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The biology and methodology of assisted reproduction in deer mice (*Peromyscus maniculatus*)

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Abstract

Although laboratory-reared species of the genus *Peromyscus*—including deer mice—are used as model animals in a wide range of research, routine manipulation of *Peromyscus* embryogenesis and reproduction has been lagging. The objective of the present study was to optimize conditions for oocyte and/or embryo retrieval and for in vitro culturing. On average, 6.4 oocytes per mouse were recovered when two doses of 15 IU of pregnant mare serum gonadotropin (PMSG) were given 24 h apart, followed by 15 IU of hCG 48 h later. Following this hormone priming, females mated overnight with a fertile male yielded an average of 9.1 two-cell stage embryos. Although two-cell stage embryos developed to 8-cell stage in Potassium Simplex Optimized Medium (KSOM; Millipore-Chemicon, Billerica, MA, USA) in vitro, but not further, embryos recovered at the 8- to 16-cell stages developed into fully expanded blastocysts when cultured in M16 media in vitro. These blastocysts had full potential to develop into late stage fetuses and possibly into live pups. As a result of the present work, all stages of *Peromyscus* preimplantation development are now obtainable in numbers sufficient for molecular or other analyses. These advances provide the opportunity for routine studies involving embryo transfer (e.g., chimeras, transgenics), and preservation of genetic lines by cryopreservation.

Keywords: Peromyscus; ART; Induced ovulation; Embryo culture; Blastocyst; Estrous cycle

1. Introduction

There are few mammalian systems in which one can assess the effects of natural genetic variants and allelic combinations on disease susceptibility and phenotypes. This is important, as ablating gene function via transgenic technology does not predict the effects of coding-region or regulatory variants. Furthermore, common alleles underlie predisposition to many human diseases [1,2].

Rodents of the genus *Peromyscus* provide a rare opportunity for laboratory studies of such variation, due to the unique resource of laboratory stocks derived from natural populations, allowing direct comparison with animals in their native habitat [3]. Commonly referred to as deer-, white-footed—or oldfield mice, *Peromyscus* are the most common group of indigenous North American mammals. Despite superficial resemblances, *Peromyscus* diverged from both laboratory mice (*Mus*) and rats (*Rattus*) approximately 25×10^6 yr ago [4]. Members of the genus are found from Alaska to Central America and from the Atlantic to the Pacific. There is variation in morphology, physiology, behavior, growth, coat-color, diet, and habitat [3]. The *P. manicu-*

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latus species complex is particularly widespread and variable [5,6]. Interfertility within this group makes genetic analyses of natural variants and many spontaneous mutants feasible. Furthermore, the ecological importance of these animals renders them indicators of environmental health, as well as biomedical models.

Development of *Peromyscus* systems as biomedical models has been hindered by a relative lack of resources. However, this dearth is rapidly being alleviated: a complete genetic map is nascent, > 90 000 expressed sequence tags (ESTs) are now available in the public database, and genome sequencing is in progress. Nevertheless, improvements in *Peromyscus* assisted reproductive technology are necessary for this group to reach its potential as a model system.

For example, crosses between the *Peromyscus maniculatus* stock BW and the *P. polionotus* stock PO resulted in dramatic asymmetric effects on growth and development. Although they are the same size at birth, BW females mated to PO males produce growth-retarded but viable offspring [7,8]. In contrast, PO females mated to BW males produce overgrown but dysmorphic conceptuses [9,10]; nearly 50% of these litters are entirely dead by midgestation, and the remainder typically exhibit defects (e.g., hemorrhage) consistent with nonviability [11]. Genetic analyses indicate a maternal effect which controls epigenetic regulation of gene expression [12]. An obvious test of maternal genotype on embryonic growth is to perform reciprocal embryo transfer experiments between the two species (i.e., transfer of pure BW embryos into a PO pseudopregnant female, and vice-versa).

Although induced ovulation, AI, and embryo transfer have previously been performed in *Peromyscus* [13–16], these techniques were relatively inefficient. Optimization of such techniques would not only allow for developmental studies, but would also make transgenic models feasible. These techniques could also be used in conjunction with embryo cryogenesis to preserve mutant and/or congenic strains.

