Early Embryo Development in Senescent Golden Hamsters

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Abstract: *Golden hamster embryos were recovered from aged multiparous dams (15 to 16 months old), and young nulliparous females (3 to 4 months old) after mating with fertile males. The numbers of embryos and fetuses were counted at 1-cell, morula or blastocyst, and 8-day stages, respectively. The average number of normal eggs per dam was without significant difference, although slightly larger in young nulliparous females (young group) than in senescent multiparous females (aged group). However, the average number of anomalous eggs per dam increased significantly in the aged group. The average number of the embryos at morula or blastocyst stage per dam was significantly large in the young group. The average numbers of deciduae and fetuses, which were recovered from females at 8 days after mating, decreased significantly in the aged group. The frequency of appearance of mitotic metaphases in first-cleavage embryos decreased significantly in the aged group. These results indicate that the aging of dams causes delay of fertilization, or asynchronous development of embryos and degeneration of the resultant embryos through the embryo development. In senescent golden hamster females, large numbers of unfertilized eggs, "shell eggs" without cytoplasm, which were previously ovulated and then degenerated, were recovered. They were accumulated in oviducts or uterotubal junctions.*
Key words: *Golden hamster, Embryo, Aging, Unfertilized egg.*

With the advance of years in dams, decline in their reproductive capacity appeared as reduction in litter size in polytocous animals [6], and sterility in monotocous animals. Various causes of the decline such as: reduction in the numbers of ovulated eggs and implanted embryos [1, 11], increase of embryonic mortality [4. 11], deterioration of function in ovary and/or uterus [3, 5, 8, 9, 22], fall in viability of eggs [1, 17], and increase of chromosomal aberrations in embryos [7, 21, 24], have been reported until now. It is also well known that incidence of Down syndrome children is significantly high in the aged mothers [14, 15]. Further increases of chromosomal aberrations have been shown in early embryos obtained from aged mouse and human females [7, 15], and also in first-cleavage mouse embryos derived from aged dams [12].

In the present study, the frequency of appearance of mitotic metaphases in first-cleavage embryos of aged dams was examined in comparison with embryos of young dams. Viability of their embryos at other development stages was also compared to young dams'.

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Materials and Methods

Golden hamsters purchased from a commercial breeder were housed in a room controlled at about 24°C, with a photoperiod of 12-hours light (4:00 to 16:00) and 12-hours dark. Twenty-four multiparous hamsters (15–16 months: a senescent group) and 32 virgin hamsters (3–4 months: a young group) were used. The postestrus vaginal discharge [18] of females were checked every morning, and females showing at least three recurrences of the 4-day normal estrus cycle, were selected. Females showing estrus were individually paired with fertile males overnight. In the next morning, successful mating was ascertained by the presence of sperm in the vaginal smear. For recovery of first-cleavage eggs, the mated females were killed at 16 to 17 h (16:00 to 17:00) after the estimated time of ovulation (0:00) [10]. Eggs were obtained by flushing the excised oviducts with the top of uterine horn, and then incubated at 37°C in Eagle MEM, containing 30% fetal bovine serum and 0.1 μg/ml colcemid, for 5 to 6 hours.

Embryos at morula or blastocyst stages were recovered from the uterus of females killed at 69 to 72 h after the estimated ovulation time. The postimplantation stage embryos were obtained from deciduae at 8 days post

coitus, when the sperm ascertained day was estimated as the first day. The number of eggs ovulated was individually estimated by counting corpora lutea.

Chromosome preparation of 1-cell eggs was done according to the method of Yoshizawa *et al.* [25]. The preparations of 8-day embryos were made using the method of Wroblewska and Dyban [23], which was slightly modified by us.

The obtained data were analyzed by the t-test and the χ^2-test.

Results

The recurrence of estrus cycle in senescent hamsters was normal in observation of the postestrus vaginal discharge over two weeks.

The average number of normal eggs per dam was without significant difference although slightly larger in young nulliparous females (young group); 15.1, than in senescent multiparous females (aged group); 13.4 (Table 1). However, the average number of anomalous eggs per dam, "shell eggs", was significantly larger ($P<0.01$) in the aged group, 21.9, than in the young group, 0.4, although the average number of corpus lutea in an aged dam showed a tendency of decrease with no significant difference in comparison with that of a young dam. The anomalous eggs were almost all "shell eggs", which were composed of zona pellucida only, with debris of cytoplasm, or without cytoplasm.

