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Divergent selection for in vitro developmental capacity of preimplantation mouse embryos

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Summary. Replicated divergent selection was conducted for two generations in ICR mice for in vitro developmental capacity (IVDC; percentage of fertilized one-cell zygotes developing to blastocysts in vitro per female donor). Realized heritabilities based on high and low selection were 0.03 ± 0.08 and -0.11 ± 0.09 in replicate 1, and 0.10 ± 0.11 and 0.08 ± 0.10 in replicate 2. No differences were detected between selection lines ($P > 0.2$) or replicates ($P > 0.1$). Estimate of heritability in the base population based on 332 daughter-dam pairs was 0.14 ± 0.18. These results indicate that additive genetic variance contributes little to the phenotypic variance in this trait. Considerable phenotypic variation in IVDC was observed (mean = 49.3; SD = 31.0), with a range of IVDC from 0%–100%. Utilization of donor female as a blocking factor is suggested for designs of experiments with preimplantation embryos to increase precision and power of statistical analyses.

Key words: Selection – Embryo development – Embryo culture – Mice

Introduction

The ability to sustain development of preimplantation mouse embryos in vitro is important for basic research on the requirements and processes of early development and differentiation (Brackett 1981). In addition, in vitro culture of preimplantation embryos is of practical importance for the application of new reproductive biotechnologies to domestic livestock production (Rutledge and Seidel 1983). Of immediate significance is the refinement of in vitro fertilization, cryopreservation and embryo transfer techniques. Other less developed technologies, such as oocyte maturation, gene transfer, nuclear transfer and cloning, also rely on in vitro embryo culture, and possess potential for improvement of food and fiber production in the future.

Optimally, an embryo culture system should support the development of one-cell fertilized zygotes to blastocysts that, when transferred to recipient mothers, are capable of normal development to term. Such culture systems have been developed for rabbit (Kane 1972), human (Edwards et al. 1981) and some inbred strains of mice and their F_1 crosses (Whitten and Biggers 1968). Unfortunately, relatively efficient in vitro culture systems do not yet exist for embryos of most classes of domestic livestock. Cow and sheep embryos, when cultured 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). Hamster embryos block at the 2-cell stage (Bavister et al. 1983), as do embryos from many inbred and random bred strains of mice (Whitten and Biggers 1968; Goddard and Pratt 1983). This phenomenon is commonly referred to as the "2-cell block to in vitro development" (Goddard and Pratt 1983). The cause of this in vitro defect is not well understood, although it has been determined to be of a cytoplasmic nature (Muggleton-Harris et al. 1982; Goddard and Pratt 1983).

The between-strain variability in development of preimplantation mouse embryos in vitro, as well as individual variation within strain (Pomp et al. 1988), affords

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the opportunity to utilize the mouse as a pilot organism for genetic studies of in vitro developmental capacity (IVDC; percentage of fertilized one-cell embryos developing to blastocysts in vitro per female donor). An understanding of the genetic control governing in vitro embryonic development would be useful in attempts to achieve successful IVDC across strains and species. The objectives of the present research were to utilize genetic selection procedures to quantify the additive genetic variance, estimate the degree of heritability, and analyze the among female variation of in vitro developmental capacity of preimplantation mouse embryos.

Materials and methods

Experimental animals

The base population for this study was formed from the ICR mouse stock, which has been maintained as a random bred stock since its synthesis. ICR mice were utilized because they possess large genetic variation and excellent reproductive capabilities. Mice were maintained at the Genetics Research Laboratory of the University of Wisconsin – Madison, and were housed in cages with pine shavings. A standard mouse diet (Wayne 8604 Lab Blox; distributed by Waldschmidt and Sons, Madison WI) and tap water were supplied ad libitum. Room temperature was maintained at 21 ± 2°C with a controlled light:dark cycle (14:10). Litters were standardized to nine pups (five females and four males) 1 day after birth, identified by toe-clipping at 12 days and weaned at 21 days.