Several factors render *Peromyscus* reproductive biology less tractable than that of *Mus*. The first is that they are most successfully mated in pairs, eliminating the use of a single male to produce multiple synchronized pregnancies. Second, *Peromyscus* postcoital plugs are not readily visible and, hence, are unreliable as an indicator of mating [17,18]. However, natural matings may be timed through the use a vaginal saline wash [18]; the type and proportion of cells present can be used to determine the estrous cycle phase and whether mating has occurred (i.e. presence of sperm). The objectives of the present study were to assess estrous cycle phase and/or mating timing, the effects of hormone dose and timing on oocyte release, as well as conditions necessary for embryo culture.

2. Materials and methods

2.1. Animals

Peromyscus maniculatus bairdii (BW stock) animals were obtained from the *Peromyscus* Genetic Stock Center (http://stkctr.biol.sc.edu), where they are maintained on a 16:8 h light-dark cycle. All procedures were approved by the University of South Carolina Institutional Animal Care and Use Committee (IACUC).

2.2. Chemicals

Pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) were obtained from the National Hormone & Peptide Program (Harbor-UCLA Medical Center, West Carson, CA, USA) and from Sigma-Aldrich (St. Louis, MO, USA). The Potassium Simplex Optimized Medium (KSOM [19]) was obtained from Millipore-Chemicon (Billerica, MA, USA). Both 50x essential amino acids (EAA) and 100x nonessential amino acids (NEAA) were purchased from Invitrogen (Carlsbad, CA, USA). The M2 and M16 media, embryo culture grade oil, and other chemicals were obtained from Sigma-Aldrich. None of the media used in this study was supplemented with antibiotics. All liquids were handled aseptically and were used for ≤ 2 wk.

2.3. Light microscopy

Embryos were observed and photographed using an Olympus BX41 bright field light microscope fitted with a 10 X objective and an Olympus DP71 CCD camera (Center Valley, PA, USA) attached to a computer or using a Zeiss inverted microscope IM35 (Thornwood, NY, USA) fitted with a Sony α-350 DSLR camera (New York, NY, USA).

2.4. Estrous cycle determination

The vagina of each animal was gently washed with 50 μL of PBS. This drop was collected and observed under phase-contrast microscopy. The proportion and morphology of cell types was used to determine the phase of the estrous cycle [18], based on reported stages of the deer mouse estrous cycle (Fig. 1).

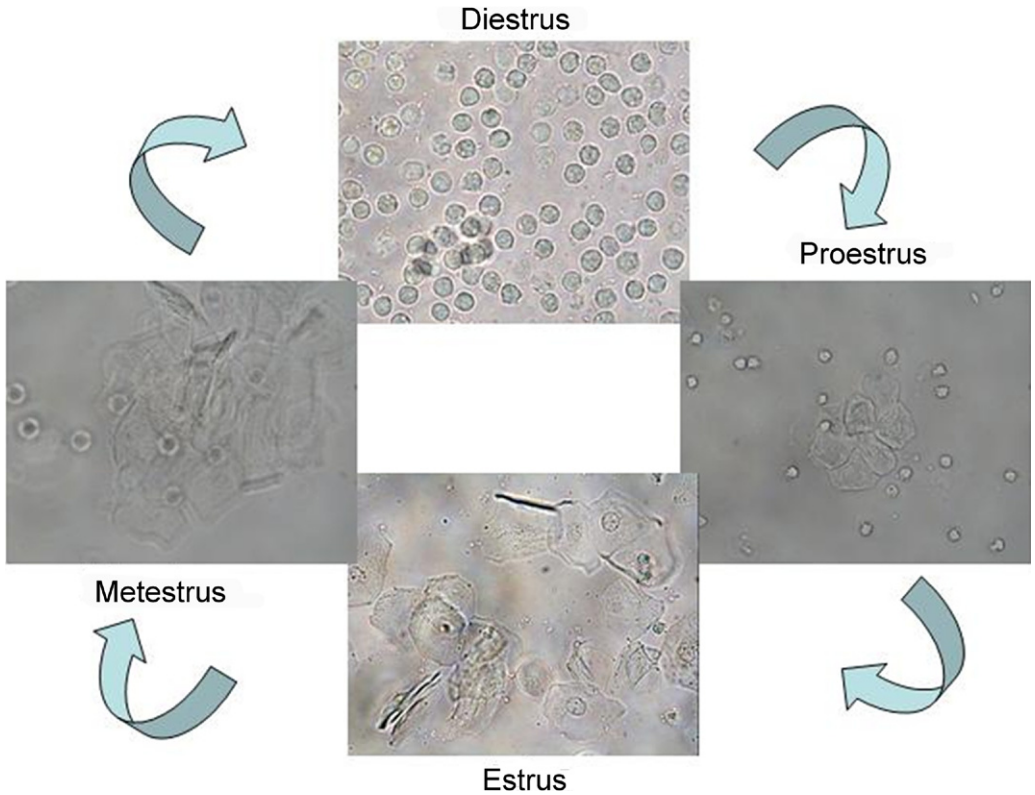

Fig. 1. Typical micrographs at various stages of the estrous cycle in deer mice. During diestrus, cells in vaginal smears were mostly leukocytes, whereas in proestrus they were a mixture of leukocytes and epithelial cells. Estrus stage was characterized by a preponderance of large, squamous-shaped epithelial cells, whereas in metestrus both leukocytes and some epithelial cells were present.

