Laparoscopy vs. laparotomy for embryo transfer to produce transgenic goats (Capra hircus)

Sang Tae Shin1,*, Sung Keun Jang1, Hong Suk Yang1, Ok Keun Lee1, Yhong Hee Shim1, Won Il Choi1, Doo Soo Lee1, Gwan Sun Lee2, Jong Ki Cho1, Young Won Lee1

1College of Veterinary Medicine, Chungnam National University, Daejeon 305-764, Korea
2Hanmi Research Center, Hwaseong 445-813, Korea

This study was performed to produce transgenic Korean native goat (Capra hircus) by laparoscopic embryo transfer (ET) to overcome the limitations of ET performed by laparotomy. Transgenic embryos were produced by DNA pronuclear microinjection of in vivo zygotes. The recipient goats were synchronized for estrus by using an introvaginal progesterone devices as a controlled internal drug-releasing insert (CIDR) for 13 days and injection of 400 IU PMSG 48 h before removal of the insert. Embryos were transferred on day 3 and 4 after removal of the insert. Recipient goats were deprived of feed for 48 h, then suspended in a laparotomy cradle at an angle of 45°. After obtaining a sufficient pneumoperitoneum, the laparoscope and forceps were inserted abdominally through 5 mm trocar sleeves. Examination of the ovaries and uterus was performed and then 213 embryos were transferred into the oviducts via the infundibula of 76 recipient goats. To compare pregnancy rates, ET was also performed by laparotomy in 82 recipient goats. The pregnancies in the recipient goats were diagnosed by ultrasound on day 30 after embryo transfer. The pregnancy rate with laparoscopic ET was significantly higher than with ET performed by laparotomy (46.1% vs. 28.6%, \( p < 0.05 \)). In addition, the pregnancy rates were compared between ovulated and non-ovulated ovaries of the recipient goats in the laparoscopic ET group. No significant difference was observed between the pregnancy rates of ovulated and non-ovulated ovaries (41.3% vs. 33.3%, \( p < 0.05 \)) suggesting that ET may also be possible in non-ovulated recipients through artificial rupture of Graafian follicles. These results suggest that laparoscopic ET is a highly efficient method for the transfer of goat embryos.

Keywords: embryo transfer, goat, laparoscopy, laparotomy, transgenic

Introduction

The mammary gland of transgenic farm animals has been proposed as the best available bioreactor for the production of human pharmaceutical proteins [4,5,13,21]. Dairy goats have been used as a bioreactor because of their relatively short gestation period and low maintenance costs compared to cattle. Many studies have reported on the production of transgenic goats [6-8,11]. The possibility of large-scale production for industrial application has been demonstrated [9,10]. In Korea, transgenic goats have been used to produce human granulocyte colony stimulating factor (G-CSF) in their milk [15,18].

To date, laparotomy methods have generally been used for goat embryo transfer (ET). However, this method can cause adhesions in the reproductive tract following repeated surgical ET and requires relatively long intervals before the re-use of a recipient female [15,18]. To overcome the limitations of laparotomy, laparoscopic ET has been performed in various species including sheep [19,20], cows [12] and pigs [3]. The laparoscopic method has also been performed in goats [1,2,16,17,24]. However, in the above mentioned studies, the laparoscopic method was used only for oocyte recovery by ovum pick-up and embryo recovery but not for embryo transfer.

Estrus synchronization is essential for successful ET and corpus luteum (CL) formation is necessary for pregnancy maintenance. However, it is difficult to know the exact status of the ovaries if they are not observed directly by exploratory surgery or ultrasonography. Therefore, if the CL is not formed during ET, artificial formation of the CL by follicle puncture is necessary for ovulation and progesterone support is required to maintain the pregnancy or the ET must be postponed until CL formation.

In the present study, we performed laparoscopic ET to overcome the limitations of laparotomy in the production of transgenic goats. The pregnancy rates resulting from the two methods were compared in the Korean native goat.
Table 1. The superovulation and estrous synchronization schedule for the donor and recipient Korean native goats

| Day | Donor goats | Recipient goats |
|-----|-------------|----------------|
| -14 | CIDR insert* | CIDR insert |
| -3  | PMSG 150 iu IM FSH 0.9 mg IM | PMSG 400 iu IM |
| -2  | FSH 0.9 mg IM | |
| 1   | FSH 0.9 mg IM | |
| 3   | Embryo recovery | Embryo transfer |

*Intravaginal progesterone insertion with controlled internal drug-releasing insert (CIDR).
to the injection needle. After examination of the ovaries, oviducts and uterine horns, the embryos were transferred.