Evaluation of in vitro developmental capacity (IVDC)

All embryos utilized in this study were harvested from 16 week uniparous females following natural mating. Two females were caged with one male, with successful matings determined by the presence of a copulation plug. Females were euthanized by cervical dislocation on the day of mating between 12:00 and 14:00. Oviducts were removed and placed in modified Whitten's medium (Whitten 1971) containing HEPES buffer (4.76 mg/ml), reduced $NaHCO_3$ (0.17 mg/ml), and bovine serum albumin [BSA, Fraction V (Sigma, St. Louis/MO); 3 mg/ml]. One-cell zygotes were removed from the oviducts, freed of cumulus cells by short exposure to hyaluronidase (1 mg/ml Whittens-HEPES) and transferred individually to separate microdrops (50 µl) of Whitten's medium. Unfertilized eggs and two-cell embryos were discarded, but were counted with one-cell zygotes to determine ovulation rate. Microdrops were covered with saline-equilibrated paraffin oil, and embryos were cultured in a high humidity incubator with 5% CO_2 in air at 37°C. Every 24 h embryos were examined under a dissecting microscope (60×) and scored for stage of development. After 96–120 h, in vitro developmental capacity (IVDC) was measured as the percentage of fertilized one-cell zygotes developing in vitro to blastocysts per female.

Selection procedures

Two generations of divergent mass selection for IVDC were carried out. The design of selection procedures is summarized in Fig. 1. A base population of 84 non-full sib 6–8 week old ICR female mice (GEN0) were mated to 42 random ICR males. Following weaning, litters were retained for future use. When GEN0 females reached 16 weeks of age, they were randomly allocated to 2 replicates of 42 females each and evaluated for IVDC.

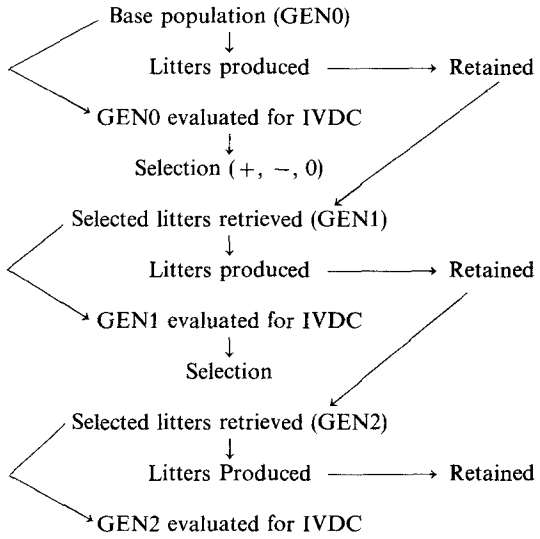

Fig. 1. Experimental design of selection for IVDC in ICR mice

Table 1. Genetic line designations and selection criteria

Line designation	Selection criteria	
1^+	High IVDC[a],	Replicate 1
2^+	High IVDC,	Replicate 2
1^-	Low IVDC,	Replicate 1
2^-	Low IVDC,	Replicate 2
1^0	Control,	Replicate 1
2^0	Control,	Replicate 2

[a] Percentage of fertilized one-cell embryos developing to blastocysts in vitro per female donor

Within each replicate, litters were randomly selected from 11 females to form control lines (1^0, 2^0). Of the remaining 31 litters, 11 from females with the highest IVDC were selected to form high selection lines (1^+, 2^+) and 11 from females with the lowest IVDC were selected to form low selection lines (1^-, 2^-). Genetic line designations and selection criteria are presented in Table 1.

Selected litters of GEN0 females were designated as generation 1 (GEN1). Within each line, 6–8 weeks old females and males were mated at random, avoiding full sib mating. These matings produced litters that were retained for future use. At 16 weeks of age, GEN1 females were evaluated for IVDC and litters from 10 females were selected in each of the high and low lines. In control lines, litters were randomly selected from 10 females. Selected litters were designated as generation 2 (GEN2).

