On fragmentation and elimination of ovarian oocytes

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Summary. Examination of numerous fragmented oocytes in the ovary revealed an asynchronous relationship between nuclear and cell divisions. The asynchrony was observed in non-fragmented oocytes as well and was considered to be one of the common processes leading towards oocyte elimination in the ovary. The present study additionally demonstrates the morphology of follicle cell degeneration observed on the surface of fragmented oocytes.

Fragmentation of ovarian oocytes is a conspicuous phenomenon and has been attracting the attention of many researchers[2,3] because of its atypical feature as a process of oocyte multiplication; although meiosis and further cell cleavage are limited, and progression as far as fertilization could not take place[4] in situ, oocyte divisions occur repeatedly (parthenogenesis). The phenomenon of fragmentation means that the oocyte per se is living, in spite of its postulated cytogenetical abnormality[5] and the fact that it is destined for elimination. The evaluation of fragmented oocytes is extremely important, because it has been suggested that fragmented oocytes can offer researchers cytological clues in considering how living oocytes in general follow the process toward elimination.

The usual methods for collection of oocytes after mechanical liberation[6,7] do not easily supply sufficient numbers of fragmented and/or non-fragmented oocytes on the way to elimination showing diversity of morphology. The efficacy of a modified whole mount method developed by Shinohara et al.[8] was further enhanced by centrifugation and omission of the staining procedure. The new method was used in the present study to elucidate morphological characteristics of fragmented oocytes.

Materials and methods. Wistar strain rats age ranging from 8 to 12 weeks old were assigned for the present study. They were kept at 24 °C with a dark-light schedule of 12 h. Only animals having sexual periodicity were supplied for experiments, without regard to the estrus cycle.

The experimental animals were killed using ether, and the bilateral ovaries were extirpated. The surrounding connective and fatty tissues were carefully removed on a clean filter paper. The ovary was placed in a plastic dish (3.5 cm in diameter) containing 5.0 ml of physiological saline solution, and randomly punctured 200 times under a stereoscopic binocular microscope. The oocyte suspension was transferred to a test tube and centrifuged at 400 rpm for 5 min. Discarding the supernatant, 0.5 ml of the sediment was gently dropped on to a glass slide with a cover slip. The slit between the glass slide and cover slip was ajusted to 300–400 µm and fixed by several drops of paraffin wax before use. A Nomarski differential interference contrast illumination with an Olympus photomicroscope (VANOX) was used for oocyte observation.

Results and discussions. Multiple nuclei in a condition expressed as germinal vesicle breakdown (GVBD) and multiple nucleoli were very frequently recognized in fragmented oocytes (figs 1 and 3). Fragmented oocytes, especially when they accompanied a relatively small number of granulosa cells, often had degenerating granulosa cells which had thickened processus penetrating the zona pellucida (ZP) and granulosa cell like structures (GCLS) at the tip of the processus in the perivitelline space (figs 2 and 3).

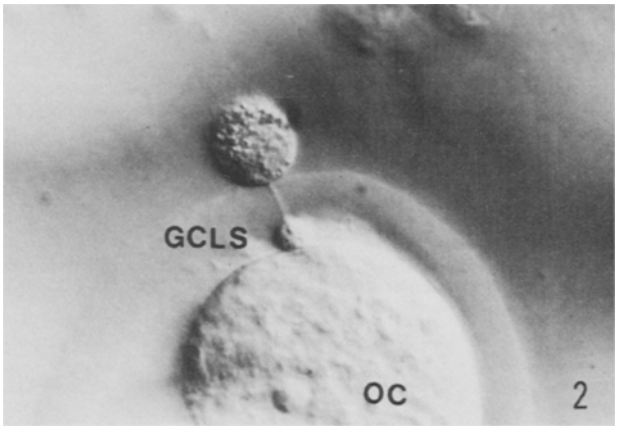

Figure 2. GCLS, granulosa cell like structure; OC, oocyte. Clear connection of the GC and GCLS via a process was demonstrated. The GCLS was situated in the perivitelline space and looked like a knob of the process.

