

## 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 blastocysts 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. © 2005 Elsevier Inc. All rights reserved.

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### 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

with a Pasteur pipette, aspirated back into the pipette and transferred to a slide to view for spermatozoa under a light microscope ( $\times 400$ ). The mating of the donor female was considered successful if sperm were detected in the sample. No spermatozoa were detected in the vaginal samples from the recipients mated with the vasectomized males.

### 2.3. Embryo recovery and transfer

To retrieve embryos from mated donor ferrets, the animals were weighed and euthanized by an overdose of pentobarbital sodium injection (50–100 mg/kg, i.p., Ovation Pharmaceuticals Inc., Deerfield, IL, USA) at Day 2 after the first mating for zygote recovery [45 h post copulation (hpc), where Day 0 is the day of mating], and at Day 7 after the first mating (165 hpc) for blastocyst recovery. The ovaries, oviducts and uteri of donors were removed and washed with 0.9% (w/v) saline supplemented with 1% (v/v) penicillin-streptomycin (Invitrogen Co.) at 37–38.5 °C. The oviducts were transected distal to the uterotubal junction. Both uterine horns were transected cranial to the bifurcation. Each uterine horn was flushed with 10 mL mPBS [Dulbecco PBS supplemented with 0.1% (w/v) D-glucose, 36 mg/L pyruvate and 0.4% (w/v) BSA], and the oviducts were flushed with 5 mL mPBS to release embryos into a Petri dish. The elapsed time between euthanasia and the start of embryo recovery was less than 5–10 min. Recovered embryos were evaluated under a stereomicroscope ( $\times 40$ ) for their developmental stage and photographed. Diameters of the embryos including the zona pellucida were immediately measured under an inverted microscope (Leica DMIRB,  $\times 100$ ). The embryos were then washed, kept in mPBS medium (on 38.5 °C warm stage), and transferred into a recipient within 10–30 min. To determine the embryo recovery rate from donor ferrets, the number embryos collected was divided by the number of corpora lutea that were visible in the dissected ovaries following ovulation.

A single stock solution of saline containing 10 mg/ml each of ketamine HCl (Abbott Laboratories, N. Chicago, IL, USA) and xylazine (Phoenix Pharmaceutical Inc., St. Joseph, MO, USA) was prepared. The recipient ferrets were routinely anaesthetized by i.p. injection of this solution to a final concentration of 20 mg/kg of ketamine and 20 mg/kg xylazine. If the depth of anaesthesia was insufficient, an additional dose of the stock solution was administered up to a total dose of 30 mg/kg. During the surgery, a 3–4 cm incision was made along the midline of the abdomen to expose the

oviducts and uteri. Embryos were either transferred into unilateral (left) and bilateral oviducts (for zygotes), or unilateral (left) and bilateral uteri (for blastocysts) using a fine glass pipette. In >60% of the ferrets examined, the left oviduct was found to be slightly larger and less convoluted than the right oviduct. This difference made zygotic transfer into the left oviduct somewhat easier than transfer into the right oviduct. There was no technical difference when transferring blastocysts into either the left or the right uterus. Therefore, we choose the left uterine horn for unilateral ET to match unilateral transfers into the left oviduct. The ferret ETs usually lasted 30–60 min. After the incision was sutured, the ferrets were returned to their cages and closely monitored until they were awake.

### 2.4. Assessment of *in vivo* development of transferred embryos

The ferret gestational period is  $42 \pm 1$  day post copulation (dpc) [18]; therefore, all recipients were examined at 21 dpc (mid-term after their first mating) for signs of implantation and fetal formation in uteri. The fetuses derived from two naturally-mated pregnant ferrets at 21 dpc were used as controls. To perform these examinations, the ferrets were euthanized by an overdose of sodium pentobarbital (as described above). The uteri of recipient ferrets were removed, and signs of implantation and fetal formation were carefully observed, counted, dissected and photographed. The size and morphology of transferred and control (naturally-mated) fetuses were compared. The size and morphology of fetuses from transferred embryos were very similar to the fetuses from naturally-mated embryos, and therefore, were considered normally developed fetuses.

