SELECTION FOR IN VITRO DEVELOPMENTAL COMPETENCY OF PREIMPLANTATION ICR MOUSE EMBRYOS

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SUMMARY

Data from 332 female ICR mice were utilized to study in vitro developmental competency (IVDC; percentage of fertilized one cell embryos developing to blastocysts in vitro per female donor). A mean ± SD of 49.28% ± 31.0 was found, displaying a very large between female variation for this trait. The distribution of IVDC ranged from 0% to 100% competency. Two generations of replicated divergent selection for IVDC in ICR mice were carried out. No significant pattern of response was observed. Estimation of heritability by daughter - dam regression yielded an estimate of .12 ± .18, indicating that the large between female variation in IVDC is not of an additive genetic nature.

INTRODUCTION

In vitro culture of preimplantation embryos is a critical step in applying gene transfer and nuclear manipulation technologies to livestock. A culture system supporting development from one cell zygotes to blastocysts which are capable of development to term following transfer to recipient mothers is required. Unfortunately, an efficient in vitro system does not yet exist for most domestic livestock. Cow and sheep embryos, when cultured in vitro from the one cell stage, exhibit a block to development at the 8 to 16 cell stage (Wright and Bondioli, 1981; Camous et al., 1984), while pig embryos block at the 4 cell stage (Herrmann and Holtz, 1981). Extensive studies with mouse embryos have led to the present capabilities of sustaining embryonic development in vitro from one cell to blastocyst stages in certain inbred lines and some of their F1 crosses (Brinster, 1965; Whitten and Biggers, 1968; Gwatkin, 1972). However, in other strains embryos block at the 2 to 8 cell stage, a phenomenon commonly referred to as the "two cell block" (Goddard and Pratt, 1983). In addition, within some mouse strains variation is exhibited in the ability of embryos to develop to blastocysts. The variation in in vitro developmental competency (IVDC) between and within strains makes the mouse a useful model for study of the genetics of developmental competence.

Between donor female variability in IVDC has been observed in our laboratory when working with mice from the outbred ICR strain. The objectives of the present research were to 1) measure between female variance and 2) attempt to select for IVDC to determine if this trait is heritable.

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EXPERIMENTAL PROCEDURE

Evaluation of In Vitro Developmental Competency

Mice were housed under controlled light:dark cycle (14:10) with feed and water available ad libitum. Sixteen wk uniparous ICR females were caged with random ICR males with successful matings determined by the presence of a vaginal plug. Females were sacrificed by cervical dislocation the day of mating. Oviducts were removed and placed in Whitten's media (Whitten, 1971) containing Hepes buffer (20 mM), reduced $NaHCO_3$ (0.2 mM) and bovine serum albumin (BSA; 3 mg/ml). One cell embryos enclosed in a cumulus cell mass were obtained by incising the oviductal wall at the embryo-cumulus bulge. Cumulus cells were removed by treatment with hyaluronidase (1 mg/ml Whittens-Hepes) and transferred to pre-equilibrated microdrops (50 μl) of Whitten's media (3 mg/ml BSA) under saline saturated paraffin oil. Embryos were cultured in a high humidity incubator with 5% CO_2 in air at 37° C. Every 24 hr embryos were observed under a dissecting microscope and stage of development recorded. After 96 to 120 hr embryos were classified as blastocysts or blocked (2 to 8 cell stage). Competency was measured as percentage of fertilized one cell embryos developing to blastocysts in vitro per female donor. As a control for the culture procedure, females from the competent C57BL/6J X ICR cross were cultured and evaluated each day in the same manner as the ICR females.

Experiment 1 - Parameters of IVDC in a Large ICR Population

Two non-contemporary replicates were conducted to measure mean and variance of IVDC in a large population of ICR females, using 147 and 185 females in the first and second replicates, respectively. Means and standard deviations were 48.18% $\pm$ 30.64 and 50.16 $\pm$ 31.29, showing a large, repeatable between female variation for this trait. A frequency histogram displaying the distribution of IVDC for the two replicates pooled is presented in Figure 1. A relatively flat distribution is observed, with values ranging from 0 to 100% competency.

