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Factors affecting the efficiency of embryo transfer in the domestic ferret (*Mustela putorius furo*)

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Abstract

Embryo transfer (ET) to recipient females is a foundational strategy for a number of assisted reproductive technologies, including cloning by somatic cell nuclear transfer. In an attempt to develop efficient ET in domestic ferrets, factors affecting development of transferred embryo were investigated. Unilateral and bilateral transfer of zygotes or blastocysts in the oviduct or uterus was evaluated in recipient nulliparous or primiparous females. Developing fetuses were collected from recipient animals 21 days post-copulation and examined. The percentage of fetal formation was different ($P < 0.05$) for unilateral and bilateral transfer of zygotes (71%) in nulliparous females with bilateral transfer (56%) in primiparous recipients. The percentage (90%) of fetal formation in nulliparous recipients following unilateral transfer of blastocysts was higher ($P < 0.05$) than that observed in primiparous recipients with bilateral ET (73%). Notably, the percentage of fetal formation was higher ($P < 0.05$) when blastocyts were transferred as compared to zygotes (90% versus 71%). Transuterine migration of embryos occurred following all unilateral transfers and also in approximately 50% of bilateral transfers with different number of embryos in each uterine horn. These data will help to facilitate the development of assisted reproductive strategies in the ferret and could lead to the use of this species for modeling human disease and for conservation of the endangered Mustelidae species such as black-footed ferret and European mink.

Keywords: Domestic ferret; Embryo transfer; Embryo development; Nulliparous female; Primiparous female

1. Introduction

Embryo transfer (ET) involves the placement of in vivo- or in vitro-produced embryos into a surrogate female for gestation and delivery. This process was first accomplished in domestic rabbits [1] and research in this area with many species has since contributed greatly to our knowledge and understanding of mammalian reproductive biology and development [2]. Furthermore, ET has become a cornerstone for a number of assisted reproduction technologies, including cloning by somatic cell nuclear transfer (SCNT). A systematic analysis of factors that govern the production of live, healthy offspring from transferred embryos is paramount to fully realize the potential of this and related technologies.

Efficient ET depends upon several embryo- and recipient-related factors, as well as the method for embryo delivery to the recipient female. In the pig, for example, the number of embryos transferred to a recipient female plays an important role in embryo development and maintenance of pregnancy. Indeed, it

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has been reported that pregnancy maintenance following ET requires a critical threshold signal generated by a minimum of four embryos at around Day 12 of gestation [3]. Furthermore, since nuclear transfer (NT) embryos are generally of lower quality than naturally produced embryos, successful pregnancy in the pig also requires the transfer of a large number (>100) one-cell stage NT embryos [4], presumably to offset attrition. In cattle, by contrast, single NT embryos that develop to morula or blastocyst stages are most often transferred non-surgically into the uterine lumen [5]. In the rabbit, the developmental potential of in vivo fertilized embryos is rapidly compromised after 1 day of in vitro culture, partly because of the lack of a mucin coat covering the zona pellucida [6]. Thus, only reconstructed embryos that quickly reach the 4–6-cell stage in vitro are transferred into recipient females and only a single NT fetus is usually observed in rabbits, a favorable condition for full-term development in this species [7]. By contrast, mice produce multiple cloned animals in a litter. These, and other species-specific differences that are required for the developmental success of NT embryos, have profoundly influenced the progress of ET technology and its applications in SCNT.

Efforts to apply ET to mustelid species began in the 1960s and later the domestic ferret (*Mustela putorius furo*) became the first carnivorous mammalian species in which this technology was successfully applied [8]. In this study by Chang, 51 ferret eggs were transferred into the uteri of eight ferrets, 13 living fetuses and four young (33%, 17/51) were obtained after the transfer of morula and blastocysts [8]. Kidder et al. [9] reported that 26% (65/251) live births resulted from the nonsurgical collection of embryos from donor ferrets, followed by nonsurgical transfer of those same embryos to synchronous recipient ferrets. Our previous report demonstrated that 32% (12/38) live births came from successful transfers of in vivo produced (collected at zygote stage) and in vitro cultured embryos (to 9–16 cell stage) into uterus of domestic ferret [10]. Embryo transfer has also been reported in other members of the Mustelidae family: the American mink (*Mustela vison*) [8], the European mink (*Mustela lutreola*) [11], and the European polecat (*Mustela putorius*) [12–14].

The ferret has been used extensively as an animal model in biomedical research involving virology, reproductive physiology, and endocrinology [15]. This species is also considered an excellent model for human lung diseases, such as influenza infection [16] and cystic fibrosis (CF) [17]. To fully realize the ferret as a genetic model for human lung disease, it is essential to optimize ET efficiency in this species as an integral part of SCNT technology. The aims of this study were to determine how the following factors affect the efficiency of ET in domestic ferrets: (1) the developmental stage of the transferred embryo (i.e., zygote or blastocyst); (2) the regional placement of embryos in the reproductive tract (i.e., unilateral or bilateral deposition of zygotes or blastocysts within the oviduct(s) or uterine horns, respectively); and (3) the reproductive history of the recipient female (i.e., nullipara or primipara).

2. Materials and methods

2.1. Chemicals and animals

Chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Invitrogen Co. (Grand Island, NY, USA) unless otherwise noted. Ferrets were purchased from Marshall Farms (North Rose, NY, USA). Female sable coat-color ferrets (nullipara, 6–7 months of age, weight 610–851 g) and albino coat-color ferrets (primipara, 9–12 months of age, weight 469–680 g) were in estrus when delivered. Breeder male ferrets (10–12 months of age) were used for mating female ferrets for embryo production, and vasectomized males were used to induce pseudopregnancy. Vasectomized males were confirmed as sterile at Marshall Farms by the lack of spermatozoa in ejaculates and inability to reproduce following several mating attempts. Nulliparous ferrets were used as embryo donors for all experiments, whereas embryo recipients utilized both nulliparous ferrets and primiparous ferrets (i.e., females that have undergone a single previous pregnancy). All ferrets were housed in separate cages under controlled temperatures (20–22 °C) and a long day light cycle (16 h light, 8 h dark). The use of animals in this study was carried out according to a protocol approved by the University of Iowa animal care review committee and conformed to or exceeded National Institutes of Health standards.

2.2. Mating of embryo donors and recipients

Female ferrets with maximal vulva swelling were confirmed ready for mating. The embryo donor ferret was placed into the breeder male cage for 24 h, and the recipient was mated with a vasectomized male the next day for 24 h, providing a 24 h interval between the two matings. When left together overnight, each pair may mate many times and each mating lasted from 10 min to 3 h. Matings were confirmed in all ferrets by observation and by sampling vaginal fluid. Specifically, a small amount of warm saline was injected inside the vagina