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Ovary transplantation method resulting in high reproductive performance in mice

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Introduction

A number of mutant and transgenic mouse strains do not breed. The infertility can be due to non-functional germ cells, prepubertal lethality, inability to mate or incapacity to complete a successful fertilization, gestation or parturition. When the reason for infertility is other than non-functional germ cells ovary transplantation and / or artificial insemination can be used. Such techniques are necessary for maintaining the genotype of strains with dominant inheritance of any of these breeding dysfunctions. The techniques are very useful also for maintaining the genotype of strains that recessively inherit the breeding dysfunction. They allow breeding with homozygous rather than heterozygous mice resulting in twice the ratio of abnormal versus normal offspring in the litter. In addition, the use of heterozygous genotype for both parentals would necessitate progeny testing to assure presence of the mutant genotype in the offspring with which the strain maintenance is to be continued. Often, the only way of testing this is to breed the offspring to find out whether the second generation litter include any phenotypically mutant mouse. Depending on when the phenotype can be ascertained, the progeny testing can be very consuming regarding time, labour and number of animals needed. In outcrosses between a normally functional strain and a breeding dysfunctional strain where an affected sex is to be used, the profits of ovary transplantation and artificial insemination are the same as in incrosses.

Ovary transplantation methods with moderate success were described in several publications in the 1960s (*Stevens, 1957*). Applications of these methods, or of modifications of them, showing varying success have been reported until recently (*Parkening et al, 1985; Brem et al, 1990; Cox et al, 1996; Gunasena et al, 1997a; Gunasena et al, 1997b*). The ovary transplantation method presented here is similar to what has previously been described by Stevens (1957) and Cunliffe-Beamer (1983). This method is used at the Jackson Laboratory, Maine, and has recently been established also at the Karolinska Institute, Sweden. In this publication we evaluate the performance of this method using an example with the non-breeding mouse mutant megencephaly, *mceph*, with a BALB/cByJ background.

Materials and Methods

Animals:

Three females of inbred BALB/cByJ mice homozygous for the spontaneous mutation megencephaly, *mceph*, (Jackson Laboratory, Bar Harbor, Maine) were used as donors of ovaries (*Donahue et al, 1996*). These homozygous, phenotypically mutant mice do not breed but they have fully functional ovaries. Ovary recipients were 10 females of the congenic strain C.B6-$+^{Tyr\text{-}c}$ *Hbb* ^{s} (Jackson Laboratory, Bar Harbor, Maine). The genome of this strain is a background of BALB/c-$+^{Tyr\text{-}c}$ *Tyrp1* ^{b} with the *Hbb* locus originating from the C57BL/6By strain. The alleles of this locus differ between the BALB/cByJ and the C57BL/6By strain allowing them to be used as markers for determination of maternal germ-line in the offspring. The animals were housed in a barrier animal facility and kept at 12 h light:12 h darkness, a temperature of 21 - 22° C, and relative humidity of 40 - 50%. They were caged in individually ventilated microisolators in Vent-A-Cage

racks (*Iwarsson & Norén, 1992*) with Beekay Bedding (B&K Universal AB, Sollentuna, Sweden). Rodent breeding diet R36 (protein 18.5 %, fat 4.0 %, ash 6.3 %, vitamin and mineral fortified; Lactamin AB, Stockholm, Sweden) and water were provided *ad libitum*.

Ovary transplantation:
Both ovary recipient and ovary donor mice were divided into age groups: recipients 42-45 and 95 days old, donors 45 and 70 days old. Ovary transplantation was performed for three age combinations. The ovary transplantation technique was carried out essentially as previously described (*Cunliffe-Beamer, 1983*). The donor mice were euthanized by cervical dislocation. The two ovaries from each mouse were removed under aseptic conditions. They were placed in a petri dish containing 2 ml of M2 medium (Sigma, St. Louis, MO) at room temperature. The ovaries were cleaned from the attached fat pad and the covering bursa under microscope at 2 X magnification. The cleaned ovaries were cut in halves. The recipient mice were anaesthetized with 0.2 ml / 10 g body weight of 2.5 % tribromo-ethanol and tert-amyl alcohol ("Avertin") solution. After preparing for aseptic surgery, a single incision of the skin was made at dorsal, transversally across the lumbar region, making both ovaries accessible. The ovary-attached fat pad was grasped and pulled out with the ovary following. Under microscope at 2 X magnification a small incision was made in the bursa which then was peeled back over the surface of the ovary, allowing removal of the whole ovary. Both native recipient ovaries were removed and one half of a donor-ovary was placed into only the right bursa of the recipient mouse. Thus, ovaries from 1 donor was enough for transplanting 4 recipients.