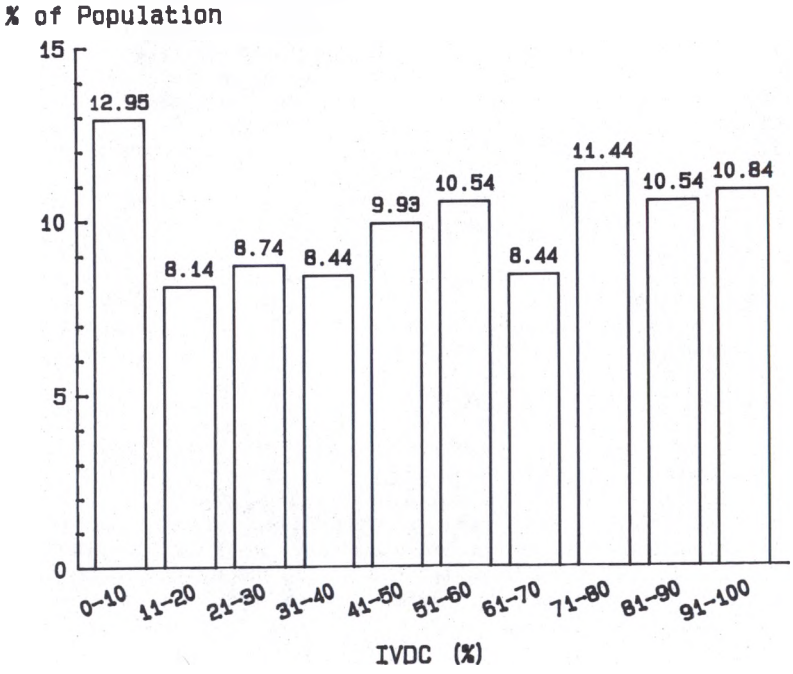
Experiment 2 - Selection for In Vitro Developmental Competency

Two generations of divergent selection for IVDC were carried out. A base population (GEN0) of 84 ICR females were randomly mated to 42 ICR males. Litters were standardized at birth to five females and four males, toe notched at 12 D and weaned at 21 D. At 16 wk of age, GEN0 females were evaluated for IVDC and randomly allocated to two replicates of 42 females each. Eleven females in each replicate were randomly selected to form control lines (1^0,2^0). Of the remaining 31 females in each replicate, 11 with the highest competency were selected to form upward lines (1^+,2^+), and 11 with the lowest competency were selected to form downward lines (1^-,2^-). Litters of the selected females became Generation 1 (GEN1). Within each line of GEN1, females and males were mated (two females per male, avoiding full sib mating) with the males taken from litters of the best five or six selected GEN0 females in an attempt to increase selection intensity. These matings produced litters which later became Generation 2 (GEN2). At 16 wk GEN1 females were evaluated for IVDC and 10 females were selected in each of the upward and downward lines. In control lines 10 females were selected at random. GEN2 mice were mated in the same manner as GEN1 mice and the litters produced were discarded after weaning. At 16 wk GEN2 females were evaluated for IVDC.

Weighted selection differentials for GEN0 and GEN1 were obtained by taking the deviation of the mean of the selected females from the mean of the population, weighted by the number of progeny produced by each female which contributed to the following generations (Becker, 1975). Standardized selection intensities were calculated by dividing weighted selection differentials by the standard deviation of the population.

Realized heritabilities for upward and downward selection lines were calculated by dividing cumulative response to selection (as a deviation from control) by cumulative weighted selection differential (Falconer, 1981). Heritability of IVDC in the base population was estimated by daughter - dam regression with the dam's record repeated for each of her daughter's records (Kempthorne and Tandon, 1953). The entire data set was utilized regardless of replicate according to the model: $Co = \mu + Gdh + Li + bCd + e$, where Co = IVDC of offspring; μ = overall means; Gdh = effect of dam's generation (h = 0,1); Li = effect of line (i = 1-6); Cd = IVDC of dam; e = residual error. The regression coefficient and its standard error were doubled to estimate heritability.

FIGURE 1. DISTRIBUTION OF IN VITRO DEVELOPMENTAL COMPETENCY (IVDC) (332 ICR FEMALE MICE)



RESULTS

Selection for In Vitro Developmental Competency

Weighted selection differentials and standardized selection intensities for GEN0 and GEN1 are presented in Table 1. The rather large selection intensities in the control lines may be due in part to the small sample size of each of the lines.

