Peri-implantation Embryonic Loss and Its Related Uterine Factors in Aged Hamsters

Shixiong XI, Hiroyuki SUZUKI and Koji TOYOKAWA

Faculty of Agriculture and Life Sciences, Hirosaki University, Hirosaki 036-8561, Japan

Abstract. The embryonic development and changes of the uterine tissue during pregnancy were examined morphologically to reveal causes of the age-related decline in litter size in the golden hamster. Uteri were obtained from young (9–12-week old, $n=48$) and aged hamsters (43–53-week old, $n=47$) at estrus and on Days 2 to 16 of pregnancy. Viability of conceptuses were examined in half of implants from Day 6 onwards by a dissecting microscope. The remaining implants were evaluated histologically. The number of resorbing implants was significantly larger in the aged hamsters than in the young females on Day 6 (4.4 vs. 1.2, $p<0.05$), although no difference was found in the overall mean number of ova ovulated (14.8 vs. 14.2, respectively). Delays in the embryonic development and uterine response were observed in all aged hamsters throughout gestation. Resorbing implants noted in the aged hamsters were accompanied with stunted decidual cells having pale cytoplasm (incomplete decidualization) and heavy infiltration of endometrial granulocytes. It is suggested that the embryonic wastage may occur much crucially during the peri-implantation period rather than late gestational period, due to the retarded development of embryos and less-developed decidualization, and that endometrial granulocytes may function in resorption of greatly retarded embryos in the aged hamsters.

Key words: Aged hamsters, Embryonic loss, Uterus, Resorbing implants, Endometrial granulocytes. (*J. Reprod. Dev.* 45: 197–204, 1999)

It is well known that the female reproductive capacity declines as age increases, and a progressive reduction in litter size has been reported in hamsters, mice, rats and rabbits [1–6]. In the hamsters aged 14–19 months, the reduction in litter size depends upon increases in preimplantation loss and in resorption of implanted embryos [8–12]. Since no difference in the ovulation rate was found between the young and aged hamsters [9, 10], it seems that the decline in litter size is not caused primarily by the decreased number of ova. Several studies have revealed that embryos from old hamsters show a developmental delay before and during implantation [10–14], and that a pro-

gressive deterioration in uterine adaptability to pregnancy is noted in the aged hamsters [15].

Our previous studies revealed that about 25–30% of decline was observed in litter size of 43-week old hamsters as compared with young females [16] and that a reduced ability to reproduce in the aged hamster may be due to an increased proportion of abnormal ova before implantation [17]. In order to clarify the causes of embryo wastage in the aged hamster, the morphological changes of the conceptuses and uterine compartments were examined in both young and aged hamsters. In the present study, we compared the embryo viability and uterine response in the young and aged hamsters.

Materials and Methods

Animals

Golden hamsters (*Mesocricetus auratus*) were kept under constant temperature (21–23 °C) and a cycle of 14 h light (0500 h–1900 h) and 10 h darkness. They were given laboratory chow and water *ad libitum*. The vaginal discharge of each female was checked prior to mating to assure that the females had normal estrous cyclic pattern [18]. A total of 95 virgin females were mated at 9–12 weeks of age and randomly assigned to young (9–12 weeks of age, $n=48$) and aged groups (43–53 weeks of age, $n=47$). The females in the aged group were repeatedly mated at intervals of 10–12 weeks until examination at 43–53 weeks of age according to our previous studies [16, 17]. In the evening of estrus, the females were caged with young fertile males and the day when spermatozoa were found in the vaginal smear was designated as Day 1 of pregnancy (Day 1).

Viability of embryos and histological examination

Females were sacrificed by an intraperitoneal overdose of pentobarbital sodium and the uteri were dissected out at estrus and on Days 2, 4, 5, 6, 8, 9, 10, 11, 13 and 15 in young and aged groups (4–5 animals each, except for $n=2$ at estrus and on Day 9). Females in the aged group were also examined on Day 16, since all aged hamsters delivered young on Day 17, one day later than did young females (our unpublished data). The number of ruptured follicles or corpora lutea was counted and considered as the number of ova ovulated. Uterine tissues were obtained from the upper-, middle- and lower-portions of each uterine horn at estrus and until Day 5. After Day 6, half of implants in both uterine horns were cut open and classified as viable or resorbing ones under a dissecting microscope according to Thorneycroft and Soderwall [9]. Resorbing implants contained greatly retarded embryos, fragile embryos, or only blood clots. The remaining implants were examined histologically. Uterine tissues with or without implants (viable or resorbing) were immersed into Bouin's fixative or a mixture of 2% glutaraldehyde and 4% formaldehyde in 0.1 M phosphate buffer (pH=7.3). After fixation, the tissues were dehydrated in a graded series of ethanol and embedded in paraffin. Trans-

