TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2014) 38: 253-256
© TÜBİTAK
doi:10.3906/vet-1310-22

Investigation of conception rates achieved with the transfer of sexed and unsexed bovine embryos

Tahir KARAŞAHİN[1], Numan AKYOL[2,*], Muharrem SATILMIŞ[3], Sedat Hamdi KIZIL[3], Mustafa Numan BUCAK[4], Kenan ÇOYAN[5]

[1]Department of Physiology, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Turkey
[2]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Kırıkkale University, Kırıkkale, Turkey
[3]Lalahan Livestock Central Research Institute, Lalahan, Ankara, Turkey
[4]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Selçuk University, Konya, Turkey
[5]Department of Histology and Embryology, Faculty of Medicine, Pamukkale University, Denizli, Turkey

Received: 09.10.2013 • **Accepted:** 23.01.2014 • **Published Online:** 21.04.2014 • **Printed:** 20.05.2014

Abstract: The objective of this study was to investigate the conception rates achieved with the transfer of in-vivo–derived sexed and unsexed Holstein bovine embryos to appropriate recipients and to determine the accuracy of nonelectrophoretic PCR sexing. Seven-day-old embryos were derived in vivo by the nonsurgical flushing of the uterus. Before being vitrified and frozen some of the embryos obtained were sexed, while others were not sexed and were maintained as the control group. After thawing, the sexed and unsexed embryos were transferred to 23 and 21 bovine recipients, respectively. The conception rates achieved with the transfer of the sexed and unsexed embryos were 30.4% (7/23) and 42.9% (9/21), respectively. The difference between conception rates achieved in the 2 groups was not statistically significant ($P > 0.05$). For the sexed embryos the conception rates achieved with the transfer of male and female embryos were 27.2% (3/11) and 33.3% (4/12), respectively. The accuracy of embryo sexing with nonelectrophoretic PCR was 66.6% for male embryos and 100% for female embryos postdelivery. The mean rate of accuracy determined for embryo sexing at the end of the study was 83.33%.

Key words: Bovine embryo sexing, PCR, vitrification, transfer

1. Introduction

Embryo transfer technology represents a powerful tool for the acceleration of breeding programs in cattle. It is important to know the sex of the embryos in order to improve the genetic potential of the herd. Embryo sexing allows a clear return on investment due to the higher degree of security in obtaining offspring of the desired sex from a given mating, and savings are also achieved through better management of recipients. However, the reliability, cost, practicality, and time required for this approach must be considered as well (1,2).

The PCR technique enables determination of the sex of the embryo within a short time period and with high accuracy (95%) by means of the amplification of the X and Y chromosomes of in-vivo– and in-vitro–derived embryos (3–5). It has been reported that embryo sexing produces accuracy rates of 92%, 94%, and >96% with, respectively, the use of 1 blastomere, 2 blastomeres, and 3 blastomeres which are biopsied from an embryo (6–8). In previous research the accuracy of embryo sexing with nonelectrophoretic PCR and electrophoretic PCR was determined at 93% and 89%, respectively (9). The nonelectrophoretic PCR technique is an efficient method that produces results within a shorter period of time and at lower cost than the electrophoretic PCR method, which has been used frequently in the past several years; the nonelectrophoretic technique even avoids DNA contamination because electrophoresis is not conducted in this method (3,6). In previously conducted studies the conception rates achieved with the transfer of sexed fresh embryos have ranged from 49% to 60%, while the conception rates achieved with the transfer of frozen and thawed embryos have ranged between 25% and 60% (6,10,11).

In a study by Plummer and Beckett (12), in which frozen embryos were thawed for sexing, the conception rate achieved was 56%. In the same study the accuracy of embryo sexing was 75%. In another investigation the accuracy of nonelectrophoretic PCR sexing was 93%, while the accuracy of electrophoretic PCR was 89% (9). Hasler

* Correspondence: numanakyol@gmail.com

et al. (6) reported that conception rates achieved with the transfer of biopsied embryos was 50% when the embryos were frozen in glycerol and 26.9% when they were frozen in ethylene glycol and 49.8% when fresh embryos were transferred. The same researchers reported the accuracy of embryo sexing to be 98.7% for male embryos and 94.4% for female embryos. In a study in which the embryos were sexed prior to transfer, the conception rates achieved were 46.9% for the transfer of vitrified and frozen embryos and 51.4% for the transfer of fresh embryos (11). In another study it was reported that a conception rate of 62.5% had been achieved with the direct transfer of frozen embryos (13).

