Kawasaki Med. J. Vol. 1 : 77—83, 1975.

FETAL WEIGHT AND VARIABILITY IN YOUNG ADULT AND AGED PREGNANT MICE

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Accepted for publication on Dec. 23, 1974.

Abstract

The influence of maternal aging on the reproductive function, especially on the fetal growth, was studied in compairing the number of ova per ovulation, implants and of live fetuses, and the fetal weights on 17th day of pregnancy and their variances, in young adult and aged female mice of CFW strain. It was revealed that the maternal aging not only reduced the number of ova per ovulation and implants and the fetal live rate but diminished the fetal growth. It was also shown that the remarkable increase of the variance of fetal weight in aged pregnant mice and this was principally owing to the enlargement of the within-litter variation. The increase of the embryonic mortality, slow fetal growth and the enlarged within-litter variation of fetal weight in the aged female mice suggested that the maternal environment of the aged pregnant females must be less comfortable than it of young adult pregnant females. And therefore the maternal aging seemed to intensely influence on the maternal environment for fetuses.

INTRODUCTION

Recently, the physiological and pathological changes with advancing age have attracted attention and to investigate them becomes an important subject to study.

Many reports were made up to this time on degenerations of the reproductive function in the aged female animals. In those reports, it has been demonstrated that polytococus species (e.g. mouse, rat and golden hamster) markedly reduce their litter size owing to the reduction of number of ova per ovulation[1,2], increase of the implantation failure[3], and increase of the embryonic mortality[4,5]. They gave following points as the causes of upper evidences that were fall of the ovarian function[3,6], lowering of the uterine function[1,7], endocrine

unbalance[8], and drop of the viability of ova[1,9], in the aged females. Talbert[10] pointed out the need to clarify that those decrease of the implantation rate and increase of embryonic mortality in aged females, derived from whether the degeneration of uterus itself, or the other causes of degenerations of endocrine, circulatory, nutritional and metabolic functions. In this respect, some further results were reported[11,12,13]. However, on the relation between the maternal aging and the fetal growth, no paper was ever issued but the one reported by Kita and Ino[2] and many subjects remain unstudied.

It may be considered that in polytococus species when the maternal environment during pregnancy are growing worse with maternal aging, the fetuses in the uterus will be suffered from various influences. This study was aimed to investigate the influences of the maternal aging on the fetal growth in mice.

MATERIALS and METHODS

Mice: CFW strain, maintained by the closed colony in the National Institute of Animal Health (JAH), were used. One group was young adult nulliparous female of 90-120 day-old and the other was aged same ones of 250-280 day-old.

Environmental conditions: Environmental conditions of animal room were controlled at temperature 25±2°C, relative humidity 50-60 per cent.

Diets: Oriental CMF (Oriental Yeast Co., Tokyo) was used, and food and water were available *ad libitum*.

Cage: Alminium cage, size 20×30×10 cm, 6 mice per cage were placed.

Mating: Female mice were mated to male for the first time on the 90-120th day and 250-280th day without male after the weaning (21 day-old). Young adult male mice, 90-120 day-old, were mated to both the 90-120 day-old nulliparous females and the 250-280 day-old ones at the ratio of ♀4 : ♂1.

Check of copulation: Check of copulation was done by visualization of the vaginal plug and checking the presence of the sperm in the vaginal smear, and the day of their appearance was made the 0 day of pregnancy.

Number of ova per ovulation: Number of ova per ovulation was culculated from the ova counted in the ampulla of oviducts under microscope at 10 : 00 a.m. ~ 1 : 00 p.m. on the day copulation was confirmed.

Number of implants and live fetuses: Number of implants and live fetuses were counted in the sacrificed mice on the 17th day of pregnancy and the post-implantational embryonic mortality was calculated.

Litter size: Litter size was represented as the number of newborn mice (including the dead at the observation).

Fetal weight: The live fetal weight was weighed on the 17th day of pregnancy in which the fetal growth was investigated. And the variability of fetal weight was compared by calculating the variance and the coefficient of variance (C. V. per cent) of the fetal weight. Furthermore, by means of the variance analysis, the variance of fetal weight was divided into the between-litter variance and the within-litter variance and compared among the young adult pregnant mice and the aged ones by each contribution rate.

RESULTS

Number of ova per ovulation, implants, live fetuses, embryonic mortality, litter size: Number of ova per ovulation, implants and of live fetuses and litter size in young adult and aged femal mice were shown in Table 1 and graphically in Fig. 1. As presented in Table 1 and Fig. 1, it was plain that the number of ova per ovulation, implants and of live fetuses and litter size were lower in aged female mice than young adult ones and in every cases the statistically significant differences were obtained ($0.001 < P < 0.01$). These data were cited from our previous report[2].

