

Prolongation of duration of ovulation in ageing mice

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In female mice, fertility and fecundity decrease progressively with ageing for unknown reasons. The time of day at which ovulation occurred and the time required for all the follicles to ovulate in young (10–14 weeks), middle-aged (9–11 months) and old (13–15 months) female mice were compared under controlled lighting conditions (12 h dark to 12 h light) to determine the relationship between maternal age and reproductive loss. The number of oocytes present in the follicles and the ampullae were counted at intervals of 1 h after mating. In the groups of young and middle-aged mice, the percentage of oocytes ovulated into the ampullae increased gradually and reached almost 100% at 7 h after the midpoint of the dark period. Whereas, in the group of old mice, it took twice as long (15 h) to reach 100%. However, the mean number of total oocytes remained relatively unchanged (young, 14.8; middle-aged, 16.2; old, 13.8). The prolongation in the time required for all the follicles to ovulate in old female mice may therefore be associated with a low fertilization rate and consequently the age-related decrease in number of offspring produced.

Introduction

It is generally assumed that ovulation in mice occurs around, or just after, the midpoint of the dark period under natural or quasi-natural lighting conditions. However, some studies have shown a variety of ovulation times in several different strains of mice (Snell *et al.*, 1940; Braden and Austin, 1954; Whitten and Dagg, 1961; Bingel and Schwartz, 1969; Krishna and Generoso, 1977; Albanese, 1987; Boerjan and de Boer, 1990). In an earlier experiment (Ishikawa *et al.*, 1992), it was found that in young female mice of the Jcl/ICR strain ovulation started around the midpoint of the dark period under a controlled 12 h light:12 h dark cycle. These different ovulation times may reflect differences among strains or various lighting conditions. Furthermore, it is possible that some of the apparent discrepancies are due to the ages of the mice rather than to differences among strains.

Several reports have demonstrated that there is not an age-related decrease in the numbers of ovulated oocytes in mice (Biggers *et al.*, 1962; Harman and Talbert, 1970; Gosden, 1975), rats (van der Schoot, 1976; Matt *et al.*, 1987a; Day *et al.*, 1989) and hamsters (Thornycroft and Soderwall, 1969; Mizoguchi and Dukelow, 1981). Furthermore, our literature review failed to reveal any reports comparing the duration of ovulation (the time required for all the follicles to ovulate) in mice at different ages.

In this study, the numbers of oocytes present in the follicles and the ampullae were examined more precisely at intervals of 1 h around the presumed time of ovulation in the three different age groups of Jcl/ICR mice exposed to a 12 h dark:12 h light cycle and the duration of ovulation was

compared. This study is, to our knowledge, the first to examine whether the duration of spontaneous ovulation in mice is affected by increasing maternal age.

Materials and Methods

Retired breeder Jcl/ICR female mice were obtained at 7–9 months of age from CLEA Japan Co. (Tokyo) and housed five or six per cage in light-controlled (12 h light:12 h dark cycle; lights on from 14:00 h–02:00 h daily) and temperature-controlled (24–26°C) rooms with food and drinking water available *ad libitum*. Two different aged groups were established by continuing to breed some mice up to 9–11 months (middle-aged) and others up to 13–15 months (old group). A group of young (10–14 weeks) virgin female Jcl/ICR mice was also obtained from CLEA Japan Co. and acclimatized to the same conditions for at least 2 weeks before mating (Snell *et al.*, 1944).

Female mice were housed with male mice from the beginning of the dark period, and those with vaginal plugs were killed by cervical dislocation at appropriate intervals before and after the midpoint (08:00 h) of the dark period. Previous studies have shown that mating does not affect the onset of ovulation (Snell *et al.*, 1940; Edwards and Gates, 1959). The ovaries were placed in an embryological watch-glass containing physiological saline solution and dissected at $\times 20$ magnification under a microscope. When mature follicles were found in the ovaries (large swollen follicles with large blood vessels), they were punctured with a 25-gauge sterile hypodermic needle. When oocytes with attached cumulus cells were found in the ampullae and fully grown oocytes surrounded by many layers of follicle cells in the follicles, the numbers of oocytes from both right