GEN2 mice were mated in the same manner as in GEN1, and the litters produced were retained. At 16 weeks of age, GEN2 females were evaluated for IVDC.

Statistical analysis

Selection differentials were calculated as the deviation of the mean of selected females from the population (replicate for GEN0, selection line for GEN1) mean. Parental contributions to the selection differential were weighted by the number of progeny that were measured in the following generations (Becker 1975). Standardized selection intensities were obtained by divid-

ing weighted selection differentials by the phenotypic standard deviation of the corresponding population.

Realized heritabilities in selected lines were estimated as the ratio of total response to cumulative weighted selection differential, and corresponding standard errors were calculated according to the method of Hill (1971, 1972). Response to selection was calculated as a deviation from control (within replicates) to remove environmental sources of variation (Falconer 1981).

Least-squares procedures for unequal subclass numbers (Harvey 1979) were utilized to test for differences between selection lines (pooled across replicates) and between replicates. To adjust for heterogeneity of variance of data generated as proportions and for differing numbers of embryos donated per female, IVDC rates were transformed to weighted angles (Freeman and Tukey 1950) before analysis. The statistical model included an overall mean, a fixed selection line (pooled) effect, a random replicate within line effect, a fixed generation effect, a line × generation interaction, a generation × replicate within line interaction, and a random residual. The replicate within line mean square was used as an error term for testing selection lines. All other mean squares were tested by the residual mean square.

Heritability of IVDC in the ICR base population was estimated by daughter-dam regression within generation and line, with the dam's record repeated for each of her daughters' records (Bohren et al. 1961). Rates of IVDC were transformed to weighted angles before analysis by least-squares procedures for unequal subclass numbers. Heritability of IVDC in the base population was estimated as twice the regression coefficient of daughter on dam, and the standard error of this estimate was calculated as twice the standard error of the regression coefficient.

Table 2. Means and SD of IVDC[a] and ovulation rate for selection lines[b] (GEN1, GEN2)[c]

Line	GEN	No. females	Ovulation rate	SD	IVDC (%)	SD
1^+	1	22	14.5	2.5	45.9	25.4
	2	27	13.1	2.2	54.6	33.3
1^-	1	26	14.3	3.1	48.4	28.8
	2	35	12.8	3.4	58.9	31.5
1^0	1	20	14.2	2.2	54.3	34.5
	2	27	13.9	2.9	52.0	27.5
2^+	1	27	13.9	3.6	45.2	33.3
	2	33	12.7	3.7	58.7	30.8
2^-	1	26	14.7	2.6	46.8	33.3
	2	32	13.8	3.2	46.1	30.0
2^0	1	26	14.0	2.4	49.3	29.8
	2	31	12.4	2.9	51.9	27.2

[a] Percentage of fertilized one-cell embryos developing to blastocysts in vitro per female donor

[b] Lines are defined as follows: (1) first replicate, (2) second replicate; (+) high IVDC, (−) low IVDC, (0) control

[c] Means ± SD for the base population (GEN0) were 25.0 ± 31.2 and 27.0 ± 32.1 for IVDC and 14.4 ± 4.1 and 12.9 ± 3.7 for ovulation rate in the first and second replicates, respectively

Results

Means and standard deviations of ovulation rate and IVDC in the base (GEN0) population and generation means and standard deviations for GEN1 and GEN2 are presented in Table 2. There were no significant differences in IVDC among selection lines ($P > 0.2$) or replicates ($P > 0.1$). Line × generation interaction was significant ($P < 0.05$). There was approximately a twofold increase in IVDC rates between GEN0 and subsequent generations. This may have been due, in part, to improved embryo culture techniques between these time periods. For example, mean elapsed time between removal of oviducts and placement of embryos in the incubator was 32 ± 8 min in GEN0, while in GEN1 and GEN2 this interval was reduced to 22 ± 6 min. A significant negative phenotypic correlation was detected between this elapsed time and IVDC rates ($r = -0.094$, $P = 0.05$, $df = 406$), corrected for line and generation effects. While the actual explanation for this phenomenon cannot be known with certainty, the use of unselected control lines protected the experiment from bias by such environmental fluctuations.