2.5. Oocyte and/or embryo collection

Oocyte and/or embryo collection was performed as described [20,21]. Briefly, females were cervically dislocated and oviducts with an attached fragment of the uterus were excised. A 32-gauge blunted needle connected to a 1- or 3-cc glass syringe was inserted into the infundibulum and the oocytes and/or embryos were flushed with M2 medium prewarmed to 37°C into medium of the same temperature.

2.6. Hormonal priming and regimes

Hormones were freshly prepared from a 10X frozen stock solution to 100 IU/mL concentration. Based on a dose of 15 IU/animal, 0.1 mL of 150 μL working solution was administered intraperitoneally. The time of hormone administration relative to the light cycle was not optimized. However, for practical reasons, injections were performed 8 h after the onset of light (1400).

Previous research established that 10 to 15 IU of PMSG and 10 to 15 IU of hCG hormone resulted in a yield of 2 to 6 embryos per female [14–16,22]. Therefore, higher doses of each hormone were tested. The optimal time for embryo and/or oocyte harvest relative to hCG administration was also investigated, comparing yields 12, 15, and 18 h postinjection.

Two hormonal regimes for ovulation induction were compared. Regime 1 included 15 IU of PMSG, followed by 15 IU of hCG 48 h later. Regime 2 consisted of administering a second dose of PMSG (15 IU) 24 h after the first dose, and 48 h before hCG. Mice subjected to Regime 1 were artificially inseminated (see section 2.5) 12 h after hCG administration, whereas those receiving Regime 2 were artificially inseminated 18 h after hCG injection. In both cases, embryos were harvested 48 h post AI.

To assess the effects of the hormone source on embryo and/or oocyte harvest, the above experiments were performed using hormones from the National Hormone & Peptide Program. Regime 2 was then repeated with 12 animals using PMSG and hCG from Sigma-Aldrich.

2.7. AI

Male deer mice were euthanized by cervical dislocation (without anesthesia to avoid potential effects on sperm motility [23]). The epididymides from two males were minced in 1 mL of 9% skim milk/PBS solution and sperm were allowed to swim out into the media and capacitate for 10 min [24]. Each hormone-primed female was placed under mild isoflurane anesthesia and was inseminated with 50 μL semen using a 22-gauge blunted needle passed through the cervix. The females were held upside down for 1 min to minimize backflow.

Artificial insemination was initially performed on 11 females receiving the Regime 1 hormone treatment. Regime 1 was then repeated, except that after hCG administration the females (N = 12) were immediately paired with an experienced breeder male. The male was removed after 16 h and the oviducts were flushed 48 h later. To determine the effect that mating behavior had on assisted reproduction, 12 females were treated with hormone Regime 1 and were immediately paired with an experienced breeder male after hCG administration. After 16 h, the male was removed and the females were artificially inseminated. Oviducts were flushed 48 h post AI.

The effects of hormone Regime 2 was also assessed with overnight mating and subsequent AI on 12 females. Oviducts were flushed 48 h post AI. Additionally, a single female was subjected to the same treatment (hormone Regime 2, AI), but paired with a vasectomized male prior to AI to determine the role of female-male social interactions and its effect on AI success.

2.8. Embryo culture

The KSOM supplemented with amino acids (KSOM/AA) media was prepared as previously described [20]. The KSOM, KSOM/AA, and M16 plates were prepared 1 day before embryos were harvested. Media droplets (50 μL) were covered with oil and the plates were allowed to equilibrate in a 5% CO_2 atmosphere at 37 °C.

2.9. Two-cell stage embryo culture

Two-cell stage embryos were collected 48 h after AI from females subjected to hormone Regime 2. These embryos were collected in M2 media and then transferred into KSOM media (8 to 12 embryos per 50 μL droplet) after several initial washes. After 48 h, embryos were examined to assess development. Embryos were cultured for up to 96 h. This experiment was repeated using KSOM/AA media [25]. In addition, 30 two-cell stage embryos were incubated in M16 media (three 50 μL droplets containing 10 embryos each) for 48 h.