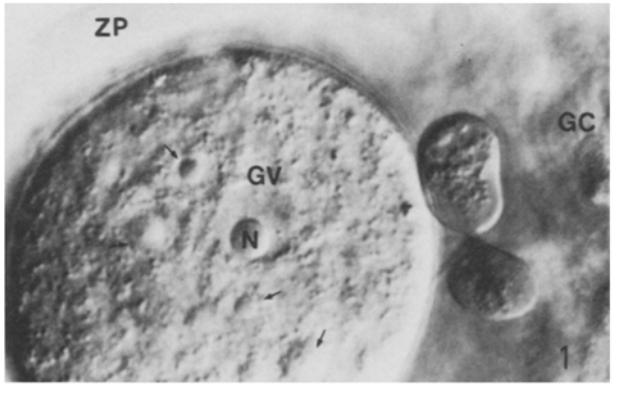

Figure 1. ZP, zona pellucida; GC, granulosa cell; GV, germinal vesicle; N, nucleolus. At least 5 nucleoli, each of which is enveloped by irregular GV (arrows).

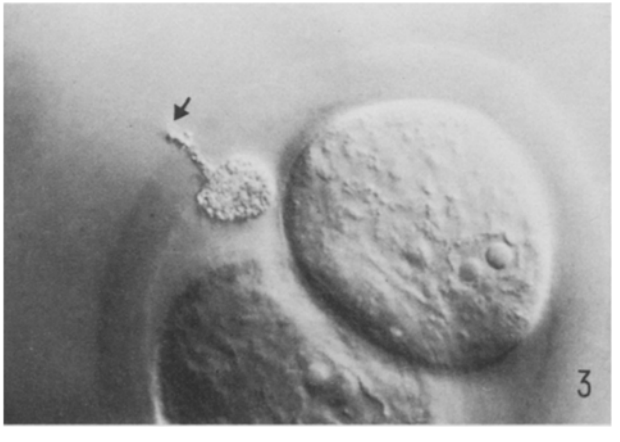

Figure 3. Note that only GCLS and a part of the process devoid of the GC (an arrow) could be observed. Multiple nucleoli and GVBDs were also seen.

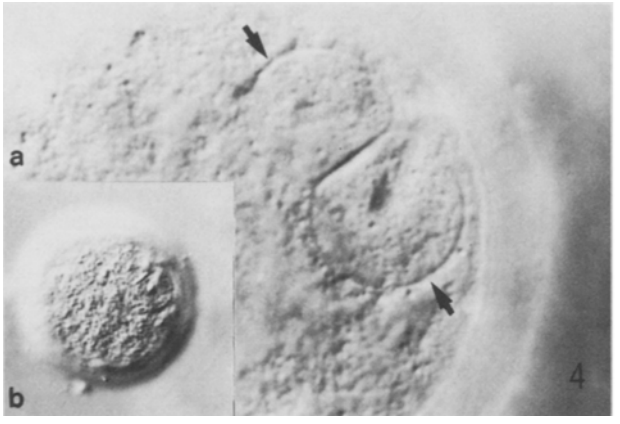

Figure 4. An almost denuded oocyte in nuclear division under a low (b) and the higher (a) magnifying power. Note the eccentricity of the nuclei adjacent to the vitelline membrane (arrows).

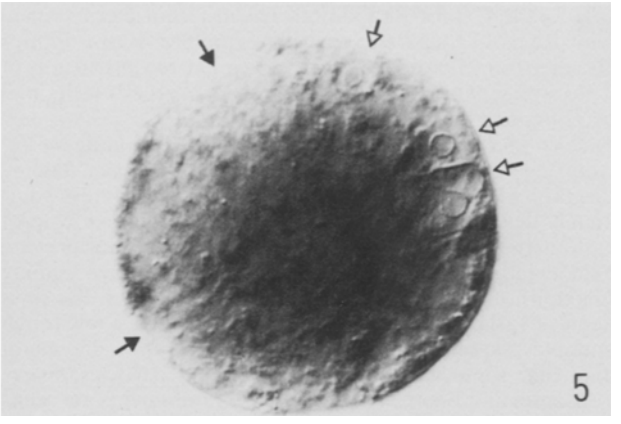

Figure 5. An oocyte which has lost the ZP and had disruptions in the vitelline membrane (closed arrows). Note the multiple nuclei situated very close to the cell membrane (open arrows).