TABLE 1.
The number of ova, implants and live fetuses and the litter size in young adult and aged female mice of CFW strain

Maternal age (days)	No. of ova (N)	No. of implants (N)	No. of live fetuses (N)	Litter size (N)
90—120	(22) 13.7±0.27*	(25) 13.9±0.45**	(25) 13.3±0.44**	(17) 13.4±0.42**
250—280	(20) 10.9±0.78	(23) 10.1±0.62	(23) 7.2±0.59	(9) 7.2±1.05

Mean±S.E.
* $0.001 < P < 0.01$
** $P < 0.001$

And, as shown in Table 2, the post-implantational embryonic mortality in aged females was 25.6 per cent and it was significantly higher than the mortality in young adult females (4.5 per cent) ($P < 0.001$).

Fetal weight and its variability: Fetal weight on the 17th day of pregnancy, its variance and coefficient of variance were shown in Table

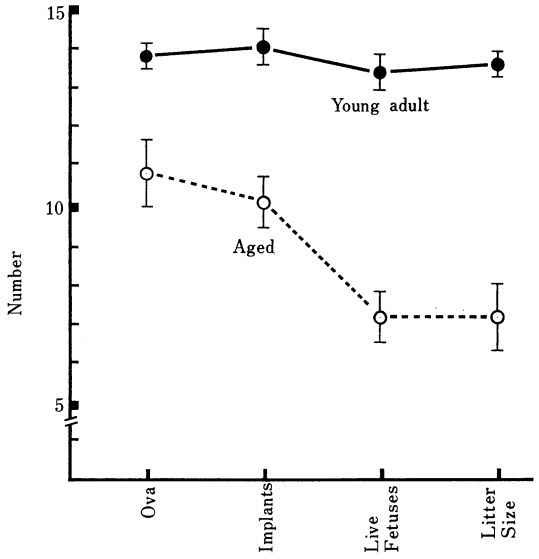

Fig. 1. Number of ova per ovulation, implants and live fetuses and litter size in young adult and aged female mice of CFW strain.

TABLE 2.

Post-implantational embryonic mortality

Maternal age (days)	Post-implantational embryonic mortality	
90—120	16/348	4.5% *
250—280	60/234	25.6%

* $P < 0.001$

3. An average of fetal weight in young adult females was 1.09 g, and 0.89 g in aged females. The latter was significantly less than the former ($P < 0.001$).

TABLE 3.

Fetal weight and its variability in young adult and aged pregnant mice of CFW strain

Maternal age (days)	(N)	Weight	Variance	C.V.
90—120	(332)	1.09g*	0.0120**	10.0%
250—280	(167)	0.89g	0.0291	19.2%

* $P < 0.001$, ** $F^{166}_{331} = 2.42 : P < 0.001$

Variance of fetal weight in aged females was also significantly larger than in young adult ones ($P < 0.001$) and coefficient of variance was also observed to show similar inclination.

Thus, it was obviously comfirmed that in aged females the fetal weight was less and the variability of it was larger than in young adult females.

Between-litter and within-litter variation of fetal weight: Table 4 and Fig. 2 represent the results which were obtained from dividing the variance of fetal weight into between-litter variance and within-litter variance, and culculating the contribution rate of each variance factor. It was obvious there that within-litter variance of fetal weight in aged females markedly increased and contribution rate of within-litter variance factor was 55.2 per cent, about twice as many as 26.3 per cent in young adult females. Therefore it may be infered from those results that the increase of variation of fetal weight in aged females principally caused by the increase of within-litter variation of fetal weight.

TABLE 4.
Analysis of variance on fetal weight

Maternal age (days)	Source of variation	SS	d.f.	MS	Contribution rate
90—120	Bet. Litter	3.01934	24	0.12580	73.7%
	W/n. Litter	0.97246	307	0.00317	26.3%
	Total	3.99180	331	0.01202	100.0%
250—280	Bet. Litter	2.50036	20	0.12501	44.8%
	W/n. Litter	2.35543	146	0.01613	55.2%
	Total	4.85579	166	0.02907	100.0%

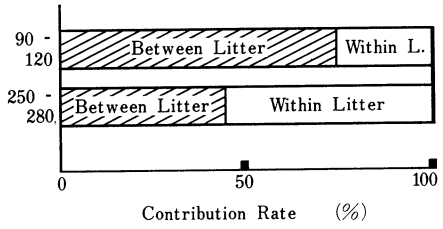

Fig. 2. Contribution rate of between and within litter variance factor to fetal weight variability in young adult and aged pregnant mice of CFW strain.

DISCUSSION

In polytococus species, the decline in litter size associated with

increasing maternal age was a wellknown fact[10]. As the cause of this fact, decrease in number of ova per ovulation[1], decrease of the implantation rate[3], increase of embryonic mortality[4,5], all owing to aging, were cited. And it was shown that embryonic death was prone to occur particularly after the implantation[6,14].