2.10. Cleavage-stage embryo culture

Eight- to 16-cell stage embryos were collected from females subjected to Regime 2 hormones, 68 h post AI. These embryos were again collected into M2 media and immediately transferred to KSOM (8 to 12 embryos per 50 μL droplet). Following 48 h of incubation, samples were examined to assess development. The experiment was then repeated with embryos transferred to and incubated in KSOM/AA media in place of KSOM media. Next, the experiment was repeated with the glucose-rich M16 media. Forty 8- to 16-cell stage embryos were placed into four 50μL droplets and were incubated for a total of 48 h. Based on previous observations, a two-step culturing protocol by transferring in vitro developed eight-cell stage embryos (using KSOM, N = 60) into M16 media was attempted.

2.11. Blastocyst transfer

Preparation of foster mothers was essentially carried out as described previously [14]. Briefly, the oviducts of the females were ligated close to the uterus and then cut near the ovary, allowing a free flow of fluids into the peritoneal cavity. After a 2-wk recovery period, females were paired with fertile males 1 day before AI was performed on the donor animals. Vaginal smears were obtained on the subsequent 2 days. Presence of sperm in these specimens indicated successful copulation. Blastocysts were implanted into the uterus of these potentially pseudopregnant females.

2.12. Microsatellite DNA amplification

Genomic DNA was isolated, microsatellite loci (5227, 5323, 5334, 5411, 5441, 5471, 5477) were amplified, and fragment sizes were determined as described previously [26].

3. Results

3.1. Age and estrous cycle

An extended estrous cycle was detected in 75% of prepubescent females. These younger females had an estrous peak of approximately 24 h (Fig. 2). Conse-

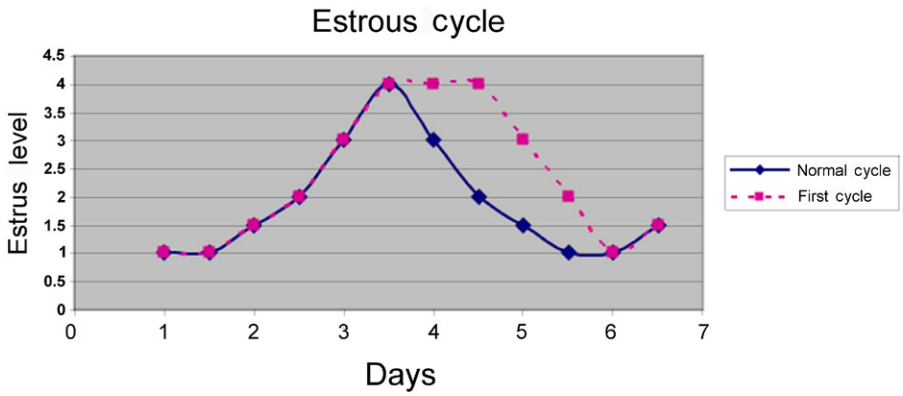

Fig. 2. Estrous cycle charted for a normal adult female deer mouse that had cycled previously and a first-cycle female. The x-axis denotes days. The y-axis denotes the level in the estrous cycle. Level 1 of the estrous cycle is equal to diestrus, level 2.5 is equal to proestrus, and level 4 is equal to estrus. The first-cycle female has an extended estrus, or perhaps reverted to proestrus briefly before returning to estrus.

quently, 12- to 24-wk-old (mature) females were used in subsequent experiments.

3.2. Hormonal priming and regimes

Doses exceeding 10 to 15 IU of PMSG and 10 to 15 IU of hCG hormone did not increase oocyte yield (data not shown). The optimal time for oocyte harvest was 18 h post hCG injection. In the initial study, at 18 h post hCG, the average number of oocytes per animal was approximately five (Table 1). However, in a more comprehensive study of 10 experiments the average number of oocytes recovered from six deer mice ranged from 3.1 to 8.2 and the overall mean was 6.4 oocytes per animal.

In comparison with hormones from the National Hormone & Peptide Program, the use of Sigma-Aldrich hormones resulted in a 30% reduction in the number of two-cell embryos recovered. Consequently, hormones from the National Hormone & Peptide Program were used for all remaining experiments.

