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Apoptosis and ovarian follicular Atresia

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Abstract

Apoptosis or the programmed cell death is generally characterized by distinct morphological characteristics and energy-dependent biochemical mechanisms. Apoptosis is considered a vital component of various processes including normal cell turnover, proper development and functioning of the immune system, hormone-dependent atrophy, embryonic development and chemical-induced cell death. Inappropriate apoptosis (either too little or too much) is a factor in many human conditions including neurodegenerative diseases, ischemic damage, autoimmune disorders and many types of cancer. The mechanisms responsible for germ cell depletion from the ovary, either directly during the perinatal period or indirectly via follicular atresia during postnatal life, are dependent upon the activation of physiological cell death mechanisms. This is accomplished via activation of a 'universal' pathway of cellular suicide involving altered expression of a conserved cohort of genes. The goal of this review is to provide an overview of current knowledge on apoptosis including morphology, biochemistry, its role in health and disease, detection methods, and a discussion of its alternative forms of apoptosis. The identity of the hormonal and intracellular effectors responsible for the coordination of life and death decisions made by ovarian cells during development as well as the biological and clinical implications of gene-directed cell death in the ovary are explored in this review.

Keywords: Apoptosis, programmed cell death, intrinsic/extrinsic pathway, granzyme A/B, perforin, autophagy

Introduction

The term apoptosis was first used by **Kerr, Wyllie, and Currie (1972)** to describe a morphologically distinct form of cell death **(Paweletz, 2001).** It is understanding of the mechanisms involved in the process of apoptosis in mammalian cells transpired from the investigation of programmed cell death that occurs during the development of the nematode *Caenorhabditis elegans* **(Horvitz, 1999).** Apoptosis has since been recognized and accepted as a distinctive and important mode of "programmed" cell death, which involves the genetically determined elimination of cells. However, it is important to note that other forms of programmed cell death have been described and other forms of programmed cell death may yet be discovered **(Formigli et al., 2000; Sperandio et al., 2000; Debnath et al., 2005).** Apoptosis occurs normally during development and aging and as a homeostatic mechanism to maintain cell populations in tissues. **Andreu-Vieyra and Habibi (2000)** have mentioned that in adults, apoptosis is observed mainly in those tissues undergoing active differentiation such as the hematopoietic system, testis, ovary, and intestinal epithelium. Although there are a wide variety of stimuli and conditions, both physiological and pathological, that can trigger apoptosis, not all cells will necessarily die in response to the same stimulus. Irradiation or drugs used for cancer chemotherapy results in DNA damage in some cells, which can lead to apoptotic death through a *p53*-dependent pathway (Abbr. list). Some hormones, such as corticosteroids, may lead to apoptotic death in some cells (e.g., thymocytes) although other cells are unaffected or even stimulated.

Some cells express Fas or TNF receptors that can lead to apoptosis via ligand binding and protein cross-linking. Other cells have a default death pathway that must be blocked by a

survival factor such as a hormone or growth factor. There is also the issue of distinguishing apoptosis from necrosis, two processes that can occur independently, sequentially, as well as simultaneously **(Hirsch et al., 1997; Zeiss, 2003)**. In some cases it is the type of stimuli and/or the degree of stimuli that determines if cells die by apoptosis or necrosis. At low doses, a variety of injurious stimuli such as heat, radiation, hypoxia and cytotoxic anticancer drugs can induce apoptosis but these same stimuli can result in necrosis at higher doses. Finally, apoptosis is a coordinated and often energy-dependent process that involves the activation of a group of cysteine proteases called "caspases" and a complex cascade of events that link the initiating stimuli to the final demise of the cell.

During ovarian follicular development, only limited numbers of follicles are selected for ovulation, whereas the rest undergo atresia at various stages of development **(Byskov, 1978)**. Previous studies indicate that the death of granulosa cells (GC) triggers atresia of the follicles (**Hughes and Gorospe , 1991 and Rajakoski, 1996**). Although it has been suggested that alterations in steroidogenesis might be involved in the initiation of follicular atresia **(Jolly et al., 1994; Hsueh et al., 1994 and Rosenfeld et al., 2001)**, but the exact pattern of steroid hormone during atresia has yet to be established. It has been shown that Insulin-like growth factors (IGFs) play an important role in regulating follicular development and granulosa cell apoptosis **(deMoura et al., 2000 and Armstrong et al., 2001)** and thus could block apoptosis. **Yuan Song et al., (2004)** have studied the relationship between the levels of IGFs and steroids and granulosa cell apoptosis in goat ovary. They indicated that significant apoptosis occurs in atretic, but not healthy follicles. Apoptosis of granulosa cells is related to the imbalance between estradiol and progesterone in the follicular fluid. The level of IGF-I, but not IGF-II, is the crucial factor in deciding whether a follicle will mature or undergo atresia.

Apoptosis in General

Morphological Alterations of Apoptosis

Although the morphological events of apoptosis are rapid and the fragments are quickly phagocytized, considerable apoptosis may occur in some tissues before it is histologically apparent.

Light (Figs 1-2) and electron microscopy (Figs 3-6) have identified the various morphological changes that occur during apoptosis **(Hacker, 2000).** This method detects the later events of apoptosis, so cells in the early phase of apoptosis will not be histologically apparent. Phagocytosis of apoptotic bodies can also be appreciated with TEM. However, it is difficult to detect apoptotic cells at the earliest stages due to their transient nature.

In **1972, Kerr et al.,** have described the cells undergoing apoptosis by light microscopy. The cell appears smaller in size with dense cytoplasm. Pyknosis is the most characteristic feature of apoptosis. It results from chromatin condensation. Examination of H&E stained sections revealed that apoptosis involves single cell or small clusters of cells. The apoptotic cell appears as a round or oval mass with dark eosinophilic cytoplasm and dense purple nuclear chromatin fragments.

Semi-ultrathin sections stained with toluidine or methylene blue revealed intensely stained apoptotic cells when seen by light microscopy. This methodology depends on the nuclear and cytoplasmic condensation that occur during apoptosis. Smaller apoptotic bodies will not be detected and healthy cells with large dense intracellular granules can be mistaken for apoptotic cells or debris.

Electron microscopy can better define the subcellular changes. This is because categorization of an apoptotic cell is irrefutable if the cell contains certain ultra-structural morphological characteristics (**White and Cinti, 2004**). These characteristics are: (1) electron-dense nucleus; (2) nuclear fragmentation; (3) intact cell membrane even late in the cell disintegration phase; (4) disorganized cytoplasmic organelles; (5) large clear vacuoles; and (6) blebs at the cell surface. As apoptosis progresses, the cell-to-cell adhesions is lost and the cell will separate from neighboring cells. During late phase of apoptosis, the cell

fragments into apoptotic bodies with intact cell membranes.

Extensive cell membrane blebbing occurs followed by karyorrhexis and separation of cell fragments into apoptotic bodies during a process called "budding." Apoptotic bodies or plasma membrane-bound vesicles containing the cellular constituents of the dying cell which are then phagocytized by macrophages, parenchymal cells, or neoplastic cells and degraded within phagolysosomes. No inflammatory reaction was associated with the process of apoptosis nor with the removal of apoptotic cells because: (1) Apoptotic cells do not release their cellular constituents into the surrounding interstitial tissue; (2) They are quickly phagocytosed by the surrounding cells thus likely preventing secondary necrosis; and, (3) The engulfing cells do not produce anti-inflammatory cytokines *(Savill and Fadok, 2000; Kurosaka et al., 2003)*.

Biochemical Features

Apoptotic cells exhibit several biochemical modifications such as protein cleavage, protein cross-linking, DNA breakdown, and phagocytic recognition **(Hengartner, 2000)**. Caspases are inactive proenzyme form in most cells. They have proteolytic activity and are able to cleave proteins. Once caspases are initially activated they can often activate other procaspases, allowing initiation of a protease cascade. This proteolytic cascade amplifies the apoptotic signaling pathway and thus leads to rapid cell death.

To date, ten major caspases have been identified and broadly categorized into initiators (caspase-2,-8,-9,-10), effectors (caspase-3,-6,-7) and inflammatory (caspase-1,-4,-5) caspases **(Cohen, 1997; Rai et al., 2005)**. The other caspases that have been identified include caspase-11, which is reported to regulate apoptosis and cytokine maturation during septic shock, caspase-12, which mediates endoplasmic-specific apoptosis and cytotoxicity by amyloid-β, caspase-13, which is suggested to be a bovine gene, and caspase-14, which is highly expressed in embryonic tissues but not in adult tissues **(Hu et al., 1998; Nakagawa et al., 2000, Koenig et al., 2001; Kang et al., 2002)**.

Extensive protein cross-linking is another characteristic of apoptotic cells and is achieved through the expression and activation of tissue transglutaminase **(Nemes et al., 1996)**. DNA breakdown by Ca^{2+}-and Mg^{2+}-dependent endonucleases also occurs, resulting in DNA fragments of 180 to 200 base pairs **(Bortner et al., 1995)**. A characteristic "DNA ladder" can be visualized by agarose gel electrophoresis with an ethidium bromide stain and ultraviolet illumination.

Another biochemical feature is the expression of cell surface markers that result in the early phagocytic recognition of apoptotic cells by adjacent cells, permitting quick phagocytosis with minimal compromise to the surrounding tissue. This is achieved by the movement of the normal inward-facing phosphatidylserine of the cell's lipid bilayer to express on the outer layers of the plasma membrane **(Bratton et al., 1997)**. Externalization of phosphatidylserine is a well-known recognition ligand for phagocytes on the surface of the apoptotic cell. Recent studies have shown that other proteins are also being exposed on the cell surface during apoptotic cell clearance. These include Annexin I and calreticulin.

Annexin V is a recombinant phosphatidylserine-binding protein that interacts strongly and specifically with phosphatidylserine residues and can be used for the detection of apoptosis (**Arur et al., 2003)**. Calreticulin is a protein that binds to an LDL-receptor related protein on the engulfing cell and is suggested to cooperate with phosphatidylserine as a recognition signal **(Gardai et al., 2005)**.

Distinguishing Apoptosis from Necrosis

Elmore (2007) mentioned that necrosis is the alternative to apoptotic cell death. It is considered to be a toxic or degradative process that occurs after cell death. Necrosis leads to cell death with cell swelling and karyolysis. Apoptosis leads to cell death with cell shrinkage, pyknosis, and karyorrhexis.

Because the mechanism and morphology of apoptosis and necrosis differ, there is overlap

between these two processes. Evidence indicates that necrosis and apoptosis represent morphologic expressions of a shared biochemical network described as the "apoptosis-necrosis continuum" (**Zeiss, 2003**). For example, two factors that will convert an ongoing apoptotic process into a necrotic process include a decrease in the availability of caspases and intracellular ATP (**Leist et al., 1997; Denecker et al., 2001**). Whether a cell dies by necrosis or apoptosis depends in part on the nature of the cell death signal, the tissue type, the developmental stage of the tissue and the physiologic milieu (**Fiers et al., 1999; Zeiss, 2003**).