verse sections (5–7 μm) were stained with hematoxylin and eosin or reacted with the periodic acid-Schiff (PAS) technique (with or without previous diastase digestion and counterstained with hematoxylin). Numbers of mitotic figures in the antimesometrial and mesometrial stromal cells adjacent to morphologically normal embryos were counted in every seven serial sections up to 10–15 sections per animal at $\times 400$ magnification.

The number of embryos lost during the preimplantation period was estimated from the difference between the average number of ova ovulated and that of implants (viable and resorbing) on Day 6. Postimplantation wastage of embryos was calculated by subtracting the mean number of viable implants on the day before parturition from the mean number of implants (viable and resorbing) on Day 6.

Statistical analysis

Statistical significance of these data was analyzed by Student's *t*-test.

Results

Viability of conceptuses

The numbers of ova ovulated and viability of conceptuses in young and aged hamsters were summarized in Tables 1 and 2. The overall mean number of ova ovulated did not differ significantly between the young (14.2, $n=46$) and aged hamsters (14.8, $n=45$). But significant differences in the number of viable implants were found between the young and aged hamsters on Days 6, 11, 13 and 15. In contrast, more resorbing implants was found in the aged females than in the young females on Day 6 (4.4 vs. 1.2, $p<0.05$) and Day 8 (4.2 vs. 1.0, $p<0.05$). The number of embryos lost before implantation was estimated at 1.8 (13%) and 4.0 (27%) for the young and aged hamsters, respectively. Estimated numbers of the postimplantation loss of embryos in the young and aged hamsters were 0.3 (2%) and 2.8 (19%), respectively.

As shown in Table 2, the incidence of implants containing fragile embryos and only blood clots in the aged hamsters was much higher on Days 6 (77%, 17/22) and 8 (86%, 18/21), respectively. Greatly retarded embryos were observed only in the aged females.

Table 1. Numbers of ova ovulated and implants during pregnancy in the young and aged hamsters (mean $\pm$ SEM)

Days of pregnancy	No. animals		No. ova ovulated		No. viable implants		No. resorbing implants	
	Young	Aged	Young	Aged	Young	Aged	Young	Aged
2	5	4	14.0 $\pm$ 0.7	14.8 $\pm$ 1.3	–	–	–	–
4	5	4	14.4 $\pm$ 0.9	15.3 $\pm$ 1.0	–	–	–	–
5	5	4	14.2 $\pm$ 0.8	15.5 $\pm$ 1.7	–	–	–	–
6	5	5	13.6 $\pm$ 1.3	15.0 $\pm$ 1.6	10.6 $\pm$ 0.5 ^a	6.6 $\pm$ 3.1 ^c	1.2 $\pm$ 0.8 ^a	4.4 $\pm$ 2.6 ^c
8	5	5	14.2 $\pm$ 0.4	15.4 $\pm$ 1.1	12.2 $\pm$ 0.8	8.2 $\pm$ 3.6	1.0 $\pm$ 0.7 ^a	4.2 $\pm$ 2.4 ^c
9	2	2	14.0 $\pm$ 1.4	15.5 $\pm$ 0.7	11.5 $\pm$ 0.7	8.0 $\pm$ 2.8	1.0 $\pm$ 1.4	3.0 $\pm$ 2.8
10	5	4	13.6 $\pm$ 1.7	14.5 $\pm$ 1.3	10.0 $\pm$ 2.0	7.0 $\pm$ 2.1	1.6 $\pm$ 1.1	3.2 $\pm$ 1.7
11	5	5	14.8 $\pm$ 0.4	14.8 $\pm$ 0.8	12.2 $\pm$ 1.6 ^a	7.2 $\pm$ 1.7 ^b	1.6 $\pm$ 1.1	2.8 $\pm$ 1.6
13	5	4	14.8 $\pm$ 0.8	14.5 $\pm$ 0.6	11.6 $\pm$ 1.6 ^a	7.5 $\pm$ 1.9 ^c	1.6 $\pm$ 1.5	2.5 $\pm$ 1.7
15	4	4	14.5 $\pm$ 0.6	14.0 $\pm$ 0.8	11.5 $\pm$ 1.3 ^a	7.5 $\pm$ 0.5 ^b	1.5 $\pm$ 1.2	2.7 $\pm$ 1.2
16*	–	4	–	13.8 $\pm$ 1.0	–	8.2 $\pm$ 2.0	–	1.0 $\pm$ 0.8

Means with different superscripts were significantly different between young and aged hamsters (a,b, $p < 0.01$; a,c, $p < 0.05$).

