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Original Article

Pilot Test of Embryo Transfer in Murine at the National University of La Plata, Argentina

Maria Alfonsina Lizárraga[1*], Guillermo Giovambattista[1], Rocío Mola[2], Fabricio Maschi[3], and Martin Rumbo[4]

[1] Veterinary Genetics Institute "Eng. Fernando Noel Dulout" of the National University of La Plata (IGEVET-UNLP-CONICET), La Plata, Argentina
[2] Faculty of Veterinary Sciences of the National University of La Plata, La Plata, Argentina
[3] Laboratory of Experimental Animals, Faculty of Veterinary Sciences of the National University of La Plata, La Plata, Argentina
[4] Institute of Immunological and Physiopathological Studies of the National University of La Plata (IIFP-UNLP-CONICET), La Plata, Argentina

* ***Corresponding author:*** Maria Alfonsina Lizárraga, Veterinary Genetics Institute "Eng. Fernando Noel Dulout" of the National University of La Plata (IGEVET-UNLP-CONICET), La Plata, Argentina. Email: mariaalfonsina007@yahoo.com.ar

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ABSTRACT

Introduction: Embryo transfer in murine models is a fundamental technique in biomedical studies, particularly in reproductive biology, genetic modification, and transgenic animal production. Successful implementation of this method requires appropriate laboratory infrastructure, technical expertise, and careful standardization of procedures. The present study aimed to report the first pilot embryo transfer in mice at the National University of La Plata, Argentina, and to evaluate the technique's initial feasibility and efficiency.

Materials and methods: Embryos were generated by ovarian superovulation in an 8-week-old female C57BL/6J donor mouse using a single intraperitoneal injection of 10 IU of equine chorionic gonadotropin (eCG), followed 48 hours later by 10 IU of human chorionic gonadotropin (hCG). The donor female mouse was subsequently mated with a fertile C57BL/6J male mouse. The following day, the mucus plug in the donor female was examined and then euthanized by cervical dislocation. Single-cell embryos (zygotes) were obtained. Thirteen fresh embryos were surgically transferred into a 12-week-old pseudopregnant Swiss recipient female mouse using the conventional oviductal transfer technique.

Results: A pregnancy rate of 61.5% was achieved following embryo transfer. The transferred embryos were one-cell zygotes collected at 0.5 days post-coitum. Eight live pups were delivered at 19.5 days post-coitum and exhibited normal postnatal development. The pups were weaned at 21 days of age. The litter consisted of five males and three females. The administration of preoperative analgesia and the use of a standardized anesthesia protocol contributed to a favorable postoperative recovery in the recipient female mouse.

Conclusion: This successful pilot study can help establish local murine strain banks in Argentina, where such infrastructure is currently lacking, and highlights the potential to fully implement and benefit from cryopreservation technologies.

1. Introduction

In Argentina, the use of laboratory animals with certified genetic and microbiological quality remains an unresolved challenge. Opportunities for routine genetic and microbiological monitoring, as well as for modernizing and renewing laboratory animal production centers, have been limited or absent. Additionally, national economic policies, high costs, and excessive administrative procedures are major factors that hinder the import of breeding stock from international sources[1]. Consequently, biomedical studies in Argentina encounter limited access to high-quality, certified biological resources.

To address current limitations in laboratory animal science in Argentina, establishing a national murine embryo bank as a strain repository is highly significant. A study by