3.3. AI

Artificial insemination of 11 females receiving only hormone Regime 1 resulted in the recovery of only oocytes. However, immediate pairing with an experienced breeder male following hormone Regime 1 and without AI resulted in the recovery of 90% oocytes and 10% embryos. The combination of AI and pairing with experienced male breeders of females treated with only hormone Regime 1 resulted in the recovery of $>$ 5% embryos at the two- to three-cell stage.

Overnight mating and subsequent AI of 12 females treated with only hormone Regime 2 resulted in a greater than five-fold increase in the number of two-cell stage embryos recovered in comparison with the number of two-cell stage embryos recovered for mice on Regime 1 (Table 2). Six embryos and nine oocytes were collected when a single female was subjected to the same treatment (Regime 2, AI), but paired with a vasectomized male prior to AI.

3.4. Two-cell stage embryo culture

After 48 h in culture, 50% of the two-cell stage embryos collected 48 h after AI (Fig. 3A) reached the eight-cell stage (Fig. 3B). Additional culture did not result in further development (embryos had signs of

Table 1
Time course study of oocyte release after hCG administration in deer mice.

Interval after hCG, h	Mice, N	Oocytes, N
12	5	6
15	6	14
18	7	35

An additional 6 h relative to *Mus* protocols resulted in a five-fold increase in number of harvested oocytes.

Table 2
Yield of two-cell stage embryos in deer mice at 48 h post AI using single and two consecutive PMSG injection protocols.

Procedure	Mice, N	Two- to three-cell embryos, N	Embryos per mouse, N
Single PMSG injection	11	19	1.7
Two consecutive PMSG injections	12	109	9.1

PMSG, pregnant mare serum gonadotropin.

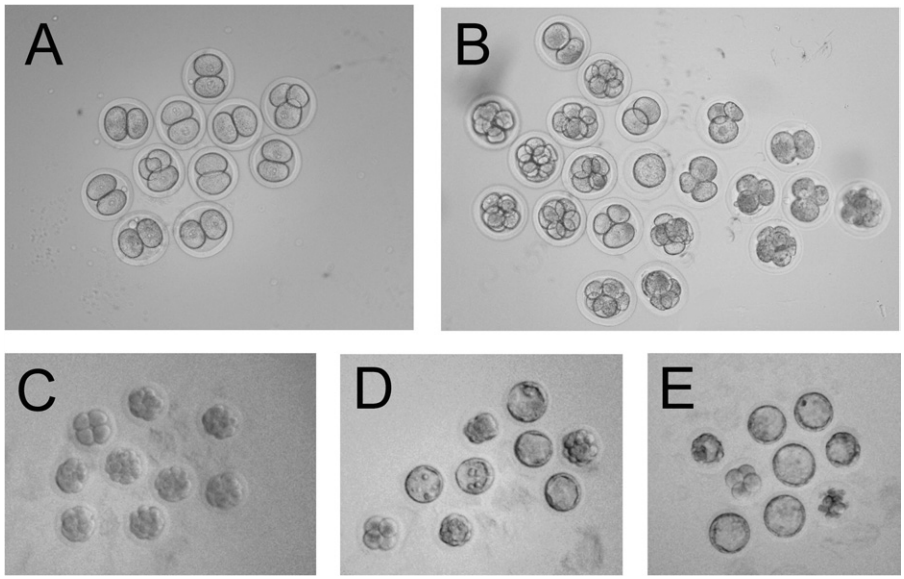

Fig. 3. In vitro development of deer mouse embryos. (A) Embryos flushed from the uteri 48 h after AI. (B) Embryos after 48 h culture in KSOM. (C) Embryos flushed 68 h after AI. (D) After 24 h culture in M16, embryos developed into early blastocysts. (E) After an additional 24 h culture, they developed into fully expanded blastocysts. KSOM, Potassium Simplex Optimized Medium (Millipore-Chemicon, Billerica, MA, USA).

disintegration). Similar results, including lack of development past the eight-cell stage, were found in embryos incubated in KSOM/AA media after 48 h. No cell division occurred for embryos incubated in M16 media.

3.5. Cleavage-stage embryo culture

Following 48 h in KSOM media, no development occurred in embryos harvested 68 h post AI. Furthermore, embryos had signs of disintegration after 48 h of culture. Likewise, the use of KSOM/AA in place of KSOM yielded the same results.

In contrast, 35 of the 40 embryos (8- to 16-cell stage; Fig. 3C) incubated in glucose-rich M16 media reached the early blastocyst stage after 24 h (Fig. 3D). After an additional 24 h, development progressed to the fully expanded blastocyst stage (Fig. 3E). In total, approximately 80% to 90% of cleavage stage embryos reached the fully expanded blastocyst stage under these conditions. A summary of the experimental timeline is shown (Fig. 4).