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Table 1. The number of oocytes in the ampullae and follicles in young, middle-aged and old ovulating mice

Time from midpoint of dark period (h)	Young				Middle-aged				Old			
	Number of females	Ampullae	Follicles	Total	Number of females	Ampullae	Follicles	Total	Number of females	Ampullae	Follicles	Total
- 2	10	0	13.0	13.0	7	0	14.3	14.3				
		(0-0)	(11-15)	(11-15)		(0-0)	(11-18)	(11-18)				
- 1	10	0.9	13.0	13.9	5	0	15.8	15.8				
		(0-9)	(2-21)	(9-21)		(0-0)	(14-18)	(14-18)				
0 (midpoint)	11	3.7	9.4	13.1	13	2.3	9.7	12.0	10	0	14.1	14.1
		(0-17)	(0-16)	(8-17)		(0-16)	(1-14)	(8-17)		(0-0)	(10-18)	(10-18)
+ 1	12	5.2	7.4	12.6	10	0	15.4	15.4	9	2.6	13.3	15.9
		(0-18)	(0-14)	(9-18)		(0-0)	(11-18)	(11-18)		(0-13)	(4-21)	(13-21)
+ 2	14	3.4	9.7	13.1	13	5.9	8.9	14.7	11	1.8	11.5	13.3
		(0-15)	(0-17)	(6-17)		(0-20)	(0-17)	(9-20)		(0-9)	(4-16)	(10-16)
+ 3	13	8.6	5.4	14.0	12	6.7	7.3	14.0	13	9.2	6.4	15.5
		(0-19)	(0-15)	(10-21)		(0-16)	(0-16)	(9-17)		(0-19)	(0-16)	(11-19)
+ 4	10	11.5	3.0	14.5	8	12.5	3.1	15.6	8	8.3	5.6	13.9
		(0-18)	(0-13)	(11-18)		(0-19)	(0-14)	(8-19)		(0-14)	(0-18)	(9-21)
+ 5	11	14.2	1.1	15.3	7	10.7	4.4	15.1	6	12.8	2.5	15.3
		(8-19)	(0-4)	(10-22)		(0-16)	(0-13)	(11-17)		(9-15)	(0-6)	(13-18)
+ 6	8	16.5	0.4	16.9	5	17.4	0.2	17.6	6	11.0	3.0	14.0
		(14-18)	(0-2)	(14-20)		(16-19)	(0-1)	(16-19)		(0-16)	(0-10)	(10-18)
+ 7	7	15.1	0.1	15.3	5	16.6	0	16.6	5	6.4	4.0	10.4
		(13-18)	(0-1)	(13-18)		(14-19)	(0-0)	(14-19)		(0-10)	(0-7)	(7-15)
+ 8									5	10.8	3.6	14.4
										(3-17)	(1-9)	(10-19)
+ 9									5	10.2	4.0	14.2
										(6-16)	(1-10)	(10-17)
+ 10									5	10.6	3.0	13.6
										(8-14)	(0-6)	(8-17)
+ 11									9	7.8	2.4	10.2
										(1-16)	(0-6)	(5-16)
+ 12									6	8.2	4.0	12.2
										(3-16)	(1-7)	(5-19)
+ 13									5	11.2	1.2	12.4
										(7-18)	(0-3)	(8-19)
+ 14									5	12.8	1.2	14.0
										(9-15)	(0-3)	(10-17)
+ 15									6	14.7	0	14.7
										(8-19)	(0-0)	(8-19)
+ 16									6	15.7	0	15.7
										(12-20)	(0-0)	(12-20)

The range is given in parentheses.