Microscopically, it is not always easy to distinguish apoptosis from necrosis. They can occur simultaneously depending on the intensity and duration of the stimulus, the extent of ATP depletion and the availability of caspases (**Zeiss, 2003**). Necrosis is an uncontrolled and passive process that usually affects large fields of cells whereas apoptosis is controlled and energy-dependent and can affect individual or clusters of cells. Necrotic cell injury is mediated by two main mechanisms; interference with the energy supply of the cell and direct damage to cell membranes.

Some of the major morphological changes that occur with necrosis include cell swelling; formation of cytoplasmic vacuoles; distended endoplasmic reticulum; formation of cytoplasmic blebs; condensed, swollen or ruptured mitochondria; disaggregation and detachment of ribosomes; disrupted organelle membranes; swollen and ruptured lysosomes and eventually disruption of the cell membrane (**Kerr et al., 1972; Majno and Joris, 1995**; **Trump et al., 1997**). The loss of cell membrane integrity results in the release of the cytoplasmic contents into the surrounding tissue, sending chemotatic signals with eventual recruitment of inflammatory cells. Because apoptotic cells do not release their cellular constituents into the surrounding interstitial tissue and are quickly phagocytosed by macrophages or adjacent normal cells, there is essentially no inflammatory reaction (**Savill and Fadok, 2000; Kurosaka et al., 2003**). It is also important to note that pyknosis and karyorrhexis are not exclusive to apoptosis and can be a part of the spectrum of cytomorphological changes that occurs with necrosis (**Cotran et al., 1999**).

Apoptosis can be an Irreversible Process

Metzstein et al.., (1998) mentioned that many of the genes that control killing and engulfment processes of programmed cell death have been identified. Until recently, apoptosis has traditionally been considered an irreversible process with caspase activation committing a cell to death and the engulfment genes serving the purpose of dead cell removal. **Hoeppner et al. (2001)** have shown that blocking engulfment genes in C. elegans embryos enhances cell survival when cells are subjected to weak pro-apoptotic signals. **Reddien et al., (2001)** have demonstrated that, in C. elegans, mutations that cause partial loss of function of killer genes allow the survival of some cells that are programmed to die via apoptosis. There is some evidence of a potential role for macrophages in promoting the death of cells in some tissues. **Diez-Roux and Lang (1997)** mentioned that elimination of macrophages in the anterior chamber of the rat eye resulted in the survival of vascular endothelial cells that normally undergo apoptosis. **Geske and coworkers (2001)** demonstrated that early p53-induced apoptotic cells can be rescued from the apoptotic program if the apoptotic stimulus is removed. Their research suggests that DNA repair may be involved in reversing the cell death pathway in some circumstances.

Mechanisms of Apoptosis

Researchs indicate that there are two main apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. However, there is now evidence that these two pathways are linked and that molecules in one pathway can influence the other (**Igney and Krammer, 2002**). There is an additional granzyme B pathway that involves T-cell mediated cytotoxicity and perforin-granzyme-dependent killing of the cell. The extrinsic, intrinsic, and granzyme B pathways converge on the same terminal, the execution pathway. The latter is initiated by the cleavage of caspase-3 and results in DNA fragmentation, degradation of cytoskeletal and nuclear proteins,

cross-linking of proteins, formation of apoptotic bodies, expression of ligands for phagocytic cell receptors and finally uptake by phagocytic cells. The granzyme A pathway activates a parallel, caspase-independent cell death pathway via single stranded DNA damage (**Martinvalet et al., 2005**).

Extrinsic Pathway

The extrinsic signaling pathways that initiate apoptosis involve transmembrane receptor-mediated interactions. These involve death receptors that are members of the tumor necrosis factor (TNF) receptor gene superfamily (**Locksley et al., 2001**). Members of the TNF receptor family share cysteine-rich extracellular domains and have a cytoplasmic domain of about 80 amino acids called the "death domain" (**Ashkenazi and Dixit, 1998**). This death domain plays a critical role in transmitting the death signal from the cell surface to the intracellular signaling pathways. To date, the best-characterized ligands and corresponding death receptors include FasL/FasR, TNF-α/TNFR1, Apo2L/DR4, Apo2L/DR5 and Apo3L/DR3 (**Chicheportiche et al., 1997; Peter and Kramer, 1998; Suliman et al., 2001; Rubio-Moscardo et al., 2005**).

The sequences of events that define the extrinsic phase of apoptosis are best characterized with the FasL/FasR and TNF-α/TNFR1 models. In these models, there is clustering of receptors and binding with the homologous trimeric ligand. Upon ligand binding, cytplasmic adapter proteins are recruited which exhibit corresponding death domains that bind with the receptors. The binding of Fas ligand to Fas receptor results in the binding of the adapter protein FADD and the binding of TNF ligand to TNF receptor results in the binding of the adapter protein TRADD with recruitment of FADD and RIP (**Hsu et al., 1995 and Wajant, 2002**). FADD then associates with procaspase-8 via dimerization of the death effector domain. At this point, a death-inducing signaling complex (DISC) is formed, resulting in the auto-catalytic activation of procaspase-8 (**Kischkel et al., 1995**).

Once caspase-8 is activated, the execution phase of apoptosis is triggered. Death receptor-mediated apoptosis can be inhibited by a protein called c-FLIP which will bind to FADD and caspase-8, rendering them ineffective (**Kataoka et al., 1998; Scaffidi, 1999**). Another point of potential apoptosis regulation involves a protein called Toso, which has been shown to block Fas-induced apoptosis in T cells via inhibition of caspase-8 processing (**Hitoshi et al., 1998**).

Perforin/granzyme Pathway

The cytotoxic T lymphocytes (CTLs) are able to kill target cells via the extrinsic pathway and the FasL/FasR interaction is the predominant method of CTL-induced apoptosis (**Brunner et al., 2003**). However, they are also able to exert their cytotoxic effects on tumor cells and virus-infected cells via a novel pathway. The latter involves secretion of transmembrane perforin molecule with a subsequent release of cytoplasmic granules through the pore and into the target cell (**Trapani and Smyth, 2002**). The serine proteases granzyme A and granzyme B are the most important component within the granules (**Pardo et al., 2004**).

Granzyme B activates procaspase-10 and cleaves proteins. As well it can cleave Inhibitor of Caspase Activated DNAse or ICAD (**Sakahira et al., 1998**). Also granzyme B can utilize the mitochondrial pathway for amplification of the death signal by specific cleavage of Bid and induction of cytochrome c release (**Barry and Bleackley, 2002; Russell and Ley, 2002**). Granzyme B can directly activate caspase-3. In this view, the upstream signaling pathways are bypassed resulting into direct induction of the execution phase of apoptosis.

It is suggested that both the mitochondrial pathway and direct activation of caspase-3 are critical for granzyme B-induced killing (**Goping et al., 2003**).

Recent findings indicate that this method of granzyme B cytotoxicity is critical as a control mechanism for T cell expansion (**Devadas et al., 2006**). Moreover, findings indicate that neither death receptors nor caspases are involved with

the T cell receptor-induced apoptosis. On the other hand, Fas-Fas ligand interaction, adapter proteins with death domains and caspases.
Granzyme A is also important in cytotoxic T cell induced apoptosis. It activates caspase independent pathways by DNA nicking via DNAse NM23-H1, a tumor suppressor gene product **(Fan et al., 2003)**. This DNAse has an important role in preventing cancer through the induction of tumor cell apoptosis. Granzyme A protease cleaves the nucleosome assembly protein SET complex, resulting in apoptotic DNA degradation **(Lieberman and Fan, 2003)**. Therefore, inactivation of this SET complex by granzyme A most likely also contributes to apoptosis by blocking the maintenance of DNA and chromatin structure integrity.

Intrinsic Pathway

This pathway initiates apoptosis and involves stimuli that produce intracellular signals. The intrinsic pathway is a mitochondrial-initiated events. The stimuli that initiate the intrinsic pathway produce intracellular signals that may act in either a positive or negative fashion. Negative signals can lead to failure of suppression of death programs and thereby triggering apoptosis. They involve certain growth factors, hormones and cytokines.
Other stimuli that act in a positive fashion include radiation, toxins, hypoxia, hyperthermia, viral infections, and free radicals. All of these stimuli cause changes in the inner mitochondrial membrane that results in formation of mitochondrial permeability transition (MPT) pore, loss of the mitochondrial trans membrane potential and release of two main groups of pro-apoptotic proteins from the intermembrane space into the cytosol **(Saelens et al., 2004)** to activate the caspase-dependent mitochondrial pathway. Cytochrome c binds and activates Apaf-1 as well as procaspase-9, forming an "apoptosome" **(Chinnaiyan, 1999; Hill et al., 2004)** and promote apoptosis by inhibiting IAP (inhibitors of apoptosis proteins) activity **(van Loo et al., 2002a; Schimmer, 2004)**.
The second group of pro-apoptotic proteins, AIF, endonuclease G and CAD, are released from the mitochondria after the cell has committed to die, during apoptosis. Endonuclease G translocates to the nucleus where it cleaves nuclear chromatin to produce oligonucleosomal DNA fragments **(Li et al., 2001)**. Both AIF and endonuclease G function in a caspase-independent manner. CAD is subsequently released from the mitochondria and translocates to the nucleus where, after cleavage by caspase-3, it causes oligonucleosomal DNA fragmentation and a more pronounced chromatin condensation "stage II" condensation **(Enari et al., 1998 and Susin et al., 2000)**.
The Bcl-2 is a family of proteins governs the mitochondrial membrane permeability and can be either pro-apoptotic or anti-apoptotic **(Cory and Adams, 2002)**. This family have been identified into 25 genes. Their anti-apoptotic proteins include Bcl-2, Bcl-x, Bcl-XL, Bcl-XS, Bcl-w, BAG, and their pro-apoptotic proteins include Bcl-10, Bax, Bak, Bid, Bad, Bim, Bik, and Blk. These proteins can determine if the cell commits to apoptosis or aborts the process. It is thought that the main mechanism of action of the Bcl-2 family of proteins is the regulation of cytochrome c release from the mitochondria via alteration of mitochondrial membrane permeability. The tumor suppressor protein p53 has a critical role in regulation of the Bcl-2 family of proteins, however the exact mechanisms have not yet been completely elucidated **(Schuler and Green, 2001)**. Bad with Bcl-Xl or Bcl-2, promoting cell death **(Yang et al., 1995)**. Reports indicate that Bcl-2 and Bcl-XL inhibit apoptotic death by controlling the activation of caspase proteases **(Newmeyer et al., 2000)**. "Aven" an additional protein designated appears to bind both Bcl-Xl and Apaf-1, preventing activation of procaspase-9 **(Chau et al., 2000)**. Puma and Noxa are two members of the Bcl2 family that are also involved in pro-apoptosis. Puma plays an important role in p53-mediated apoptosis. It was shown that, in vitro, overexpression of Puma is accompanied by increased BAX expression, BAX conformational change, translocation to the mitochondria, cytochrome c release and reduction in the mitochondrial membrane potential **(Liu et al., 2003)**. Noxa is also a candidate mediator of p53-induced apoptosis. Studies show that this protein can localize to the mitochondria and interact with anti-apoptotic

Bcl-2 family members, resulting in the activation of caspase-9 (**Oda et al., 2000**).