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Diaz et al.[2], funded by the Argentine Ministry of Science and Technology, evaluated laboratory animal science and technology across 27 rat and mouse breeding institutions within Argentina's National System of Animal Establishments. Over half of these facilities were breeding centers that had relied on founder populations imported at least a decade earlier[2]. Diaz et al.[2] indicated the critical need for systematic genetic monitoring programs to certify the genetic quality of rodents produced in these centers. Additionally, Diaz et al.[2] emphasized the importance of establishing a national repository of wild-type strains, including genetically modified lines. Such a repository would safeguard genetic integrity and provide a sustainable local source of laboratory animals, thereby reducing reliance on costly international imports. Despite these identified needs, the implementation of advanced reproductive technologies to support the establishment of a national strain bank has not yet been formally developed or reported within the Argentine system. The adoption of these technologies provides significant benefits, notably aligning with the principles of the 3Rs (Reduce, Refine, Replace)[3], particularly by decreasing the number of actively maintained breeding colonies through the long-term storage of cryopreserved embryos in liquid nitrogen. Notably, murine embryos can remain viable after being cryostored for up to 20 years and can be transported with relative ease[4]. The present study aimed to indicate the first pilot embryo transfer in mice at the National University of La Plata, Argentina, and to evaluate the technique's initial feasibility and efficiency.

2. Materials and methods

2.1. Ethical approval

All procedures were conducted in accordance with international guidelines for the care and use of laboratory animals[5]. The experimental protocol (Protocol No. 241018-2) was approved by the Committee for the Care and Use of Laboratory Animals at the Faculty of Veterinary Sciences, National University of La Plata, La Plata, Argentina.

2.2. Animals and housing

The mice used in this experimentere bred and maintained in the specific pathogen-free (SPF) animal facility of the Laboratory of Experimental Animals (LAE), Faculty of Veterinary Sciences, National University of La Plata, La Plata, Argentina. Animals were housed in ventilated rack systems under controlled environmental conditions (20 ± 1°C; 40–60% relative humidity; 14 hours light/10 hours dark photoperiod). Standard commercial rodent chow (Gepsa, Buenos Aires, Argentina) and water were provided *ad libitum*. Environmental enrichment, including paper nesting material and cardboard tubes, was supplied. The experimental design included one 12-week-old Swiss recipient female (28.7 g) and one 8-week-old C57BL/6J donor female (19 g)[6]. In addition to the standard diet, the donor female received sunflower seeds as supplemental nutritional support during the experimental period.[7]

2.3. Superovulation in embryo donor mice

Superovulation was induced in the 8-week-old C57BL/6J donor female by intraperitoneal administration of 10 IU equine chorionic gonadotropin (eCG; Novormon®, Syntex, Buenos Aires, Argentina), followed 48 hours later by 10 IU human chorionic gonadotropin (hCG; Ovusyn®, Syntex, Argentina) to trigger ovulation. At the time of hCG injection, the female was paired with a fertile C57BL/6J male for natural mating[7].

2.4. Embryo collection

The day after the mating, the female donor after mating, the donor female was euthanized by cervical dislocation. The reproductive tract was aseptically exposed, and the oviducts, with a small portion of the uterine horn, were excised, carefully removing surrounding adipose tissue[8].

The oviducts were placed in M2 medium (Sigma-Aldrich, M7167, USA), which was suitable for manipulation of preimplantation murine embryos under non-controlled atmospheric conditions. Each oviduct was transferred to a 10-cm Petri dish containing multiple 50 µL drops of hyaluronidase solution. The ampulla was gently ruptured using fine forceps to release the cumulus–oocyte complexes (Figure 1). Zygotes were incubated in hyaluronidase for 3-5 minutes until complete dispersal of cumulus cells was observed. Denuded zygotes were collected using a glass capillary pipette and washed several times in fresh drops of M2 medium. Embryos were maintained on a heated stage for up to 30 minutes prior to transfer into the recipient female[8].

A total of 18 embryos were recovered, including 13 single-cell embryos with intact zona pellucida and 5 degenerated embryos, which were discarded. Only morphologically normal one-cell embryos with intact zona pellucida were selected for embryo transfer (Figure 2).