### 2.5. Statistical analysis

For statistical analysis of embryo transfer and development data (Tables 1 and 2), the mean percentage ( $\pm$ S.E.M.) was calculated for each group. The normality assumption of the percentages for each data set was checked by the Kolmogorov–Smirnov test, using statistical Minitab 13 software (Minitab Inc., State College, PA, USA). In addition, the Bartlett test (also using Minitab 13 software) was performed to justify the equal variance assumption of the percentages for each data set. When these two tests suggested that the two assumptions were valid, two-way ANOVA was used for statistical analysis with two independent variables (i.e., evaluating the effects of oviduct versus uterine delivery

Table 1  
Ferret ovulation and embryo recovery

| Embryo stages recovered | No. ferrets examined | No. embryos recovered | No. corpora lutea counted | Recovery rate <sup>a</sup> |           |
|-------------------------|----------------------|-----------------------|---------------------------|----------------------------|-----------|
|                         |                      |                       |                           | % (Mean ± S.E.M.)          | Range     |
| Zygote                  | 8                    | 132                   | 146                       | 90.0 ± 4.2                 | 71.4–100  |
| Blastocyst              | 8                    | 107                   | 132                       | 80.4 ± 3.9                 | 64.7–88.9 |

<sup>a</sup> No difference ( $P > 0.05$ ) in recovery rate between the zygote and blastocyst stage.

Table 2  
Ferret embryo size

| Embryo stages recovered | No. of ferrets used | No. of embryos examined | Embryo size with zona pellucida <sup>a</sup><br>( $\mu\text{m}$ in diameter) |         |
|-------------------------|---------------------|-------------------------|--|---------|
|                         |                     |                         | Mean ± S.E.M.  | Range   |
| Zygote                  | 4                   | 50                      | 172.8 ± 1.1 a  | 160–190 |
| Blastocyst              | 4                   | 48                      | 321.6 ± 13.6 b   | 210–510 |

(a–b) Within a column, percentages without a common superscript are different ( $P < 0.05$ ).

<sup>a</sup> Zona pellucida = 20  $\mu\text{m}$ .

Table 3  
Zygotic transfer and development in the ferret

| ET method  | Recipient type | Replicates | No. embryos transferred | Percentage (mean ± S.E.M.)<br>developing embryos |                 |
|------------|----------------|------------|-------------------------|--|-----------------|
|            |                |            |                         | Implantation                                     | Fetal formation |
| Unilateral | Nullipara      | 3          | 42                      | 71.6 ± 1.4                                       | 69.5 ± 3.5 ab   |
| Bilateral  | Nullipara      | 3          | 45                      | 72.3 ± 4.6                                       | 71.2 ± 4.4 ab   |
| Unilateral | Primipara      | 3          | 38                      | 72.5 ± 5.1                                       | 62.3 ± 4.2 bc   |
| Bilateral  | Primipara      | 3          | 37                      | 61.2 ± 5.8                                       | 56.2 ± 3.4 c    |

(a–c) Within a column, percentages without a common superscript are different ( $P < 0.05$ ).

Table 4  
Blastocyst transfer and development in ferrets

| ET method  | Recipient type | Replicates | No. embryos transferred | Percentage (mean ± S.E.M.)<br>developing embryos |                 |
|------------|----------------|------------|-------------------------|--|-----------------|
|            |                |            |                         | Implantation                                     | Fetal formation |
| Unilateral | Nullipara      | 3          | 34                      | 95.6 ± 4.4                                       | 90.0 ± 5.8 a    |
| Bilateral  | Nullipara      | 3          | 36                      | 92.8 ± 3.9                                       | 84.6 ± 5.7 ab   |
| Unilateral | Primipara      | 3          | 31                      | 83.8 ± 1.9                                       | 83.8 ± 1.9 ab   |
| Bilateral  | Primipara      | 3          | 31                      | 80.5 ± 5.2                                       | 73.3 ± 2.7 bc   |

(a–c) Within a column, percentages without a common superscript are different ( $P < 0.05$ ).

of embryos and recipient types on ferret ET efficiency; Tables 3 and 4). When ANOVA demonstrated a significant difference, the follow-up Tukey multiple comparison test was performed to determine  $P$ -values for all possible two-group comparisons within the data set. The Student's  $t$ -test was applied to data sets with

only two groups (i.e., Tables 1, 2 and 5). For all statistical analysis including ANOVA, Tukey, and Student's  $t$ -tests, a difference was considered to be significant when the  $P$ -value was  $< 0.05$ . All statistical analysis was performed using SAS 8.0 statistical software (SAS Institute Inc., Cary, NC, USA).