Execution Pathway

It is the final pathway of apoptosis. The extrinsic and intrinsic pathways both end at the point of the execution phase. Execution caspases activate cytoplasmic endonuclease, which degrades nuclear material, and proteases that degrade the nuclear and cytoskeletal proteins. Caspase-3, -6 and -7 function as effector or "executioner" caspases, cleaving cytokeratins, the plasma membrane cytoskeletal protein, the nuclear protein NuMA and others, causing the morphological and biochemical changes seen in apoptotic cells (**Slee et al., 2001**). Caspase-3 is the most important of the executioner caspases. It is activated by any of the initiator caspases (caspase-8,-9, or-10). In apoptotic cells, activated caspase-3 cleaves ICAD (endonuclease inhibitors) to release CAD (**Sakahira et al., 1998**). CAD then degrades chromosomal DNA within the nuclei and causes chromatin condensation. Caspase-3 also induces disintegration of the cell into apoptotic bodies. Phagocytic uptake of apoptotic cells is the last component of apoptosis. The appearance of phosphotidylserine on the outer leaflet of apoptotic cells then facilitates noninflammatory phagocytic recognition, allowing for their early uptake and disposal (**Fadok et al., 2001**). This process of early and efficient uptake with no release of cellular constituents, results in essentially no inflammatory response.

Physiologic Apoptosis

Physiologically, apoptosis demonstrates an opposite role to mitosis and cell proliferation thus regulates of various cell populations. It also maintains homeostasis in the adult human body, (**Renehan et al., 2001**).

Apoptosis is critically important during various developmental processes e.g., the ovary arise through overproduction of cells. This initial overproduction is then followed by the death of those extra-cells.

Apoptosis is central to remodeling in the adult, such as the follicular atresia of the postovulatory follicle and post-weaning mammary gland involution (**Tilly, 1993; Lund et al., 1996**). Furthermore, as organisms grow older, some cells begin to deteriorate at a faster rate and are eliminated via apoptosis.

Pathologic Apoptosis

Abnormalities in cell death regulation can be a significant component of diseases such as cancer, autoimmune lymphoproliferative syndrome, ischemia, and neurodegenerative diseases. Some conditions feature insufficient apoptosis whereas others feature excessive apoptosis. Cancer is an example where the normal mechanisms of cell cycle regulation are dysfunctional, with either an overproliferation of cells and/or decreased removal of cells (**King and Cidlowski, 1998**). In fact, suppression of apoptosis during carcinogenesis is thought to play a central role in the development and progression of some cancers (**Kerr et al., 1994**). Tumor cells can acquire resistance to apoptosis by the expression of anti-apoptotic proteins such as Bcl-2 or by the down-regulation or mutation of pro-apoptotic proteins such as Bax. The expression of both Bcl-2 and Bax is regulated by the *p53* tumor suppressor gene (**Miyashita et al., 1994**). Certain immune cells (T cells and natural killer cells) normally destroy tumor cells via the perforin/granzyme B pathway or the death-receptor pathway. Alterations of various cell signaling pathways can result in dysregulation of apoptosis and lead to cancer. The *p53* tumor suppressor gene is a transcription factor that regulates the cell cycle and is the most widely mutated gene in human tumorigenesis (**Wang and Harris, 1997**). The critical role of *p53* is evident by the fact that it is mutated in over 50% of all human cancers. *p53* can activate DNA repair proteins when DNA has sustained damage, can hold the cell cycle at the G_1/S regulation point on DNA damage recognition, and can initiate apoptosis if the DNA damage proves to be irreparable (**Pientenpol and Stewart, 2002**). Excessive apoptosis may also be a feature of some conditions such as autoimmune diseases, neurodegenerative diseases, and ischemia-associated injury. Alzheimer's disease is a neurodegenerative condition that is thought to be caused by

mutations in certain proteins such as APP (amyloid precursor protein) and presenilins. Presenilins are thought to be involved in the processing of APP to amyloid β. This condition is associated with the deposition of amyloid β in extracellular deposits known as plaques and amyloid β is thought to be neurotoxic when found in aggregated plaque form. Amyloid β is thought to induce apoptosis by causing oxidative stress or by triggering increased Fas ligand expressions in neurons and glia. It may also activate microglia, which would result in TNFα secretion and activation of the TNF-R1, leading to apoptosis (**Ethell and Buhler, 2003**).

Inhibition of Apoptosis

A short list of potential methods of anti-apoptotic therapy includes stimulation of the IAP (inhibitors of apoptosis proteins) family of proteins, caspase inhibition, PARP (poly [ADP-ribose] polymerase) inhibition, stimulation of the PKB/Akt (protein kinase B) pathway, and inhibition of Bcl-2 proteins (**Nicholson, 2000**). The IAP family of proteins is perhaps the most important regulators of apoptosis due to the fact that they regulate both the intrinsic and extrinsic pathways (**Deveraux and Reed, 1999**). Eight human IAP proteins have now been identified although XIAP (X-linked mammalian inhibitor of apoptosis protein) and survivin remain the better-known members (**Colnaghi et al., 2006**). So far, members of the IAP family have been investigated as therapeutic targets for the treatment of stroke, spinal cord injuries, multiple sclerosis as well as cancer. Specific inhibitors of caspase activity may also prove beneficial. ICE (Interleukin-1 beta-converting enzyme), also called caspase I, is a cysteine protease that appears to mediate intracellular protein degradation during apoptosis (**Livingston, 1997**). ICE inhibitors have been developed to treat rheumatoid arthritis and other inflammatory conditions via reduction of interleukin 1β (**Le and Abbenante, 2005**). Due to the dual role of PARP-1 in both DNA repair and apoptosis, the pharmacological use of PARP-1 inhibitors may be able to attenuate ischemic and inflammatory cell and organ injury or may be able to enhance the cytotoxicity of antitumor agents (**Graziani and Szabo, 2005**).

Recent research with transgenic models of cardiac ischemia and global brain ischemia indicate that inhibiting the expression and/or function of Bax can prevent cytochrome c release from mitochondria, inhibit the decrease in the mitochondrial membrane potential, and protect cells against apoptosis (**Hochhauser et al., 2003; Hetz et al., 2005**).

Regulators, and Inhibitors

There are more than 13 known caspases (procaspases or active cysteine caspases) that can be detected using various types of caspase activity assays (**Gurtu et al., 1997**). There are also immunohistochemistry assays that can detect cleaved substrates such as PARP and known cell modifications such as phosphorylated histones. (**Love et al., 1999; Talasz et al., 2002**). Fluorescently conjugated caspase inhibitors can also be used to label active caspases within cells (**Grabarek et al., 2002**). Caspase activation can be detected in a variety of ways including western blot, immunoprecipitation and immunohistochemistry. Both polyclonal and monoclonal antibodies are available to both procaspases and active caspases (**Love et al., 1999**).

Apoptosis in The Ovary

At a time when the terms 'apoptosis' and 'programmed cell death' were unknown, a detailed morphological characterization of granulosa cells within degenerating rabbit ovarian follicles offered the first glimpse into the pathway by which follicular atresia is probably accomplished. Only recently, however, has the role of physiological cell death in ovarian function re-emerged as an intriguing and exciting research area that encompasses almost every aspect of ovarian physiology, from early oogenesis to the demise of the corpus luteum.

Several lines of evidence now support the possibility that there is a final common ovarian cell death pathway composed of genes encoding intracellular effector proteins that have been conserved, both structurally and functionally, across species and cell types. While the intracellular signaling mechanisms may vary in different cells, they all display similar

morphological and biochemical features at the later stages of the apoptotic process. Apoptosis can be triggered by many factors, such as hormones, cytokines, and drugs, depending on the type of the cell **(Hirshfield, 1991)**.

Identification of apoptosis during atresia:

The first reported observation of apoptosis in the ovary was made by **Flemming (1885)** after morphological analysis of granulosa cells in degenerated antral follicles in rabbit ovary. A process referred to as 'chromatolysis' was proposed as a mechanism by which granulosa cell loss was mediated. Since this initial observation, many studies on the morphological changes that occur in granulosa cells and theca-interstitial cells of follicles progressing through atresia have documented that apoptosis is the primary mechanism by which cell loss is mediated during follicle degeneration **(Tsafriri and Braw, 1984; Hirshfield, 1991; Tilly and Ratts, 1996)**. The first biochemical evidence that endonucleases responsible for the generation of DNA oligonucleosomes could be detected in nuclei isolated from rat granulosa and luteal cells was published by **Zeleznik et al ., (1989)**. Despite these data, the role of apoptosis in either atresia or luteolysis at that time continued to be an area of research that attracted very little attention. However, two simultaneous reports documented the occurrence in vivo of internucleosomal DNA fragmentation in granulosa cells isolated from atretic follicles of both avian and mammalian species were made (**Hughes and Gorospe, 1991; Tilly et al ., 1991**). This revealed a tremendous increase in research efforts to understand the roles and regulation of apoptosis in the ovary.

Ovarian cyclicity and atresia

A review of the literature indicates that apoptosis plays a fundamental role in ovarian cyclicity: (1) Oogonium and oocyte attrition, (2) follicular atresia and (3) luteolysis. Although the vast majority of available information pertains to the loss of granulosa cells during follicle demise, an increasing number of studies have implicated apoptosis in the degeneration of germ cells and luteal cells (**Tilly et al ., 1991**).

Germ cell attrition:

In most vertebrate species, during embryonic and early neonatal, mitotically active primordial germ cells migrate to and colonize the developing genital ridge. In the newly formed embryonic ovary, these dividing germ cells (oogonia) then begin to leave the mitotic cycle, initiate meiotic division and, as oocytes, become arrested in the first prophase **(Peters, 1970; Hirshfield, 1991)**. During both mitosis and meiosis, large numbers of germ cells are culled from the ovary for as yet unknown reasons, resulting in less than one-third of the total number of potential germ cells being endowed in the ovary within primordial follicles shortly after birth. Detailed analyses of germ cells depletion in rodents suggest that there are several discrete peaks of degeneration of both oogonia and oocytes in the embryonic ovary (**Beaumont and Mandl, 1961; Borum, 1961**)

The largest peak of germ cell loss occurs within the dividing oogonial population during the later stages of fetal development, although cellular degeneration continues into the first few days after birth and is observed in both mitotic and post-mitotic germ cells.

Morphometric and cytological studies made by **Baker (1963)** revealed that at least three discrete waves of germ cell loss probably occur in the human fetal ovary: (1) attrition of dividing oogonia, (2) degeneration of pachytene stage oocytes, and (3) loss of diplotene stage oocytes. Unequivocal evidence that apoptosis is the underlying mechanism responsible for the loss of germ cells in mouse ovary comes cellular and nuclear morphology by microscopy. This is supported by the findings of **Coucouvanis et al ., (1993)** who demonstrated that degeneration of mouse oogonia and oocytes in vivo occurs via apoptosis.