No visual implants were found on Days 2–5.

*, Young hamsters delivered early in the morning on Day 16.

Table 2. Number of resorbing implants in the young and aged hamsters

Days of pregnancy	Total No.		No. resorbing implants containing:					
			greatly retarded embryos		fragile embryos		blood clots	
	Young	Aged	Young	Aged	Young	Aged	Young	Aged
6	6	22	0	0	0	17	6	5
8	5	21	0	0	0	3	5	18
9	2	6	0	0	1	1	1	5
10	8	13	0	2	3	1	5	10
11	8	14	0	3	3	2	5	9
13	8	10	0	0	2	1	6	9
15	6	11	0	0	0	0	6	11
16*	–	4	–	0	–	0	–	4

*, Young hamsters delivered early in the morning on Day 16.

Histological observation on the uterine tissue and viable implants

Aged females had thicker uterine wall than did young females. At estrus, the uterine lumen was irregular and wide, and the endometrium was infiltrated with occasional leukocytes and plasma cells in the young and aged females. Pigment-containing macrophages were found in the aged uterus, especially in the mesometrial triangle, but not in the young uterus.

On Days 2 and 4, uterine lumen became a narrow slit in the young females (Fig. 1a), but in the aged females it remained relatively wide (Fig. 1d). On Day 4, blastocysts attached to the antimesometrial epithelium in the young females (Fig. 1a), but in the aged females they were still free within the uterine lumen (Fig. 1d). Leukocytes and plasma cells in the endometrium increased until Day 4,

but decreased thereafter in both young and aged females.

On Day 5, early gastrulae invaded uterine epithelium in the young females (Fig. 1b), whereas in the aged females embryos were still at the early primitive endoderm stage (Fig. 1e). Decidua capsularis was formed in the young females, but in the aged females the decidual cells were less-developed (Figs. 1b vs. 1e). On Day 6, aged females possessed smaller gastrula embryos than did young ones (Figs. 1c vs. 1f). Decidua basalis was formed in the young females, where typical decidual cells were closely packed and contained glycogen-rich cytoplasm stained by PAS reaction (Fig. 1c). In the aged females, however, fibroblast-like stromal cells with wide intercellular spaces were still observed mesometrially. Mono- or binucleate endometrial granulocytes, characterized by their glycoprotein-containing cytoplasmic granules, were

Fig. 1. Transverse sections of viable implants from young (a, b, c) and aged hamsters (d, e, f). Antimesometrial side is on the right of photographs.

- a) An implantation site of a young female on Day 4. A blastocyst has attached to the antimesometrial epithelium. No remarkable change is found in the uterine stroma. Hematoxylin and eosin. $\times 165$.
- b) An early gastrula-stage embryo in a young female on Day 5. Accumulation of decidual cells is evident and endometrial granulocytes (arrows) are identified among them. PAS and hematoxylin. $\times 165$.
- c) An advanced gastrula embryo in a young female on Day 6. Note typical decidual cells with glycogen-rich cytoplasm (arrows). PAS and hematoxylin. $\times 180$.
- d) A blastocyst freed within the uterine lumen of an aged female on Day 4. No remarkable change is found in the endometrium. Hematoxylin and eosin. $\times 165$.

initially noted in the antimesometrial stroma on Day 5 and in the mesometrial stroma on Day 6 in young females, whereas in aged females they occurred in the antimesometrial stroma on Day 6 and in the mesometrial stroma on Day 8. Macrophages were hardly noticed in the endometrium after Day 6 in the aged group.

The maternal blood space was formed on Day 8, the primitive chorioallantoic placenta was established on Day 9 and the trophospongium and labyrinth were defined with the central arterial space on Day 10 in the young females, but one day delay was noticed in the aged females. On Days

11–13, the fetuses developed progressively, the spiral arteries were greatly enlarged, and the decidua basalis became thinner. After Day 15, decidual cells began to degenerate and endometrial granulocytes disrupted to release their granules into the intercellular space. During the late pregnancy, a delay in embryonic development was evident in the aged hamsters. The course of pregnancy in the young and aged hamsters was summarized in Table 3.

