, their numbers are diminishing because of damage to their environment (MA and WU, 2002). Mithun (*Bos frontalis*) is a semi-wild ruminant species that is widespread throughout the north-east hill region of India and some parts

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of Bhutan, Myanmar, Bangladesh, and China. In China, Mithun, called “Dulong cattle”, inhabit the rainforests of the Gaoligongshan Mountains, located in north-west Yunnan Province, feeding on bamboo and the leaves of various trees and shrubs. It has been estimated that the total Dulong cattle population is about 3,000 head in Yunnan Province and that extinction of the animals can be expected to occur within several decades because of natural disasters, which may lead to a lack of food, or death from low temperatures (QU et al., 2008). Embryo transfer is a valuable technique for the production of offspring from animals with high genetic value. Moreover, interspecies embryo transfer can be a tool for the conservation and management of endangered species when there is a lack of a reasonable number of same-species recipients (NIASARI-NASLAJI et al., 2009). Interspecies transfer of gaur (*Bos gaurus*) embryos into domestic cows (*Bos taurus*) has produced pregnancies and live offspring (HRADECKY et al., 1988). However, little information exists in relation to the use of interspecies embryo transfer for the production of semi-wild, rare *Bos* species. This study was conducted to determine whether transferable embryos from superovulated Dulong cows could be collected and their embryos develop into offspring after interspecies transfer into cross-bred recipients.

Materials and methods

Animals. Ten semi-wild Dulong cows that inhabited forest land at 2,400-3,500 m above sea level were brought to a research farm at 1,980 m above sea level and tamed for about 7 months. Five of the ten semi-wild Dulong cows (4-5 years old) survived after taming and were superovulated for embryo collection. Nine Red Angus cows (4-8 years old) with regular estrous cycles were used as controls. Cross-bred cows of Brahman × Murray Grey × Yunnan Yellow (BMY; 3-10 years old) with regular estrous cycles were used as recipients. All donor cows were fed with corn silage, hay, and concentrated feeds. The recipients were fed ad libitum with grass in pastureland.

Superovulation and embryo recovery. CUE-MATE™ with a progesterone suppository (Bioniche Animal Health Pty. Ltd., Australia) was inserted randomly into the vagina of each donor. Nine days later, superovulation treatment was performed by intramuscular administration of 36 AU follicle-stimulating hormone (FSH, Antrin; Kawasaki Mitaka Seiyaku KK, Kawasaki, Japan), administered in a series of decreasing doses over a 4-day period. Donors were given 0.6 mg and 0.2 mg of prostaglandin F₂ alpha (PGF₂α; cloprostenol, Shanghai Institute of Planned Parenthood Research, China), respectively, along with the fifth and the sixth FSH treatment. CUE-MATE was withdrawn at the time of the first PGF₂α injection. At 12 h after detection of estrus, each donor was artificially inseminated with frozen-thawed semen from each of the same species (Dulong or Red Angus bull). Embryos were recovered non-surgically using a balloon catheter 7 days after insemination. After collection, the embryos were examined for their morphology under a

stereomicroscope and classified according to criteria defined by the International Embryo Transfer Society (ROBERTSON and NELSON, 1998). Embryos graded as 1 and 2 were defined as transferable embryos.

Embryo transfer. Cross-bred BMY cows received intravaginal CUE-MATE™ for 11 days. The cows received 0.6 mg of PGF_{2α} im 9 days after the start of the estrous synchronization treatment. The embryos (one embryo per recipient) from each donor were transferred non-surgically into the uterine horn, ipsilateral to the ovary bearing the corpus luteum, on day 7 after estrus. The assessment of pregnancy was performed on days 40, 60, and 90 after embryo transfer by transrectal ultrasonography (5.0-MHz transducer, Honda HS-101V; Products Group International, Inc., Colorado, USA).

Statistical analysis. Differences between Dulong and Red Angus cows with regard to the ovarian response and embryo yield were analysed using an independent Student t-tests. Differences in probability values (P) of 0.05 or fewer were considered statistically significant.

Results

As shown in Table 1, the ovarian response parameters did not differ between Dulong and Red Angus cows. Two of the three (66.7%) recipients of Dulong embryos were diagnosed as pregnant at Day 40 of gestation. The fetus was no longer detected in one recipient at the 60-day recheck. The other remained ongoing at the Day 60 pregnancy determination, but the fetus was no longer detected at the Day 90 recheck. Four of the nine (44.4%) recipients of Red Angus embryos were diagnosed as pregnant at Day 60 of gestation, but one recipient lost the fetus by Day 90, while the other three delivered live offspring.