Breeding:
The transplanted mice were observed at standard conditions for 32 days. They were then mated with a reproductive male of the strain CAST/Ei (Jackson Laboratory, Bar Harbor, Maine). They were mated in 2 - 3 periods to allow for more significant evaluation of reproducibility, since it is common that the birth rate per mating is lower at the first mating period than at subsequent periods (*Gunasena et al, 1997a*). The offspring were counted 12 - 72 h after delivery and at weaning. The numbers of offspring given in results are from the counting at weaning. The pups were mated with eachother and the size of the F2 litters was determined at weaning.

Typing of the hemoglobin ß-chain (Hbb):
Whole blood (50 µl) was taken from the tip of the tail of each offspring mouse at 3 weeks of age. The blood was diluted with water (0.5 ml), and sample buffer (10 µl of 0.5 M cystamine (Sigma, St. Louis, MO), 0.01 M DTT, 0.74 M NH_4OH) was added. The Hbb pattern was analyzed with polyacrylamide gel electrophoresis (T = 5 %, C = 3 %) at 50 V in electrophoresis buffer (0.025 M Tris(hydroxymethyl)aminomethane, 0.19 M glycin pH 8.6) using the Mini-Protean II Electrophoresis System (BIO-RAD Laboratories, Richmond, VA). Under these conditions the relative migration distance of the Hbb from the donor (BALB/cByJ) as compared to the Hbb from the recipient (C57BL/6By) is approximately 80%, allowing unambiguous typing. Having also Hbb from CAST/Ei in the blood sample does not interfere with the distinguishing between BALB/cByJ Hbb and C57BL/6By Hbb (Figure 1).

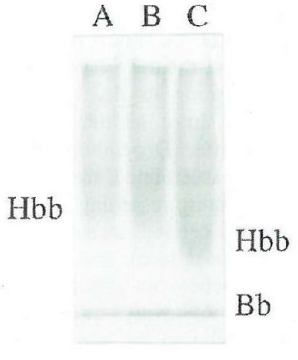

Figure 1. Electrophoresis pattern of the hemoglobin β-chain (Hbb). A = CAST/Ei, B = donor (BALB/cByJ), C = recipient (C57BL/6By). Bb = Bromphenol blue.

Results

Table 1 shows the outcome from the first two mating periods for each transplanted mouse. All transplanted mice produced at least one litter. The mean litter size for all the transplanted mice from the first two mating periods was 6.0 ± 2.7 (mean ± SD) offspring per litter. For untransplanted BALB/cJ the mean litter size is reported to be 5.2 (*Green & Witham, 1991*). The mean litter size for the transplanted mice when grouped by age of recipient and age of donor mice as 42-45 / 70 days, 95 / 70 days and 42 / 45 days (recipient age / donor age) was 6.0 ± 3.7, 6.4 ± 2.2 and 5.0 ± 2.3 (mean ± SD) offspring per litter, respectively. There was no significant difference in mean litter size between the age combinations ($p > 0.05$, *t*-test). The time from start of the mating period until delivery for the transplanted mice ranged between 17 and 54 days, with a mean of 28.9 ± 10.4 (mean ± SD) days. Laboratory female mice are receptive at intervals of 4 - 6 days with gestation periods of 19 - 20 days. For BALB/cJ only 47% of matings result in a delivery (*Green & Witham, 1991*). Thus, for the transplanted mice productive matings occurred within a normal time range of the breeding pair being together.

Eight of the 10 transplanted mice produced offspring that survived beyond 3 weeks. To determine the maternal origin of these offspring they were typed for hemoglobin ß-chain variant.

Table 1. Reproductive performance of the transplanted mice from the first two mating periods.

				First mating period			Second mating period		
ID of recipient	Age of recipient (days)	ID of donor	Age of donor (days)	ID of male	No live offspring	No dead offspring	ID of male	No live offspring	No dead offspring
1A*	42	1425	70	V	0	0	V	0	ND
2A	42	1425	70	V	0	0	Y	0	ND
3A	42	1425	70	Z	0	0	Z	1	1
4A	42	1425	70	Z	2	0	Y	9	0
1C	45	1426	70	X	8	0	X	8	1
1B	95	1426	70	V	6	0	V	0	ND
2B	95	1426	70	V	7	0	Z	3	0
3B	95	1426	70	V	6	1	V	9	0
2C	42	1431	45	X	7	0	V	0	0
3C	42	1431	45	Y	6	0	Y	2	0

* Euthanized by cervitcal dislocation at the first delivery due to delivery complications.
ND Whole litter dead. Number of dead offspring not determined.