Ovulation rates fluctuated slightly throughout the study. From GEN0 to GEN1, ovulation rates were stable in replicate 1, while an increase of 1 to 2 eggs was observed in lines of replicate 2. In contrast, all selection lines in both replicates exhibited decreased ovulation rates of 1 to 2 eggs from GEN1 to GEN2. These fluctuations were consistent across selection treatments and controls, and are attributed to random environmental trends rather than to a possible correlated response to selection for IVDC. Interestingly, a negative phenotypic correlation was detected between ovulation rate and IVDC ($r = -0.10$, $P = 0.07$), utilizing data from 332 females from GEN1 to GEN2 corrected for line and generation effects. While this negative relationship may be due to an unknown biological phenomenon, it should be pointed out that increased ovulation rate led to increased time during which embryos were handled prior to culture. Thus the negative phenotypic correlation between ovulation rate and IVDC may be environmental in nature.

A large phenotypic variance in IVDC was observed within lines and generations. A frequency distribution of IVDC over GEN1 and GEN2 is presented in Fig. 2. Mean IVDC was 49.3% with a SD of 31.0. A nearly uniform distribution was observed encompassing the range of 0%–100%. The weighted angular transformation reduced the degree of between female variability, and allowed for a more normal distribution of IVDC (Fig. 2).

Weighted and standardized selection differentials by generation and cumulative are presented in Table 3. Because of large phenotypic variation in IVDC, large selection differentials were obtained despite moderate selection intensities. The slight selection intensity in the control lines may have been due to random sampling variance. While the design of this study was expected to

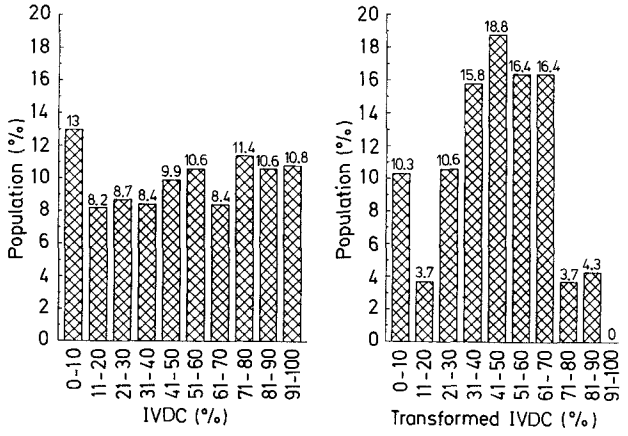

Fig. 2. Frequency distribution of IVDC and transformed (weighted angular) IVDC for 332 ICR mice

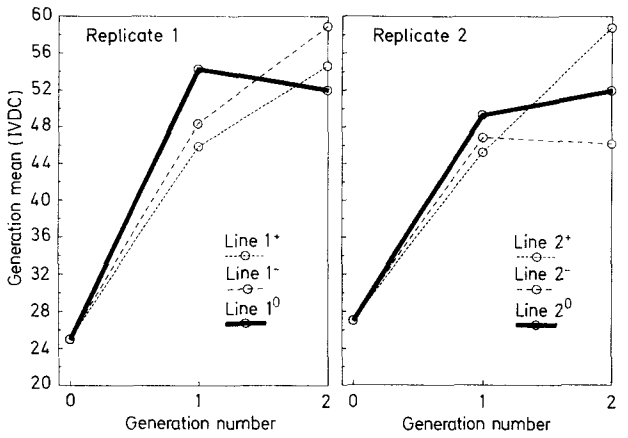

Fig. 3. Generation means versus generation number in selection for IVDC in ICR mice

yield approximately 40 females per line each generation for evaluation of IVDC, the realized treatment sizes were lower (20–35 females). Several phenomena contributed to this, including infertile matings, possible unobserved copulation plugs, and matings that resulted in harvest of only two-cell embryos at time of evaluation. This lower yield of evaluated females probably limited possible increases in selection intensity.