Mitosis in the endometrial stroma: Table 4 showed numbers of mitotic figures in the endometrial stromal cells in the young and aged hamsters. On Days 4 and 5, numbers of mitoses on the mesometrial side in the aged females were significantly lower than those in the young females ($P < 0.01$), but not on the antimesometrial side. From Day 8 onwards, the mitoses on the mesometrial side decreased, followed by the full differentiation into the decidua basalis.

Histological observation on resorbing implants

Three types of resorbing implants, which contained greatly retarded embryos, fragile embryos or only blood clots, were noted (Table 2). Fragile embryos were characterized by an incomplete decidualization on the antimesometrial and mesometrial sides. The decidual reaction was found in very limited area and the decidual cells contained paler cytoplasm than those observed at viable implants (Fig. 2a).

Table 3. Comparison of implantation and placentation processes in the young and aged hamsters

Events	Days of pregnancy	
	Young	Aged
Blastocyst attachment	4	<5*
Formation of decidua capsularis	5	6
Formation of decidua basalis	6	8
Formation of maternal blood space	8	8
Development of primitive chorioallantoic placenta	9	10
Formation of central arterial space	10	11
Establishment of trophospongium and labyrinth	10	11
Parturition **	16	17

*, Blastocysts attached by Day 5.

**, Our unpublished data.

Table 4. Number of mitosis in endometrial stromal cells in the young and aged hamsters during pregnancy (mean $\pm$ SEM)

Days of pregnancy	No. animals		Mesometrium		Antimesometrium	
	Young	Aged	Young	Aged	Young	Aged
2	5	4	<0.10	<0.10	<0.10	<0.10
4	5	4	3.9 $\pm$ 2.2 ^a	0.4 $\pm$ 0.6 ^b	3.7 $\pm$ 1.9	3.7 $\pm$ 1.7
5	5	4	10.6 $\pm$ 3.0 ^a	2.6 $\pm$ 1.5 ^b	10.2 $\pm$ 4.6	8.2 $\pm$ 2.9
6	5	5	11.4 $\pm$ 2.3	10.4 $\pm$ 2.5	0.7 $\pm$ 0.8	6.2 $\pm$ 1.9
8	5	5	5.8 $\pm$ 1.6	7.1 $\pm$ 1.4	<0.10	<0.10
9	2	2	0.8 $\pm$ 0.8	4.1 $\pm$ 1.7	<0.10	<0.10
10	5	4	<0.10	0.8 $\pm$ 0.6	<0.10	<0.10
11	5	5	<0.10	<0.10	<0.10	<0.10

a,b, Means with different superscripts were significantly different between young and aged hamsters ($p < 0.01$).

e) An embryo at early primitive endoderm stage in an aged female on Day 5. Note a delayed decidualization compared to Fig. 1b. PAS and hematoxylin. $\times 165$.

f) A gastrula-stage embryo in an aged female on Day 6. Decidual cells (arrows) are noted on the antimesometrial side of the embryo. PAS and hematoxylin. $\times 180$.

Fig. 2. Transverse sections of resorbing (a, b, d, e) or viable implants (c).

- a) A fragile embryo (E) noted in an aged hamster on Day 6. The embryo is merely remnant of darkly stained, degenerated trophoblastic cells. Decidual cells contain pale cytoplasm without glycogen. PAS and hematoxylin. $\times 180$.
- b) Decidua capsularis adjacent to a resorbing embryo (E), containing blood clots (on the left of the photograph), in an aged hamster on Day 8. Note that macrophages (arrows) are migrating. Hematoxylin and eosin. $\times 825$.
- c) A viable fetus in a young hamster on Day 11. N, neural tube; A, atrium. Hematoxylin and eosin. $\times 33$.
- d) A greatly retarded embryo in an aged hamster on Day 11. The development seems to be corresponding with that of embryos in the young hamsters on Day 9. Note primitive chorioallantoic placenta (P). Hematoxylin and eosin. $\times 33$.
- e) Decidua basalis adjacent to the embryo in Fig. 2d. Note endometrial granulocytes (small arrows) and their released cytoplasmic granules (large arrows). Diastase, PAS and hematoxylin. $\times 825$.