The stage and quality of the embryos were evaluated under a stereomicroscope, and the embryos were loaded into a polyethylene tube (SP65; Nastume, Japan) attached to the injection needle (Fig. 1). With the forceps, the infundibulum was grasped (Fig. 2A), the polyethylene tube was inserted into the oviduct via the infundibulum (Fig. 2B), and 2 to 3 embryos were then transferred (Fig. 2C). After transferring the embryos, the back flow of the medium, into the abdominal cavity, was prevented by grasping the infundibulum with the forceps (Fig. 2D). The polyethylene tube was washed with medium, then checked for any remaining embryos. Recipient goats were used up to three to four times if no pregnancy was established after the ET.

To compare the pregnancy rates, ET by laparotomy was performed as described previously [18]. Briefly, 2-3 embryos were surgically transferred into 1 oviduct ipsilateral to the ovulated ovary, using a syringe connected to a sterile polyethylene tube, which was inserted into the oviduct lumen via the fimbria. The pregnancies were diagnosed by transrectal ultrasound scanning (SonoVet 600; Medison, Korea) using a transrectal 5-MHz linear array probe on day 30 and 40 following ET in both groups.

**Experimental design**

The pregnancy rates following ET were compared between the laparoscopy and laparotomy groups in experiment 1. In experiment 2, the recipient goats were classified into two groups based on whether they had ovulated or non-ovulated ovaries (GF; ovary with Graafian follicle that was non-ovulated, CH; ovary with corpus hemorrhagicum after ovulation). The pregnancy rates were compared after the laparoscopic ET. In the GF group, the non-ovulated follicle was ruptured artificially by needle puncture prior to the ET. This experiment was performed to investigate the effects of artificial rupture, of non-ovulated Graafian follicles, on the efficiency of laparoscopic ET.

**Statistical analysis**

All values for each parameter were analyzed by ANOVA using a general linear model (PROC-GLM) in the SAS 8.1 program (p < 0.05).

**Results**

A comparison of the pregnancy rates between the laparoscopic ET and ET by laparotomy revealed that there was a significant difference between the two methods of ET (Table 2). Following the laparoscopic method, 213 transgenic embryos were transferred to 76 recipient goats and 35 recipients (46.1%) became pregnant. However, with the ET by laparotomy, only 22 out of the 82 recipients

---

**Table 2. Comparison of pregnancy rates between laparoscopic and laparotomic embryo transfer**

| Method of embryo transfer | Transferred embryos | Recipients | Pregnancies (%) |
|---------------------------|---------------------|------------|-----------------|
| Laparoscopic              | 213                 | 76         | 35 (46.1)       |
| Laparotomic               | 232                 | 82         | 22 (26.8)       |

<sup>a,b</sup> Values in the same column with different superscripts are different (p < 0.05). †: number.
Table 3. Comparison of pregnancy rates according to ovarian findings of the recipient goat with laparoscopic embryo transfer

| Ovarian cycle          | Transferred embryos | Recipients | Pregnancies (%) |
|------------------------|---------------------|------------|-----------------|
| Graafian follicle      | 25                  | 9          | 3 (33.3)        |
| Corpus hemorrhagicum   | 131                 | 46         | 19 (41.3)       |

†: number.

(26.8%) became pregnant. No significant difference was observed in the pregnancy rates between the ovulated (CH) and non-ovulated (GF) groups (Table 3).

Discussion

The results of this study showed a significantly higher pregnancy rate with laparoscopic ET compared to ET by laparotomy. Tittel et al. [23] noted that laparoscopic adhesiolysis resulted in a significantly reduced number of new adhesions compared to open surgery. The operation was performed only in transferable recipients after laparoscopic exploration of the ovary and uterus.