No embryo development was observed in eight-cell stage embryos developed in vitro (using KSOM) and transferred into M16 media. Furthermore, these embryos showed signs of disintegration.

3.6. Fetal development of cultured blastocysts

Three blastocysts that were the result of 48 h in vitro culture of eight-cell embryos were implanted into the uterus of a potentially pseudopregnant foster mother. Fifteen days after implantation, the female was euthanized and one anatomically normal, live fetus was recovered (Fig. 5). Microsatellite DNA was amplified at seven loci from both the foster mother and the fetus. At three loci, the DNA fragment size pattern excluded the foster mother as being a biological parent (data not shown).

4. Discussion

In the present study, giving a second dose of PMSG 24 h after the first dose significantly increased the number of *Peromyscus* embryos and/or oocyte compared with only a single dose of PMSG. In contrast, previous studies performed on *Mus* suggested that only one dose of PMSG was necessary to maximize embryo and/or oocyte yield. This discrepancy underscores apparent physiological differences between *Mus* and *Peromyscus*. Furthermore, it also provides a greater understanding of the conditions necessary to optimize *Peromyscus* ovulation and opportunities to increase the number of embryos and/or oocytes harvested.

Not only was the number of PMSG doses important in the maximization of embryos and/or oocytes yield, but also the interval between hCG treatment and collection. In this study, the number of days

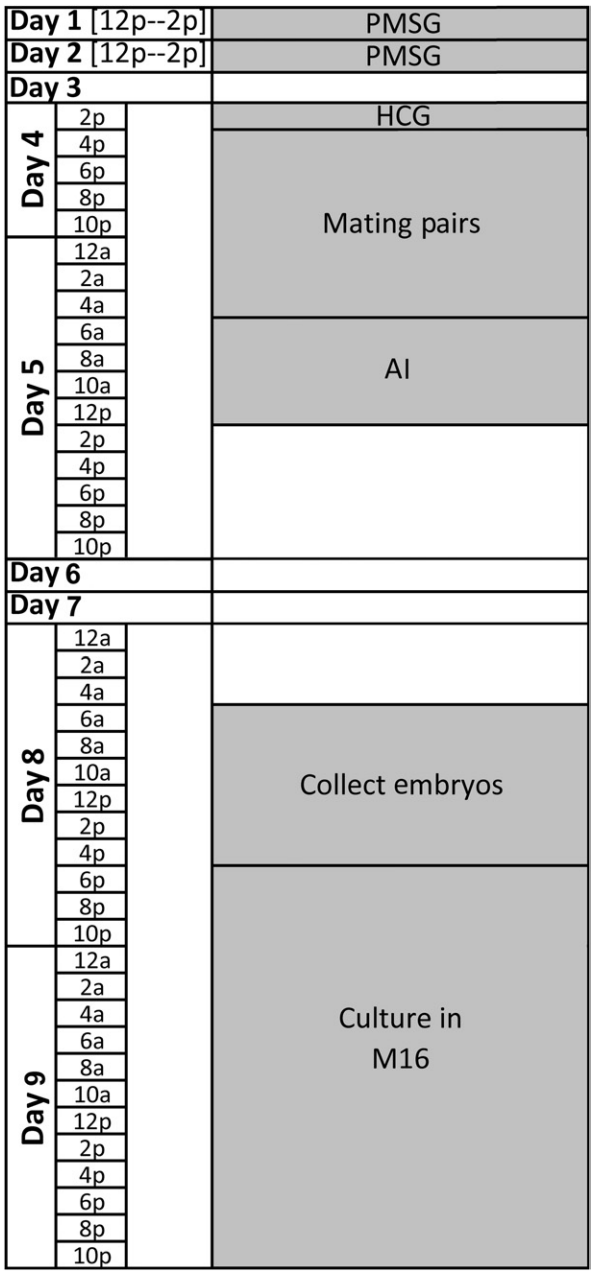

Fig. 4. Timeline of obtaining fully expanded blastocysts in deer mice.

PMSG was administered (2 days) corresponded to approximately half of the length of the estrous cycle (4 to 5 days). This interval is typical of hormone treatment in human IVF protocols [27]. Forty-eight hours after AI, a majority of the embryos were at the two-cell stage in deer mice, whereas 48 h after fertilization, *Mus* embryos were at the four-cell stage [20]. Perhaps *Peromyscus* spend an additional 12 h at the two-cell stage, or have delayed fertilization.

