ABSTRACTS

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OBJECTIVES AND METHODS: The assisted reproductive techniques (ARTs) such as in vitro fertilization, embryo transfer and cryopreservation of gametes have considerably contributed to the development of biomedical sciences in addition to improved breeding in domestic animals and infertility treatment in humans. However, ARTs used in canine species have strictly limited utility when compared with other mammalian species including humans. Although successful somatic cell cloning has been reported, artificial insemination by frozen semen is only available for the improved breeding and reproduction for companion and working dogs. The first mammalian species to have embryos successfully cryopreserved was the mouse in the early 1970s, and over the next three decades this has been extended to embryos of most species including humans. However, cryopreservation of canine embryos has not progressed far enough. Similar to the other mammals, embryo cryopreservation and subsequent transfer in canines would be centrally important in reproductive technologies by allowing optimal utilization of genetic resources in the development of breeding programs in animals by permitting genetic banking (for example, of rare or economically and socially important breeds), and by enhancing the clinical approach to infertility and sexually transmitted disease treatment. This report describes successful cryopreservation of embryos and subsequent embryo transfer with a nonsurgical technique in dogs. The day on which the plasma concentration of progesterone exceeded 2 ng/ml was designated as Day 0 post-LH surge. Embryos at 1-cell to blastocyst stages were collected from excised reproductive organs of Labrador retrievers after artificial insemination and subsequently cryopreserved by a vitrification method. The cryopreserved embryos at 4-cell to morula stages were non-surgically transferred into the uterus of recipient bitches by using a cystoscope. The pregnant bitches allowed to delivery by natural labor or cesarean section. Paternity testing based on microsatellite polymorphisms for the delivered pups was performed by using 23 micro-satellite markers. The animals used in this study were treated and cared for in accord with the Guiding Principles for the Care and Use of Research Animals established by Obihiro University of Agriculture and Veterinary Medicine (Obihiro, Japan).

RESULTS: A total 474 embryos were collected from 80 bitches (average 5.9; range 0-9). Embryos at the morula stage migrated from the oviduct into the uterus on Day 10 post-LH surge. This transport might be complete within 24 h. By Day 12 post-LH surge, all of the developing embryos were localized in the uteri. Embryos developed to the morula stage and to the blastocysts by Days 11-12 and Days 12-13 post-LH surge, respectively. Examination of the embryos at the 1-cell to blastocyst stages vitrified in the ethylene glycol-based vitrification solution on a cryotop sheet and then warmed revealed that over 80% of the embryos that were younger than the blastocyst stage exhibited a normal morphology. However, the majority of cryopreserved embryos at the blastocyst stage showed abnormal morphology after warming. To assess the membrane integrity of the cryopreserved embryos, the vitrified-warmed embryos were stained with propidium iodide. The viability of the embryos at 4- to 16-cell, morula, and blastocyst stages was 90-100%, 50%, and 40%, respectively. Of nine embryo transfer experiments five recipients became pregnant and four of them delivered seven pups in total. Percentages of development to newborn after the transfer of cryopreserved canine embryos were 9.1% (7/77). A very lower percentage of embryos disappeared after detection of fetal sacs in recipients. The suitable combination of stages between transferring embryo and estrus of recipient might be when 8-cell to 16-cell stage of embryos were transferred to recipients on day 8-9 post LH surge. The microsatellite genotypes clearly demonstrated that those delivered pus were derived from donor embryos but not from the recipient animal (1).

CONCLUSION: These results clearly indicate that cryopreserved canine embryos are able to develop to term, even if developmental rates after the transfer were lower. Vitrification is suitable for cryopreservation of canine embryos, and transcervical embryo transfer using a cystoscope is a potentially useful nonsurgical method that can be used in the field. These techniques may contribute to the control of genetic diversity in canine.