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Author: Ukeshima, Atsumi

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# Germ Cell Death in the Degenerating Right Ovary of the Chick Embryo

Atsumi Ukeshima

*Kumamoto University, College of Medical Science, Kumamoto 862, Japan*

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**ABSTRACT**—The right ovary of chick embryos is known to degenerate during embryogenesis, while the left one advanced its normal growth. The present study demonstrates germ cell death in the degenerating right ovary. In the process of germ cell death, chromatin condensation, reduction of cell size, and formation of apoptotic bodies were observed. The cell organelles were almost normal in the early stages of the process. Fragments of broken germ cells were phagocytosed by neighboring cells or macrophages. From these observations, it may be assumed that germ cell death in the right ovary of chicks is related to apoptosis. In the normal left ovary, similar cell death was only observed in the germ cells in the medulla, but not in the cortex. Therefore, germ cell death seems to be a phenomenon common to the medullae of both the right and left ovaries.

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## INTRODUCTION

Chick germ cells originate in an extraembryonic area during the early stages of development, and then migrate into the forming right and left gonadal anlagen via the blood circulation network (Fujimoto *et al.*, 1976; Ukeshima and Fujimoto, 1984). After the arrival of the germ cells, the gonadal primordium begins to differentiate into either an ovary or a testis according to the genetic sex. In female chicks, however, the ovary develops only on the left side, and the right one degenerates. Eventually, only the left side functions as an ovary. This is due to the different manner of the ovarian differentiation between the right and left sides (Romanoff, 1960). The normal left ovary consists of the cortex and the medulla, the former being the major functional tissue of the ovary. On the other hand, the degenerating right ovary is only composed of the medulla.

Corresponding to the degeneration of the right ovary, the germ cells in the right ovary behave differently from those in the normal left ovary. We previously showed that germ cells in the medulla of the right and left ovaries are eliminated, in contrast with those of proliferating in the cortex of the left ovary (Ukeshima and Fujimoto, 1991; Ukeshima, 1994). In regards to germ-cell elimination, two modes have been demonstrated, consisting of cell death in the lacunal epithelium and cell abandonment into the medullary lacunae. On the cell death, however, there were poor descriptions in the previous reports. In the present study, germ cell death in the degenerating right ovary was focused and examined ultrastructurally.

## MATERIALS AND METHODS

Materials used for the present study were chick ovaries from 7–17 day embryos, since the ovarian differentiation begins at 7 days.

The degenerating right ovaries were excised from these embryos. Some of the developing left ovaries were also prepared for comparison. They were routinely fixed in 2.5% glutaraldehyde (0.1 M phosphate buffer, pH 7.4) for 1.5 hr at 4°C, and postfixed in 1% osmic acid (0.1 M phosphate buffer, pH 7.4) for 1 hr at 4°C. After washing in the same buffer, they were dehydrated in a graded ethanol series and embedded in epoxy resin. Semithin sections were stained with toluidine blue for light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed with a Hitachi HU-12A transmission electron microscope (TEM).

## RESULTS

The rudimentary right ovary, only composed of the medulla, was occupied by many lacunae. Germ cells were located in the epithelium of the medullary lacunae. They possessed large and round nuclei with fragmented or thread-like nucleoli (Fig. 1). These morphological characteristics were commonly observed at each stage examined.

Some of the germ cells, however, showed an unusual profile, having condensed chromatin masses in the nucleus (Fig. 2). These masses resembled the chromosomes seen commonly in mitosis. Their electron density was, however, not as high as that of metaphase chromosomes. Besides the condensed chromatin, no structural differences were seen in both the nucleus and cytoplasm. The nuclear membrane was distinct and the nucleolus showed a fragmented or thread-like structure. The germ cells showing these characteristics were sometimes found from 8 days onward. The shape and size of the condensed chromatin masses were variable, and their contour was not distinct. In some germ cells, the chromatin was more condensed, but the nuclear matrix was conversely more lucent than in others.