The present study was aimed at determining the conception rates achieved with the transfer of Holstein embryos, either sexed with nonelectrophoretic PCR or unsexed, to appropriate recipients (surrogate animals) and calculating the accuracy of nonelectrophoretic PCR sexing. The present study is the first research conducted on this subject in Turkey, and it will contribute to future efforts such as work towards the conservation of local animal breeds, acceleration of genetic improvement, and establishment of embryo banks.

2. Materials and methods

To obtain the embryos used in the study, 5 primiparous Holstein cows were synchronized. Progesterone was administered to the animals for 9 days by intravaginal progesterone releasing device (CUE-MATE containing 1.38 g of progesterone; Bioniche, Australia), and the onset of this administration was considered day zero (0). From day 7 onwards, for 4 days, decreasing doses of FSH were also administered to the animals (Folltropin-V, Bioniche, Ireland) twice a day in the morning and evening (100:100 IU, 75:75 IU, 50:50 IU, 25:25 IU) by injection. On day 9 the animals were injected with 500 µg of cloprostenol (Estrumate, Sanofi Dif, Turkey) in the morning, and the intravaginal progesterone releasing device was removed from the animals in the evening. Subsequently, the animals were monitored for signs of estrus. Each donor was inseminated into the uterine body at 12 and 24 h after estrus detection. On day 7 following the artificial insemination of the recipient animals, embryos were collected by flushing the uterus with lactated Ringer's solution containing 1% calf serum (CS, N4762; Sigma-Aldrich, USA) and an antibiotic (125 mg/L of kanamycin). Following flushing, the resulting filtrate was examined under a microscope. The embryos detected at this microscopic examination were recovered from the filtrate and immediately transferred into a solution (holding medium) containing PBS + 20% CS + 0.4% bovine serum albumin (BSA-A8022; Sigma-Aldrich, USA) and assessed under an inverted microscope. Only embryos of high quality constituted the material of the study. Before being vitrified and frozen, 21 of the embryos derived were sexed and 23 were not sexed. The vitrification procedure was based on the treatment of the embryos first with vitrification solution 1 (VS1: 0.1 M sucrose + 0.1 M xylose + 1% polyethylene glycol + 10% glycerol) and then with VS2 (0.2 M sucrose + 0.2 M xylose + 2% polyethylene glycol + 10% glycerol + 10% ethylene glycol), each for 5 min, followed by treatment with VS3 (0.3 M sucrose + 0.3 M xylose + 3% polyethylene glycol + 20% glycerol + 20% ethylene glycol) for 1 min. Subsequently, the embryos were aspirated into French straws, and these were plunged directly into liquid nitrogen for freezing (10,14).

The biopsy of the embryos by microsurgical bisection was performed using microsurgical blades attached to a micromanipulator (Nikon, Narishige, Japan). The embryos were bisected in a protein-free D-PBS (Gibco, 14080, Scotland) solution (holding medium) containing 20% fetal calf serum (F9665, Sigma-Aldrich, USA) and 100 IU/mL of crystallized penicillin-G potassium (İ.E. Ulagay, Turkey). The bisected embryos were cultured using TCM 199 (Gibco, 12340030, Scotland) medium containing 20% fetal calf serum and 100 IU/mL of crystallized penicillin-G potassium. Petri dishes (35 mm diameter) containing 30 µL droplets of this medium were placed into an incubator with an adjusted temperature of 38.5 °C and a relative humidity rate above 95% and perfused with air containing 5% CO_2. The biopsy material taken from the embryos for sexing at a rate of 10%–30% was transferred into a reaction tube containing 10 µL of Ampli-Y A-solution (Finnzymes, Finland) and placed into a PCR device (Eppendorf Mastercycler Gradient, Germany). Apart from these tubes, male and female control tubes were also placed inside the device. The control tubes did not contain the Ampli-Y A-solution. Following the transfer of samples belonging to the bisected embryos into the reaction tubes, the PCR device was operated to perform lysis at 55 °C for 5 min and denaturation at 95 °C for 5 min. To initiate the DNA amplification program, 15 µL of Ampli-Y B-solution was added to each reaction tube found in the device. Following the addition of Ampli-Y B-solution, the first amplification process was performed in 10 cycles at 94 °C for 20 s, at 50 °C for 40 s, and at 75 °C for 20 s. The second amplification process involved 35 cycles at 96 °C for 10 s, at 60 °C for 30 s, and at 75 °C for 25 s. Later, the final extension process was realized at 75 °C for 5 min, and the tubes were maintained at 4 °C until analysis. After removing them from the PCR device, the tubes were observed under an UV lamp emitting light at a wavelength of 302–312 nm. Accordingly, the reaction tubes in which male DNA had been amplified produced a bright pink fluorescence (positive signal) under UV light, and the embryos to which these DNA samples belonged were considered male; the tubes that did not produce any fluorescence were