The percentage of recovered morulae or blastocysts was about 88% of the eggs recovered in the young group, while about 31% in the aged group (Table 2). The average number of embryos per dam was significantly larger in the young group, 12.4, than in the aged group, 4.1. However, the average number of anomalous eggs per dam was significantly larger in the aged group, 9.1, than in the young group, 1.8, although the average number of corpora lutea in an aged dam showed no significant difference in comparison with that of a young dam.

The average number of corpora lutea per dam in the aged group showed a tendency of decrease with no significant difference in comparison with that of the young group at 8-day embryonic stage (Table 3). However, the average numbers of deciduae and fetuses, which were recovered from females at 8 days after mating, were 13.4 and 12.6 in the young group, and 1.4 and 0.5 in the aged group, respectively. There were significant differences between both groups in the numbers of deciduae and embryos. The incidence of "embryonic loss";

Table 1. Numbers of corpus lutea and eggs recovered from young nulliparous and aged multiparous female golden hamsters at 16 to 17 h after the estimated ovulation

	No. of		No. of normal eggs		No. of anomalous eggs		
	dams	c. l.	eggs	1-cell stage	shell	others	total
Young dams	12	182	192	181 (94.3%)	5	6	11 (5.7%)
Per dam ± SD		15.2 ± 1.46	16.0* ± 2.52	15.1 ± 1.80	0.4* ± 1.38		
Aged dams	7	92	248	94 (37.9%)	153	1	154 (62.1%)
Per dam ± SD		13.1 ± 2.10	35.4* ± 20.74	13.4 ± 2.44	21.9* ± 20.52		

c. l. : corpus lutea. Young dams: at age 3–4 months, Aged dams: at age 15–16 months. * $P<0.01$ (t-test).

Table 2. Number of corpus lutea and embryos recovered from young nulliparous and aged female golden hamsters at 69 to 72 h after the estimated ovulation

	No. of				No. of anomalous eggs			
	dams	c. l.	eggs	m. & bl.	1-cell	shell	others	total
Young dams	8	115	113	99 (87.6%)	7	0	7	14 (12.4%)
Par dam ± SD		14.4 ± 1.80	14.1 ± 1.54	12.4* ± 3.04				1.8** ± 2.59
Aged dams	7	83	93	29 (31.2%)	15	36	13	64 (68.8%)
Par dam ± SD		11.9 ± 2.36	13.3 ± 6.43	4.1* ± 4.26				9.1** ± 7.02

c. l. : corpus lutea, m. & bl.: morulae and blastocysts. Young dams: at age 3–4 months, Aged dams: at age 15–16 months. * $P<0.01$, ** $P<0.05$ (t-test).

or deciduae without an embryo, was higher in the aged group than in the young group.

Frequency of mitotic metaphases in 1-cell stage eggs was 97.2% in the young group, while significantly lower at 6.7% in the aged group (Table 4). The percentage of 8-day fetuses with mitotic metaphases was 86.5% in the young group, while 100% in the aged group, although only occurring in two embryos. Both embryos which survived, were chromosomally normal.

Discussion

The normality of recurrence of senescent hamsters' estrus cycles in the present study agreed with the result of Kita and Ino [11], which showed little difference between young adult and aged nulliparous female mice in the estrus cycle.

Many abnormal eggs, or "shell eggs", were found in the present study. Because the number of corpora lutea was comparable with that of normal eggs, we considered the "shell eggs" to be eggs ovulated previously. We also have frequently observed similar eggs in experiments with mice. However, the number of the eggs in mice was not as many as that of the aged hamster. It is known well that in the female horse, unfertilized horse eggs are left in the isthmus of the uterine tube, while fertilized eggs are transferred to the uterus [16]. The result of the present study suggests the presence of similar phenomenon in the golden hamster.

In the present study, there was a very low recovery rate of morulae or blastocysts within a large number of anomalous eggs in the aged group. In golden hamsters, Blaha [1] showed a significantly small number of eggs recovered from old (14 to 18 month) females at 63 to 68 hours after ovulation. Furthermore, Parkening and Soderwall [19] demonstrated approximately 40 % of the eggs ovulated by senescent females to be nonviable (unfertile, abnormal, and degenerating) at the time of implantation. Shimizu [21] also found decreases of the egg-recovery rate and the number of ovulated eggs per rat, and an increase of abnormal eggs in aged rats. Onodera and Ishijima [17] reported that the percentage of anomalous eggs in aged female mice (7 to 9 and 10 to 12 months old) was significantly higher than that of young female mice (2 or 4 months old) at about 36 to 38 hours after ovulation. They concluded that the viability of eggs of aged female mice, was lower than that of the eggs of young adult females. This was because the percentage of the eggs degenerated in culture was significantly higher in the aged mice than in the young mice.