Figures 4 and 5 represent non-fragmented oocytes with multiple nuclei and nucleoli. The oocyte in figure 4 is finishing nuclear division without synchronizing cell division. The oocyte in figure 5 has disruptions of the vitelline membrane and is being eliminated. In both oocytes it is noteworthy that the nuclei are very close to the vitelline membrane.

Success in experimental induction of fragmentation of oocytes in the Fallopian tubes[9] as well as in the ovaries[10] indicates that fragmented oocytes are formed by repeated cell divisions, whether they may be meiotic or mitotic. However, the synchrony between cell and nuclear divisions is completely lost in fragmented oocytes. Moreover, the asynchrony also functions in the process of elimination of non-fragmented oocytes in the ovary.

Presence of follicle cells in the ZP and perivitelline space was reported by Zamboni and Thompson[11]. However, existence of GCLS in the perivitelline space requires consideration of the relationship between GC and GCLS. In the present study GCLS in the perivitelline space almost invariably accompanied the thickened processus (fig. 3). Such a processus was thick enough (up to a few µm in diameter) to allow a cytoplasmic shift from GC to GCLS. It is possible that granulosa cells can change their position from the outside of the ZP to the perivitelline space by amoeboid movement.

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2 L. Loeb, J. Am. med. Ass. *56*, 1327 (1911).
3 M.C. Chang, Anat. Rec. *108*, 31 (1950).
4 R.G. Edwards, Sci. Am. *215*, 72 (1966).
5 K. Mikamo, Cytogenetics *7*, 212 (1968).
6 R.P. Donahue, J. exp. Zool. *169*, 237 (1968).
7 W.J. Swartz and A.W. Schuetz, Am. J. Anat. *144*, 365 (1975).
8 H. Shinohara, S. Okoyama, K. Akasofu and E. Nishida, Experientia *36*, 1329 (1980).
9 N.W. Fugo and R.L. Butcher, Fert. Steril. *17*, 807 (1966).
10 H. Shinohara, Experientia *37*, 1353 (1981).
11 L. Zamboni and R.S. Thompson, Biol. Reprod. *7*, 425 (1972).

Transendothelial transport of lipids in the absorbing lymphatic vessel

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Summary. Intraendothelial channels have been shown in the lacteal vessels under normal conditions and during experimentally induced stasis, using ultrastructural and tridimensional reconstruction methods. Through the channels of the lymphatic endothelial wall, free lipid drainage from the interstitium into lymph was detected, although the intercellular junctions did not appear to be modified.

In spite of frequent and recent ultrastructural studies the question of transendothelial transport in the absorbing lymphatic vessel is still debated. As far as fluids and plasma proteins are concerned, significant roles are played by vesicular transport, by diffusion mechanisms[2-6] and by normal intercellular channels[7]. On the other hand the 'open junctions' route among contiguous endothelial cells[8-16], and the presence of temporary intraendothelial channels[17], have been suggested in the transport of small and large molecules. In order to identify and characterize the mechanisms intervening during the passage of large sized molecules through lymphatic endothelium, the absorbing lymphatic vessels of the intestinal villi have been studied in normal and experimental conditions.

Materials and methods. A total of 20 Chiroptera *(Vesperugo savi)* were used in the present study. The animals were killed under ether anesthesia, 6 during the lethargic winter fasting period, and 14 during the summer. Of the latter, 8 were sacrificed 2-3 h after feeding and six 5 h after lymphatic stasis had been induced by mesenteric trunk ligature. The duodenum and jejunum were removed from each animal. Fragments of duodenal and jejunal mucosa, 2-4 mm in thickness, were dehydrated in acetone and embedded in Durcupan after 1% osmium tetroxide solution