Additional experiments using light and electron microscopy, as well as biochemical analyses, of mouse primordial germ cells cultured in vitro revealed that germ cells deprived of trophic factor support degenerate with many of the morphological and biochemical features characteristic of apoptotic cell death, including cellular and nuclear condensation,

internucleosomal DNA cleavage and increased expression of tissue transglutaminase (**Pesce et al ., 1993**).

b- Follicular atresia

Once the primordial follicle pool is established, depletion of most of the remaining oocytes occurs indirectly as a result of atretic degeneration of follicles not selected for ovulation (**Tsafriri and Braw, 1984; Hirshfield, 1991**). Although still controversial, it appears that in most mammals the majority of follicles undergo atresia during the late preantral to the early antral stage when continued growth is dependent upon gonadotrophin. This observation is based on several pieces of evidence, although the most convincing data are derived from analysis of apoptosis via in situ end-labelling of DNA fragments in fixed ovarian sections. In all mammals studied thus far, specific labelling of apoptotic cells by this approach (which has also been confirmed by morphological analyses) is apparently confined to granulosa cells of follicles undergoing maturational transition to the antral stage of development or to large subordinate antral follicles not selected for ovulation (**Tilly, 1993; Tilly et al., 1995a, 1996**). These histochemical data are supported by qualitative assessments of DNA oligonucleosomes in ovarian extracts throughout postnatal development, which confirm the presence of apoptotic DNA fragments in ovaries only after the first wave of developing antral follicles has formed (**Ratts et al ., 1995**). It is possible that degeneration of immature (primordial, primary, small preantral) follicles does occur, but that the process remains inconspicuous owing to the efficient and rapid removal of the cellular debris (apoptotic bodies) by resident macrophages.

The hormonal regulation of apoptosis in granulosa cells during follicular atresia appears very complex, and probably involves a classic mesenchymal (theca–interstitial)–epithelial (granulosa) cell interaction (**Chun et al., 1994; Flaws et al., 1995a; Tilly et al., 1995b**). Much of our current knowledge of the endocrine, paracrine and autocrine regulation of apoptosis has been derived from studies of rat antral follicles incubated in vitro under defined conditions (**Tilly et al., 1992a**).

Data derived from studies of rat follicles incubated in vitro have revealed that, as anticipated from in vivo observations (**Tsafriri and Braw, 1984; Hirshfield, 1991**), gonadotrophins are effective inhibitors of apoptosis that occurs in granulosa cells of follicles deprived of trophic support (**Chun et al ., 1994; Tilly and Tilly, 1995**). The anti-apoptotic actions of FSH and LH can also be mimicked by treatment of follicles with neuropeptides, such as vasoactive intestinal peptide (VIP), and protein kinase A activators (**Flaws et al ., 1995a**).

It has been proposed that intrafollicular-derived insulin-like growth factor I (IGF-I), and paracrine factors produced by theca–interstitial cells, such as transforming growth factor α , keratinocyte growth factor and hepatocyte growth factor, which bind specific receptors expressed by the adjacent granulosa cells, mediate the suppression of apoptosis by gonadotrophins and other endocrine factors in granulosa cells of antral follicles (**Tilly et al ., 1992a, 1995b; Chun et al ., 1994; Eisenhauer et al ., 1995; Flaws et al ., 1995a**). To add another level of complexity to these findings, the actions of growth factors on apoptosis in rat granulosa cells may in turn involve progesterone as a mediator (**Luciano et al ., 1994**). Lastly, it should be pointed out that pathways distinct from those activated by the binding of hormones to their receptors, such as extracellular matrix protein interactions (**Peluso et al ., 1996**) and gap junction maintenance (**Weisen and Midgley, 1993**), may play key roles in determining the fate of granulosa cells.

Investigations of the regulatory events surrounding survival of less mature follicles within the ovary, and possible differences in the apoptotic potential of cells within follicles at various developmental stages, will also be of great interest.

c-Luteolysis:

Luteolysis, relative to atresia . Little is known of the requirement for apoptosis in the process of luteal regression. The majority of studies published thus far have identified the presence of apoptotic cells at the time of structural degeneration of the corpus luteum (**Orlicky et al ., 1992; Juengel et al ., 1993;**

Rueda et al ., 1995a, b), however, Dharmarajan et al ., (1994) links the timing of apoptosis to functional luteolysis in rabbits.
Few published reports on the regulation of cell death within the corpus luteum could be found. Prostaglandin F2α (PGF2α), a known luteolysin that has been implicated in the process of apoptosis in the corpus luteum (**Orlicky et al ., 1992**).
By comparison, the luteotrophic factor, hCG, enhances expression of anti-oxidant factors, such as superoxide dismutase, in the rat corpus luteum (**Laloraya et al ., 1988**) and prevents apoptosis in rabbit luteal tissue (**Dharmarajan et al ., 1994**). The ability of hCG to protect luteal cells from reactive oxygen species may also involve paracrine mediators, such as progesterone, which directly inhibit superoxide radical generation by mononuclear phagocytes in the rat corpus luteum (**Sugino et al .,1996**).

Factors controlling ovarian apoptosis

Apoptotic TRAIL signaling

There are two main pathways that initiate the apoptotic cascade: the extrinsic and the intrinsic pathway. The extrinsic pathway is triggered when TRAIL binds to TRAIL R1 or TRAIL R2. Receptor trimerization, along with the sub-sequent oligomerization and clustering of the receptors, leads to the recruitment of the adaptor protein Fas-associated protein with death domain (FADD). FADD allows the recruitment of the inactive pro-caspase-8 or –caspase-10 via a shared death effector do main (DED) leading to the formation of the DISC. In some cells, upon autoactivation at the DISC, activated caspase-8 and 10 cleave and directly activate the effector caspases (caspase-3, -6, -7) leading to the exe-cution of apoptosis in cluding membrane bleb-bing, inter-nucleosomal DNA fragmentation and nuclear shrinkage (type I cells) (**Goping et al., 2003**). A protein called cellular FLICE-inhibitory protein (c-FLIP) shares structural homology with pro-caspase-8 and possesses a death effector domain that lacks protease activity. In specific conditions, its structure allows c-FLIP to be recruited to the DISC where it inhibits the processing and activation of pro-caspase-8. Although many isoforms of c-FLIP have been identified, only three are expressed in human cells (**Gozuacik and Kimchi, 2004**). They consist of two short variants, c-FLIPS and c-FLIP R , and a long splice variant, c-FLIPL . Both c-FLIPL and c-FLIPS contain two DEDs an d compete with pro-caspase-8 for association with FADD (**Grabarek et al., 2002**). De-pending on the level of c-FLIPL expression, its function at the DISC will vary. When present in high amounts, c-FLIPL will exert an anti-apoptotic effect at the DISC (**Graziani and Szabo, 2005**). When present in low amounts, it may heterodimerize with caspase-8 at the DISC and promotes apoptosis (**Greenhalgh, 1998**). c-FLIP is thus seen as a major inhibitor of the extrinsic pathway of apoptosis. In other cells (so called type-II cells), amplification of the signal via the intrinsic or mitochondrial pathway is necessary for efficient apoptosis. The intrinsic pathway is usually triggered in response to DNA damage, hypoxia or oncogene overexpression.
As a sensor of cellular stress, p53 is a critical initiator of the intrinsic pathway. In response to cellular damage, p53 translocates from the cytoplasm to the nucleus where it promotes the transcription of pro-apoptotic members of the Bcl-2 family. Pro-apoptotic Bcl-2 family members such as Bax and Bak form pores in the outer mitochondrial membrane causing the release of cytochrome c and other apoptogenic factors such as apoptosis inducing factor (AIF) and SMAC/DIABLO into the cytoplasm. Cytochrome c, along with apoptosis protease activating factor-1 (APAF-1) and pro-caspase-9 form the apoptosome. Within the apoptosome, clustered pro-caspase-9 gets activated and it cleaves downstream effector caspases, leading to the hallmarks of apoptosis (**Gu et al., 2001**). The release of SMAC/DIABLO from the mitochondria pro-motes apoptosis by binding to and neutralizing members of the family of inhibitor of apoptosis proteins (IAPs), which can block caspase-3 ac-
tivity through its baculovirus IAP repeat domains. Although the extrinsic and intrinsic pathways are activated by different mechanisms, these two pathways are interconnected.

Intracellular effectors of ovarian apoptosis

The bc2 *gene family*

The initiation of apoptosis in various ovarian cell lineages probably depends upon cell-specific stimuli received via hormonal signals, the absence or presence of which activates an intracellular cascade of events that now appears to share many common features regardless of the cell type examined. Much of our recent knowledge of the cytoplasmic and nuclear effectors of the apoptotic pathway in the ovary has come from studies of genes believed to regulate apoptosis in cells of various nonreproductive tissues **(Korsmeyer, 1995; Stellar, 1995; Wyllie, 1995)** or from genetic mutation studies of invertebrate species such as the nematode, *Caenorhabditis elegans* **(Horvitz, 1999).** One such gene in vertebrates is *bcl-2*, a proto-oncogene that encodes a membrane-anchored intracellular protein that prevents apoptosis induced by a variety of stimuli, including trophic hormone deprivation, ionizing radiation, hyperoxia, hypoxia and receptor-linked death signals, such as those elicited by tumour necrosis factor α **(Reed, 1994; Korsmeyer, 1995).**

Although the role of BCL-2 in ovarian function remains to be fully elucidated, a growing number of reports have implicated this anti-death factor and other related members of this gene family in the three instances of ovarian cell death discussed in the previous section. For example, expression of the *bcl-2* gene has been detected in the ovary of many species **(Johnson *et al.*, 1993; Rodger *et al.*, 1995; Tilly *et al.*, 1995a)**, and ablation of functional BCL-2 through targeted disruption of the gene in mice (gene 'knock-out') leads to significantly fewer oocytes and primordial follicles in the postnatal ovary **(Ratts *et al.*, 1995)**. By comparison, the extent of granulosa cell apoptosis does not appear to be affected by a loss of BCL-2, suggesting that there are cell type-specific differences in the requirement for various genes to carry out or suppress the cell death command appropriately. In any case, the commonality of the ovarian phenotype observed in SCF, SCF receptor (*c-kit*) and BCL-2-deficient mice has raised the possibility that the downstream survival actions of SCF on developing oogonia and oocytes are linked to enhanced expression of the *bcl-2* gene **(Ratts *et al.*, 1995).** Data to prove unequivocally that this association occurs *in vivo* are not yet available. However, development of defined *in vitro* models, such as isolated primordial germ cells or intact fetal ovaries placed in culture **(Pesce *et al.*, 1993; Martimbeau *et al.*, 1996)**, should permit future testing of this hypothesis.

Another member of the *bcl-2* gene family which warrants discussion in the context of ovarian function is the death-susceptibility gene, *bax*. The BAX protein was originally identified via its ability to noncovalently interact with BCL-2 in cells (**Oltvai *et al.*, 1993**). This interaction is thought to blunt BCL-2 bioactivity and thus may serve as one of its mechanisms of action as a death-inducing factor. However, BAX probably also acts independently of BCL-2 heterodimerization to induce apoptosis **(Korsmeyer, 1995),** although its precise function in this regard remains to be fully clarified. Recent studies suggest that this protein plays a pivotal role in the life-and-death decision ultimately faced by ovarian cells. For example, expression of the *bax* gene in the ovary has been reported from studies of both the mRNA (**Rueda *et al.*, 1994; Tilly *et al.*, 1995a**) and the protein **(Krajewski *et al.*, 1994a).** In addition, enhanced expression of the *bax* death-susceptibility gene in rat granulosa cells temporally coincides with the occurrence of apoptosis and the onset of follicular atresia *in vivo* and *in vitro* **(Tilly *et al.*, 1995a).**

Convincing evidence that BAX plays a fundamental role in ovarian somatic cell death has been derived from studies of genetically manipulated mice that do not express functional BAX protein **(Knudson *et al.*, 1995)**. Disruption of the *bax* gene leads to the development of grossly misshapen follicles which possess degenerating oocytes indicative of atresia. However, granulosa cells within degenerating follicles of BAX-deficient mice appear to be resistant to the induction of apoptosis **(Knudson *et al.*, 1995),** substantiating reports that increased expression of *bax* coincides with and may herald the onset of follicle demise under

normal physiological conditions (**Tilly *et al*., 1995a**). Further evaluation of the potential effects of *bax* knock-out on numbers of oocytes and follicles, as well as on alterations in the ovarian follicle response to apoptotic stimuli, should yield new clues to aid in our understanding of the precise role of BAX in the ovary.