In addition to the cells mentioned above, obviously dying germ cells were often found in the right ovarian tissue

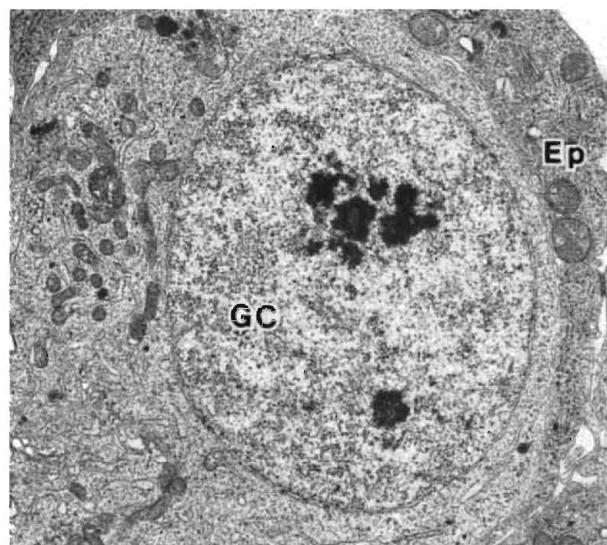


Fig. 1. A profile of a normal germ cell (GC) encircled by epithelial cell (Ep) in the right ovary of a 16-day embryo. A fragmented nucleolus is one of the structural characteristics of a chick germ cell.  $\times 8,000$ .

throughout the stages examined, especially in later stages. Chromatin was strongly condensed and shifted to the periphery of the nucleus (Fig. 3). The cytoplasm became sparse, and cytoplasmic components were somewhat aggregated. Mitochondria and other membranous structures, however, were almost normal in appearance. The cell size was rather diminished.

Extremely condensed chromatin was also detected in dying germ cells (Fig. 4). The nuclei containing highly electron-dense chromatin were usually round, but were sometimes deformed. The nuclear matrix was extremely lucent. The highly compact chromatin masses were large in size and diminished in number. They usually shifted to the nuclear membrane. The cytoplasm became very rough and inhomogeneous. Mitochondria and other membranous structures were broken. The dying germ cells exhibiting these characteristics were usually seen included by an adjacent lacunar epithelial cell.

The fragments of the dead germ cells were also found. Each fragment bounded with a membrane was phagocytosed by macrophages or adjoining cells (Fig. 5). Some of the dying germ cells were abandoned into the medullary lacunae. Such germ cells were sometimes encircled by macrophages within the lacunae (Fig. 6).

## DISCUSSION

In chicks, the right ovary degenerates during embryonic development. This is due to the failure of the cortex formation on the right side. Initially, almost the same number of germ cells enters the right and left ovarian primordia. At a later period, however, the right ovary without a cortex has a reduced number of germ cells caused by cell-death or cell-abandonment (Ukeshima and Fujimoto, 1991; Ukeshima, 1994). Although similar germ cell elimination possibly takes place in other animal species, there is no information on this.

The dying feature of germ cells was frequently observed in the right ovarian tissue composed only of the medulla. They