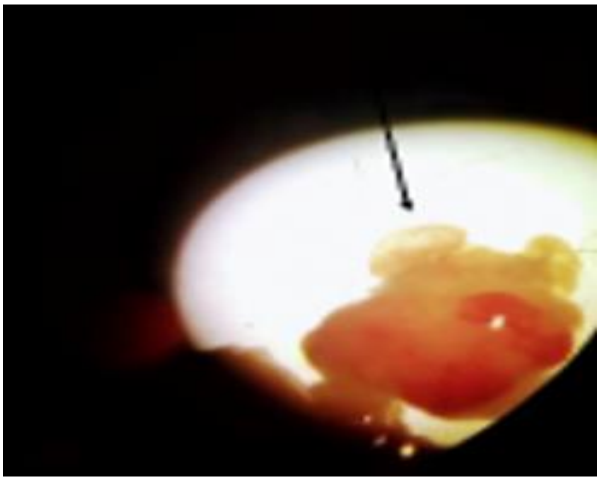

Figure 1. Oviduct of an 8-week-old female embryo donor C57BL/6J mouse. The arrow marks the ampulla (swollen, transparent part of the oviduct) containing one-cell embryos, 40x.

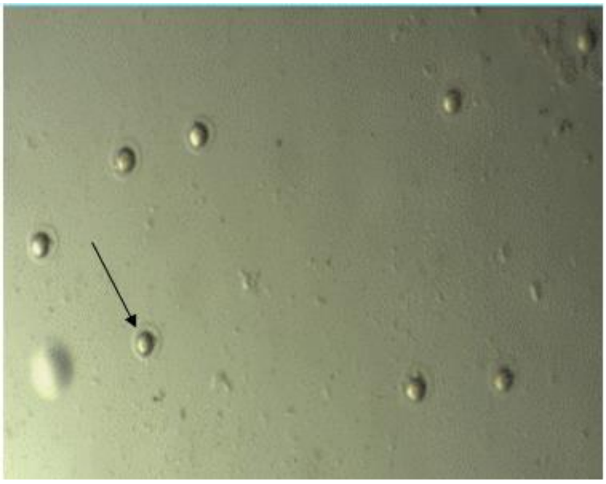

Figure 2. Fresh embryos of the C57BL/6J mouse at the one-cell stage (zygote). The arrow indicates the intact zona pellucida of the 20 μm embryo.

2.5. Embryo transfer

A 12-week-old Swiss female mouse (28.7 g) was used as the recipient of the embryo. Pseudopregnancy was induced by mating the female with a proven vasectomized male. Male sterility had been previously confirmed by mating with a fertile female. To synchronize estrus and boost receptivity, soiled bedding from adult males was placed in the recipient's cage three days before mating to induce the Whitten effect. The morning following mating, successful copulation was confirmed by the presence of a vaginal plug (P+; Figure 3). Upon confirmation of pseudopregnancy, embryo transfer was performed[9].

Figure 3. Vaginal plug examination in a 12-week-old Swiss recipient female mouse. The arrow indicates a positive vaginal plug, formed at 0.5 days post-coitum.

2.6. Surgical oviductal embryo transfer

The recipient female was anesthetized via intraperitoneal injection of ketamine (110 mg/kg, Richmond, Vet pharma, Buenos Aires, Argentina) and xylazine (13 mg/kg, Richmond, Vet pharma, Buenos Aires, Argentina). Buprenorphine (0.03 mg/kg, subcutaneous, Richmond, Vet pharma, Buenos Aires, Argentina) was administered perioperatively for analgesia. Body temperature was maintained throughout the procedure and recovery using a heating pad. In this pilot study, 13 fresh one-cell stage embryos (zygotes) were transferred using the conventional oviductal transfer technique. Following aseptic preparation, a small dorsolateral incision (~1 cm) was made to exteriorize the ovary, oviduct, and associated fat pad. The ovarian bursa was carefully opened using fine forceps and microscissors (number 5) without damaging the ovary. The infundibulum was gently exposed and stabilized, and its opening was slightly dilated using watchmaker's forceps. Embryos were loaded into a finely drawn glass capillary pipette containing M2 medium and introduced through the infundibulum into the oviductal ampulla (Figure 4). After embryo deposition, the reproductive tract was returned to the abdominal cavity. The skin incision was closed using 7-0 silk sutures (Balphin medical´s, Buenos Aires, Argentina), without suturing the muscle layer (Figure 5). The ambient temperature was kept at 25°C to prevent anesthesia-induced hypothermia and aid recovery.

