Sex Difference in X-Linked Glucose 6-Phosphate Dehydrogenase Activity of Mouse Embryos

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Summary. The activity of an X-linked enzyme, glucose 6-phosphate dehydrogenase (G6PD), was measured in preimplantation mouse embryos by a simple *in situ* colorimetric assay (Williams, 1986). Morulae and blastocysts were sufficiently stained by brilliant cresyl blue to determine the enzymatic activity, whereas 1- to 8-cell embryos were not. The distribution of the enzymatic activities at the blastocyst stage were bimodal, although the activity was lower than that obtained at the morula stage. Morulae or blastocysts grouped according to the G6PD activity were transferred separately to pseudopregnant females. Sex ratio (number of males/total number of pups) of newborns derived from morulae with high (putative female) and low (putative male) activity were 0.407 and 0.593, respectively, and those derived from blastocysts with high and low activity were 0.405 and 0.649.

KEY WORDS: G6PD, X-LINKED ENZYME, SEX RATIO, BLASTOCYST, EMBRYO TRANSFER.

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In female somatic cells of mammals, one of the X-chromosomes is inactive, while in germ cells both X-chromosomes are active during a certain period (Lyon, 1961, 1974). In the mouse, both X-chromosomes are functional in oocytes during the growth phase (Epstein, 1969, 1972). After fertilization, activation of both maternal and paternal X-chromosomes in female embryos occurs from the 2-cell stage (Schultz, 1986) and lasts up to the blastocyst stage when one of the X-chromosomes becomes inactive (Epstein, 1972; Adler *et al.*, 1977; Kratzer and Gartler, 1978). It has been reported that X-linked enzymes, such as hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and α -galactosidase (α -Gal), exhibit twice as much activity in female embryos as compared to male embryos during this period (Epstein *et al.*, 1978; Adler *et al.*, 1977).

Recently, the measurement of X-linked enzyme activity has been applied to the sexing of preimplantation embryos. Williams (1986) measured the activity of an X-linked enzyme, glucose 6-phosphate dehydrogenase (G6PD), in Day 4 mouse embryos using a simple colorimetric assay, and demonstrated that 64% of the embryos were correctly sexed prior to transfer. In this study, we determined an appropriate developmental stage of mouse embryo for the G6PD colorimetric assay, and examined the relationship between the G6PD activity of embryos and the sex ratio of newborns derived from embryos grouped according to the activity.

Materials and Methods

Experiment 1

Female ICR mice were injected intraperitoneally with 5 IU PMSG (Teikoku Zoki,

Japan) followed 48 hr later by 5 IU hCG (Sankyo Zoki, Japan). Treated mice were mated with ICR males, and 1-, 2-, 4- and 8-cell embryos, morulae and blastocysts were collected at 16, 44, 60, 68, 78 and 92 hr after hCG injection, respectively. Embryos were washed in 0.1 ml of M2 medium (Quinn *et al.*, 1982) supplemented with 0.05 mg brilliant cresyl blue (BCB, Sigma, USA)/ml, and stained in the medium for 20 min at 37°C. They were transferred into 0.1 ml of the reaction mixture: M2 medium supplemented with 0.5 mM β -nicotine adenine dinucleotide phosphate (Olyental, Japan) and 0.5 mM Na-glucose 6-phosphate (Olyental, Japan). The stained embryos in the medium were examined every 6 min, and the number of embryos displaying decolorized BCB were recorded. Activity of G6PD was represented by the time required for decolorization of BCB.

Experiment 2

Female ddY mice were mated with mature vasectomized male ddY mice, and females with a vaginal plug on the next morning (day 1) were used for recipients. Morulae were recovered from female ICR mice as described above. After measurement of G6PD activity, 167 embryos with high activity (putative females) and 166 with low activity (putative males) were transferred separately into the uteri of 24 recipients on day 3 of pseudopregnancy. Control morulae (n=92) cultivated for 2 to 3 hr in M2 medium were transferred to 7 females in the same manner.

Blastocysts were also divided into two groups after measurement of G6PD activity, and 133 putative female embryos and 134 putative male embryos were transferred separately into the uteri of 34 pseudopregnant mice on day 4 of pseudopregnancy. Control blastocysts (n=79) were transferred to 5 females. The sex of the resulting newborns was examined at birth. The statistical analysis was performed by χ^2 -test.