For efficient production of transgenic goats, by pronuclear microinjection, the pregnancy rate following ET is important. Relatively higher pregnancy rates have been recorded when transferring non-transgenic embryos by laparotomy ET [14], compared to the pregnancy rates after the transfer of transgenic embryos [18]. In previous studies using Korean native goats as recipients, the pregnancy rate following laparoscopic ET was lower than the rate observed in our study (25.7% and 36.8% vs. 46.1%).

The findings of our study suggest that by decreasing the disadvantages of ET by laparotomy, we achieved better results with laparoscopic ET. However, our pregnancy rate with ET by laparotomy was lower than reported in a previous study [11], suggesting that additional studies might lead to an improvement in pregnancy rates of ET by laparotomy.

We also compared the pregnancy rates between the GF and CH groups to evaluate the effects of artificial rupture on the non-ovulated Graafian follicles. When Graafian follicles were identified by laparoscopic ET, they were ruptured artificially for formation of the CL, essential for the maintenance of a pregnancy. We then investigated the effects of the artificially ruptured Graafian follicles by comparison of the pregnancy rates. The most appropriate period for transferring an embryo is within 24 h after ovulation. Although the recipient goats were synchronized with progesterone and PMSG for the ET, some of the recipient goats had not ovulated at the time of the ET. Out of 55 recipient goats, nine goats (16.4%) had not yet ovulated with the Graafian follicle and 46 goats (83.6%) were estimated to have passed beyond the 24 h after ovulation by observation of the corpus luteum (CL). In comparison of the pregnancy rates, there was no significant difference between the CH and GF groups (41.3% vs. 33.3%, respectively, $p > 0.05$). Although the pregnancy rates were lower than in the CH group, an acceptable pregnancy rate was achieved by artificial rupture in the GF group. Therefore, in cases with a non-ovulated Graafian follicle, artificial rupture was efficient for the formation of the CL, essential for pregnancy maintenance after embryo transfer. However, if artificial rupture was not performed in the Graafian follicle, medical induction of ovulation or additional embryo transfer after CL formation was needed.

The results of this study demonstrated that laparoscopic ET was a reliable and effective technique for efficient production of transgenic goats after pronuclear DNA microinjection. In addition, we found that artificial rupture of the Graafian follicle was an efficient method for the formation of the CL for pregnancy maintenance. More work is needed to better understand the factors involved in this process for further improvement of the pregnancy rate in caprine laparoscopic ET.

Acknowledgments

This study was supported by the High-Technology Development Project (No. 2003-6628) and Research Project on the Production of Bio-organs (No. 200606031401) from Ministry of Agriculture and Forestry, and the BioGreen 21 Program from Rural Development Administration, Korea.

References

1. Baldassarre H, Wang B, Kafidi N, Keefe C, Lazaris A, Karatzas CN. Advances in the production and propagation of transgenic goats using laparoscopic ovum pick-up and in vitro embryo production technologies. Theriogenology 2002, 57, 275-284.
2. Baldassarre H, Wang B, Kafidi N, Gauthier M, Neveu N, Lapointe J, Sneek L, Leduc M, Duguay F, Zhou JF, Lazaris A, Karatzas CN. Production of transgenic goats by pronuclear microinjection of in vitro produced zygotes derived from oocytes recovered by laparoscopy. Theriogenology 2003, 59, 831-839.
3. Besenfelder U, Mödl J, Müller M, Brem G. Endoscopic embryo collection and embryo transfer into the oviduct and the uterus of pigs. Theriogenology 1997, 47, 1051-1060.
4. Clark AJ. The mammary gland as a bioreactor: expression, processing, and production of recombinant proteins. J Mammary Gland Biol Neoplasia 1998, 3, 337-350.
5. Colman A. Production of proteins in the milk of transgenic livestock: problems, solutions, and successes. Am J Clin Nutr 1996, 63, 639S-645S.
6. Denman J, Hayes M, O’Day C, Edmunds T, Bartlett C, Hirani S, Ebert KM, Gordon K, McPherson JM. Transgenic expression of a variant of human tissue-type...
plasminogen activator in goat milk: purification and characterization of the recombinant enzyme. Biotechnology 1991, 9, 839-843.

7. Ebert KM, Selgrath JP, DiTullio P, Denman J, Smith TE, Memon MA, Schindler JE, Monastersky GM, Vitale JA, Gordon K. Transgenic production of a variant of human tissue-type plasminogen activator in goat milk: generation of transgenic goats and analysis of expression. Biotechnology 1991, 9, 835-838.

