J Anim Reprod Biotechnol 2023;38:84-88
pISSN: 2671-4639 • eISSN: 2671-4663
https://doi.org/10.12750/JARB.38.2.84

Clinical Report

A case report of embryo transfer with air-transported fresh bovine embryo produced by multiple ovulation in Hanwoo

Sang-Yup Lee[1,2,#], Seong-Eun Heo[3,#] and Won-Jae Lee[1,*]

[1]College of Veterinary Medicine, Kyungpook National University, Daegu 41566, Korea
[2]Soohyun Large Animal Hospital, Gumi 39133, Korea
[3]Gyeongsangbuk-Do Livestock Research Institute, Yeongju 36052, Korea

Received June 4, 2023
Revised June 13, 2023
Accepted June 15, 2023

***Correspondence**
Won-Jae Lee
E-mail: iamcyshd@knu.ac.kr

[#]These authors contributed equally to this work.

Author's Position and Orcid no.
Lee S-Y, PhD candidate,
https://orcid.org/0009-0001-9815-7879
Heo S-E, Veterinary officer,
https://orcid.org/0009-0007-3526-2536
Lee W-J, Associate professor,
https://orcid.org/0000-0003-1462-7798

ABSTRACT Because multiple ovulation embryo transfer (MOET) in cattle includes several benefits such as wide spreading of genetically superior offspring for long distance, this biotechnological method has been widely applied to Hanwoo. When the recipients are not stayed close after embryo recovery from donor, the embryos are moved to other farms via several vehicles (car, train, and airplane). However, air travel induces lesser oxygen level, increased vibration, lower air pressure, higher noise, and increased exposure of cosmic radiation to living things than ground level. It was still unknown that fresh embryos obtained from multiple ovulation of Hanwoo could maintain their fertility after being transported via air plane, the present case report introduced a clinical case of MOET in Hanwoo after shipping fresh embryos via air transportation. The donor was multi-ovulated via follicle-stimulating hormone series of injection, which was followed by a gonadotrophin-releasing hormone injection and artificial insemination twice. The embryos were recovered by the uterine flushing, packed in ministraws, transported to recipients for 6 h including 1 h air flight, and then transferred to the synchronized recipients. During pregnancy diagnosis of early gestation period, 5 of 7 recipients (71.4%) presented no heat signs and showed fetal sacs with fluid under transrectal ultrasonography. After normal gestation period, all recipients naturally delivered healthy calves (male n = 2 and female n = 3) without abortion, stillbirth, and premature birth. The present case report indicated that transportation of fresh embryos for MOET via domestic flight in Korea did not affect to their fertility.

Keywords: air plane, embryo transfer, Hanwoo, multiple ovulation

INTRODUCTION

Since multiple ovulation embryo transfer (MOET) in cattle was first introduced at the late 1970s, this reproductive biotechnology has been widely researched in Korea so as to apply this technique to advanced reproduction in Korean beef cattle (Hanwoo) (Son et al., 2006; Song et al., 2012; Yeom et al., 2013; Ideta et al., 2015). Although MOET technique has limitations with respect to lower pregnancy rates (below 50%) than artificial insemination (AI)-based reproduction and necessity of hormonal treatment, it has several benefits in terms of less-invasive

protocol to animal, rapid and wide spreading of genetically superior offspring at the same time and for long distance, allowing to select desired donor female cow for breeding, and delivering valuable purebred calves from other breed's dam (recipient) (Park et al., 2009; Ideta et al., 2015). During MOET, the recovered embryos from the donor should be transferred into recipients immediately as fresh embryos or cryopreserved into liquid nitrogen (LN_2). Because the latter induces damage to embryos during freezing and thawing procedure, decreases pregnancy rates lesser than fresh ones, and is necessary for special means for storage and transportation (LN_2 tank), most veterinary clinicians and researchers prefer to use the embryos as fresh (unfrozen) as possible during MOET (Ideta et al., 2015).

During transportation of fresh embryos from donor's farm to recipients' farm, several vehicles (car, train, and airplane) can be used. In general, it has been known that air travel induces lesser oxygen level, increased vibration, lower air pressure, higher noise, and increased exposure of cosmic radiation to living things than ground level (Buckett et al., 1999; Petrikovsky et al., 2018). Therefore, there have been several reports for investigating the effect of shipping *in vitro* fertilized (IVF) embryos in the portable incubator via air plane in several species including cattle and humans (Ambrose et al., 1999; Buckett et al., 1999; Morotti et al., 2014). However, it has been still unknown that fresh embryos obtained from multiple ovulation of Hanwoo and packed in ministraws can maintain their fertility with normal gestation period to recipients upon transporting via airplane. Therefore, the present case report introduced a clinical case of MOET in Hanwoo after shipping fresh embryos via air vehicle to Jeju island.

