Seasonal variation in cell cycle during early development of the mouse embryo

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Summary. Studies of the cell cycle of mouse embryos before implantation were conducted using Giemsa and DAPI stains. The time of embryo recovery did not affect the success rate of cultures during the winter, but embryos cultured during the summer showed the 'two-cell block' phenomenon at the early two-cell stage, 30–37 h after the injection of human chorionic gonadotrophin. There was no significant difference in the number of embryos collected per mouse between summer and winter, but cleavage from the two-cell to the four-cell stage occurred later in the summer than in the winter. Cell cycle of mouse embryos may therefore show seasonal variation.

Keywords: two-cell block; seasonal variation; mouse; embryo culture

Introduction

Many studies have revealed the relationship between DNA replication and morphogenetic events during early mouse embryogenesis (Luthardt & Donahue, 1973, 1975; Abramczuk & Sawicki, 1975; Holliday & Pugh, 1975; Siracusa *et al.*, 1975; Sawicki *et al.*, 1978; Johnson *et al.*, 1984; Howlett & Bolton, 1985). Several papers have reported embryonic cleavage rates (Bowman & McLaren, 1970; Gamow & Prescott, 1970; Allen & McLaren, 1971; McLaren & Bowman, 1973; Mukherjee, 1976; Krishna & Generoso, 1977; Streffer *et al.*, 1980; Goddard & Pratt, 1983; Newport & Kirschner, 1984; Aimar, 1988; Houliston *et al.*, 1988), but only endogenous maternal and genetic events have been taken into account (Allen & McLaren, 1971; Nicol & McLaren, 1974; Goddard *et al.*, 1983; Molls *et al.*, 1983; Newport & Kirschner, 1984; Aimar, 1988; Houliston *et al.*, 1988), and the scope has been limited to the first two or three cleavages, when cell number can be estimated by direct inspection (Domon, 1983). The cleavage rate of embryos also depends on the genotype of the mother (Longo & Anderson, 1969; McLaren & Bowman, 1973).

Seasonal reproductive behaviour is a well-documented phenomenon in many mammalian species (Laguichev, 1958; Elbling, 1975; Watson & Chaykin, 1987), but there have been relatively few reports dealing with the effects of the external environment on the cell cycle and developmental programme in the laboratory mouse (*Mus musculus*) during early cleavage. Several papers have reported that cultured embryos show a block to development at the early two-cell stage (30–37 h after human chorionic gonadotrophin (hCG)) (Biggers *et al.*, 1962; Abramczuk *et al.*, 1977; Goddard & Pratt, 1983; Loutradis *et al.*, 1987). In an extensive series of cultures of embryos recovered from virgin ICR mice over a period of four years (1988–91), we have found that, from July to September, it is very difficult to obtain consistently successful cultures from two-cell to blastocyst stages. Cleavage was slower during the summer than during the winter.

Materials and Methods

Animals

Institute for Cancer Research (ICR) mice were bred in a room in which temperature and lighting were not controlled artificially, but varied with the seasons. The daylight period was over 14 h in summer and 10 h in winter, the mean monthly relative humidity was 85% in summer and 77.5% in winter, the mean temperature was 30°C in summer and 17.5°C in winter.

Collection of embryos

Six–twelve-week-old mice were superovulated by a 5 i.u. injection of pregnant mares' serum gonadotrophin (PMSG) (PEAMEX: Zoki Co. Ltd, Tokyo, Japan) and this was followed by a 5 i.u. injection of hCG (Gona-500: China Chemical & Pharmaceutical Co. Ltd, Taipei, Taiwan) 48 h later. At the time of the second injection, the females were placed with males, and they were examined for vaginal plugs on the following morning.

Females were killed by cervical dislocation and embryos were collected into phosphate-buffered saline (PBS) with 1% fetal cord serum at specific times after hCG injection, rinsed twice, and then transferred to the culture medium described by Whittingham (1971). To obtain one- to eight-cell embryos, oviducts were removed at intervals from 24–60 h after hCG and the ampulla nicked with a cataract knife at the point of distension. Eight-cell embryos and morulae were obtained 60 h after hCG by inserting a 30-gauge needle through the wall of the uterus at the tubal end and flushing the uterine lumen with PBS.

Embryo culture

All embryos were cultured in Petri dishes with 0.05 ml of freshly prepared culture medium (Whittingham, 1971), pre-equilibrated overnight at 5% CO₂ in air, 37°C, ≥95% humidity at pH 7.3–7.4 under liquid paraffin (Brinster, 1965). Bovine serum albumin (BSA) (Sigma Chemical Co., Poole, Dorset, UK) was added at 4 mg ml⁻¹ before sterile filtration.

Giemsa staining

One-cell embryos were treated with hyaluronidase in 2% sodium citrate to remove the follicular cells. Embryos were assessed for normality by their morphology and expected developmental stage. The denuded embryos were washed in fresh 2% citrate and treated with a hypotonic solution of 0.7% sodium citrate for 3 min. After excessive solution was removed, about 0.1 ml of 25% acetic acid in ethanol was added to the slide to cover the embryos. The air-dried preparations were placed in 25% acetic acid in alcohol for 10 min. Fixed preparations were stained with 10% Giemsa stain for 30 s.

DAPI stain and determination of number of nuclei per embryo

4',6'-Diamidino-2-phenylindole (DAPI) is used as a fluorescent dye for nuclear stain. Embryos fixed on glass slides were stained with DAPI solution (Hamada & Fujita, 1983) for 15 min at room temperature. Finally, the slide was washed with the same PBS and the edge of the coverslip was sealed with nail polish. Fluorescence-emitting embryos were photographed with a Zeiss model epi-illuminated fluorescent microscope for DAPI stained slides.