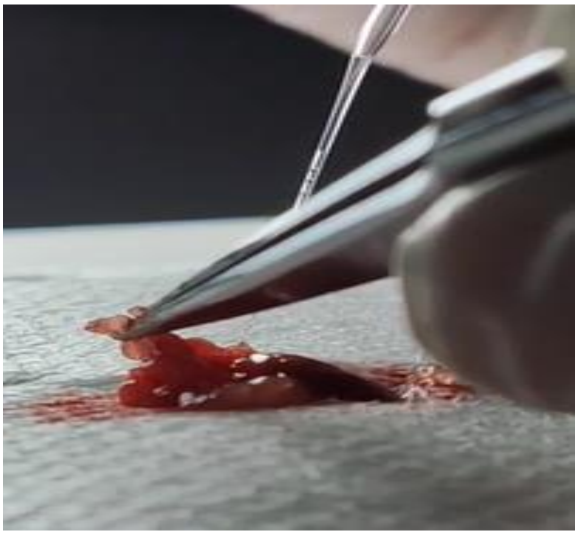

Figure 4. Embryo transfer in a 12-week-old Swiss female recipient mouse. The embryos were loaded into a glass pipette with M2 medium, and 13 fresh single-cell embryos (zygotes) were then transferred using the conventional oviductal transfer technique.

Figure 5. Skin suture of a 12-week-old female Swiss recipient mouse with sterile 0.7 mm silk thread.

3. Results

Following surgery, the recipient female was housed individually in a clean cage provided with paper nesting material, cardboard enrichment tubes, and supplemental sunflower seeds. Recovery from anesthesia was uneventful. Spontaneous movement was observed approximately 40 minutes after surgery, and normal feeding behavior resumed within 60 minutes postoperatively. Postoperative welfare assessment was performed using appropriate, validated, and practical indicators based on species-specific behavioral and physiological signs of pain or distress (Table 1). To minimize additional stress, body weight was not monitored during the postoperative period. The recipient female was evaluated three times per week throughout the 19-day gestation period. The cumulative welfare score remained at zero across all assessments, indicating no detectable pain or distress. Overall postoperative recovery was considered satisfactory (Figure 6). A total of 13 one-cell-stage embryos were transferred, resulting in eight live pups delivered at 19.5 dpc, corresponding to a gestation rate of 61.5% (8/13). All offspring exhibited normal postnatal development (Figure 7A and 7B). At 21 days of age, the litter was successfully weaned, comprising five males and three females.

Table 1. Scoring system for monitoring postoperative welfare in C57BL/6J donor and Swiss recipient mouse

Observation	Score	Description
Piloerection	0	Normal coat; clean, smooth, and well-groomed
	1	Mild or moderate piloerection
	4	Marked piloerection, damaged skin, or unkempt/dirty hair
Skin condition	0	Skin fully covered by healthy hair
	1	Minor sores or scabs without infection or signs of pruritus
	4	Self-mutilation, biting of cage mates, or signs of infection
Appetite	0	Normal food and water intake
	1	Reduced interest in food; water and food intake decreased by <25%
	4	No interest in food; signs of dehydration present
Respiration	0	Normal breathing
	1	Intermittent mouth breathing
	4	Open-mouth breathing, abdominal breathing, panting, or audible respiratory noises

Source: Fernández et al.[10]

Figure 6. The 12-week-old female Swiss mouse observed waking up from anesthesia after the embryo transfer was completed. Normal locomotion, with appetite, and no piloerection was observed.

Figure 7. Development of the 8 C57BL/6J pups, born by embryo transfer from a pseudopregnant Swiss female mouse. A: Development of the young on day 1, skin color red, internal organs visible (stomach full of milk). B: Development of the young mice on day 6, the skin takes on pigmentation on the back, and the ears are completely clear.