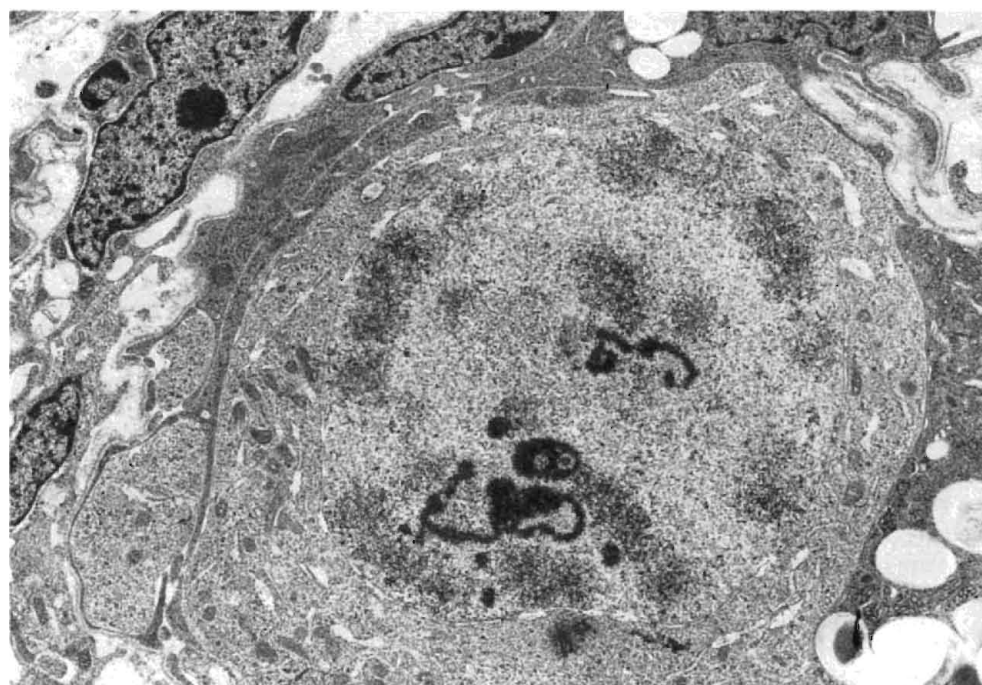


Fig. 2. A germ cell with relatively condensed chromatin masses. The condensation of chromatin seems to be an initial step in the degeneration of germ cells. In this germ cell, thread-like nucleolus is seen. 17-day right ovary.  $\times 8,600$ .