8. Ebert KM, Schindler JES. Transgenic farm animals: progress report. Theriogenology 1993, 39, 121-135.

9. Ebert KM, DiTullio P, Barry CA, Schindler JE, Ayres SL, Smith TE, Pellerin LJ, Denman J, Roberts B. Induction of human tissue plasminogen activator in the mammary gland of transgenic goats. Biotechnology 1994, 12, 699-702.

10. Edmunds T, Van Patten SM, Pollock J, Hanson E, Bernasconi R, Higgins E, Manavalan P, Ziomek C, Meade H, McPherson JC, Cole ES. Transgenically produced human antithrombin: structural and functional comparison to human plasma-derived antithrombin. Blood 1998, 91, 4561-4571.

11. Gootwine E, Barash I, Bor A, Dekel I, Friedler A, Heller M, Zaharoni U, Zenuke A, Shani M. Factors affecting success of embryo collection and transfer in a transgenic goat program. Transgenic Res 1997, 4, 285-299.

12. Fayrer-Hosken RA, Younis AI, Brackett BG, McBride CE, Harper KM, Keefer CL, Cabaniss DC. Laparoscopic oviductal transfer of in vitro matured and in vitro fertilized bovine oocytes. Theriogenology 1989, 32, 413-420.

13. Houldhine LM. Production of pharmaceutical proteins from transgenic animals. J Biotechnol 1994, 34, 269-287.

14. Kiessling AA, Hughes WH, Blankevoort MB. Supervolution and embryo transfer in the dairy goat. J Am Vet Med Assoc 1986, 188, 829-832.

15. Ko JH, Lee CS, Kim KH, Pang MG, Koo JS, Fang N, Koo DB, Oh KB, Youn WS, Zheng GD, Park JS, Kim SJ, Han YM, Choi JY, Lim J, Shin ST, Jin SW, Lee KK, Yoo OJ. Production of biologically active human granulocyte colony stimulating factor in the milk of transgenic goat. Transgenic Res 2000, 9, 215-222.

16. Koeman J, Keefer CL, Baldassarre H, Downey BR. Developmental competence of prepubertal and adult goat oocytes cultured in semi-defined media following laparoscopic recovery. Theriogenology 2003, 60, 879-889.

17. Köhler B, Müller S, Besenfelder U, Prokofiev MI, Ernst IK, Brem G. Laparoscopic recovery of pronuclear-stage goat embryos. Vet Rec 1998, 142, 16-22.

18. Lee CS, Fang NZ, Koo DB, Lee YS, Zheng GD, Oh KB, Youn WS, Han YM, Kim SJ, Lim JH, Shin ST, Jin SW, Lee KS, Ko JH, Koo JS, Park CS, Yoo OJ, Lee KK. Embryo recovery and transfer for the production of transgenic goats from Korean native strain, Capra hircus aegagrus. Transgenic Res 2000, 9, 215-222.

19. McKeelvey WA, Robinson JJ, Aitken RP. A simplified technique for the transfer of ovine embryos by laparoscopy. Vet Rec 1985, 117, 492-494.

20. Mutiga ER, Baker AA. Transfer of sheep embryos through a laparoscope. Vet Rec 1984, 114, 401-402.

21. Rudolph NS. Biopharmaceutical production in transgenic livestock. Trends Biotechnol 1999, 17, 367-374.

22. Takahashi Y, First N. In vitro development of bovine one-cell embryos: Influence of glucose, lactate, pyruvate, amino acids and vitamins. Theriogenology 1992, 37, 963-978.

23. Wing A, Treutner KH, Titkova S, Ottenger A, Schumpelick V. New adhesion formation after laparoscopic and conventional adhesiolysis: a comparative study in the rabbit. Surg Endosc 2001, 15, 44-46.

24. Wang B, Baldassarre H, Tao T, Gauthier M, Neveu N, Zhou JF, Leduc M, Duguay F, Bilodeau AS, Lazaris A, Keefer C, Karatzas CN. Transgenic goats produced by DNA pronuclear microinjection of in vitro derived zygotes. Mol Reprod Dev 2002, 63, 437-443.