In addition to *bcl-2* and *bax*, other members of the *bcl-2* gene family have been cloned, and several of these factors are expressed in the ovary (**Krajewski *et al*., 1994b; Tilly *et al*., 1995a; Johnson *et al*., 1996**). One of these genes, *bcl-x*, is unique among the other members of this family as it is alternatively processed to yield both positive ('short' isoform, death inducer) and negative ('long' isoform, death suppressor) regulators of the cell death pathway (**Boise *et al*., 1993**). Data currently available indicate that *bcl-x*$_{long}$ is the primary, if not only, isoform of the message expressed in ovarian granulosa cells of mammalian (**Tilly *et al*., 1995a**) and avian (**Johnson *et al*., 1996**) species. In agreement with the reported antiapoptotic actions of BCL-X$_{long}$ protein, recent observations have revealed that ex-pression of *bcl-x*$_{long}$ mRNA in the avian ovary is most abundant in granulosa cells of follicles destined for ovulation (**Johnson *et al*., 1996**). At this point, however, the precise requirement for either isoform of BCL-X in ovarian cell fate is uncertain. This question may remain unanswered since disruption of the *bcl-x* gene in genetically manipulated mice results in embryonic lethality between day 12 and day 13 after coitus (**Motoyama *et al*., 1995**).

Anti-oxidant pathways

The mechanisms by which BCL-2 and related proteins direct cell fate in any tissue are not well understood. The membrane localization of BCL-2 within mitochondria (**Hockenberry *et al*., 1990**) prompted early investigations into the potential inter-actions of this cell death protein with metabolic function. Use of gene-transfer techniques with cultured cells showed that overexpression of *bcl-2* protected cells from oxidative stress-induced death and reduced accumulation of certain reactive oxygen species or their intermediates (**Hockenberry *et al*., 1993; Kane *et al*., 1993**). A follow-up study demonstrated that BCL-2 may cause a slight pro-oxidant state which then serves to trig-ger oxidative stress defence mechanisms of the cell in the form of increased activity of enzymes such as superoxide dismutase (**Steinman, 1995**). The link between BCL-2 and oxidative stress in cells of nonreproductive tissues spawned a study on the potential role of oxygen free radicals in the process of granulosa cell apoptosis during atresia (**Tilly and Tilly, 1995**). Data from these investigations revealed that gonadotrophin-mediated follicular survival coincides with enhanced expression of anti-oxidant factors. Moreover, provision of inhibitors of oxidative stress to follicles cultured *in vitro* mimics the ability of FSH to prevent apoptosis in granulosa cells (**Tilly and Tilly, 1995**), which is consistent with data suggesting that uncontrolled free radical damage induces apoptosis in diverse cell types (**Buttke and Sandstrom, 1994**).

The physiological significance of these findings may be related to the stage of follicular development at which the majority of atresia is thought to occur. The transition of developing follicles to the antral stage is associated with a marked increase in metabolic function of granulosa cells, most notably in the production of steroids via enhanced activity of cytochrome P450 enzymes (**Hirshfield, 1991; Richards, 1994**). Electron transport associated with steroidogenic output is a primary site of free radical generation, suggesting that differentiation of gran-ulosa cells within the follicle coincides with an increased pro-oxidant status. As a consequence, it has been hypothesized that a potential trigger for atresia in follicles not selected for ovulation is an inadequate amount of protection from oxygen free radicals that accumulate in steroidogenically active granulosa cells (**Tilly and Tilly, 1995**). Along these lines, a similar role for oxidative stress has been proposed as a basis for both functional (**Sawada and Carlson, 1991; Musicki *et al*., 1994**) and structural (**Rueda *et al*., 1995a**) luteolysis.

The p53 tumour suppressor protein

One of the primary responses to cellular DNA damage, such as that elicited by reactive oxygen species, is the stabilization and nuclear translocation of the anti-oncogenic transcription factor, p53 **(Kastan *et al.*, 1991)**. In agreement with these data and the hypothesis that oxidative stress triggers atresia, accumulation
of p53 protein in nuclei of granulosa cells of antral follicles des-tined for atresia has been reported **(Tilly *et al.*, 1995c)**. Although the mechanisms of p53 action in any tissue are not fully understood, recent investigations demonstrated that p53 induces apoptosis in many cell types (**Clarke *et al.*, 1993**), including granulosa cells **(Keren-Tal *et al.*, 1995).** Furthermore, this effect may be the result of the ability of p53 to enhance transcriptional activity of the *bax* death-susceptibility gene (**Miyashita and Reed, 1995**), while concomitantly suppressing expression of the *bcl-2* survival gene (**Miyashita *et al.*, 1994).** The relationship between nuclear p53 accumulation and altered ex-pression of cell death genes in ovarian cells has not yet been explored.

The ICE *gene family*

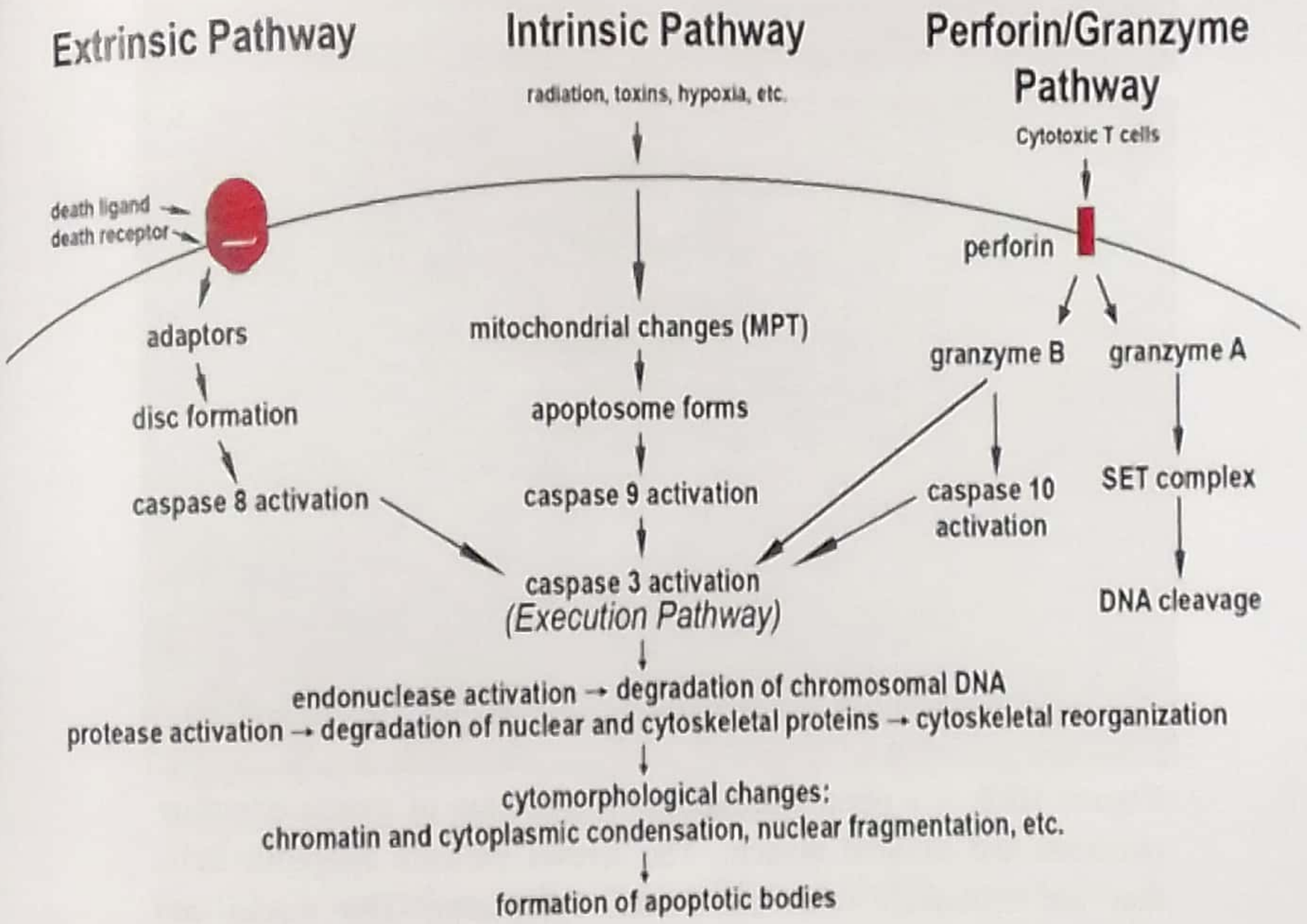
Lastly, recent evidence indicates that a newly emerging family of cell death regulators, belonging to a group of cyto-plasmic proteases that demonstrate significant homology to the *Caenorhabditis elegans* death gene, *ced-3* **(Hengartner and Horvitz, 1994),** may also contribute to the initiation or pro-gression of apoptosis in granulosa cells during atresia **(Flaws *et al.*, 1995b).** Death proteins encoded by members of this gene family in vertebrates include interleukin-1β-converting enzyme (ICE), ICE-and-*ced-3*-homologue 1 (ICH-1), cysteine protease P32 (CPP32), $ICE_{rel}II$ (TX, ICH-2), $ICE_{rel}III$ and MCH-2 **(Martin and Green, 1995)**. These proteases share a conserved active site sequence consisting of a QACRG pentapeptide motif, and appear to be unique in their ability to cleave proteins at aspar-tate residues. Members of this family of proteases attack a di-verse spectrum of specific homeostatic and structural proteins, including poly (ADP)-ribose polymerase (an enzyme involved in DNA repair), small nuclear ribonucleoproteins (factors responsible for spliceosome assembly and mRNA processing) and structural proteins of the nuclear scaffold **(Martin and Green, 1995).**
In the rat ovary, expression and gonadotrophin regulation of ICE, CPP32 and ICH-1 have been reported **(Flaws *et al.*, 1995b).** It appears that ICE *psrse* is not involved in the apoptotic death of granulosa cells as the abundance of ovarian ICE mRNA is extremely low, expression of the ICE gene in the ovary is not gonadotrophin-regulated, and follicles cultured *in vitro* to induce apoptosis and atresia do not contain detectable ICE activity **(Flaws *et al.*, 1995b).** This proposal is in agreement with recent gene knock-out data demonstrating that ablation of ICE in mice does not alter apoptosis in two well-defined model systems for cell death, the glucocorticoid-treated thymus gland and the post-lactational mammary gland (**Li *et al.*, 1995).** In contrast, expression of the death-inducing proteases, CPP32 and ICH-1, in the ovary is inhibited by gonadotrophins **(Flaws *et al.*, 1995b),** and preliminary data suggest that endonuclease activation **(Flaws *et al.*, 1995b)** and cleavage of small nuclear ribonucleo-proteins **(Trbovich and Tilly, 1995)** may be among the consequences of enhanced ICE-related protease activity in granulosa cells. Future analysis of the ovaries of genetically manipulated mice deficient in specific members of this protease gene family, complemented by *in vivo* and *in vitro* examinations of the ex-pression and actions of these cell death regulators in ovarian cells, should aid in elucidating the role of this new family of genes in ovarian function.