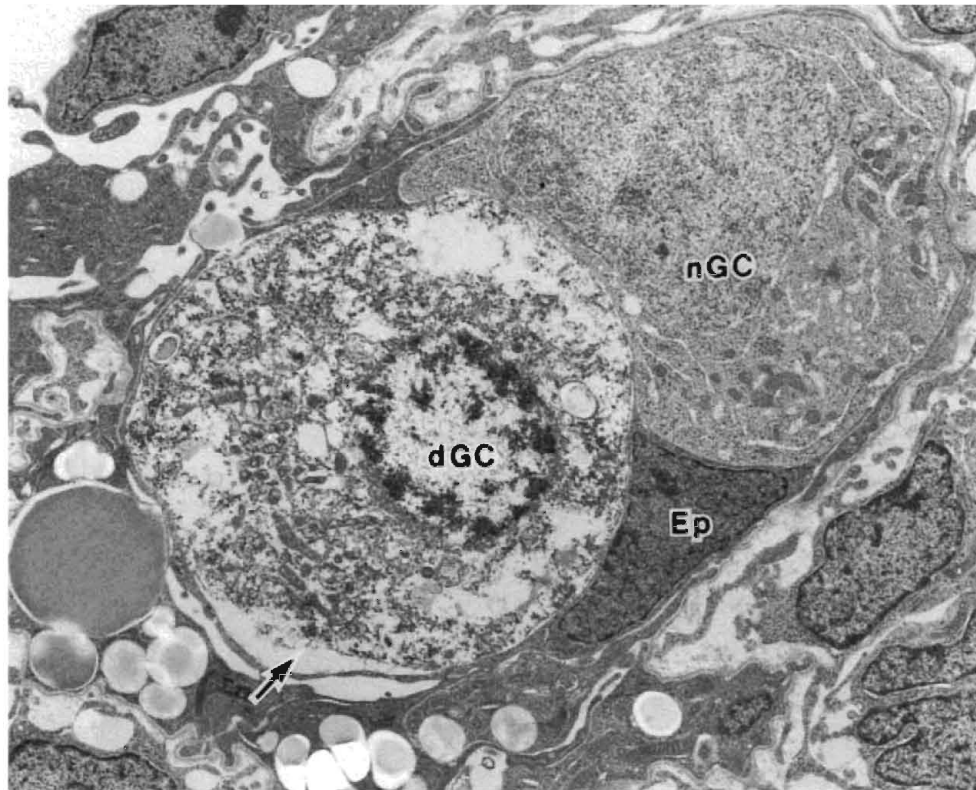


Fig. 3. A degenerating germ cell (dGC) possessing highly condensed chromatin. In contrast, the nuclear matrix is lucent. The cell size is somewhat reduced (arrow). 17-day right ovary. nGC: normal germ cell. Ep: epithelial cell.  $\times 7,000$ .

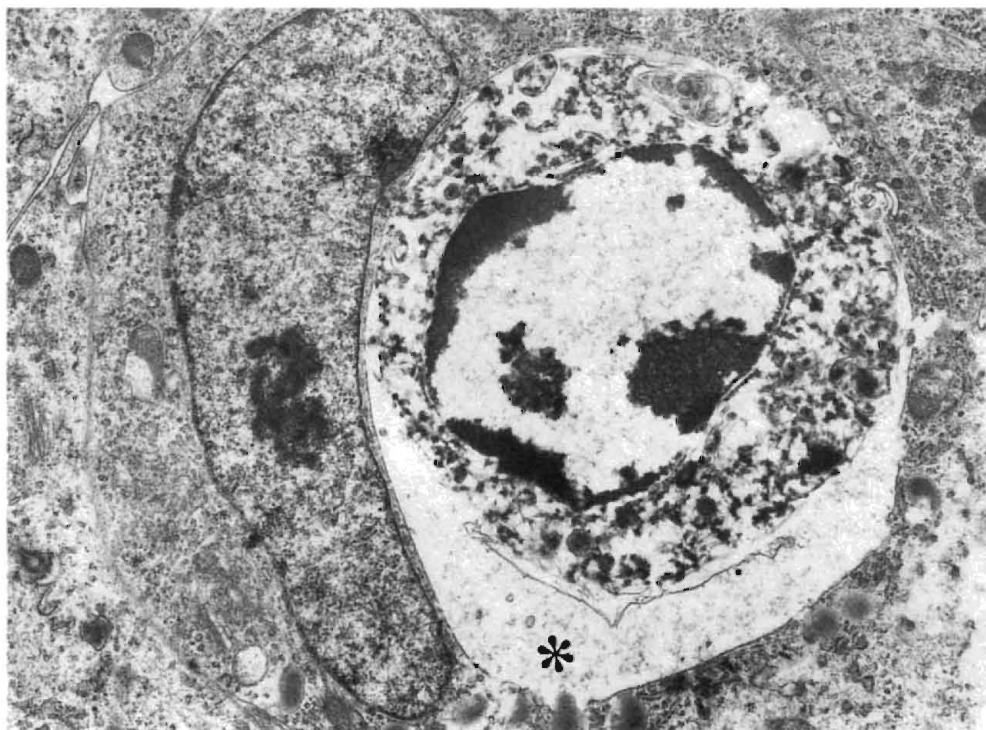


Fig. 4. The chromatin has completely aggregated and shifts at the periphery of the nucleus. In contrast, the nuclear matrix is strongly electron-lucent. The cell size is reduced and wide intercellular space (asterisk) appears. This germ cell is encircled by an adjoining cell. 8-day right ovary.  $\times 11,000$ .