Table 5  
Transuterine migration of ferret embryos following unilateral embryo transfer

| Recipient type | Recipient no. | Embryos transferred no. | Embryo migration % (mean $\pm$ S.E.M.) |                              | Recipients displaying migration (%) |
|----------------|---------------|-------------------------|--|------------------------------|-------------------------------------|
|                |               |                         | Total                                  | Fetal formation <sup>a</sup> |                                     |
| Nullipara      | 6             | 76                      | 36.8 $\pm$ 3.4                         | 93.9 $\pm$ 3.9               | 100.0 (6/6)                         |
| Primipara      | 6             | 69                      | 41.3 $\pm$ 5.5                         | 75.2 $\pm$ 2.9               | 100.0 (6/6)                         |

<sup>a</sup> Difference between nullipara and primipara in fetal formation rate ( $P < 0.05$ ).

### 3. Results

#### 3.1. Oviductal transfer

Ferret zygotes (Fig. 1A) were recovered from oviducts at 45 hpc at which time  $\geq 97.0\%$  were fertilized. The recovery rate was 90%, the average number recovered per ferret (both zygotes and blastocysts) was  $12.6 \pm 0.8$  (mean  $\pm$  S.E.M., range, 5–21,  $n = 24$ ) (Table 1). The zygotes were 160–190  $\mu\text{m}$  in diameter (Table 2). Harvested zygotes were subsequently transferred unilaterally, into the left oviduct, or bilaterally, into both oviducts, of recipient nulliparous or primiparous ferrets. Mid-term development was then

assessed at 21 dpc (Table 3). No differences in implantation percentages were observed among all groups. However, percentages of fetus formation in nulliparous recipients (70–71%) were higher than those in primiparous recipients (56–62%). This difference was apparent ( $P < 0.05$ ) when comparing unilateral and bilateral ETs in nulliparous females with bilateral ETs (56%), but not with unilateral ETs (62%), in primiparous recipients.

#### 3.2. Uterine transfer

Ferret blastocysts were all retrieved from uteri at Day 7 (165 hpc), and  $\geq 86\%$  of those recovered showed a

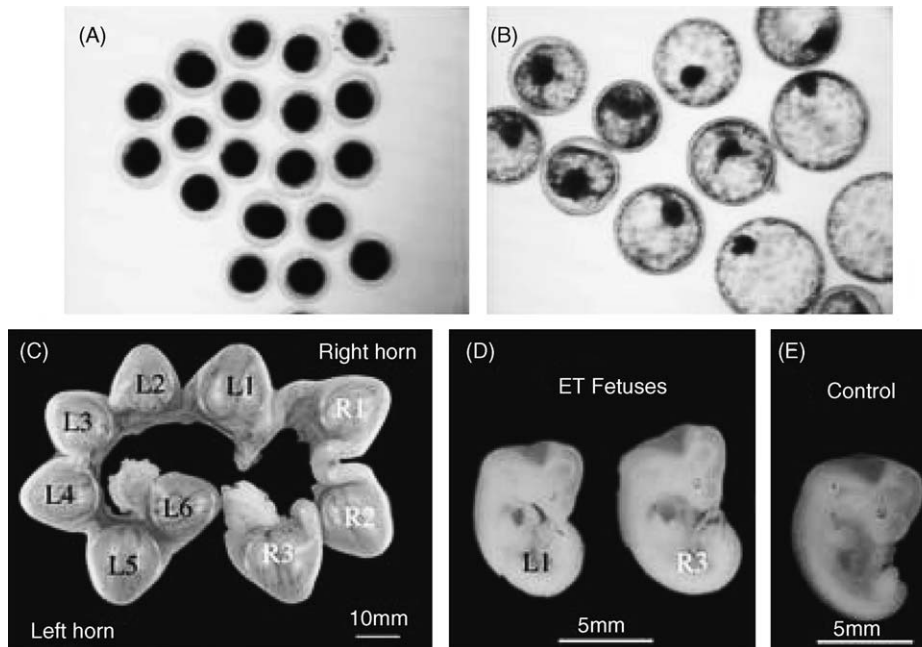


Fig. 1. Embryo development following embryo transfer in the ferret. (A) Zygotes recovered from mated Jills at 45 hpc (Day 2) for transfer into oviducts (magnification:  $\times 50$ ). (B) Blastocysts recovered from mated Jills at 165 hpc (Day 7) for transfer into uteri (magnification:  $\times 50$ ). (C) Nine blastocysts were transferred into the left uterine horn on Day 7 and development was examined at 21 dpc. Six of the nine transferred blastocysts implanted in the left uterine horn (the site of transfer), whereas 3 of 9 blastocysts migrated into the right uterine horn (scale bar, 10 mm). (D) The L1 fetus was from the left uterine horn and the R3 fetus was from the right uterine horn (scale bar: 5 mm). (E) A fetus from a natural pregnancy dissection at 21 dpc (control; scale bar, 5 mm).