Responses to selection and realized heritabilities are presented in Table 4. Little response was observed and realized heritabilities were low. Generation means are plotted against generation number in Fig. 3. In replicate 1, the low line (1^-) exhibited a greater IVDC than that of the high line (1^+). Replicate 2 showed a more expected pattern of response, with a cumulative divergence of 12.6% IVDC between the high and low lines, and realized heritabilities near 0.1 in each line.

Regression of daughter on dam, utilizing 332 pairs of records of IVDC, yielded a heritability estimate of 0.14 ± 0.18 for IVDC in the base population.

Table 3. Weighted selection differentials (S) and standardized selection intensities (i) for IVDC[a]

Line	GEN0		GEN1		Cummulative
	S	i	S	i	S
1^+	44.6	1.43	29.8	1.17	74.4
1^-	−25.0	−0.80	−36.4	−1.27	−61.4
1^0	0.4	0.01	− 4.7	−0.14	− 4.3
2^+	31.5	0.98	35.2	1.06	66.7
2^-	−27.0	−0.84	−38.9	−1.30	−65.9
2^0	− 5.7	−0.18	− 3.5	−0.13	− 9.2

[a] Percentage of fertilized one-cell embryos developing to blastocysts in vitro per female donor

Table 4. Response to selection for IVDC[a] (R) as deviation from control and realized heritabilities (h^2R)

Line	R			$h^2R \pm SE$
	GEN1	GEN2	Cummulative	
1^+	−8.4	11.0	2.6	0.03 ± 0.08
1^-	−5.9	12.8	6.9	-0.11 ± 0.09
2^+	−4.1	10.9	6.8	0.10 ± 0.11
2^-	−2.5	− 3.3	−5.8	0.08 ± 0.10

[a] Percentage of fertilized one-cell embryos developing to blastocysts in vitro per female donor

Discussion

Response to selection

Two generations of divergent selection for in vitro developmental capacity of preimplantation ICR mouse embryos resulted in little or no response in either the high or low selection lines. Heritability of IVDC from daughter-dam regression was estimated as 0.14 ± 0.18, indicating that this trait is not very heritable in the ICR mouse stock. Realized heritabilities supported this finding, with values not significantly different from zero. The short duration and moderate selection pressure may not represent the true genetic nature of IVDC, thus estimates of genetic variation and heritability may not be accurate. However, the heritability estimate from daughter-dam regression was based on 332 pairs of IVDC records, and the agreement of estimated and realized heritabilities provides support for the findings of this study.

From these results it is concluded that additive genetic variation contributes little to the phenotypic variation in IVDC for the ICR mouse stock. To the authors' knowledge, this is the first attempt to quantify the within-strain genetic variance of in vitro preimplantation embryonic development in any mammalian species. The lack of ad-

ditive genetic variation is somewhat surprising, considering the large degree of phenotypic variation (SD = 31.0) in IVDC observed in this and other studies (Pomp et al. 1988). Rates of IVDC followed a nearly uniform distribution encompassing the range of 0%–100%. However, the design of this experiment did not allow for the estimation of non-additive genetic variation in IVDC, which may have been a significant factor considering that traits related to fitness (e.g., reproduction) often have a large, non-additive genetic component. In addition, it is possible that a high degree of non-genetic variation may be masking any underlying genetic influences, rendering them difficult to quantify. It is also possible that the percentage of embryos developing to blastocysts for a given female is not a true indication of the developmental capacity of all embryos derived from that female from several ovulations, and that inherent variability may exist in IVDC from cycle to cycle (i.e., the repeatability of IVDC is unknown). While no information is yet available on repeatability of IVDC, a low value would limit the usefulness of this trait as a criterion of selection.