Conclusions and Suggestion

Apoptosis is regarded as a carefully regulated energy-dependent process, characterized by specific morphological and biochemical features. It is fundamental to many aspects of ovarian development and cyclic function

Apoptosis is a universal pathway for the execution of cell death in most tissues (Diagram-1). Its manipulation in ovarian cell populations may lead to the development of new strategies for combating a host of reproductive disorders and for novel contraceptive approaches. These data can be used to develop and protect the oocyte pool from environmental insults known to cause sterility, and to enhance success rates for oocyte retrieval and survival. Lastly, new contraceptive measures may arise from the ability to destroy selectively the quiescent germ cell pool (permanent sterility), the ovulatory cohort of antral follicles (temporary sterility), or the corpus luteum (post-conception pregnancy termination) via cell-specific manipulation of the apoptotic mechanism.

Although many of the key apoptotic proteins that are activated or inactivated in the apoptotic pathways have been identified, the molecular mechanisms of action or activation of these proteins are not fully understood and are the focus of continued research. Understanding these mechanisms and other variants of apoptosis is vital because programmed cell death, at the molecular level, is a component of both health and disease, being initiated by various physiologic and pathologic stimuli.



Schematic representation of apoptotic events. The two main pathways of apoptosis are extrinsic and intrinsic as well as a perforin granzyme pathway. Each requires specific triggering signals to begin an energy-dependent cascade of molecular events. Each pathways activates its own initiators caspase (8,9,10) which in turn activate the executioner caspase3. The execution pathway results in cell shrinkage, chromatin condensation, formation of cytoplasmic blebs, apoptotic bodies and finally phagocytosis of these bodies by the adjacent cells. (Taken after Susan Elmore (2007) Apoptosis: A Review of Programmed Cell Death. Toxicol Pathol. 2007; 35(4): 495–516).

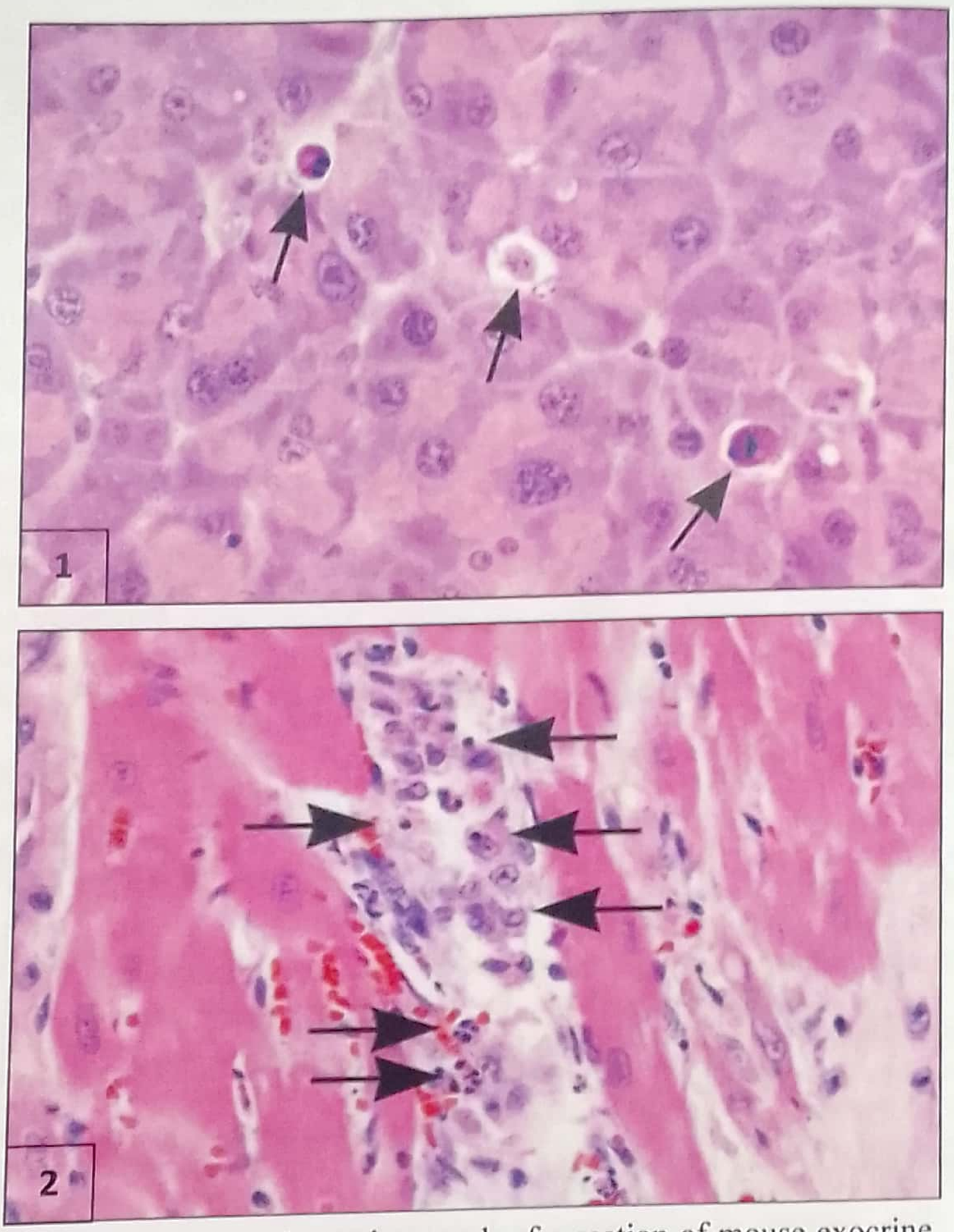


Figure 1&2: is a photomicrograph of a section of mouse exocrine pancreas and skeletal muscle. The arrows indicate apoptotic cells that are shrunken with condensed cytoplasm. The nuclei are pyknotic and fragmented. Notice the lack of inflammation.

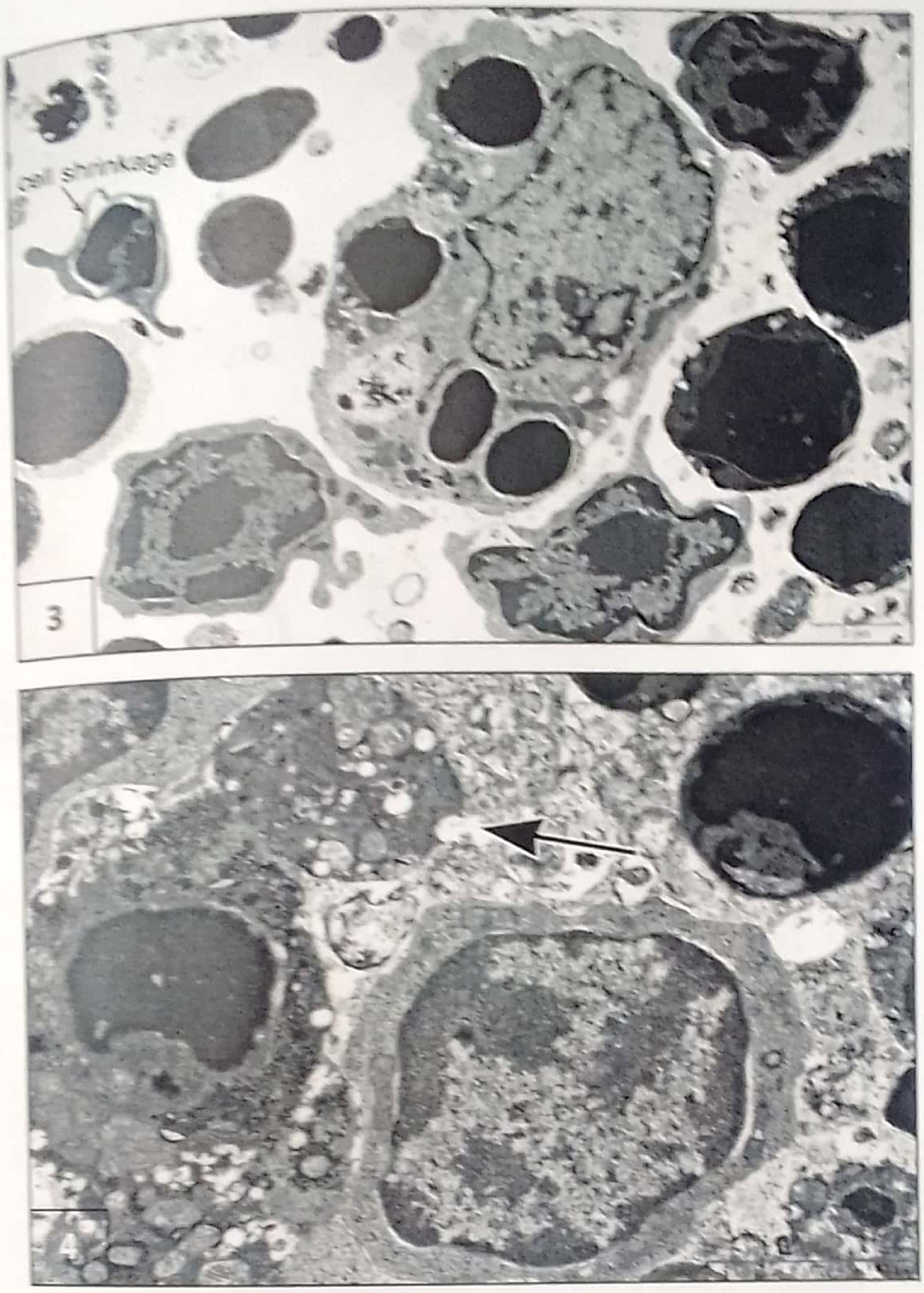


Figure 3&4: TEM of thymus tissue showing apoptotic thymic lymphocytes in an early phase of apoptosis.

Figs: 1- 4 are taken after Susan Elmore (2007) Apoptosis: A Review of Programmed Cell Death. Toxicol Pathol. 2007; 35(4): 495–516.