distinct blastocele, i.e., “blastocysts” (Fig. 1B). The remaining “pre-blastocyst” embryos were immediately cultured in TCM-199 + 10% FBS medium [10] for 1–5 days. These *in vitro* conditions have been used previously to produce blastocysts in the ferret. None of these embryos developed to the morula or blastocyst stage, indicating a loss of developmental potential. The recovery rate was approximately 80% (Table 1) and blastocyst size ranged from 210 to 510  $\mu\text{m}$  in diameter (Table 2). Blastocysts were transferred unilaterally into the left uterine horn, or bilaterally into both uterine horns, of recipient nulliparous or primiparous ferrets. Development was assessed at 21 dpc (Table 4). The percentage of fetal formation in nulliparous recipients following unilateral ET (90%) was greater ( $P < 0.05$ ) than that observed in primiparous recipients with bilateral ET (73%). However, no significant differences were observed when comparing either ET method in nulliparous recipients with unilateral ET in primiparous recipients (Table 4, Fig. 1C and D).

### 3.3. Transuterine migration of embryos

Unilateral transfer of ferret zygotes into the left oviduct, or blastocysts into the left uterine horn, resulted in the eventual migration of 37–41% of these embryos into the contralateral uterine horn (Fig. 1C and D). This transuterine migration occurred following all unilateral transfers, regardless of the developmental stage of the embryo or whether the recipient female was nulliparous or primiparous (Table 5). Nearly 94% of migratory embryos underwent normal development after transfer to nulliparous recipients, whereas in primiparous recipients, the development percentage was 75% (Table 5, Fig. 1C–E). When blastocysts were transferred bilaterally, with different numbers of embryos in each uterine horn, transuterine migration occurred in approximately 50% of recipient ferrets. However, only 17% of transferred zygotes underwent migration following bilateral transfer into oviducts.

## 4. Discussion

The domestic ferret, like the domestic cat and the rabbit, is an induced ovulator, with ovulation occurring 30–40 hpc [18]. Following fertilization, the reported rate of embryo development varies slightly among laboratories. For example, Hamilton [19] found incipient blastocysts in the uterus at 6 dpc, whereas Chang [8] reported only morulae in the uterus on Day 6, with blastocysts not found until Day 7. Results similar to those of Chang were also reported by Marston and

Kelly [20]. We recovered  $\sim 97\%$  zygotes and  $\sim 3\%$  unfertilized oocytes from ferret oviducts at Day 2 (45 hpc). By Day 7 (165 hpc), most ( $>86\%$ ) of the embryos recovered from uteri had reached the blastocyst stage consistent with the work of Chang [8], and Marston and Kelly [20]. By Day 12, implantation occurs [18,21]; thereafter, embryo collection is no longer possible.

Embryo recovery rates of approximately 70% have been reported in the domestic ferret either postmortem [22], or following non-surgical collection [9]. A similar rate of recovery was observed in the related farmed European polecat [13]. The average number of embryos reportedly recovered surgically from each ferret by Chang was 5.9 [23], whereas embryo recovery ranged from 8.2 [24] to 9.0 [22] in later studies. Non-surgical collection of embryos was 8.7/ferret [9]. In our study, recovery rates for zygotes and blastocysts were 90 and 80%, respectively (Table 1). The average number of flushed zygotes and blastocysts, collectively, per ferret, was 12.6. Our higher rates of recovery and the larger number of embryos recovered, might result from age- or strain-related differences.

In the ferret, unfertilized zona pellucida-free oocytes, and Days 7–9 blastocysts, ranged from 140 to 160  $\mu\text{m}$  [19,25], and from 200 to 850  $\mu\text{m}$  [8,26], respectively. Blastocyst expansion in the ferret occurs in distinct stages; the first stage from 400 to 700  $\mu\text{m}$  and the second stage  $>1$  mm [27]. After Day 9, the diameters of the large expanded blastocysts in mustelids tended to exceed 1 mm [20,26] and became difficult to transfer, due to their large size. However, at Days 7 and 8, expanded blastocysts rarely exceed 1 mm in diameter [8,13,26,28]. In our study with domestic ferrets, zygotes and blastocysts with the zona pellucida measured 160–190  $\mu\text{m}$  (Day 2, 45 hpc) and 210–510  $\mu\text{m}$  (Day 7, 165 hpc), respectively, well within the acceptable range for ET. We previously demonstrated the successful production of live ferret pups by transferring 8–16 cell stage embryos into the uterus of recipient females [10], similar to ET studies with the farmed European polecat [13].