4. Discussion

The present study successfully adapted standard murine embryo-transfer protocols to local laboratory conditions, demonstrating clear pregnancies and live births from transferred embryos. A previous study demonstrated that transferring 10-20 freshly collected two-cell embryos often yields pregnancy in over 90% of recipient mice[11]. Consistent with the present findings, restricting the number of embryos transferred per recipient to the recommended range of 10–20 resulted in successful outcomes. Dorsch et al.[11] reported that transfers of 10-20 embryos achieved a 90.4% pregnancy rate, whereas transfers of more than 20 embryos sharply reduced pregnancy rates. Additionally, the current study found that transferring 15 embryos per recipient maximized success, consistent with the high-yield identified by Dorsch et al.[11]. Similarly, Griffiths et al.[12] reported that 3 of 4 recipient mice (~75%) became pregnant in their optimized transfer model. This success rate is comparable to the present outcomes. The consistency of the present findings with the previous studies indicated that the present locally adapted procedures were as effective as well-established international protocols.

Notably, embryo transfer success can vary by genetic background and handling. Dorsch et al.[11] demonstrated that genetic heterogeneity had little effect on the optimal number of embryos, whereas exceeding 20 embryos per transfer dramatically reduced implantation rates. In the present study, the donor strain was not systematically varied; however, the success under local conditions suggested that standard laboratory strains can be reliably propagated in La Plata, Argentina. The high correspondence of present findings with those from well-equipped centers provided strong evidence that the present embryo-transfer technique was effective.

Beyond protocol performance, the current results have important implications for the conservation of genetic resources. Cryopreservation and embryo transfer are the cornerstones of modern mouse banking[1]. Previous studies emphasized that establishing a cryobank would improve the performance of animal experiments, meet the principles of the 3Rs (replacement, reduction, and refinement), and reduce labour and costs[3]. Likewise, Takeo et al.[13] noted that cryopreserving mouse gametes and embryos efficiently archived valuable strains; indeed, over 60,000 genetically engineered mouse strains are already stored in international repositories[14]. The successful embryo transfers in the present study demonstrated local capability to generate and implant embryos, a critical precursor to archiving.Therefore, the present study established the foundational framework for a national murine cryobank. A fully operational cryobank would become part of the global resource network. As Takeo et al.[13] concluded, advanced reproductive technologies can enable a seamless archive and supply of mouse resources across facilities and countries. In the present study, establishing embryo-transfer expertise aligned the National University of La Plata, Argentina, with international standards for mouse resource management.

Thus, the present study proved that advanced assisted reproductive technologies can be implemented effectively in the present Argentine laboratory setting. The observed pregnancy and birth rates match those reported by previous studies[11-13], validating the approach of the current study. This achievement not only validated the possibility of local embryo transfer but also established a strong murine cryobank locally, contributing to the global initiative to preserve and share important mouse resource models.

5. Conclusion

The present study demonstrated the successful local implementation of embryo transfer technology within the Argentine laboratory animal science system. Establishing this capability was an important step for institutions that maintain wild-type and genetically modified mouse strains but lacked access to cryopreservation and assisted reproductive technologies. These advances created the basis for developing a national repository of murine strains, strengthening local scientific infrastructure, and reducing dependence on external sources. Future efforts should focus on expanding the application of reproductive technologies to a larger number of strains and institutions of Argentina to establish a sustainable national murine cryobank in the country.

Declarations

Competing interests

The authors declared that they have no competing interests.

Authors' contributions

The conceptualization of the study was conducted by Alfonsina Lizárraga and Guillermo Giovambattista. The investigation was carried out by Alfonsina Lizárraga, Rocio Mola, Martin Rumbo, and Fabricio Maschi. The methodology was developed by Alfonsina Lizárraga and Rocío Mola. Project administration was overseen by Martin Rumbo and Guillermo Giovambattista. Resources were provided by Alfonsina Lizárraga, Rocio Mola, and Fabricio Maschi. All authors have read and approved the final edition of the manuscript before publication in the present journal.

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Availability of data and materials

The manuscript contains all datasets generated and/or analyzed in the current study, which are available upon reasonable request from the corresponding author.

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Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors. The authors did not use any AI applications for writing or preparing the full text of this article.

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