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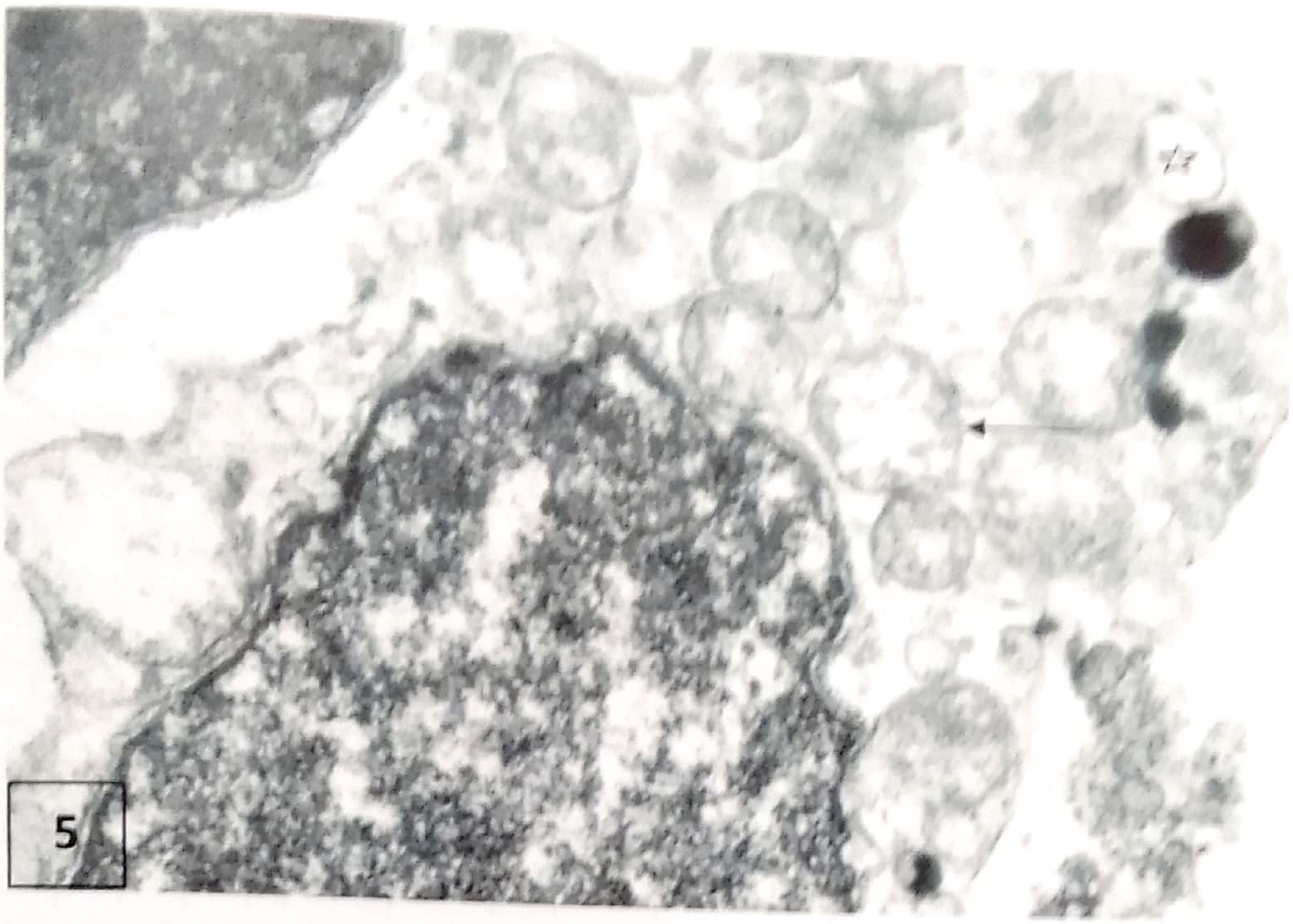

Fig. 5: Electron micrograph of granulosa cells revealing vacuolated cytoplasm and mitochondria (arrow).

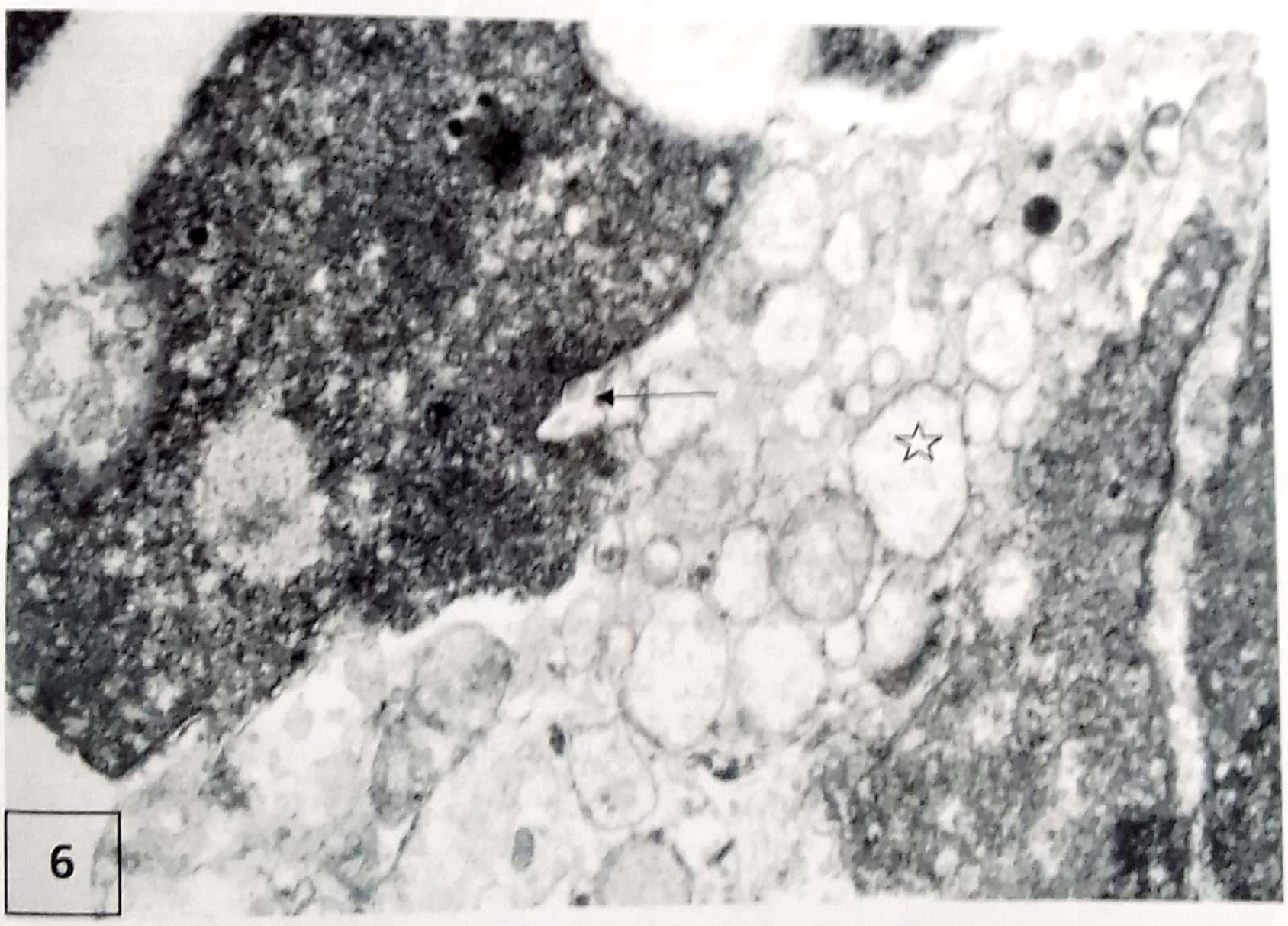

Fig. 6: Electron micrograph of apoptotic granulosa cells showing uneven wavy nuclear envelope (arrow) and increased vacuolization (star) of cytoplasm.

(Figs. 5&6 after J.K. Bhardwaj and R.K. Sharma (2012). Apoptosis and Ovarian Follicular Atresia in Mammals, Zoology, Dr. María-Dolores García (Ed.), ISBN: 978-953-51-0360-8, InTech, Available from: http://www.intechopen.com/books/zoology/apoptosis-and-ovarian-follicular-atresia-in-mammals).

Apoptosis Abbreviations

AIF	Apoptosis-inducing factor	CL	Corpus luteum
Apaf-1	Apoptosis protease-activating factor 1	DAB	Diaminobenzidine
BAD	Bcl-XL /Bcl-2-associated death promoter	DD	Death domain
Bak	Bcl-2 homologous antagonist/killer	DED	Death effector domain
Bax	Bcl-2 -associated x protein	DISC	Death-inducing signalling complex
Bcl-2	B cell leukaemia-2	DNA	Deoxyribonucleic acid
Bcl-XL	B cell leukaemia-x long	DNase	DNA ladder nuclease
Bcl-XS	B cell leukaemia-x short	E2	Estradiol
BH	Bcl-2 homology	ERK	Extracellular signal regulated kinase
BIR	Baculoviral inhibitory repeat	FADD	Fas-associated death domain
BOD	Bcl-2 -related ovarian death gene	FasL	Fas ligand
Bok	Bcl-2 -related ovarian killer	FasR	Fas receptor
bp	Base pair	FLICE	Fas ligand-interacting cell effector
CAD	Caspase-activated DNase	FLIP	FLICE-inhibitory protein
CARD	Caspase activation and recruitment domain	FSH	Follicle-stimulating hormone
cAMP	Cyclic adenosine 3',5'-monophosphate	FSHR	FSH receptor
GDF	Growth differentiation factor	IVF	*In vitro* fertilization
GnRH	Gonadotrophin releasing hormone	I B	Inhibitor of NF- B
hCG	Human chorionic gonadotrophin	kb	Kilobase
17HSD	17-Hydroxysteroid dehydrogenase	kDa	Kilodalton
IAP	Inhibitor of apoptosis	KL	Kit ligand
ICAD	Inhibitor of CAD	LH	Luteinising hormone
I B	Inhibitor of NF- B	Mcl-1	Myeloid cell leukaemia-1
IKK	Inhibitor of NF- B kinase	MAPK	Mitogen-activated protein kinase
MKK	Mitogen-activated protein kinase kinase	SSC	Saline sodium citrate
mRNA	Messenger ribonucleic acid	T	Testosterone
NF- B	Nuclear factor B	TGF-	Transforming growth factor-beta
NIK	NF- B inducing kinase	TNF-	Tumour necrosis factor
PBS	Phosphate-buffered saline	TNFR	Tumour necrosis factor receptor
PI-3K	Phophoinositide 3-kinase	TRAIL	TNF-related apoptosis inducing ligand
PKC	Protein kinase C	VDAC	Voltage-dependent anion channel
PRL	Prolactin	XIAP	X-linked inhibitor of apoptosis
P450arom	Cytochrome P450 aromatase	Z-VAD-fmk	Benzyloxycarbonyl-VAD-luoromethylketone
RIP	Receptor-interacting protein	m	Mitochondrial transmembrane potential
RNA	Ribonucleic acid	SDS	Sodium dodecyl sulphate
ROS	Reactive oxygen species	Smac	2nd mitochondria-derived activator of caspases
TNFα	Tumor necrosis factor α	IGF	insulin-like growth factor
Cyt C	cytochrome C	FGF	fibroblast growth factor
bFGF	basic fibroblastic growth factor	FGFR	fibroblast growth factor receptor
EGF	epidermal growth factor	FK	Forskolin

References

Andreu-Vieyra C.V. and Habibi H. R. (2000): Factors controlling ovarian apoptosis
Canadian Journal of Physiology and Pharmacology 78(12): 1003-1012.

Armstrong DG, McEvoy TG, Baxter G, et al. (2001): Effect of dietary energy and protein on bovine follicular dynamics and embryo production in vitro: associations with the ovarian insulin-like growth factor system. Biol Reprod; 64:1624–32.