The combination of both overnight mating and AI were necessary to maximize the number of fertilized embryos collected. If only overnight mating occurred, the number of fertilized embryos was significantly lower than in cases where overnight mating was coupled with AI. Similarly, AI alone was insufficient to produce fertilized embryos. Therefore, we inferred that mating behavior in deer mice has a marked effect on the outcome of assisted reproduction. Successful studies of females paired with vasectomized males followed by AI further indicated that female-male social interactions were crucial and sufficient for AI success in *Peromyscus*.

The present study with two-cell stage embryos in M16 media indicated the adverse effect of high glucose concentrations on early embryological development in comparison with the lower glucose concentration in KSOM medium. These findings were consistent with the effects of high glucose concentrations on the embryological development in other species [28]. However, using M16 medium resulted in 80% to 90% of cleavage stage embryos reaching the fully expanded blastocyst stage. Concurrent with the earlier findings, we inferred that by the eight-cell stage glucose was the preferred source of energy [29].

Successful progression of two-cell stage embryos in KSOM medium and of eight-cell stage embryos in M16 occurred. However, transfer of eight-cell stage embryos grown in KSOM to M16 medium failed to result in further cell development. Although previous work showed successful culturing of one-cell stage embryos to blastocysts, the rate was only 8% [14], which was not high enough for routine assisted reproduction.

The blastocyst transfer experiment presented here indicated that the eight-cell stage embryo culture conditions allowed proper in utero development up to 17 to 18 days of embryonic development. Although the foster mothers were prepared as published earlier [14], in the present study, fully expanded individual blastocytsts were transferred, in contrast to aggregated embryos previously used [14].

Taken together, this study yielded dramatic improvements in *Peromyscus* assisted reproductive technology. Key findings included: the addition of a second PMSG treatment during ovulation induction; collection of oocytes and/or embryos at 18 h post hCG administration; culturing of two-cell stage embryos in KSOM; and culturing cleavage-stage em-

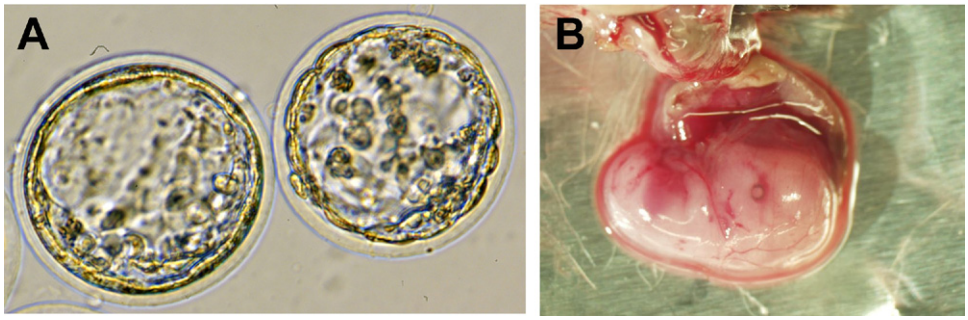

Fig. 5. Fetal development of deer mouse embryos collected at the eight-cell stage, cultured in vitro for 48 h, and transferred into the uterus of a foster mother. (A) Fully expanded blastocysts before transfer. (B) Fetus recovered 15 days post transfer.

bryos in M16 media. As a result of the optimization of culture conditions, all stages of *Peromyscus* preimplantation development are now obtainable in numbers sufficient for molecular or other analyses. These advances provided an opportunity for routine studies involving embryo transfer (e.g., chimeras, transgenics), and preservation of genetic lines through slow freezing or vitrification. These techniques, in combination with the burgeoning *Peromyscus* molecular and genetic resources, suggest a promising future for these animals as an alternative mammalian biomedical system.

