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Source: Zoological Science, 15(3) : 359-362

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.15.359

ZOOLOGICAL SCIENCE **15**: 359–362 (1998)

Influence of the Preimplantation-Embryo-Development (*Ped*) Gene On Mouse Blastocyst Differentiation

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ABSTRACT—Mouse preimplantation embryonic cleavage rate is dependent upon the presence or absence of the Preimplantation-embryo-development (*Ped*) gene; which is linked to the Qa-2 subregion of the H-2 complex. Expression of Qa-2 antigens by fast developing mouse embryos correlates with *Ped* gene phenotype: Qa-2^a. It is not known if the *Ped* gene (Qa-2^a) participates in cell differentiation in the preimplantation mouse blastocyst. Therefore, the study objective was to determine the differentiation of cells to the inner cell mass (ICM) and trophectoderm (TE) in Qa-2^a positive (*Ped*+) and Qa-2^a negative (*Ped*–) mouse blastocysts. One-cell stage embryos were recovered from the excised oviducts of PMSG (5 IU) and hCG (5 IU) primed virgin female (3-4 weeks) BALB/cByJ (Qa-2^a: *Ped*–) and BALB/cJ (Qa-2^a: *Ped*+) mice mated to fertile males (12+ weeks). Embryos were collected, 14 hr after hCG, and cultured in modified α-MEM, to the hatched blastocyst stage in an atmosphere of 5% CO_2 in air, 95% relative humidity at 37°C. Cell differentiation was determined by differential staining (bis-benzimide and propidium iodide) and fluorescence microscopy. Data were analyzed by Students t-test. There was no significant difference in total cell number between BALB/cJ (mean 139) and BALB/cByJ (mean 143) embryos. A significant difference ($p < 0.001$) was found in the number of cells differentiating to the ICM between BALB/cJ (mean 59.0) and BALB/cByJ (mean 29.0) mouse embryos. The number of cells differentiating to the TE, between BALB/cJ (mean 80.0) and BALB/cByJ (mean 114) embryos, approached significance ($p = 0.062$). The results suggest that the *Ped* gene (Qa-2^a) may have an influential role in preimplantation blastocyst cell differentiation. Additional studies are warranted to further elucidate the role of the *Ped* gene in preimplantation embryo development and blastocyst formation.

INTRODUCTION

The development of mouse preimplantation stage embryos *in vivo* and *in vitro* is affected by strain genotype (Dandekar and Glass, 1987). Genes within the mouse histocompatibility complex (H-2), e.g. the Preimplantation-embryo-development (*Ped*) gene, influence the time of the first cleavage division and subsequent rate of embryonic development (Goldbard *et al*., 1982). The *Ped* gene is located in the H-2 complex and manifests itself as two functional alleles controlling fast (dominant) or slow embryonic development (Warner *et al*., 1987). The *Ped* gene is linked to the Qa-2 subregion within the H-2 complex. Expression of Qa-2 antigens by fast developing mouse embryos correlates with *Ped* gene phenotype: Qa-2^a (Goldbard *et al*., 1982). The *Ped* gene product may be the Qa-2^a protein. Mouse Qa-2^a antigens have been detected on mouse oocytes and on two-cell, eight-cell and blastocyst stage embryos (Krco and Goldbard, 1977; Cozed and Warner, 1981). In addition, strains that are Qa-2^a positive have shorter gestation times, larger litters, and higher birth weights (Roderick, 1980).

Preimplantation blastocyst formation is characterized by the formation of a blastocoel cavity and the initial cell differentiation of two distinct cell populations: the inner cell mass (ICM) and the trophectoderm (TE). The initiating stage for preimplantation cell differentiation is the morula (Johnson, 1979) and for determination is at or just prior to the 8-cell stage (Cosby *et al*., 1988). Many factors or conditions may regulate embryonic differentiation, including, but not limited to, nucleocytoplasmic ratios, cellular apposition, or epigenetic interactions. It is not known if the *Ped* gene participates in blastocyst cell differentiation during mouse preimplantation embryo development.

Therefore, the study objective was to determine the differentiation of cells to the ICM and TE in Qa-2^a positive (Qa-2^a:*Ped*+) and Qa-2^a negative (Qa-2^a:*Ped*–) mouse blastocysts.

MATERIALS AND METHODS

Cumulus complexes (CC) were recovered from the excised oviducts of pregnant mare's serum gonadotropin (PMSG; 5 IU; Sigma

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Chem. Co., St. Louis, MO, USA) and human chorionic gonadotropin (hCG; 5 IU; Sigma) primed BALB/cJ (Qa-2^{a}:*Ped*+) and BALB/cByJ (Qa-2^{a}:*Ped*–) female mice (3-4 weeks of age). The interval between PMSG and hCG injections was 48 hr. Primed females were mated with fertile BALB/cJ or BALB/cByJ males (12+ weeks of age) immediately following the administration of hCG. CC were collected 14 hr post-hCG and cultured in modified Minimum Essential Medium-alpha modification (α-MEM; Sigma), as previously described (Roudebush *et al.*, 1994; Roudebush and Duralia, 1996), in an atmosphere of 5% CO_2 in air, 95% relative humidity at 37°C to the hatched blastocyst stage.