Mouse husbandry, age of donor female and embryo culture conditions were relatively constant throughout the selection experiment. As no superovulation techniques were employed, time of ovulation, mating and fertilization may have varied widely. Gates (1965) reported an appreciable degree of developmental variability among embryos from females of the same strain mated overnight, suggesting that this may be due to differences between females in time of ovulation and time of coitus. Dickson (1967) suggested a similar explanation after observing non-uniform blastocyst development in individual mice of the Swiss Webster albino strain. Auerbach and Brinster (1968) theorized that variation in the age of embryos at time of culture may have been the cause of variation in response observed in their research on effects of oxygen concentration on preimplantation embryonic development in mice. Chisolm et al. (1985) reported that as much as 7–8 h of within strain asynchrony among embryos at first cleavage may result from differences in timing of ovulation and/or fertilization. Thus, the time ovulated oocytes and fertilized embryos reside in the oviduct prior to culture may vary substantially among females. The oviduct plays an active and important role in preimplantation embryonic development (Whittingham 1968; Reinius 1970; Glass 1981; Nieder and Corder 1983). Embryos may acquire the ability to develop in vitro as a result of prolonged exposure to the oviductal environment prior to culture at the one-cell stage. This possibility, while entirely an environmental influence on the embryo, is under control of the maternal genotype, and would fit within the framework of the model of maternal-cytoplasmic control of in vitro developmental capacity of preimplantation mouse embryos (Muggleton-Harris et al. 1982; Goddard and Pratt 1983). Perhaps if efforts to control timing of ovulation had been employed in the present study, stronger results may have been obtained.

Implications of results

Large phenotypic variance in IVDC for the ICR stock emphasizes the importance of proper experimental design in preimplantation embryo studies. Biggers and Brinster (1965) suggested harvesting embryos from all donor females of a given genotype in a common pool, in order to equalize potential developmental variation due to inherent differences between mice and non-synchronous ovulatory responses to superovulation. Whittingham (1971) claimed that for comparative experiments, it is essential to randomize embryos to treatments from a common pool, due to variation in the quality of embryos from different females. These methods attempt to evenly distribute phenotypic variation among experimental treatments. However, they do not remove among-female variation from the variance due to experimental error, and this may reduce the precision of the analysis. An alternative design, especially for species that ovulate a large number of oocytes such as mice, rats and pigs, and for other species where females can be superovulated, is to utilize "donor female" as a blocking factor. The effect of this in the analysis of variance is to place as large a portion as possible of variance due to female heterogeneity in the variance between blocks, which is eliminated from the experimental error (Cochran and Cox 1957). Any remaining variation among donor females can be randomized by allocating embryos from each female randomly among treatments, so as to provide a valid estimate of the errors to which the experiment is liable (Fisher 1935). This design is more precise than pooling embryos from all donor females, and will provide for a more powerful statistical analysis. In addition, more information is obtained from the same raw materials. When embryos are pooled, we obtain information regarding the effects of experimental treatments on embryos for a general population only.

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References

Auerbach S, Brinster RL (1968) Effect of oxygen concentration on the development of 2-cell mouse embryos. Nature 217: 465–466

Bavister BD, Leibfried ML, Lieberman G (1983) Development of preimplantation embryos of the Golden Hamster in a defined culture medium. Biol Reprod 28:235–247

Becker WA (1975) Manual of quantitative genetics, 4th edn. Washington State University Press, Pullman WA, p 7

Biggers JD, Brinster RL (1965) Biometrical problems in the study of early mammalian embryos in vitro. J. Exp Zool 158:39–47

Bohren BB, McKean HE, Yamada Y (1961) Relative efficiencies of heritability estimates based on regression of offspring on parent. Biometrics 17:481–491

Brackett BG (1981) In vitro culture of the zygote and embryo. In: Mastroianni L Jr, Biggers JD (eds) Fertilization and embryonic development in vitro. Plenum Press, New York