Counting cell number

The mean number of cells per embryo was counted directly with the inverted microscope before 55 h after hCG treatment (embryos less than four cells) and evaluated by DAPI stain after 55 h after hCG (embryo more than four cells). The number of cell nuclei was taken as the equivalent of the cell number.

Statistical analysis

All traits studied were described by general linear models and analysed using analysis of covariance. Year and season effects were included as qualitative factors, and time after hCG injection as quantitative factor. Polynomial regression models with one independent variable, that is the time of collecting embryos after hCG injection, were used to analyse the data within seasons.

Chi-square analysis was used to test whether the percentage of embryos from two-cell to four-cell stage is independent of season at different times after hCG injection.

Results

Collection of embryos

A total of 4273 embryos from 382 females was recovered at various times after ovulation. Three thousand six hundred and seventy four embryos were viable while 599 embryos were dead. The numbers of mice and embryos investigated was 156 and 1895 in summer, 226 and 2378 in winter, respectively. There was no significant difference in the average number of embryos collected per mouse between summer (14.8 ± 1.2) and winter (11.6 ± 1.1) using least-squares estimates.

In the one- and two-cell stages, there were significant differences ($P < 0.05$) in terms of percentages between seasons and the corresponding least-squares estimates were ($33.3 \pm 3.0\%$, $66.5 \pm 3.0\%$) and ($23.9 \pm 2.8\%$, $76.1 \pm 2.8\%$) for summer and winter, respectively. However, the significant effects of seasons on embryo development vanished at later stages ($P > 0.05$).

The first cell cycle

The number of nuclei per embryo was examined either by phase-contrast microscopy, DAPI staining or Giemsa staining.

The first polar body is extruded from the primary oocyte 12 h after hCG, which is also the time of ovulation. The chromosomes of the second maturation division are aggregated on the metaphase plate 18 h after hCG. The sperm nucleus swells and its chromatin begins to decondense very rapidly as the sperm is incorporated and the male pronucleus formation begins. Both pronuclei form at 18–28 h after hCG and migrate centrally. Five to six hours after pronuclear formation, the embryos enter the second cell cycle (i.e. the first cleavage division) and the entire first cell cycle is completed by 33.3 h after hCG treatment in winter and 42.6 h after hCG treatment in summer.

Two-cell embryo culture

The number of embryos developing from the two- to four-cell stage was significantly different in winter and in summer, up to 37 h after hCG (Table 1). However, the level of significance decreased with time and no significant difference was observed at 42 h after hCG. In summer, two-cell embryos showed a 'two-cell block' if they were collected 30–37 h after hCG, but no block was found if embryos were collected 42 h after hCG.

Table 1. Relationship between the proportion of embryos developing from two-cell to four-cell stages and the time of collection during winter and summer

Time after hCG injection when embryos collected (h)	Winter		Summer		χ^2 (Summer versus winter)
	No. of embryos in culture	No. of embryos developing to four-cell stage	No. of embryos in culture	No. of embryos developing to four-cell stage	
30	46	32	37	4	28.83**
31	80	54	168	36	49.76**
33	97	53	62	12	19.50**
37	122	86	37	18	5.98*
42	49	37	33	23	0.34
46	31	26	40	31	0.45

**: $P < 0.01$; *: $P < 0.05$.

The second and third cell cycles

A comparison of the rates of cleavage of the 4273 embryos collected from summer and winter time is given (Fig. 1). From the fitted regression equations, the times after hCG required for at least 50% of embryos to reach the two-, four- and eight-cell stages are calculated as 37, 58 and 71 h, and 35, 52 and 67 h, for summer and winter, respectively.

Fig. 1. The growth curves of embryos in relation to time after hCG injection in summer (□) and in winter (■).

Discussion

The number of embryos recovered was similar in summer and winter. Our data on the timing of maturation divisions, ovulation, fertilization and the first cleavage division are similar to those in previous studies (Edwards & Gates, 1959; Donahue, 1968, 1972a,b; Kaufman, 1973), but we found that embryos need a longer time to reach the four- and eight-cell stage in summer than in winter. Seasonal conditions are an important factor in the first two cleavage divisions.

We also found an effect of the time of embryo collection on early embryonic development in summer and winter. Embryos collected 30–37 h after hCG during the summer showed a ‘two-cell block’, but those collected later than 40 h after hCG showed no such block. No ‘two-cell block’ was observed during the winter, even for embryos recovered 30–37 h after hCG.

Seasonal effects on reproductive behaviour are a well-documented phenomenon in many mammalian species (Laguichev, 1958; Elbling, 1975; Watson & Chaykin, 1987). It has been suggested that ovarian inactivity (Legan *et al.*, 1977; Armstrong *et al.*, 1986) and insufficient production of gonadotrophin-releasing hormone GnRH (Hart *et al.*, 1984) may be the prime factors responsible for seasonal variation. In our study, mice were bred in a room where the environmental factors were not artificially controlled. In summer, the temperature and humidity were higher and day length was greater than in winter. We have tested the effect of day length on embryonic development. When the mice were treated with a short daylight period (10 h light:14 h dark) during summer time, their embryos showed a significantly greater chance of progressing from two-cell to four-cell than embryos obtained from untreated mice (data not shown). We have not yet studied the effect of temperature and relative humidity.

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