TABLE 1. WEIGHTED SELECTION DIFFERENTIALS (S, % IVDC) AND STANDARDIZED SELECTION INTENSITIES (i)

	GEN0		GEN1	
Line	S	i	S	i
1^+	44.63	1.43	29.83	1.17
2^+	31.59	0.98	35.27	1.06
1^-	-25.00	0.80	-36.48	1.27
2^-	-27.00	0.84	-38.91	1.30
1^o	0.41	0.01	-4.71	0.14
2^o	-5.77	0.18	-3.59	0.13

Selection line means, response to selection (as a deviation from control) and realized heritabilities for selected lines are presented in Table 2. GEN1 means display a random distribution around 50% competency with slightly negative responses to selection in both the upward and downward lines. GEN2 means are slightly higher, as are the responses to selection. However, no real pattern of response is visible except in line 2^-, where a consistent negative response is observed. Realized heritabilities in the selected lines are very low, except in line 2^- where a higher heritability of 0.14 is observed.

Estimation of heritability of IVDC in the base population by regression of daughter on dam yielded a regression coefficient of 0.06 ± 0.09, which, when doubled, yields a heritability estimate of 0.12 ± 0.18. Essentially, this heritability estimate is not different from 0.

Culture Control

Embryos from the competent C57BL/6J X ICR cross developed to blastocysts at a consistent rate of 90 to 100% in the two experiments, demonstrating that proper conditions for mouse embryo culture existed throughout. However, GEN0 competency mean of the ICR population is significantly lower than in GEN1 and GEN2. One possible explanation for this may be that in GEN0 only one investigator was evaluating IVDC, while in GEN1 and GEN2 two investigators were simultaneously evaluating IVDC, and time between sacrifice and culture was significantly reduced.

TABLE 2. IVDC MEANS[a] (%), RESPONSE TO SELECTION[b] (R, % IVDC) AND REALIZED HERITABILITIES (h^2R)

	GEN1				GEN2				
Line	Mean ± SD	(n)	R		Mean ± SD	(n)	R	h^2R	
1^+	46.7 ± 25.4	(22)	-5.93		57.1 ± 33.3	(27)	4.11	-0.02	
2^+	47.5 ± 33.3	(26)	-1.54		56.6 ± 30.9	(34)	5.06	0.07	
1^-	51.1 ± 28.8	(25)	-1.52		60.8 ± 31.6	(33)	5.39	-0.06	
2^-	47.9 ± 33.3	(26)	-1.18		44.1 ± 30.0	(31)	-8.06	0.14	
1^o	52.6 ± 34.6	(20)	--		53.0 ± 27.5	(27)	--	--	
2^o	49.1 ± 29.9	(27)	--		53.3 ± 27.2	(32)	--	--	

[a]GENO means were (REP1) 25.0 ± 31.2 (42), (REP2) 27.0 ± 32.1 (42).

[b]Calculated as a deviation from control.

DISCUSSION

A large between female variation in IVDC exists in our ICR mouse stock. This variation encompasses the entire range (0-100%) of possible competency rates. Previous embryo culture work has suggested randomizing embryos to treatments from a common pool, avoiding bias "due to variations in the quality of embryos from different females" (Whittingham, 1971). While pooling embryos from many female donors may succeed in distributing the variation among experimental treatments, it will provide information on the effect of the treatments only for the population as a whole, while masking the effect on individual females. Blocking treatments on female may be a better method when dealing with mouse stocks which portray a large between female variation in IVDC, as it would supply information pertaining to individual females as well as to the population as a whole.

Selection for IVDC was unsuccessful, with heritability estimates essentially not different from 0. It is concluded that the large between female variation in the population is not of an additive genetic nature. The ability for one cell ICR embryos to develop to blastocysts in vitro does exist, as is evidenced by the many females with 100% competency. The cause for the large variation may lie in environmental variance in the IVDC evaluation procedure. Major sources of between female environmental variance in the procedure are time of ovulation and time of fertilization. Thus, the time ovulated ova and fertilized embryos reside in the oviduct prior to evaluation of IVDC may significantly vary between females. The oviduct, being the natural environment for the preimplantation embryo, plays an important role in the embryos development. The length of time embryos are exposed to their natural oviductal environment may affect their ability to develop in vitro.

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