Assessment of the Qa-2^{a}:*Ped* gene's influence on cell differentiation, allocation of preimplantation embryonic cells to the ICM or to TE, was determined by differential labelling with polynucleotide-specific fluorochromes as previously described (Hardy *et al.*, 1989; Roudebush *et al.*, 1994). Briefly, TE cells are labelled with 0.1 mg/ml propidium iodide (Sigma) during immunosurgery and 10.0 μg/ml bisbenzimide (Hoechst 33258; Sigma). ICM cells are labelled only with bisbenzimide. Fluorescence microscopy was performed on a Nikon-Diaphot inverted microscope equipped with an epifluorescence filter combination UV-1A. Under the aforementioned conditions, ICM cells fluoresce blue and TE cells fluoresce red (Fig. 1).

Data were evaluated by Student's *t* test and 2 X 2 chi-squared contingency table.

RESULTS AND DISCUSSION

A total of 963 BALB/cJ and 1,106 BALB/cByJ mouse preimplantation mouse embryos were collected and cultured as described. The effect of the Qa-2^{a}:*Ped* gene on mouse preimplantation embryo development in α-MEM is shown in Table 1. The effect of the Qa-2^{a}:*Ped* gene on mouse preimplantation formation in α-MEM is provided in Fig. 2 and Table 2.

The Qa-2^{a}:*Ped*+ BALB/cJ mouse 2-cell embryo required 120 hr to develop to the hatched blastocyst stage, whereas, the Qa-2^{a}:*Ped*– BALB/cByJ mouse 2-cell stage embryos required 134 hr to develop to the hatched blastocyst stage. A significantly ($P < 0.001$) higher number of BALB/cJ (57.3%) than BALB/cByJ (45.8%) 2-cell stage embryos developed to the hatched blastocyst stage (Table 1), suggesting that the Qa-2^{a}:*Ped* gene may influence not only the rate of development but also the ability to develop to the hatched blastocyst stage. The BALB/cByJ mouse hatched blastocyst had a significantly ($P < 0.001$) lower number of ICM (mean 29) than the BALB/cJ (mean 59) mouse hatched blastocyst (Fig. 2). There was no significant difference in the number of TE cells be-

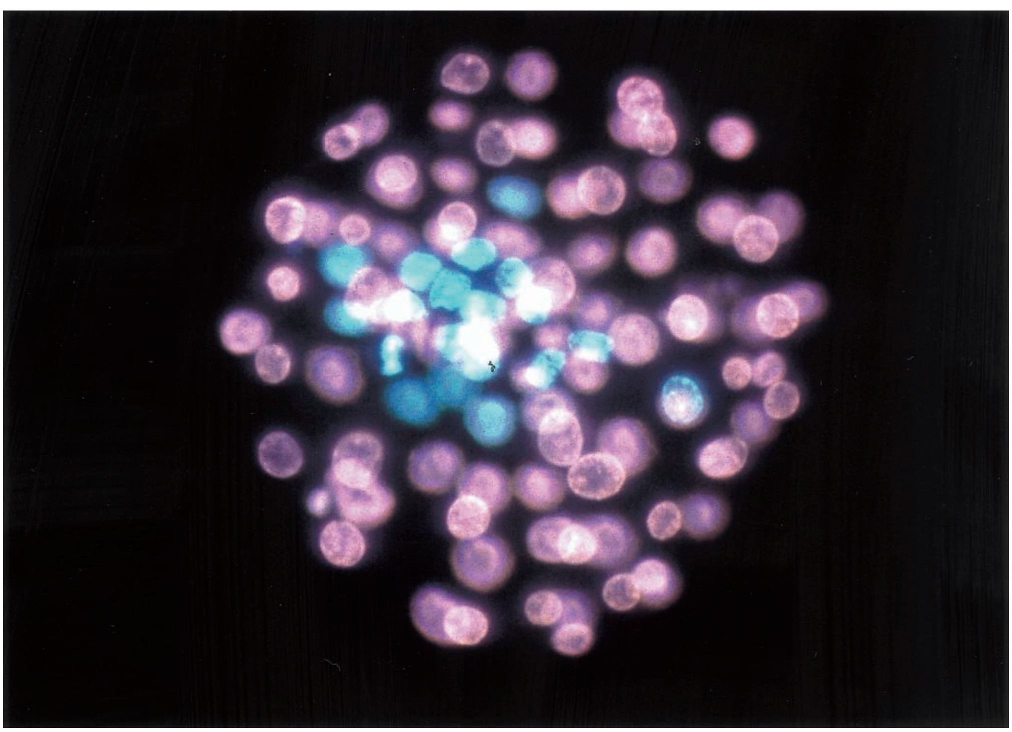

Fig. 1. Fluorescence imaging of a mouse hatched blastocyst. Blue, inner cell mass; Red, trophoectoderm.

