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Ultrastructure of granulosa cells during apoptosis in caprine antral follicles

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The molecular mechanism of atresia can be best explained on the basis of apoptosis (Palumbo and Yeh 1994, Sharma 2000). Different methods such as flow cytometry, DNA fragmentation, TUNEL technique, caspases and endonuclease are being used to investigate the process of apoptosis (Tilly *et al.* 1991, Coucouvanis *et al.* 1993, Sharma 2000). Recently, Yang and Rajamahendran (2000) have demonstrated that in atretic follicles, degenerating granulosa cells display morphological characteristics of apoptosis including nuclei with marginated chromatin, a single condensed nucleus, multiple nuclear fragments and/or membrane bound structures containing variable amount of chromatin and/or cytoplasm (apoptotic bodies) in bovine ovary. Till date no information is available on fine structural alterations during apoptosis in goat ovaries and it is a pioneer report on apoptosis in goat.

The atretic goat (*Capra hircus*) ovarian follicles obtained from the slaughterhouse materials collected from Delhi (28° 38' N, 77° 12' E) were fixed in 2.5% gluteraldehyde in 0.2M phosphate buffer saline (pH 7.2 to 7.4) at 4°C for 24 hr and processed for electron microscopic analysis (Sharma and Chowdhury 1998).

The condensation of the nuclear contents and the presence of pycnotic nuclei observed in early atretic antral population of granulosa cells of goat (Fig. 1) is an indicative of the general plan and path of atretogenic changes in mammals (Brand and de Jong 1973, Hay *et al.* 1976, Guraya *et al.* 1994). The results of electron microscopic investigations on atretic granulosa cells are similar to that observed in rodents (Peluso *et al.* 1980, Sharma and Guraya 1997). The undulations of the nuclear membrane (Fig 1) and pinching off of the apoptotic bodies containing nuclear contents strongly advocate the concept that apoptosis is the basic mechanism involved in atresia in goat. The phenomenon of formation of apoptotic bodies in goat (Fig.2) resembles with that reported in cow (Yang and Rajamahendran 2000). The vacuolization within the nucleus and condensed chromatin material are the positive indices of apoptosis. The increase in the frequency and dimensions of the nuclear pores and flattening of the nuclear membrane observed in goat follicles (Fig.2), are similar to the earlier findings on ultrastructure of apoptosis in rat and cow (Coucouvanis *et al.* 1993, Grotowski *et al.* 1997, Isobe and Yoshimura 2000, Yang and Rajamahendran 2000), thereby suggesting a common plan of apoptosis in mammals. In advanced stage of atresia, the membrane bound pycnotic chromatin carrying apoptotic vesicles were observed lying within the cytoplasm (Fig.3), further endorse the concept that apoptotic bodies are formed from condensed chromatin material, which is eventually packed in small vacuoles limited by the nuclear membrane. The condensed cytosol observed in a few cells in contrast to hyaline cytosol is possibly due to the differential functional impairment of the cytoplasmic membrane. The alteration in acidic phospholipid phosphatidyl serine content in the membrane that also act as apoptosis inducing agent (Krishnamurthy *et al.* 2000), possibly modulates the membrane chemistry that leads to a change in its permeability to water molecules. In case permeability is enhanced, the cell becomes larger and hyaline, whereas if the permeability decreases, the contents become pycnotic and electron-dense (Green and Reed 1998). The vacuolization of the cytosol and of cell organelles like mitochondria in goat granulosa cells (Fig. 4) may be because of some change in membrane biochemistry. The physical disruption of the inner membrane is because of hyperpolarization or collapse of membrane-potential by opening up of permeability transition pores. These pores have been observed at the apposed regions where voltage dependent ion-channels or the mitochondria porin and adenine nucleotide translocator interact. This is regulated by bcl-2 proteins and intracellular ATP levels (Green and Reed 1998, Adams and Cory 1998). The flattening and blebbing off of the cytoplasm in granulosa cells is possibly associated with the loss of LH-receptors (Peluso *et al.* 1977, Peluso *et al.* 1980). Since LH regulates many functions of the granulosa cells, the reduction in LH receptors would render the cell less responsive to LH, thereby results in decrease in metabolism and steroidogenesis and induces apoptosis (Hamberger *et al.* 1971, Tilly 1996, Jolly *et al* 1997, Guraya 2000).