CASE REPORT

A donor female cow (Hanwoo; raised at a farm located in Boeun, Chungcheongbuk-do; experienced for 3 parities; body condition score: 2.75; brucellosis free) was multi-ovulated in accordance with the previous articles (Son et al., 2006; Song et al., 2012; Yeom et al., 2013). In brief, when the second follicular wave was initiated with recruitment of the new primary follicles during the luteal phase [presence of mature corpus luteum (CL)] under transrectal ultrasonography (Fig. 1A), the cow was administered decreasing doses of follicle-stimulating hormone (FSH, Antorin R10, Kyoritsu Seiyaku Corporation, Japan) twice a day as 5/5 mg at the 1st day, 4/4 mg at the 2nd day, 3/3 mg at the 3rd day, and 2/2 mg at the 4th day; at 4th day, an analogue of $PGF_{2\alpha}$ (50 μg cloprostenol sodium; Repromate, Unibiotech, Korea) was injected for luteolysis. At 6th day, an analogue of gonadotrophin-releasing hormone (GnRH; 2.1 μg buserelin acetate; Buserel, Unibiotech) was injected in the morning for the ovulation of dominant follicles, followed by 1st AI during afternoon and 2nd AI after 12 h at the next day; the condition of ovaries was observed by transrectal ultrasonography (Fig. 1B). 7 days after 1st AI, embryos were gently recovered by the uterine flushing using Foley catheter with flushing media (Dulbecco's phosphate-buffered saline supplemented with 5% fetal bovine serum and 50 IU/mL penicillin-streptomycin) after the epidural anesthesia. The total number of 13 embryos was obtained after the uterine flushing and 8 embryos (grade 1: 4 embryos, grade 2: 4 embryos) were determined as transferable to recipients in accordance with standard from previous article (Fig. 1C) (Gantugs et al., 2017). Each embryo was packed in 0.25 mL ministraws (IMV technologies, France) with flushing media, and the straws were heat-sealed; from grading to packing embryos, it required 30 min. The straws were horizontally laid in a thermos (vacuum bottle) set at 38℃; the present case report did not use the portable incubator during transporting embryos. The embryos in a thermos were transported to Daegu airport by car for approximately 120 min. Then

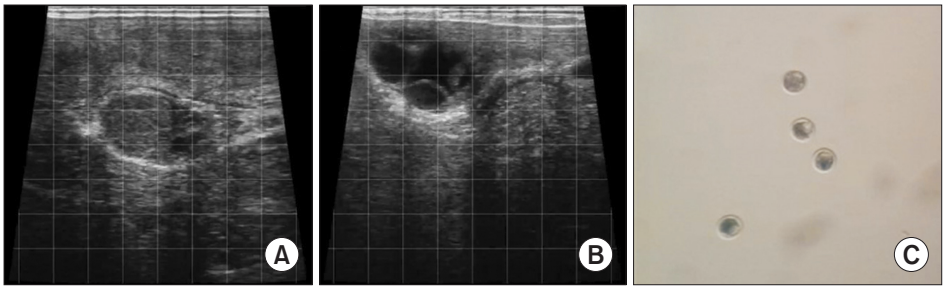

Fig. 1. Preparation of embryos from female donor. (A) The initial day of follicle-stimulating hormone series of injection, with showing mature corpus luteum and new primary follicles recruitment, (B) The ovary with multi-dominant follicles, (C) Grade 1 embryos after the uterine flushing.

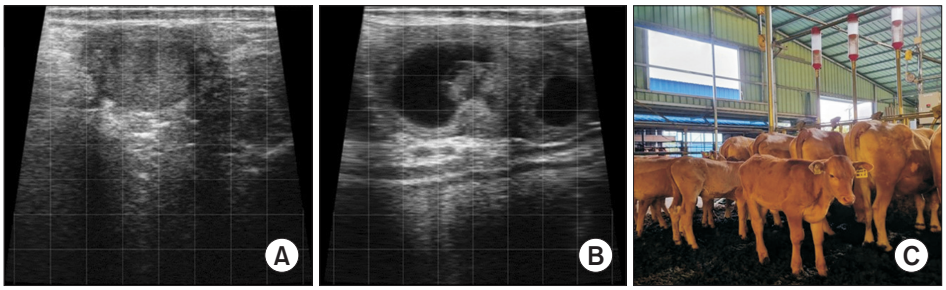

Fig. 2. Embryo transfer to recipients. (A) Assessment of recipients showing healthy and mature corpus luteum, (B) Fetal sac with fluid during early pregnancy diagnosis on Day 35, (C) Calves from the present case report.

the thermos was shipped as flight cargo to Jeju island; in general, the domestic flight information from Daegu to Jeju island was 1) waiting time before/after flight: 1 h, 2) flight time: 60–65 min, 3) flight speed: 700–800 km/h, and 4) a flight altitude: 7,620–8,840 m. From landing to a farm for ET, it took 60 min by car, and preparation of recipients for ET spent about 30 min. Overall, the recovered embryos were transported during approximately 6 h including the car and air flight.