On Days 6 and 8, plenty of macrophages were seen around resorbing gastrulae or neurulae in the aged group (Fig. 2b). Long interconceptual areas, 10–25 mm in length, were noticed in 39% (13/33) of the aged females after Day 6. These areas were characterized by either containing a blood remnant or heavy infiltration with macrophages, plasma cells and lymphocytes.

A great delay in development of the embryos was noted only in the aged group on Days 10 and 11 as compared with the embryos in the young group (Figs. 2c vs. 2d). Their organogenesis was delayed more than 2 days as compared to that of viable fetuses in the young females, on the basis of anatomical and histological observations. More endometrial granulocytes were concentrated around such retarded embryos than viable embryos even in the same female (Fig. 2e). Some of endometrial granulocytes released frequently their cytoplasmic granules.

Discussion

Age-related decline was not found in the number of ova ovulated in the present study. Decrease in the ovulation rate seemed not to be the primary cause in a decline of litter size in aged hamsters [9–12, 16, 17], mice [2, 19] and rats [20]. The present study clearly demonstrated that there was a significant difference between the numbers of implants in the young and aged hamsters and that embryonic wastage seemed to be greater in the preimplantation period than in the postimplantation period. A similar tendency was obtained by Thorneycroft and Soderwall [9], who observed a seven fold increase in preimplantation deaths and twice as many resorptions in postimplantation period in old hamsters compared with young hamsters.

Preimplantation loss may be one of important factors in the decline of litter size. Parkening and Soderwall [12, 14] have noted delayed fertilization in the majority of ova in the aged hamsters. In our previous study, a delay in the process of ovulation, a delay in the male pronuclear development and high incidence of polygyny were noticed in the aged hamsters [17]. In addition, embryos from old animals showed a developmental delay before and during implantation [10, 11, 13]. Similar results were obtained in the aged rats [21]. Increment

of retarded embryos in the aged animals may be an indication of the less viable embryos, probably resulting from the delay of ovulation [17] and abnormal fertilization [11, 12, 17]. Mizoguchi and Dukelow [22] have noted that aged hamsters tend to ovulate morphologically abnormal oocytes, incidence of which was 6.9% contrasting with 0.5% in young females. Recently, Yoshizawa [23] showed that 69% of ova recovered from the old hamsters aged 15–16 months were anomalous and that proportion of embryonic loss reached to 64% at 8 days after mating. She found asynchronous development of embryos in the old hamsters [23], too. It has been reported, in mice [24] and rats [25], that ultrastructure of the preovulatory oocytes undergoes age-related changes and some biochemical activities are also altered in aged animals, possibly leading to increase in abnormal fertilization and reduced viability in embryonic development.

Another fascinating observation from the present study is that the development of embryos was delayed about one day during postimplantation period in the aged hamsters. Similarly, the uterine response such as decidualization was also delayed. Delayed changes in the proportion of mitotic figures in the endometrium noted in the aged hamsters may be related to the delayed decidualization. Blaha [8] observed an inadequate decidual reaction in the uterus of the old hamsters aged 16 months. In our preliminary study, all aged hamsters delivered young on Day 17 and no young were obtained after Day 18 (unpublished data), suggesting that 1-day delay in the embryonic development may be critical for survival of fetuses during pregnancy. Therefore, the greatly retarded embryos, more than 2 days, noticed in the aged hamster are considered to be resorbed before parturition. In addition, plenty of endometrial granulocytes migrated around such conceptuses. Appearance of endometrial granulocytes in the hamsters seems to be a component of the decidual reaction [26]. In the mice, endometrial granulocytes play a role in surveying trophoblastic cell quality and participate in resorption of abnormal conceptuses [27].

In conclusion, the embryonic development and uterine responses were delayed approximately one day in the aged hamsters compared with the young hamsters. The embryonic wastage in the aged females was greater in the peri-implantation period

than in later gestational period. The endometrial granulocytes may function to resorb the greatly retarded embryos, although the biological signifi-

cance of their appearance is not clear in the hamster.