Results

G6PD activity in mouse embryos

Morulae and blastocysts were stained with BCB, while 1-, 2-, 4- and 8-cell embryos were not, suggesting that this colorimetric assay can be applied to the measurement of G6PD activity only at the morula and blastocyst stages. After transfer of stained embryos into the reaction mixture, BCB was gradually decolorized and the embryos eventually returned to a pre-stained state. Figure 1 shows the distributions of G6PD activity of each embryo at the morula and blastocyst stages. The average activity of 71 morulae was 40.9 ± 15.3 min, while the activity of 52 blastocysts was 51.0 ± 18.7 min. A bimodal distribution was seen at the blastocyst stage, while it was not clear at the morula stage. The average activities for the two modes of the bimodal distribution at the blastocyst stage were 33.5 ± 8.8 min (28 blastocysts with high G6PD activity) and 66.0 ± 9.5 min (24 with low activity). There was a two-fold difference between the modes (1.97 : 1).

Relationship between G6PD activity and newborn sex ratio

Two groups of morulae, with high- and low-G6PD activities, were transferred to 12 and 12 recipients, subsequently, 8 and 8

Fig. 1. Distribution of glucose 6-phosphate dehydrogenase (G6PD) activities of mouse morulae and blastocysts.

□: morula (n=71), ■: blastocyst (n=52)

Table 1. Sex ratio of newborn mice derived from embryos with high- and low-glucose 6-phosphate dehydrogenase (G6PD) activity

Developmental stage of embryos	G6PD activity	No. of embryos transferred	No. of newborn mice	No. of males	No. of females	Sex ratio ¹⁾
Morula	High	167	59	24	35	0.407
	Low	166	54	32	22	0.593
	Control	92	34	18	16	0.529
Blastocyst	High	133	42	17	25	0.405 ^a
	Low	134	74	48	26	0.649 ^b
	Control	79	38	19	19	0.500

¹⁾ Number of males/total number of newborn mice.

a, b) Values with different superscripts differ ($P < 0.05$).

mice delivered 59 and 54 newborns, respectively (Table 1). The sex ratio (number of males/total number of pups) of newborns derived from the morulae with the high activity was 0.407, while the ratio from the morulae with the low activity was 0.593. The sex ratio of newborns derived from control morulae was 0.529.

Two groups of blastocysts were transferred to 17 and 17 recipients, subsequently, 8 and 12 mice delivered 42 and 74 newborns, respectively (Table 1). The sex ratios of newborns with the high- and low-G6PD activity were 0.405 and 0.649, respectively. There was a significant difference between the sex ratios of the two groups. The ratio of newborns derived from control blastocysts was 0.500. The accuracy of the assay was 62.9% in predicting sex.

Discussion

Williams (1986) scored Day 4 mouse embryos according to BCB staining level after 30 min in reaction mixture. When we used the same method for assay of G6PD activity of morulae and blastocysts, 30 to 40% of embryos were not stained and activity could not be determined. Hence, we measured the time required for the embryos to reduce BCB to a colorless compound and represented the G6PD activity over time. Morulae and blastocysts were stained blue,

while 1- to 8-cell stage embryos were not stained even after 30 min of staining. Moreover, the activity of blastocysts were lower than that obtained in morulae. Brinster (1966) demonstrated that G6PD activity decreased as embryos developed, and subsequently, the activity of late blastocysts was decreased to 14% of the activity of unfertilized eggs. The fact that early embryos were not stained by BCB in our experimental females in the same litter (Tsunoda *et al.*, 1985, Miyano *et al.*, 1989). Since G6PD activity of embryos decreases as developmental stages progress, the activity of male embryos decreases rapidly. If the activity of embryos in the same litter is assayed at a certain developmental stage, the accuracy in sex prediction might be advanced further.

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マウス胚における X 染色体由来グルコース 6 リン酸脱水素活性の性差

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Williams (1986) の BCB 比色法によって、着床前のマウス胚における X 染色体由来グルコース 6-リン酸脱水素酵素 (G6PD) の活性測定を試みた。1～8 細胞期胚は BCB に不染であり、G6PD 活性を測定できなかった。BCB に染色された桑実胚および胚盤胞について G6PD 活性を測定した結果、胚盤胞の活性は桑実胚より低かったが、胚は二頂性の分布を示した。

桑実胚および胚盤胞を G6PD 活性の高い群と低い群

に分類した後移植し、得られた産子の性比を調べた。G6PD 活性の高い桑実胚および低い桑実胚に由来する産子の性比 (産子に占める雄の割合) は 0.407 および 0.593 であり、G6PD 活性の高い群では雌が多い傾向を示した。胚盤胞を同様に移植したところ、G6PD 活性の高い群および低い群に由来する産子の性比は 0.405 および 0.649 であり、G6PD 活性の高い群では雌が有意に多かった。