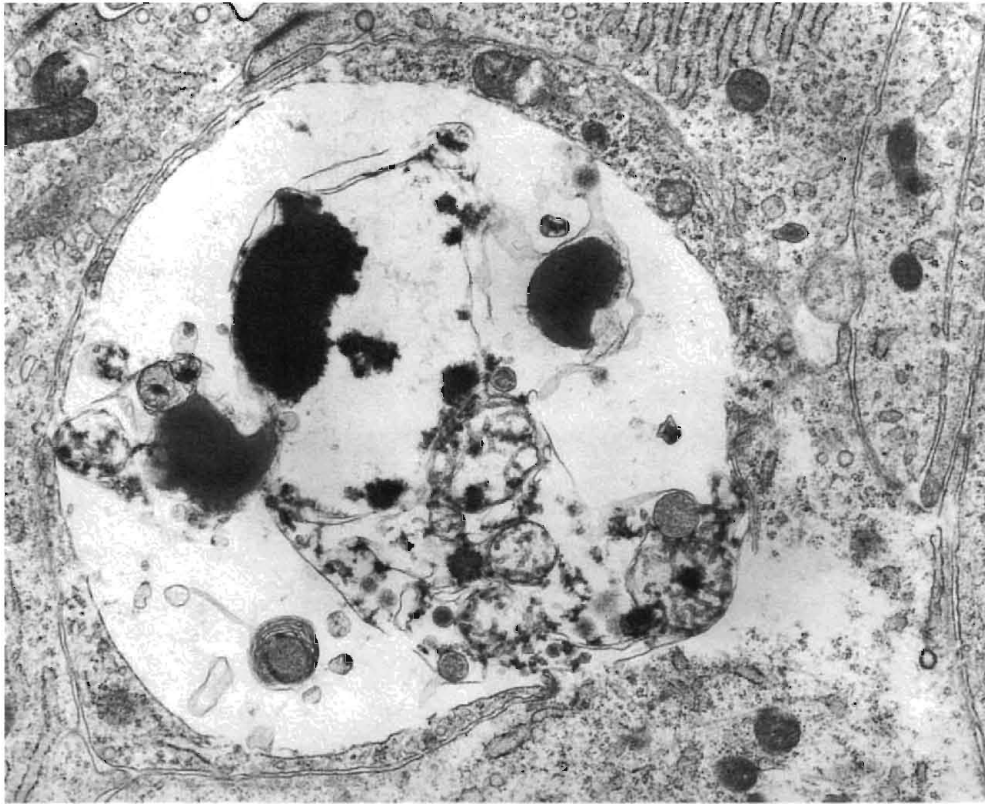


Fig. 5. A germ cell phagocytosed by a neighboring cell. The germ cell was completely destroyed, showing as several pieces. 9-day right ovary.  $\times 15,000$ .