To prepare recipient cattle (Hanwoo), estrus cycles were synchronized in advance by Ovsynch protocol (a GnRH analogue injection followed 7 days later by $PGF_{2\alpha}$ analogue and a second treatment with GnRH analogue 48 h later; day of estrus = day 0). Before a day of ET, the CLs of recipients were evaluated by transrectal ultrasonography to exclude recipient candidates presenting cystic CL but to select ones having healthy and mature CL, in accordance with a standard from a previous article (Fig. 2A); the mean diameter of CLs of selected recipients was 2.14 ± 0.23 cm (Ambrose et al., 1999). The shipped embryos were non-surgically transferred into recipients (an embryo per a recipient; n = 7) to the uterine horn ipsilateral to CL using the ET gun on days 6–8 of the estrus cycle. On days 20–22, 5 of 7 recipients did not present estrus sign, as early pregnant sign. On days 35, as pregnancy diagnosis, the fetal sacs with fluid were observed in same 5 recipients under transrectal ultrasonography (Fig. 2B). During gestation period, there was no case of abortion, stillbirth, and premature birth. After normal gestation period, all pregnant recipient cattle naturally delivered healthy calves (male n = 2 and female n = 3; Fig. 2C).

DISCUSSION

To spread genetically superior cattle to other farms by MOET, the viability of recovered embryos after transportation is a key determinant of successful reproduction. To achieve this, there are several efforts to protect fresh embryos from long distance or duration travel. Because unfrozen embryos could be stored for only 24–48 h at room temperature, the technique for hypothermic storage at 4℃ of embryos was investigated, resulting that antifreezing protein supplementation (first identified from the blood of fish species that could survive in sub-zero environment) to storage media was able to keep bovine embryos alive for 10 days (Ideta et al., 2015). When small molecules such as CHIR99021 and Y-27632 were added to culture media, bovine IVF embryos at 10℃ could survive until 96 h (Kim et al., 2017). However, in the farm condition, during MOET, most farm owners and veterinarians hope to transfer the recovered embryos to the recipients as quick and fresh as possible, rather than storing them for a long time. Therefore, the present case report introduced that fresh embryos for MOET could be transported for 6 h including air flight with maintaining their fertility to offspring. Of note, compared with the results from previous articles investigating ET efficiency of MOET in Hanwoo, the fresh embryos shipped via air plane in the present case report exhibited higher pregnancy rate compared with previous articles, indicating normal fertility of fresh embryos even after air flight (71.4% as 5/7 in the present case report vs. 33.3–63.6% in previous articles) (Kim et al., 2004; Son et al., 2006; Yeom et al., 2013).

Aside from the present case report with fresh bovine embryos during MOET, transportation effect to another type of bovine embryos (IVF embryos) has been studied. When IVF bovine embryos were shipped during *in vitro* culture (IVC) after IVF in a portable incubator (developing fresh IVF embryos) by car or airplane up to 2,000 km distance area for 24–48 h in Brazil, a 41% average pregnancy rate was observed after ET (Morotti et al., 2014). During investigating the effect of transporting time course including flight in Korea, fresh IVF bovine embryos in mini-straws presented higher pregnancy rate in a group that

embryos were transferred within 4 h (60%) than the other which was required more 6 h (26.3%) (Park et al., 2009). In USA, bovine IVF embryos shipped by airplane for 2–3 h and car for 1 h in the portable incubator could make 14.3% recipients pregnant (Ambrose et al., 1999).

In human clinics, similar trials to reveal the effect of air transportation of embryos have been performed. For the purpose of determining the capability of a transport IVF program including flight shipment of oocytes, when IVF and intracytoplasmic sperm injected (ISCI) embryos were transported for ET by airplane for 1 h, there were some clinical cases confirmed as pregnancy (Buckett et al., 1999). In addition, when ICSI embryos during IVC in the portable incubator were traveled by air plane between a branch hospital and the central laboratory (almost 1,000 miles) for genetically test, the returned embryos maintained the ability for clinical pregnancy (Langley et al., 2001).

CONCLUSION

There is real concern about whether the rough conditions during air transportation of fresh embryos (lesser oxygen level, increased vibration, lower air pressure, higher noise, and increased exposure of cosmic radiation) induce adverse effect to their fertility. In this context, the present case report introduced a case that transportation of fresh embryos for MOET via domestic air flight in Korea did not affect to fertility of embryos. We hope that this result can contribute to the field of veterinary clinics and livestock industry.

Author Contributions: Conceptualization, S-Y.L., S-E.H.; data curation, S-Y.L., S-E.H.; formal analysis, S-Y.L., S-E.H.; funding acquisition, W-J.L.; investigation, S-Y.L., S-E.H.; methodology, S-Y.L., S-E.H.; project administration, W-J.L.; resources, S-Y.L., S-E.H.; software, W-J.L.; supervision, W-J.L.; validation, W-J.L.; visualization, W-J.L.; writing original draft, S-Y.L., S-E.H.; review & editing, W-J.L.

Funding: This work was supported by a grant from the National Research Foundation (NRF) of Korea, funded by the government of the Republic of Korea (RS-2023-00251171).

Ethical Approval: Not applicable.

Consent to Participate: Not applicable.

Consent to Publish: Not applicable.

Availability of Data and Materials: Not applicable.

Acknowledgements: None.

Conflicts of Interest: No potential conflict of interest relevant to this article was reported.

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