and left follicles and ampullae were counted. At intervals of 1 h the mean number of oocytes either in the follicles or in the ampullae were measured for each animal. The percentage of oocytes in the ampullae was calculated as follows: (the number of oocytes in the ampullae/the total number of oocytes both in the follicles and in the ampullae) \times 100 (%). For further analysis, when the percentage of oocytes in ampullae was > 50% or 100% the female was defined as an individual with 50% or 100% ovulation, respectively. Subsequently the number of females with 50% or 100% ovulation at respective timepoints was determined by calculating the cumulative percentage of females with 50% or 100% ovulation as follows: cumulative percentage of females with 50%

ovulation = [(number of females that had ovulated 50% or more than 50% of total matured oocytes from the follicles into the ampullae by the time indicated)/(number of females that had ovulated 50% or more than 50% before that timepoint + females examined at that timepoint)] \times 100 (%), and cumulative percentage of females with 100% ovulation = [(number of females that had ovulated 100% of total matured oocytes from the follicles into the ampullae by the time indicated)/(number of females that had 100% ovulated before that timepoint + females examined at that timepoint)] \times 100 (%).

Some of the data from the young group were reported by Ishikawa *et al.* (1992) and have been reanalysed.

Results

Female mice in the old group were not examined at 2 h and 1 h before the midpoint of the dark period as few females had ovulated in either the young (10–16 weeks old) or middle-aged (9–11 months old) groups at these timepoints, and no females appeared to ovulate until the midpoint of the dark period in the old group (Table 1). In the young and middle-aged groups, the percentage of oocytes ovulated into the ampullae increased gradually and reached almost 100% at 7 h after the midpoint of the dark period; whereas in the old group at this timepoint, about 62% of oocytes ovulated into the ampullae. The percentage of oocytes that ovulated into the ampullae in the old group increased more slowly and it reached 100% at 15 h after the midpoint of the dark period.

In all three age groups, the cumulative percentage of females with 50% ovulation reached more than 90% by 5 h after the midpoint of the dark period (Fig. 1). However, although the cumulative percentage of females with 100% ovulation in the young and middle-aged groups reached more than 90% by 6 h after the midpoint of the dark period, it was not until 13 h or 15 h after the midpoint that the cumulative percentage of females with 100% ovulation in the group of old mice reached more than 90%.

In female mice with 100% ovulation, the mean number of total oocytes (the mean number of all oocytes ovulated into the ampullae) was similar in the three groups (young, 14.8; middle-aged, 16.2; old, 13.8).

Discussion

The study reported here demonstrates that the duration of ovulation is greater in old female mice than in younger mice, and that the numbers of ovulated oocytes remain relatively unchanged in ageing mice. Several reports have shown that in ageing female mice embryonic development is delayed and that the number of offspring produced is lower, although the numbers of ovulated oocytes is similar (Biggers *et al.*, 1962; Harman and Talbert, 1970; Gosden, 1975). Ishikawa and Endo (1995) reported that, in middle-aged (9–11 months) and old (13–15 months) female mice, numbers of live fetuses at mid-gestation (day 8 of gestation) were lower, and embryonic development was delayed compared with those from young females, although numbers of corpora lutea were almost equal to that of young females.

The findings reported here indicate that in old female mice more time is required for all the follicles to ovulate compared with young and middle-aged mice. Consequently, oocytes from old female mice that ovulated at the end of the ovulation period would become overripe in the follicles. Parkening and Soderwall (1973) have observed that a large proportion of the oocytes released from old female hamsters had undergone degenerative changes before fertilization. In addition, several reports have shown that preovulatory over-ripeness of the ovum affects fertilization rate and results in abnormal embryonic development, chromosomal aberrations, failure of implantation, and embryonic death (Fugo and Butcher, 1966, 1971; Kamiguchi *et al.*, 1979). These reports indicate that the pre-ovulatory overripeness of ova in old female mice may be associated with fewer offspring.