References

1. **Talbert GB.** Effect of maternal age on reproductive capacity. *Am J Obstet Gynecol* 1968; 102: 451-477.
2. **Jones EC.** The aging ovary and its influence on reproductive capacity. *J Reprod Fertil Suppl* 1970; 12: 17-30.
3. **Foote RH.** The gametogenic function of the aging ovary in the mammal. In: Blandau RJ (ed.), *Aging Gametes*. Basel: Karger; 1975: 175-200.
4. **Butcher RL, Page RD.** Role of the aging ovary in cessation of reproduction. In: Schwartz NB, Hunzicker-Dunn M (eds.), *Dynamics of Ovarian Function*. New York: Raven Press; 1981: 253-271.
5. **Adams CE.** Reproductive senescence. In: Austin CR, Short RV (eds.), *Reproduction in Mammals, Book 4: Reproductive Fitness*. Cambridge: Cambridge University Press; 1984: 210-233.
6. **vom Saal FS, Finch CE.** Reproductive senescence: phenomena and mechanisms in mammals and selected vertebrates. In: Knobil E, Neill J (eds.), *The Physiology of Reproduction*. New York: Raven Press; 1988: 2351-2413.
7. **Soderwall AL, Kent HA, Turbyfill CL, Britenbaker AL.** Variation in gestation length and litter size of the golden hamster, *Mesocricetus auratus*. *J Geront* 1960; 15: 246-248.
8. **Blaha GC.** Reproductive senescence in the female golden hamster. *Anat Rec* 1964; 150: 405-412.
9. **Thorneycroft IH, Soderwall AL.** The nature of the litter size loss in senescent hamsters. *Anat Rec* 1969; 165: 343-348.
10. **Connors TJ, Thorpe LW, Soderwall AL.** An analysis of preimplantation embryonic death in senescent golden hamsters. *Biol Reprod* 1972; 6: 131-135.
11. **Parkening TA, Soderwall AL.** Delayed embryonic development and implantation in senescent golden hamsters. *Biol Reprod* 1973; 8: 427-434.
12. **Parkening TA, Soderwall AL.** Delayed fertilization and preimplantation loss in senescent golden hamsters. *Biol Reprod* 1975; 12: 618-631.
13. **Parkening TA, Soderwall AL.** Preimplantation stages from young and senescent golden hamsters: presence of succinic dehydrogenase and non-viable ova. *J Reprod Fertil* 1973; 35: 373-376.
14. **Parkening TA, Soderwall AL.** Delayed fertilization in senescent golden hamsters. *Nature* 1974; 251: 723-724.
15. **Blaha GC.** Effect of age of the donor and recipient on the development of transferred golden hamster ova. *Anat Rec* 1964; 150: 413-416.
16. **Suzuki H, Kida R, Moriguchi M.** Effects of age and repeatedly mating on litter size in golden hamsters. *Bull Fac Agric Hirosaki Univ* 1995; 59:88-96.
17. **Suzuki H, Moriguchi M, Kida R, Moro Y.** Delay in ovulation and fertilization and asynchronous pronuclear development in aged hamsters. *J Reprod Dev* 1996; 42: 15-22.
18. **Orsini MW.** The external vaginal phenomena characterizing the stages of the estrus cycle, pregnancy, pseudopregnancy, lactation and the anestrus hamster, *Mesocricetus auratus*. *Proc Animal Care Panel* 1961; 11: 193-206.
19. **Jones EC, Krohn PL.** The relationships between age, numbers of oocytes and fertility in virgin and multiparous mice. *J Endocrinol* 1961; 21: 469-494.
20. **Matt DW, Sarver PL, Lu JKH.** Relation of parity and estrous cyclicity to the biology of pregnancy in aging female rats. *Biol Reprod* 1987; 37: 421-430.
21. **Day JR, La Polt PS, Morales TH, Lu JKH.** An abnormal pattern of embryonic development during early pregnancy in aging rats. *Biol Reprod* 1989; 41: 933-939.
22. **Mizoguchi H, Dukelow WR.** Fertilizability of ova from young or old hamsters after spontaneous or induced ovulation. *Fertil Steril* 1981; 35: 79-83.
23. **Yoshizawa M.** Early embryo development in senescent golden hamsters. *J Mamm Ova Res* 1995; 12: 107-111.
24. **Narita A.** Endogenous factors affecting sterility in oocytes of aged animals. *Jpn J Fertil Steril* 1995; 40: 57-65.
25. **Peluso JJ, Hutz R, England-Charlesworth C.** Age-related alteration in preovulatory oocytes. In: Schwartz NB, Hunzicker-Dunn M (eds.), *Dynamics of Ovarian Function*. New York: Raven Press; 1981: 303-308.
26. **Bulmer D, Stewart I, Peel S.** Endometrial granulocytes of the pregnant hamster. *J Anat* 1983; 136: 329-337.
27. **Croy BA.** Granulated metrial gland cells: hypotheses concerning possible functions during murine gestation. *J Reprod Immunol* 1994; 27: 85-94.