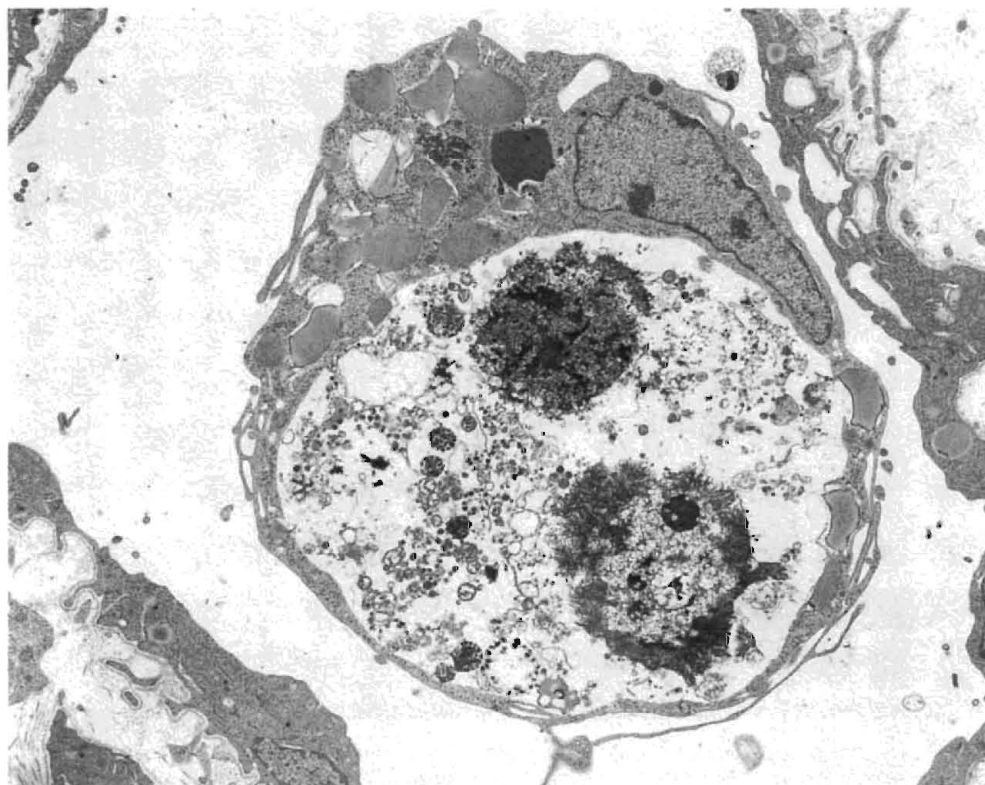


Fig. 6. A degenerating germ cell found in the medullary lacunae of 17-day left ovary. This germ cell has been phagocytosed by a macrophage-like cell.  $\times 5,400$ .



were mainly characterized by the condensation of chromatin and the reduction of cell size. Membranous structures including mitochondria and endoplasmic reticulum (ER) were kept normal during the early stages of the death process. The dead germ cells were seen to be phagocytosed by adjacent lacunar epithelial cells or macrophages. These findings may suggest that the death of germ cells in the right ovary is caused by apoptosis.

Apoptosis is a type of cell death named by Kerr *et al.* (Kerr *et al.*, 1972; Wyllie *et al.*, 1980). Unlike necrosis, apoptosis is morphologically characterized by cell shrinkage and condensation of chromatin. However, organelles keep a normal appearance. In necrosis, on the other hand, the cell body is rather swollen with dilation of mitochondria and ER. Finally, the nucleus, organelles and plasma membranes rupture, and inflammation develops in the adjoining tissue. In the later stage of apoptosis, the dying cells are often split into membrane-bounded pieces, and finally phagocytosed by macrophages or adjacent cells.

In the present study, the condensed chromatin masses resembling chromosomes in mitosis were sometimes found in the germ cells. Unlike mitosis, however, the nucleus of these cells had a nucleolus and a distinct nuclear membrane. Therefore, such chromatin figures may be associated with an initial sign of the germ cell death.

Generally, highly compact chromatin masses occur in the early stage of apoptotic process. In the present study, however, the cells with highly compact chromatin had already phagocytosed by other cells. From this observation, the progress of apoptosis in germ cells seems to proceed rapidly. This may be characteristic of germ cell death in chicks.

The normally developing left ovary was also examined as a comparison. The left ovary is composed of the medulla and the cortex, and the latter develops into the functional tissue (Romanoff, 1960). As in the right ovary, germ cell death was also found in the left medulla. In the cortex, however, no germ cell death was seen. From these results, it may be assumed

that the germ cells located in the medulla of both the right and left ovaries are fated to degenerate by apoptosis.

In the present study, germ cell death was estimated only by morphological characteristics under the TEM. As mentioned above, the criteria were the condensation of chromatin, the reduction of cell size and the integrity of cell organelles. In addition to these results, however, the TUNEL method (Gavrieli *et al.*, 1992) should be applied for the identification of apoptosis by light microscopy. Although apoptosis is not always determined by the TUNEL method alone, TEM observations in conjunction with the TUNEL method can confirm apoptosis (Yaginuma *et al.*, 1995). This is a next problem in the study of germ cell apoptosis.

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