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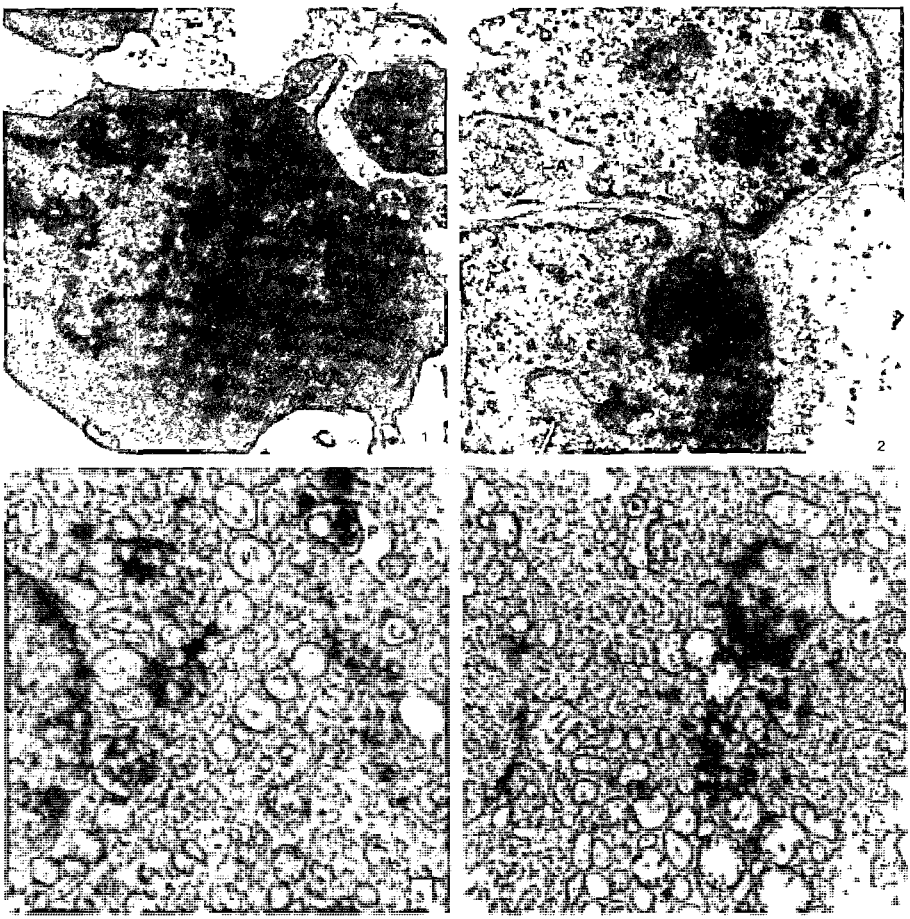

Figs 1-4. **1**. Low power view of the fine structure of apoptotic granulosa cell showing uneven nuclear membrane with clumps of vacuolated chromatin material (arrow). The cytoplasm is dense having a few microextensions. ×3400. **2**. A portion of early atretic granulosa cell showing apoptotic bodies (A) both within the nucleus and the cytoplasm having condensed chromatin material with vacuoles. ×3400. **3**. A magnified view of the cytoplasm of advanced atretic granulosa cell revealing membrane-bound apoptotic bodies (A), pleiomorphic bodies (P) and vacuolated mitochondria (V). ×4200. 4. A granulosa cell at an advanced stage of atresia revealing apoptotic body (A) containing chromatin material lying in highly vacuolated cytosolic organelles like mitochrondria, endoplasmic reticulum and other structures. ×2900.

SUMMARY

The fine structural changes during apoptosis in goat ovaries have been reported here. The nuclear contents condensed and pycnotic nuclei were present in early atretic antral population of granulosa cells of goat. The apopotic bodies formed from the chromatin material have also been observed in the cytoplasm of atretic granulosa cells.

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