Camous L, Heyman Y, Meziou W, Menzio Y (1984) Cleavage beyond the block stage and survival after transfer of early bovine embryos cultured with trophoblastic vesicles. J Reprod Fertil 72:479–485

Chisolm JC, Johnson MH, Warren PD, Flemming TP, Pickering SJ (1985) Developmental variability within and between mouse expanding blastocysts and their ICMs. J Embryol Exp Morphol 86:311–336

Cochran WG, Cox GM (1957) Experimental Designs, 2nd edn. Wiley, New York

Dickson AD (1967) Variations in development of mouse blastocysts. J Anat 101:263–267

Edwards RG, Purdy JM, Steptoe PC, Walters DE (1981) The growth of human preimplantation embryos in vitro. Am J Obstet Gynecol 141:408–416

Falconer DS (1981) Introduction to quantitative genetics. 2nd edn. Longman, New York

Fisher RA (1935) The design of experiments. Hafner, New York, 1971

Freeman MF, Tukey JW (1950) Transformations related to the angular and to the square root. Ann Math Stat 21:607–611

Gates AH (1965) Rate of ovular development as a factor in embryonic survival. In: Wolstenholme GEW (ed) Preimplantation stages of pregnancy. Churchill, London, pp 270–293

Glass LE (1981) Transmission of maternal proteins into oocytes. Adv Biosci 6:29–58

Goddard MJ, Pratt HPM (1983) Control of events during early cleavage of the mouse embryo: an analysis of the "2-cell block". J Embryol Exp Morphol 73:111–133

Harvey WR (1979) Least squares analysis of data with unequal subclass numbers. USDA ARS H-4, Beltsville

Herrmann HH, Holtz W (1981) Culture of pig embryos collected in situ or after slaughter. Anim Reprod Sci 4:143–147

Hill WG (1971) Design and efficiency of selection experiments for estimating genetic parameters. Biometrics 27:293–311

Hill WG (1972) Estimation of realized heritabilities from selection experiments: I. Divergent selection. Biometrics 28:747–765

Kane MT (1972) Energy substates on culture of single cell rabbit ova to blastocysts. Nature 238:468

Muggleton-Harris A, Whittingham DG, Wilson L (1982) Cytoplasmic control of preimplantation development in vitro in the mouse. Nature 299:460–461

Nieder GL, Corder CN (1983) Pyruvate and lactate levels in oviducts of cycling, pregnant and pseudopregnant mice. Biol Reprod 28:566–574

Pomp D, Critser ES, Rutledge JJ (1986) Selection for in vitro developmental competency of preimplantation ICR mouse embryos. Proc 3rd World Congr Genet Appl Livestock Prod, Lincoln NE, 12:109–114

Pomp D, Critser ES, Rutledge JJ (1988) Lower sodium-lactate levels in Whitten's medium improves in vitro developmental capacity of one-cell mouse embryos. Theriogenology 29: 1019–1025

Reinius S (1970) Morphology of oviduct, gametes, and zygotes as a basis of oviductal function in the mouse. I. Secretory activity of oviductal epithelium. Int J Fertil 15:191

Rutledge JJ, Seidel GE Jr (1983) Genetic engineering and animal production. J Anim Sci 57:265–271

Whitten WK (1971) Nutrient requirements for the culture of preimplantation embryos. Adv Biosci 6:129–140

Whitten WK, Biggers JD (1968) Complete development in vitro of the preimplantation stages of the mouse in a simple chemically defined medium. J Reprod Fertil 17:399–401

Whittingham DG (1968) Development of zygotes in cultured mouse oviducts. II. The influence of the estrous cycle and ovarian hormones upon the development of the zygote. J Exp Zool 169:399–406

Whittingham DG (1971) Culture of mouse ova. J Reprod Fertil 14:7–21

Wright RW, Bondioli KR (1981) Aspects of in vitro fertilization and embryo culture in domestic animals. J Anim Sci 53: 702–709