Table 1. Ovarian response and embryo yield of Dulong and Red Angus cows after superovulation treatment*

Characteristic	Cows	
	Dulong	Red Angus
Number of animals	5	9
Number of corpora lutea	7.4 ± 2.7	6.6 ± 1.5
Number of ova and embryos	6.4 ± 2.7	5.0 ± 1.7
Number of fertilized ova	3.8 ± 0.4	4.3 ± 1.8
Number of transferable embryos	2.0 ± 0.5	2.9 ± 0.8

*Values are presented as means ± SEM

Discussion

We confirmed that transferable embryos from semi-wild Dulong cows can be collected by superovulation treatment after habituation lasting about 7 months. Although two singleton pregnancies by interspecies transfer of Dulong embryos to cross-bred recipients were established, all pregnancies were lost by Day 90 of gestation.

Superovulatory treatments are widely used in embryo transfer programs to increase the supply of embryos from animals with superior genetic merits. No reports in the literature describe the superovulation treatment of Mithun or Dulong cows (*Bos frontalis*). In contrast to other bovines, estrous signs such as mucous discharge, tail raising, and loss of appetite have been suggested as less pronounced in Mithun cows (MONDAL et al., 2005; MONDAL et al., 2006). However, we found that the ovarian responses and embryo yield in Dulong cows were comparable to those in Red Angus cows. In the estrous synchronization protocol, a progesterone-impregnated controlled internal drug release (CIDR) device in combination with GnRH and PGF₂α provides a more effective method for synchronizing estrus and ovulation than PGF alone or with GnRH, especially in anestrus postpartum cows (LARSON et al., 2006). Moreover, the inclusion of CIDR in the synchronization protocol increased plasma progesterone concentration and the proportion of cows detected in estrus (ECHTERNKAMP and THALLMAN, 2011). PRAKASH et al. (2008) reported that the CIDR treatment in cyclic and post-partum Mithun cows showed more prominent behavioural signs than spontaneous estrus. In this study, each donor was artificially inseminated with frozen-thawed semen after detection of behavioural estrus. Therefore, the treatment using CUE-MATE with a progesterone suppository during superovulation treatment in Dulong cows might improve embryo yield by prominent estrous signs. These results indicate that transferable embryos from semi-wild Dulong cows can be collected through superovulation treatment using a device with a progesterone suppository.

It has been demonstrated that interspecies transfer of gaur (*Bos gaurus*) embryos into domestic cows (*Bos taurus*) produces pregnancies and live offspring (HRADECKY et al., 1988; HAMMER et al., 2001). However, interspecies embryo transfer from water buffalo (*Bubalus bubalis*) to domestic cows was not able to establish pregnancy (DROST et al., 1986). It has been suggested that placentae resulting from interspecies embryo transfers have fewer cotyledons, which are much larger than cotyledons from normal placentae. HAMMER et al. (2001) reported poor branching of the villi and inability to penetrate maternal caruncular crypts sufficiently, suggesting that feto-maternal incompatibility between the embryos and the uterus of recipients retards fetal growth. The reason for the loss of the two pregnancies established in this study remains unclear, but a possible explanation for the failure to maintain pregnancy might be the result of poor development of utero-placental compatibility, such as poor penetration of the villi.

HRADECKY et al. (1988) reported that the success of interspecies embryo transfer depends not only on the proper matching of species, but also on the individual characteristics of the parental pair and the surrogate mother. Cross-bred offspring have been produced by artificial insemination of Brahman cows (*Bos indicus*) with Dulong bull semen (HE et al., 2011). Moreover, HE et al. (2011) reported that the cross-bred females (F1 generation) had full reproductive capacity including puberty expression. Therefore, the selection of a recipient, such as females cross-bred with Dulong bull semen, might maximise opportunities for interspecies embryos transfer (DURRANT and BENIRSCHKE, 1981).

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Z. X. He et al.: Superovulatory response and interspecies embryo transfer in semi-wild Dulong

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SAŽETAK

Istraživanje je provedeno da bi se utvrdio superovulacijski odgovor krava poludivljeg Dulong goveda (*Bos frontalis*) i da bi se procijenilo da li embriji tih krava opstaju tijekom bređosti nakon što su preneseni u primateljice druge vrste (*Bos taurus*). Embriji su dobiveni nakon superovulacije izazvane kod pet Dulong krava i devet krava pasmine crveni angus (kontrolna skupina). Prosječni broj žutih tijela, jajašaca i prenosivih embrija kod Dulong krava davateljica bio je sličan kao kod davateljica crvene angus pasmine. Embriji Dulong davateljica preneseni su u tri primateljice - križanke od kojih su dvije ostale bređe, no jedan je embrij izgubljen između 40. i 60. dana bređosti. Drugi embrij izgubljen je između 60. i 90. dana bređosti. U slučaju krava pasmine crveni angus, 44,4% (4/9) primateljica ostalo je bređe, od kojih su se otelile njih tri. Može se ustvrditi da Dulong krave reagiraju na postupak superovulacije. Prijenos embrija Dulong krava u primateljice - križanke rezultirao je gravidnošću, ali ne i živooteljenom teladi.

Ključne riječi: međuvrste, prijenos embrija, superovulacija, divlje životinje