considered to contain DNA samples belonging to female embryos. After aspiration into 0.25-mL French straws the sexed embryos were vitrified and frozen (3,6).

The embryos were transferred to the appropriate recipients on day 7 of estrus. Embryo transfer was performed by removing the French straws from liquid nitrogen, keeping them first in air for 5–6 s and then in water at 20 °C until completely thawed (approximately 10 s). The embryos expelled from the French straws were devitrified first in 0.5 M sucrose solution for 5 min and then in 0.25 M sucrose solution for another 5 min. Devitrified embryos were transferred to PBS solution containing 20% CS. The embryos were transferred to the surrogate animals under epidural anesthesia to the uterine horn confirmed to have a corpus luteum. The embryos included in the control group were transferred into the surrogate animals after being frozen and thawed and without bisection; in other words, without being sexed. The examination of the surrogate animals for pregnancy was performed between days 50 and 60 after embryo transfer by rectal palpation (14). By determining the sex of the newborn calves postdelivery, the accuracy of embryo sexing with the nonelectrophoretic PCR technique was measured. The data obtained in this study were analyzed using the chi-square test.

3. Results

In this study 23 nonsurgical uterus irrigations were performed in 5 donors, and of the 54 high-quality embryos obtained 23 were frozen after being sexed. The 21 embryos that were frozen without being sexed constituted the control group. Twenty-one embryos that were vitrified without being subjected to sexing were transferred to recipients. Examination of the recipients at day 60 after embryo transfer demonstrated that conception had been achieved in 9 of the animals, and all of these pregnancies resulted in birth. The conception rate obtained from unsexed embryos was 42.9% (9/21), and the rate from sexed embryos was 30.4% (7/23) after the embryo transfers. The conception rates achieved with the transfer of sexed and unsexed embryos to appropriate recipients did not differ significantly ($P > 0.05$). Twenty-one unsexed embryos were transferred to the recipients, and pregnancy diagnoses were performed by rectal palpation at day 60 after embryo transfer. The pregnancy rate was 42.9% (9/21); 5 (55.5%) female and 4 (44.4%) male calves were delivered from 9 pregnancies obtained with the transferred, unsexed embryos. The conception rate achieved with the transfer of sexed embryos was 30.4%. One of these pregnancies terminated as a result of early embryonic death, and the sex of the embryo that died was determined to be male. The remaining 6 pregnancies resulted in the delivery of healthy calves. Of these calves, 4 were female and 2 were male. The accuracy rates of embryo sexing were 66.6% and 100% for the male and female embryos, respectively. The sex of the newborn calves was determined at an accuracy level of 85.7% at the embryonic stage (Table).

4. Discussion

In cattle breeding systems that rely on embryo transfer, cryopreservation and sexing of embryos are among the major tools that impact and trigger the development of the dairy and beef cattle industries. Therefore, both the freezing procedures applied to sexed embryos and sexing at high accuracy rates bear great significance (1,2). Nonelectrophoretic PCR sexing is able to produce results within a much shorter time and with greater ease (6). The present study was designed to perform the sexing of in-vivo–derived bovine embryos using the nonelectrophoretic PCR technique to investigate the conception rates achieved with the transfer of these embryos after freezing and thawing and to detect the accuracy of nonelectrophoretic PCR sexing based on the determination of the sex of the calves delivered by surrogate animals.

In the present study the conception rates achieved with the transfer of embryos, which were vitrified after sexing or without sexing, did not display a statistically significant difference ($P > 0.05$). Twelve female and 11 male embryos, in which sex was determined by nonelectrophoretic PCR, were transferred to the appropriate recipients. These transfers resulted in the delivery of 3 male and 4 female calves. The sex of all female calves delivered in this study was determined accurately at the embryonic stage. On the other hand, the embryo sexing of the 3 male calves

Table. Conception rates obtained with the transfer of sexed and unsexed embryos to recipients and sex accuracy rates in sexed embryos after delivery.