The results obtained in this study concerning the number of ova per ovulation, implants and of live fetuses and litter size, well agreed with the results reported up to date and it was confirmed over again that the principal cause of reduction in litter size by maternal aging was the increase of the post-implantational embryonic death. However, regarding the cause of the increase of embryonic death in aged females, no coinside view has been published and it may be necessary to investigate particularly whether the cause of increase in embryonic death is the aging of uterus itself, as pointed by Talbert[10], or the aging of endocrine, circulatory, nutritional and metabolic systems intimate with uterus.

The inferiolity of fetal weight in aged mice to the fetal weight in young adult ones was observed. And it was also shown that the variability of fetal weight increased in aged mice and the fetal growth became uneven with maternal aging. Then, two causes of the increase in the variability of fetal weight were conceived, the one was the wide distribution of the maternal ability with aging, namely the between-litter variance factor, the other was the increase in the variability of fetal weight in the same mother, the within-litter variance factor.

The enlargement of the variability of fetal weight in aged mice may be considered to be due to the enlargement of either between-litter variation or within-litter variation, or to the enlargement of both litter variation with maternal aging, but as the result of the variance analysis of the fetal weight, the principal cause was revealed to be the enlargement of the within-litter variation.

The fetal growth largely depend on the maternal environment, and therefore will be influenced much by the minute change in the maternal environment. And as the factor enlarging the within-litter variation of fetal weight, the struggle within litter mate was principal[15] and it would become more intense when the maternal environment was unfavorable to the fetal survival and growth.

In relation to these themes, Larson and Foote[11] reported that the uterine blood flow in aged rabbits was less than the flow in mature rabbits and the reduction of uterine blood flow likely is one of the uterine factors limiting reproductive capacity in aged rabbits.

As the causes of delayed fetal growth and enlarged variation of fetal weight in aged mice, obtained in this study, the obstruction of fetal nutrition and removal of metabolic products, and further intensified struggle within litter mate with poor nutritional condition following the reduction of uterine blood flow and declined maternal metabolic function, may be considered.

In conclusion, it was revealed that the maternal aging not only reduced the number of ova per ovulation and implants, fetal live rate and litter size but diminished the fetal growth. And it was also suggested that the maternal environment may be influenced by the maternal aging.

ACKNOWLEDGEMENT

The authers wish to thank Mr. G. Suzuki and Mr. S. Hashimoto, Laboratory Animal Research Section, NIAH, Japan, for their self-sacrificing assistance on taking care of experimental animals.

REFERENCES

1. Blaha, G. C.: Effect of age of the donor and recipient on the development of transfered golden hamster ova. Anat. Rec. 150: 413-416, 1964.
2. Kita, M. and Ino, T.: Reproductive performance of aged female mice. Jap. J. Fert. Ster. 18: 216-224, 1973 (in Japanese with English summary).
3. Harman, S. M. and Talbert, G. B.: The effect of maternal aged on ovulation, corpora lutea of pregnancy and implantation failure in mice. J. Reprod. Fert. 23: 33-39, 1970.
4. Finn, C. A.: Embryonic death in aged mice. Nature 194: 499-500, 1962.
5. Thorneycroft, I. H. and Soderwall, A. L,: The nature of the litter size loss in senesent hamster. Anat. Rec. 165: 343-348, 1969.
6. Talbert, G. B.: Effect of age on post-implantation reproductive failure in mice. J. Reprod. Fert. 24: 449-452, 1971.
7. Blaha, G. C.: Reproductive senesence in the female golden hamster. Anat. Rec. 150: 405-412, 1964.
8. Ortiz, E.: The relation of advancing age to reactivity of the reproductive system in the female hamster. Anat. Rec. 122: 517-537, 1955.
9. Talbert, G. B. and Krohn, P. L.: Effect of maternal age on viability of ova and uterine support of pregnancy in mice. J. Reprod. Fert. 11: 339-406, 1966.
10. Talbert, G. B.: Effect of maternal age on reproductive capacity. Am. J. Obst. Gynec. 102: 451-477, 1968.
11. Larson, L. L., Spilman, C. H. and Foote, R. H.: Uterine uptake of progesterone and estradiol in young and aged rabbits. Pro. Soc. Exp. Biol. Med. 141: 463-466, 1972.
12. Larson. L. L. and Foote, R. H.: Uterine blood flow in young and aged rabbits. Pro. Soc. Exp. Biol. Med. 141: 67-69, 1972.
13. Shapiro, M. and Talbert, G.B.: The effect of maternal age on decidualization in the mouse. J. Gernot. 29: 145-148, 1974.
14. Maibenco, H. C. and Krehbiel, R. H.: Reproductive decline in aged female rats. J. Reprod. Fert. 32: 121-123, 1972.
15. Harfez, E. S. E. (ed.): Reproduction in farm animals. 2nd ed., Philadelphia, Lea and Febiger, 1968.