Table 1. The effect of the Qa-2^{a}:*Ped* gene on BALB/cJ and BALB/cByJ preimplantation embryo development

	No. of 2-Cells	No. of Hatched Blastocysts
BALB/cByJ (Qa-2^{a}:*Ped*–)	963	507 (45.8%)[A]
BALB/cJ (Qa-2^{a}:*Ped*+)	1,106	552 (57.3%)[A]

A: significantly different ($P < 0.001$).

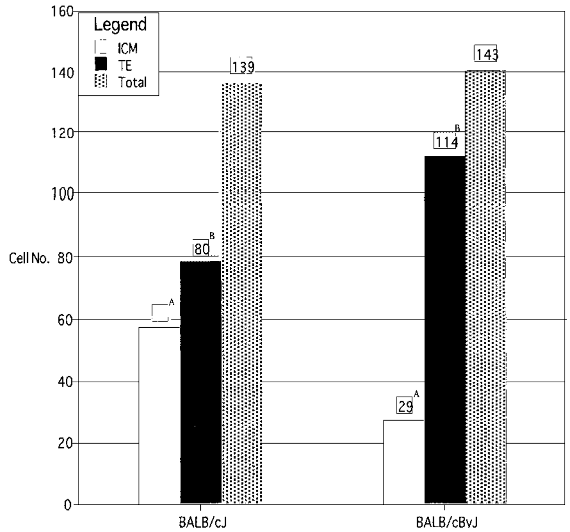

Fig. 2. The effect of the *Ped* gene on BALB/cJ and BALB/cByJ blastocyst differentiation. A, Significantly different (P < 0.001); B, approaches significance (P = 0.062).

Table 2. The effect of the *Ped* gene on per cent cell allocation in BALB/cJ and BALB/cByJ hatched blastocysts

	ICM	TE
BALB/cJ (*ped+*)	42.4	57.6
BALB/cByJ (*ped–*)	20.3	79.7

tween the two mouse strains (Fig. 2), however, the difference did approach significance (P = 0.062). There was no significant difference in the total number of hatched blastocyst cells between the two mouse strains (Fig. 2). A proportionally higher number of preimplantation cells differentiate to the ICM than to the TE in the Qa-2^a:*Ped* positive BALB/cJ (42.4%) blastocyst than in the Qa-2^a:*Ped* negative (20.3%) BALB/cByJ blastocyst (Table 2). The results suggest that the Qa-2^a:*Ped* gene may have an influential role in preimplantation embryo cell differentiation.

Preimplantation embryo development is genetically controlled by both maternal and embryonic genes. Mouse preimplantation stage embryos develop at different rates *in vivo* and *in vitro*; this difference in cleavage division rate between slow and fast developing mouse strains is maintained *in vitro* (Brownell and Warner, 1988) and is strain dependent (Streffer *et al.*, 1980; Dandekar and Glass, 1987). This effect has been ascribed to the Preimplantation-embryo-development or *Ped* gene, and may be an effect of the Qa-2 locus. Fast, but not slow, developing mouse embryos express the Qa-2^a antigen (Xu *et al.*, 1994). The embryonic *Ped* gene phenotype is an intrinsic property of the embryos themselves (Brownell and Warner, 1988). The Qa-2 locus is located within the mouse major histocompatability complex (H-2) on chromosome 17 (Green, 1989) and is a product of the H-2 Q9 gene (Xu *et al.*, 1994). However, background genes, in addition to the H-2 associated *Ped*, play a role in the control of the rate early preimplantation embryonic cleavage (Goldbard and Warner, 1982).

In this study, it was found that the embryos of two related inbred mouse strains (BALB/cJ and BALB/cByJ) cultured under similar conditions: (1) develop at different cleavage rates; the BALB/cByJ embryos requiring additional time to reach the hatched blastocyst stage; and (2) will have a proportionally different number of cells differentiating to the blastocysts inner cell mass or trophectoderm although the total cell number remained relatively constant.

Human preimplantation stage embryos, between patients, fertilized and cultured *in vivo* and recovered from the uterus have been reported to be at different stages of embryonic development (Buster *et al.*, 1984). Human *in vitro* fertilized embryos, between patients, also develop at different rates (Roudebush, unpublished observations). This suggests that some genetic component(s) regulate human embryonic development.

Other embryonic factors which have reported to effect cleavage (mitotic) rates include, but are not limited to: platelet-activating factor (Roberts *et al.*, 1993) and growth factors (Paria and Dey, 1990). Additional studies are warranted to determine if the *Ped* gene interacts with these other factors in the control and regulation of preimplantation embryo development.

ACKNOWLEDGMENTS

This research was supported in part by NICHD-1RO3HD3523301 and Medical University of South Carolina Institutional Funds of 1996-1997.

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(Received September 16, 1997 / Accepted February 16, 1998)