In the domestic ferret, the successful production of live offspring from uterine-transferred blastocysts varied slightly, depending upon the method of ET. For example, when embryos were transferred surgically, 33% developed to term [8], whereas 26% of non-surgically transferred embryos produced live offspring in later studies [9]. However, higher rates of development have been reported in related Mustelidae species. For example, the surgical transfer of embryos from the farmed European polecat resulted in a 42% success rate

[13], whereas the rate in the European mink was 50% [11]. In our study, we systematically examined the impact of several factors on mid-term embryo development following ferret ET. The efficiencies of ferret ET, 71% with zygotic transfer and 90% with blastocyst transfer, were much higher than those observed for full-term development in ferrets and other Mustelidae species (see above). However, since previous studies evaluated live births, these apparent differences are not too surprising, considering that some embryo loss would be expected during late-stage development in the ferret, resulting in a reduced rate of live births. Furthermore, we cannot rule out that the technically more challenging procedure of zygote transfer to the oviduct led to reduced viability of embryos when compared to blastocyst uterine transfers.

We found that 90% of the blastocysts developed to viable fetuses on the day of examination (21 dpc), significantly higher than that of zygote development (71%) following ET, irrespective of whether unilateral or bilateral embryo deposition was conducted. The blastocysts have successfully undergone critical stages of development prior to collection; the difference we observed may have reflected the natural attrition associated with unhealthy single-cell embryos. Indeed, 14% of the embryos recovered at 165 hpc failed to reach the blastocyst stage, a possible indication of impaired developmental potential. We also found that fetal formation of transferred embryos in nulliparous females was more efficient than in primiparous females (70% versus 56% at zygote transfer; 90% versus 73% at blastocyst transfer). In domestic ferrets, the average litter size is 8–10, but jills often give their first birth to 13–18 kits [29]. So, our results, and those from an earlier report [29], suggest that female ferrets naturally limit their litter size after their first birth.

The effect of ET methods on the efficiency of ET in ferrets was also evaluated. No significant difference was observed between unilateral and bilateral ET within the oviduct(s) or uterine horn(s). The migration of embryos within the uterus, and between uterine horns, is postulated to be an important strategy for ensuring sufficient uterine space in pregnancies [30]. Transuterine migration occurs with high frequency and is well documented in litter-producing domesticated species, e.g. the pig [31], cat [32], and dog [33], as well as in farm animals that regularly produce twins and higher order births, e.g. sheep [34] and goats [35]. Chang and colleagues reported three cases of transuterine migration of eggs in the ferret following unilateral uterine transfer [8]. In our study, transuterine migration of embryos occurred following all unilateral transfers and

also after approximately 50% of bilateral transfers when different numbers of embryos were placed in each uterine horn. We inferred that the redistribution of embryos between uterine horns may offer some developmental advantage in this species. Furthermore, perhaps transuterine migration in the ferret is influenced by the number of transferred embryos. Migration occurs at a higher frequency from the side with more embryos to the contralateral side with fewer embryos. Therefore, it appears that ferrets can successfully balance their embryo numbers in each uterine horn during implantation, with 75–94% of migrated embryos developing normally.

Taken together, our data demonstrate that the transfer of blastocysts to recipient females is technically easier and developmentally superior to the transfer of early cleavage stage ferret embryos. Therefore, we infer that uterine transfer is likely to be the preferred approach for reproductive strategies that involve naturally-produced ferret embryos. However, successful cloning in most species results from the transfer of early stage NT embryos into the oviducts of recipient females, without extended *in vitro* culture. Given the lack of similar experimental evidence in the ferret, it remains unclear if uterine transfer of ferret NT embryos is more effective than the transfer of early stage embryos into oviducts. Further insights into the requirements for efficient ET with both natural and NT ferret embryos could lead to the use of this species for modeling human disease and for conservation of the endangered Mustelidae species, e.g. the black-footed ferret and the European mink.

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