Acknowledgements

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References

[1] Peng B, Kimmel M. Simulations provide support for the common disease-common variant hypothesis. Genetics 2007;175: 763–76.
[2] Ouyang C, Smith DD, Krontiris TG. Evolutionary signatures of common human cis-regulatory haplotypes. PLoS One 2008;3: e3362.
[3] Dewey MJ, Dawson WD. Deer mice: "The Drosophila of North American mammalogy." Genesis 2001;29:105–9.
[4] Steppan SJ, Adkins RM, Anderson J. Phylogeny and divergence-date estimates of rapid radiations in muroid rodents based on multiple nuclear genes. Syst Biol 2004;53:533–53.
[5] Dice L. Speciation in *Peromyscus*. American Naturalist 1940; 74:289–98.
[6] Vrana PB. Genomic imprinting as a mechanism of reproductive isolation in mammals. J Mammalogy 2007;88:5–23.
[7] Dawson WD. Fertility and size inheritance in a *Peromyscus* species cross. Evolution 1965;19:44–55.
[8] Rogers JF, Dawson WD. Foetal and placental size in a Peromyscus species cross. J Reprod Fertil 1970;21:255–62.
[9] Dawson WD, Sagedy MN, En-yu L, Kass DH, Crossland JP. Growth regulation in Peromyscus species hybrids: a test for mitochondrial-nuclear genomic interaction. Growth Dev Aging 1993;57:121–33.
[10] Maddock MB, Chang MC. Reproductive failure and maternal-fetal relationship in a Peromyscus species cross. J Exp Zool 1979;209:417–26.
[11] Duselis AR, Vrana PB. Assessment and disease comparisons of hybrid developmental defects. Hum Molec Genet 2007; 16:808–19.
[12] Duselis AR, Wiley CD, O'Neill MJ, Vrana PB. Genetic evidence for a maternal effect locus controlling genomic imprinting and growth. Genesis 2005;43:155–65.
[13] Fukuda Y, Maddock MB, Chang MC. In vitro fertilization of two species of deer mouse eggs by homologous or heterologous sperm and penetration of laboratory mouse eggs by deer mouse sperm. J Exp Zool 1979;207:481–9.
[14] Klein MS, Markert CL. Production of chimeras in the deer mouse Peromyscus maniculatus bairdi. J Exp Zool 1981;218: 183–93.
[15] Roth VL, Klein MS. Maternal effects on body size of large insular Peromyscus maniculatus: evidence from embryo transfer experiments. J Mammalogy 1986;67:37–45.
[16] Maddock MB, Dawson WD. Artificial insemination of deer mice (*Peromyscus maniculatus*) with sperm from other rodent species. J Embryol Exp Morphol 1974;31:621–34.
[17] Joyner CP, Myrick LC, Crossland JP, Dawson WD. Deer mice as laboratory mice. ILAR J 1998;39:322–30.
[18] Bradley EL, Terman CR. Ovulation in Peromyscus-Maniculatus-Bairdi under laboratory conditions. J Mammalogy 1979;60: 543–9.
[19] Erbach GT, Lawitts JA, Papaioannou VE, Biggers JD. Differential growth of the mouse preimplantation embryo in chemically defined media. Biol Reprod 1994;50:1027–33.

[20] Nagy A. Manipulating the Mouse Embryo: A Laboratory Manual, Third Edition. Cold Spring Harbor Laboratory Press, 2003.
[21] Duselis AR, Vrana PB. Retrieval of mouse oocytes. J Vis Exp 2007;3:185.
[22] Marston JH, Chang MC. Morphology and timing of fertilization and early cleavage in Mongolian gerbil and deer mouse. J Embryol Exp Morphol 1966;15:169–75.
[23] Oropeza-Hernandez LF, Quintanilla-Vega B, Albores A, Fernandez-Guasti A. Inhibitory action of halothane on rat masculine sexual behavior and sperm motility. Pharmacol Biochem Behav 2002;72:937–42.
[24] Duselis AR, Vrana PB. Harvesting sperm and artificial insemination of mice. J Vis Exp 2007;3:184.
[25] Ho Y, Wigglesworth K, Eppig JJ, Schultz RM. Preimplantation development of mouse embryos in KSOM: augmentation by amino acids and analysis of gene expression. Mol Reprod Dev 1995;41:232–8.
[26] Weber JN, Peters MB, Tsyusko OV, Linnen CR, Hagen C, Schable NA, et al. Five hundred microsatellite loci for Peromyscus. Conserv Genet 2010;11:1243–6.
[27] Nargund G, Waterstone J, Bland J, Philips Z, Parsons J, Campbell S. Cumulative conception and live birth rates in natural (unstimulated) IVF cycles. Hum Reprod 2001;16:259–62.
[28] Summers MC, Biggers JD. Chemically defined media and the culture of mammalian preimplantation embryos: Historical perspective and current issues. Hum Reprod Update 2003;9:557–82.
[29] Leese HJ, Barton AM. Pyruvate and glucose uptake by mouse ova and preimplantation embryos. J Reprod Fertil 1984;72:9–13.