Table 2 shows the maternal origin of the offspring from the 2 first mating periods, except for female 1B where the data are from the 3 first mating periods. 1B, but no one else, had 3 mating periods. For 6 of the 8 transplanted mice all their offspring, 50 mice, were derived from the transplanted ovaries. The 2 remaining transplanted mice produced 14 out of 15 and 12 out of 16 offspring mice originating from the donor ovary. A total of 5 offspring consequently represented the recipients natural progeny. In total 76 of the 81 surviving offspring mice, 94%, were derived from the transplanted ovaries. The offspring mice did not appear to have any defects and the mean size of the F2 litters from the first mating period of the offspring was 9.4 ± 2.2 (mean ± SD).

Table 2. Distribution of donor versus recipient maternal germ-line in the offspring.

ID recipient	Number of Offspring from donor ovary	Offspring from recipient ovary	Dead offspring
1A*	0	0	ND
2A	0	0	ND
3A	1	0	1
4A	11	0	0
1C	12	4	1
1B	13	0	ND
2B	10	0	0
3B	14	1	1
2C	7	0	0
3C	8	0	0
Total	76	5	ND

* Euthanized by cervical dislocation at the first delivery due to delivery complications.
ND Number of dead offspring not determined.

Discussion

This evaluation showed a very high performance of the ovary transplantation method using the protocol presented here, also compared to previously published techniques (*Stevens, 1957; Parkening et al, 1985; Brem et al, 1990; Cox et al, 1996; Gunasena et al, 1997a; Gunasena et al, 1997b*). All transplanted mice were reproductive and 94% of the offspring were derived from the transplanted ovaries. In addition, the mean litter size for the females was normal, despite the fact that the two native ovaries were replaced by only one half of an ovary. Previously reported percentages of ovary transplanted mice becoming pregnant or producing offspring are 33% (*Cox et al, 1996*), 48% (*Parkening et al, 1985*), 60% (*Brem et al, 1990*), 66% (*Gunasena et al, 1997b*), 80% (*Stevens, 1957*) and 100% (*Gunasena et al, 1997a*). These data were obtained after bilateral ovariectomy of the recipients and replacement with one half or two whole ovaries per female. The mean litter size for transplanted mice has previously been reported to be similar (*Cox et al, 1996*) or reduced (*Gunasena et al, 1997a*) compared to control mice.

One of the CAST/Ei males, Z, produced only small litters of 2 - 3 offspring. All the other males gave litters of 6 offspring or more, with the same females as the male Z had produced litters with. There was no apparent delay by the transplantation in the production of litter. Four of the transplanted mice had productive matings at the age of 9 weeks. The average age at first mating for female BALB/cJ is 8 weeks (*Green& Witham, 1991*). All the transplanted mice became pregnant within a normal time range after being put together with a male. From the first mating period 70% of the females delivered, whereas 90% delivered from the second mating period. These values are normal, especially since 2 - 3 females simultaneously were together with 1 male. The birth rate per mating result is similar to what is reported by Gunasena et al (1997a).

There is a risk of not completely removing the native ovaries during transplantation and thus of the transplanted mouse producing her natural progeny. Delivery of natural progeny occurred in 6% of the offspring. Four of these 5 endogenous offspring were delivered from one and the same female, suggesting that incomplete ovary removal

is infrequent. However, to be sure of the genotype of the offspring it is important to type the progeny for the maternal germ-line. This can often be performed easily with coat colour markers. With the protocol used, only one donor mouse is needed to provide ovary transplants to four recipient mice. Surplus ovaries can be frozen for transplantation at a later time. In summary, the ovary transplantation technique used proved to be an efficient tool regarding time, labour, cost and number of animals needed for the maintenance of mouse strains that do not breed but have functional ovaries.

Summary

There are mutant and transgenic mouse strains which lack the ability to breed, but where the females have functional ovaries. Ovary transplantation is an important tool for maintaining and producing crosses with these non-breeding strains. We have evaluated an ovary transplantation method by transplanting ovaries from females belonging to a non-reproductive BALB/cByJ mutant mouse strain. All transplanted mice, 10 BALB/c.C57BL/6By, produced offspring and 94% of the progeny originated from the transplanted ovaries. The mean litter size and the mating period needed for productive mating to occur were similar to what is observed for corresponding control mice.

Sammanfattning

Det finns mutanta och transgena musstammar som saknar förmågan att reproducera sig, men där honorna har fungerande äggstockar. Äggstockstransplantation är ett viktigt instrument för att behålla och skapa korsningar med dessa icke-reproduktiva stammar. Vi har utvärderat en äggstockstransplantationsmetod genom att transplantera äggstockar från honor tillhörande en icke-reproduktiv mutant musstam uppkommen i BALB/cByJ. Alla transplanterade möss, 10 BALB/c.C57BL/6By, gav avkomma och 94% av den härstammade från de transplanterade äggstockarna. Medelvärdet på kullstorlek och tidsåtgång av hane och hona tillsammans för produktiv parning var snarlika med värden för motsvarande kontroll möss.

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