		Number of transferred embryos	Conception rates (%)	Sex accuracy rates after delivery (%)
Sexed embryo	Male	11	27.3 (3/11)	66.6
	Female	12	33.3 (4/12)	100
	Total	23	30.4 (7/23)	83.33
Unsexed embryo		21	42.9 (9/21)	-

delivered in this study produced accurate results in 2 but failed in 1. The false result in 1 of the male calves after embryo sexing was attributed to the misinterpretation of the findings observed under UV light postamplification. Thus, the accuracy of embryo sexing was 85.7%. This rate is in agreement with accuracy rates previously indicated in the literature (6,9,12). The conception rate achieved with the transfer of frozen and thawed sexed embryos was 30.4%. This rate was lower than the rate achieved by freezing embryos in glycerol and higher than the rate achieved by freezing embryos in ethylene glycol as reported by Hasler et al. (6) and was similar to the rate reported by Tominaga (11). In the present study the conception rate achieved with the transfer of in-vivo–derived embryos that were vitrified without being sexed was 42.9%, and this percentage was lower than the rate reported by Kızıl et al. (13) and similar to the rate reported by Tominaga (11).

In conclusion, the method used in the present study for in vivo embryo production was efficient in obtaining high quality embryos. Furthermore, it was ascertained that embryo sexing with the nonelectrophoretic PCR technique produced reliable results within a short period of time and with an acceptable rate of accuracy.

Acknowledgments
This study was financed by a project supported by TAPGEM, Turkey (project no.: 07/01/01/03).

References

1. Thibier M, Nibart M. The sexing of bovine embryos in the field. Theriogenology 1995; 43: 71–80.
2. Geshi M. Current status and the future of bovine embryo transfer using sex determined embryos. J Mammal Ova Res 2012; 29: 124–127.
3. Bredbacka P, Kankaanpaa A, Peippo J. PCR-sexing of bovine embryos: a simplified protocol. Theriogenology 1995; 44: 167–176.
4. Machaty Z, Paldi A, Csaki T, Varga Z, Kiss I, Barandi Z, Vajta G. Biopsy and sex determination by PCR of IVF bovine embryos. J Reprod Fertil 1993; 98: 467–470.
5. Saito N, Imai K, Tomizawa M. Effect of sugars-addition on the survival of vitrified bovine blastocysts produced in vitro. Theriogenology 1994; 41: 1053–1060.
6. Hasler JF, Cardey E, Stokes JE, Bredbacka P. Nonelectrophoretic PCR-sexing of bovine embryos in a commercial environment. Theriogenology 2002; 58: 1457–1469.
7. Jafar SI, Flint APF. Sex selection in mammals. Theriogenology 1996; 46: 191–200.
8. Park JH, Lee JH, Choi KM, Joung SY, Kim JY, Chung GM, Jin DI, Im KS. Rapid sexing of preimplantation bovine embryo using consecutive and multiplex polymerase chain reaction (PCR) with biopsied single blastomere. Theriogenology 2001; 55: 1843–1853.
9. Findlay I, Corby N, Rutherford A, Quirke P. Comparison of FISH, PRINS, and conventional and fluorescent PCR for single-cell sexing: suitability for preimplantation genetic diagnosis. J Assist Reprod Genet 1998; 15: 258–265.
10. Shea BF. Determining the sex of bovine embryos using polymerase chain reaction results: a six-year retrospective study. Theriogenology 1999; 51: 841–854.
11. Tominaga K. Cryopreservation and sexing of in vivo and in vitro produced bovine embryos for their practical use. J Reprod Dev 2004; 50: 29–38.
12. Plummer WE, Beckett D. Development of successful sex determination method of bovine embryos utilizing embryo biopsy and PCR. California State University Agricultural Research Initiative Final Report, 2006.
13. Kızıl SH, Akyol N, Karaşahin T, Satılmış M. Transfer of in vivo embryos frozen by direct transfer method with ethylene glycol in cattle. Kafkas Univ Vet Fak Derg, 2011; 17: 721–724.
14. Gordon I. Controlled Reproduction in Cattle and Buffaloes. Wallingford, UK: CABI; 1996.