However, the results of *in-vitro* fertilization of eggs recovered from senescent mice and hamsters indicated that there was no significant difference between young

Table 3. Numbers of corpus lutea, deciduae and embryos recovered from young nulliparous and aged multiparous female golden hamsters at 8 days after mating

		No. of				
	dams	c. l.	deciduae	embryos	embryonic loss	rudiments
Young dams	12	179	161	151 (93.8%)	10 (6.2%)	0
Par dam ± SD		14.9 ± 1.64	13.4* ± 2.50	12.6* ± 2.66		
Aged dams	10	125	14	5 (35.7%)	9 (64.3%)	2
Par dam ± SD		12.5 ± 3.14	1.4* ± 2.2	0.5* ± 0.81		

c. l. : corpus lutea. Young dams: at age 3–4 months, Aged dams: at age 15–16 months. *P<0.001 (t-test).

Table 4. Results of obsevation of chromosomes in embryos recovered from young nulliparous and aged multiparous femal golden hamsters

	1-cell stage eggs		8-day stage embryos	
	perpared	in mitosis	prepared	with mitosis
Young dams	177	172* (97.2%)	148	128 (86.5%)
Aged dams	89	6* (6.7%)	2	2 (100%)

Young dams: at age 3–4 months, Aged dams: at age 15–16 months. *P<0.001 (χ^2-test).

and aged animals in the proportions of eggs fertilized [20]. By egg transfer experiments with young and old multiparous female mice, Gosden [8] showed that eggs from old donors had the same development potential as eggs from young donors, and that the age of the recipient had a significant effect on embryonic survival. Maurer and Foote [13] also reported similar results using the rabbit. The decline of viability of eggs observed in the aged group in the present study might have resulted from their own low viability and from the maternal environment.

The high incidence of embryonic loss in the aged group in the present study agreed with the results shown by Kita and Ino [11], in which the numbers of implantation sites and live fetuses were significantly larger in young adult female mice than in aged females.

Frequency of mitotic metaphases in 1-cell stage eggs of the aged dams was significantly low, as indicated by an increase of the pronuclear eggs in the aged group. We attributed that to the delay of fertilization, because Parkening and Soderwall [19] reported that the majority of embryos recovered from aged hamsters (14–17 months of age) exhibited a 2 to 5 hour delay in fertilization at about 84 hour postovulation. Furthermore, they attributed this to the prolonged penetration by the spermatozoon into the zona pellucida and vitellus, i.e. extending the time of fertilization, and then they explained that the higher levels of circulating progesterone in plasma of senescent hamsters [2] might cause the delayed fertilization as the result of slowed spermatozoon transport in senescent hamsters.

The results of the present study indicate that the aged dam causes delay of fertilization, and that the resultant embryos degenerate through the development into fetus. Furthermore, it was found also that many unfertilized eggs, "shell eggs", which were previously ovulated and then degenerated, were accumulated in oviducts or uterotubal junctions of senescent golden hamster females.

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高齢ゴールデンハムスターにおける胚の初期発生能について

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15～16か月齢の経産高齢ゴールデンハムスター24頭および3～4か月齢の未経産ゴールデンハムスター32頭を用い，母体の加齢が胚の初期発生能に与える影響について調査した．排卵卵子数，受精卵子数，桑実胚および胚盤胞への発生数および着床胚数を調べ，併せて第1分割期の染色体標本を作製し，分裂中期像の出現率を比較検討した．1細胞期卵子の1頭あたりの平均回収卵子数は，若齢群15.1個，高齢群13.4個で両群間に有意な差は見られなかった．また，透明帯だけの異常卵子 (shell eggs) が，高齢群において数多く観察された．これらの卵子は，以前に排卵された未受精卵子が退行したものと考えられ，卵管あるいは卵管子宮接合部のどこかに滞留していたものと思われた．排卵推定時より69～72時間後に，子宮灌流を行うことにより得られた正常な桑実胚もしくは胚盤胞の平均数は，若齢群では12.4個，高齢群では4.1個で，高齢群において有意に少なかった ($P<0.01$)．交配後8日目における脱落膜の平均数は，若齢群で13.4個，高齢群では1.4個であり，正常胚の平均数は，12.5個，高齢群0.5個で，いずれも高齢群において有意に少なかった ($P<0.001$)．第1分割期の染色体像を示す卵子は，若齢群では97.2%であったが，高齢群ではわずかに6.7%にとどまった．これらのことから，高齢群では，受精が遅延され，その結果生じた胚の発生能力も若齢群の卵子より劣ることが推察された．

キーワード：ゴールデンハムスター，胚，加齢，未受精卵．