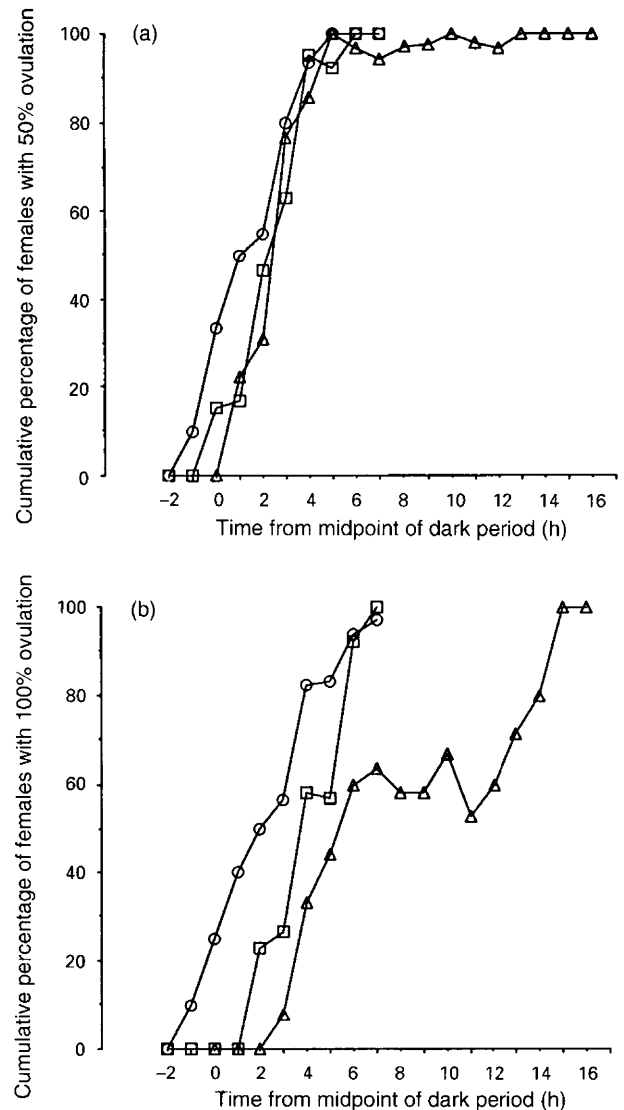


Fig. 1. The cumulative percentage of females with (a) 50% and (b) 100% ovulation for each hour before or after the midpoint of the dark period. Upper and lower line charts indicate cumulative percentage of 50% ovulation and 100% ovulation, respectively. (○) young; (□) middle-aged; (△) old.

Several reports have shown that the steroid-induced surge of LH is lower in middle-aged and old female rats than in younger female rats (Shaar *et al.*, 1975; Matt *et al.*, 1987b; Gray *et al.*, 1980) and in mice (Mobbs *et al.*, 1984). Furthermore, Parkening *et al.* (1980) suggested that there is an alteration in the mouse LH molecule with age. Thus, altered gonadotrophin secretion may also explain the findings reported here; any reduction in FSH, LH or prolactin concentrations and delay in onset of secretion might affect follicular development, final maturation, rupture of follicles, and development of oocytes. Although circadian rhythm changes have not been reported in ageing mice, it is possible that in old mice there is some irregularity of circadian rhythm of hormones related to ovulation. The fertilization rate may also decrease at the end of the time at which ovulation is occurring, because it has been shown that the fertilizing life of mouse spermatozoa in the female

tract was from 6 h to 12 h and their fertilizing capacity apparently decreased after 4 h (Merton, 1939; McGaughey *et al.*, 1968).

Martin *et al.* (1976) showed that the incidence of chromosome anomalies in the oocytes increased with maternal age from young to middle-aged female mice but declined in the oldest maternal age group. In addition, Ishikawa and Endo (1995) examined cytogenetic effects of delayed fertilization in three different aged groups. However, a stepwise increase of chromosome anomalies with advancing age was not observed: the incidence in the old group was lower than that in the middle-aged group. In this study, there was a delay of 6 h in all groups. This seemingly paradoxical finding may be understood if it is noted that the true time interval from ovulation to fertilization in 'delayed-fertilized embryos' from the old female was not so great as had been anticipated because of prolongation of ovulation.

In conclusion, the prolongation of ovulation was demonstrated in aged females and this may have an effect on fertilization rate, abnormal embryonic development and embryonic death in early pregnancy and may thus account for the lower number of offspring produced.

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