Ashkenazi A, Dixit VM. (1998): Death receptors: signaling and modulation. Science.;281:1305–8.

Baker TG (1963): A quantitative and cytological study of germ cells in human ovaries. *Proceedings of the Royal Society of London (B)* **158** 417–433

Barry M, Bleackley RC. Cytotoxic T lymphocytes: all roads lead to death. Nat Rev Immunol.;2:401–9.

Beaumont HM and Mandl AM (1961): A quantitative and cytological study of oogonia and oocytes in the foetal and neonatal rat *Proceedings of the Royal Society of London (B)* **155** 557–579

Boise LH, Gonzalez-Garcia M, Postema CE, Ding L, Lindsten T, Turka LA, Mao X, Nuñez G and Thompson CB (1993): Bcl-x, a *bcl-2*-related gene that functions as a dominant regulator of apoptotic cell death *Cell* 4 597–608

Bortner CD, Oldenburg NB, Cidlowski JA. The role of DNA fragmentation in apoptosis. Trends Cell Biol. 1995;5:21–6.

Borum K (1961): Oogenesis in the mouse: a study of the meiotic prophase. *Experimental Cell Research* **24** 495–507

Bratton DL, Fadok VA, Richter DA, Kailey JM, Guthrie LA, Henson PM. Appearance of phosphatidylserine on apoptotic cells requires calcium-mediated nonspecific flip-flop and is enhanced by loss of the aminophospholipid translocase. J Biol Chem. 1997;272:26159–65.

Brunner T, Wasem C, Torgler R, Cima I, Jakob S, Corazza N. (2003): Fas (CD95/Apo-1) ligand regulation in T cell homeostasis, cell-mediated cytotoxicity and immune pathology. Semin Immunol.;15:167–76.

Buttke TM and Sandstrom PA (1994): Oxidative stress as a mediator of apop-tosis *Immunology Today* **15** 7–10

Byskov AG. (1978): Follicular atresia. In: Jones RE editor, The Vertebrate Ovary. Plenum Press, New York. p533–562.

Chun S-Y, Billig H, Tilly JL, Furuta I, Tsafriri A and Hsueh AJW (1994): Gonadotropin suppression of apoptosis in cultured preovulatory follicles: mediatory role of endogenous insulin-like growth factor-I *Endocrinology* **135** 1845–1853

Clarke AR, Purdie CA, Harrison DJ, Morris RG, Bird CC, Hooper ML and Wyllie AH (1993): Thymocyte apoptosis induced by p53-dependent and -independent pathways *Nature* **362** 849–852

Cohen GM. Caspases: the executioners of apoptosis. Biochem J. 1997;326(Pt 1):1–16.

Cohen GM, Sun X-M, Snowden RT, Dinsdale D and Skilleter DN (1992): Key morphological features of apoptosis may occur in the absence of inter-nucleosomal DNA fragmentation *Biochemical Journal* **286** 331–334

Cotran RS, Kumar V, Collins T. Cellular pathology I. (1999): cell injury and cell death. In: Cortan RS, Kumar V, Collins T, editors. Robbins Pathologic Basis of Disease. 6. W.B. Saunders Co; Philadelphia, PA:. pp. 1–29.

Coucouvanis EC, Sherwood SW, Carswell-Crumpton C, Spack EG and Jones PP (1993): Evidence that the mechanism of prenatal germ cell death in the mouse is apoptosis *Experimental Cell Research* **209** 238–247

deMoura MD, Chamoun D, Resnick CE, Adashi EY. (2000): Insulin-like growth factor (IGF)-I stimulates IGF-I and type 1 IGF receptor expression in cultured rat granulosa cells: autocrine regulation of the intrafollicular IGF-I system. Endocrine; 13:103–10.

Debnath J, Baehrecke EH, Kroemer G. (2005): Does autophagy contribute to cell death? Autophagy.;1:66–74.

Denecker G, Vercammen D, Declercq W, Vandenabeele P. (2001): Apoptotic and

Kastan MB, Onyekwere O, Sidransky D, Vogelstein B and Craig RW (1991): Participation of p53 protein in the cellular response to DNA damage. *Cancer Research* **51** 6304–6311

Kaver-Tal I, Suh B-S, Dantes A, Lindner S, Oren M and Amsterdam A (1995): Involvement of p53 expression in cAMP-mediated apoptosis in im-mortalized granulosa cells *Experimental Cell Research* **218** 283–295

Kerr JFR, Wyllie AH and Currie AR (1972): Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics *British Journal of Cancer* **26** 239–257

Knudson CM, Tung KSK, Tourtellotte WG, Brown GAJ and Korsmeyer SJ (1995): Bax-deficient mice with lymphoid hyperplasia and male germ cell death *Science* **270** 96–99

Korsmeyer SJ (1995): Regulators of cell death *Trends in Genetics* **11** 101–105

Krajewski S, Krajewska M, Shabaik A, Miyashita T, Wang H-G and Reed JC (1994a): Immunohistochemical determination of *in vivo* distribution of Bax, a dominant inhibitor of Bcl-2 *American Journal of Pathology* **145** 1323–1336

Krajewski S, Krajewska M, Shabaik A, Wang H-G, Irie S, Fong L and Reed JC (1994b): Immunohistochemical analysis of *in vivo* patterns of Bcl-x expression *Cancer Research* **54** 5501–5507

Laloraya M, Pradeep KG and Laloraya MM (1988): Changes in the levels of superoxide anion radical and superoxide dismutase during the estrous cycle of *Rattus norvegicus* and induction of superoxide dismutase in the rat ovary by lutropin *Biochemical and Biophysical Research Communications* **157** 146–153

Li P, Allen H, Banerjee S, Franklin S, Herzog L, Johnston C, McDowell J, Paskind M, Rodman L, Salfeld J, Towne E, Tracey D, Wardwell S, Wei F-Y, Wong W, Kamen R and Seshadri T (1995): Mice deficient in IL-1ß-converting enzyme are defective in production of mature IL-1ß and resistant to endotoxic shock *Cell* **80** 401–411

Luciano AM, Pappalardo A, Ray C and Peluso JJ (1994): Epidermal growth factor inhibits large granulosa cell apoptosis by stimulating progesterone synthesis and regulating the distribution of intracellular free calcium *Biology of Reproduction* **51** 646–654

Majno G and Joris I (1995): Apoptosis, oncosis, and necrosis. An overview of cell death *American Journal of Pathology* **146** 3–15

Martimbeau S, Marganaro TK, Donahoe PK and Tilly JL (1996): Preliminary characterization of an *in vitro* model to elucidate the regulation of germ cell apoptosis in the intact fetal rat ovary *Journal of the Society for Gynecologic Investigation Supplement* **3** 216A

Martin SJ and Green DR (1995): Protease activation during apoptosis: death by a thousand cuts? *Cell* **82** 349–352

Miyashita T and Reed JC (1995): Tumor suppressor p53 is a direct transcriptional activator of the human *bax* gene *Cell* **80** 293–299

Miyashita T, Harigai M, Hanada M and Reed JC (1994): Identification of a p53-dependent negative response element in the *bcl-2* gene *Cancer Research* **54** 3131–3135

Motoyama N, Wang F, Roth KA, Sawa H, Nakayama K-I, Nakayama K, Negishi I, Senju S, Zhang Q, Fujii S and Loh DY (1995): Massive cell death of immature hematopoietic cells and neurons in Bcl-x-deficient mice *Science* **267** 1506–1509

Musicki B, Aten RF and Behrman HR (1994): Inhibition of protein synthesis and hormone-sensitive steroidogenesis in response to hydrogen peroxide in rat luteal cells *Endocrinology* **134** 588–595

Oltvai ZN, Milliman CL and Korsmeyer SJ (1993): Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death *Cell* **74** 609–619

Orlicky DJ, Fisher L, Dunsomb N and Miller GJ (1992): Immunohistochemical localization of $PGF_{2\alpha}$ receptor in the rat ovary *Prostaglandins, Leukotrienes and Essential*

Fatty Acids **46** 223–229

Peluso JJ, Pappalardo A and Trolice MP (1996): N-Cadherin mediated cell contact inhibits granulosa cell apoptosis in a progesterone-independent manner *Endocrinology* **137** 1196–1203

Pesce M, Farrace MG, Piacentini M, Dolci S and De Felici M (1993): Stem cell factor and leukemia inhibitory factor promote primordial germ cell survival by suppressing programmed cell death (apoptosis) *Development* **118** 1089–1094

Peters H (1970): Migration of gonocytes into the mammalian gonad and their differentiation *Proceedings of the Royal Society of London (B)* **259** 91–101

Pietenpol JA, Stewart ZA. (2002): Cell cycle checkpoint signaling: cell cycle arrest versus apoptosis. Toxicology. 181–182:475–81.

Ratts VS, Flaws JA, Kolp R, Sorenson CM and Tilly JL (1995): Ablation of *bcl-2* gene expression decreases the numbers of oocytes and primordial fol-licles established in the post-natal female mouse gonad *Endocrinology* **136** 3665–3668

Reed JC (1994): Bcl-2 and the regulation of programmed cell death *Journal of Cell Biology* **124** 1–6

Reddien PW, Cameron S, Horvitz HR. (2001): Phagocytosis promotes programmed cell death in C. elegans. Nature;412:198–202.

Richards JS (1994): Hormonal control of gene expression in the ovary. *Endocrine Reviews* **15** 725–751

Rodger FE, Fraser HM, Duncan WC and Illingworth PJ (1995): Immunolocalization of Bcl-2 in the human corpus luteum *Human Reproduction* **10** 1566–1570

Rueda BR, Tilly KI, Hoyer PB, Hansen TR and Tilly JL (1994): Superoxide dismutase and cell death gene expression in the ovine and bovine corpus luteum: a role for oxidative stress in luteolysis *Proceedings from the Xth Ovarian Workshop, Serono Symposia (USA)*, 27

Rueda BR, Tilly KI, Hansen TR, Hoyer PB and Tilly JL (1995a): Expression of superoxide dismutase, catalase and glutathione peroxidase in the bovine corpus luteum: evidence supporting a role for oxidative stress in luteolysis *Endocrine* **3** 227–232

Rueda BR, Wegner JA, Marion SL, Wahlen DD and Hoyer PB (1995b): Internucleosomal DNA fragmentation in ovine luteal tissue associated with luteolysis: *in vivo* and *in vitro* analyses *Biology of Reproduction* **52** 305–312

Rosenfeld CS, Wagner JS, Roberts RM, Lubahn DB (2001): Intraovarian actions of oestrogen. Reproduction; 122:215–26. |

Rajakoski E. (1996): The ovarian follicular system in sexually mature heifers with special reference to season, cyclical and left-right variation. Acta Endocrinol Suppl; 52:1–68.

Sawada M and Carlson JC (1991): Rapid plasma membrane changes in superoxide radical formation, fluidity, and phospolipase A_2 activity in the corpus luteum of the rat during induction of luteolysis *Endocrinology* **128** 2992–2998

Steinman HM (1995): The Bcl-2 oncoprotein functions as a pro-oxidant *Journal of Biological Chemistry* **270** 3487–3490

Stellar H (1995): Mechanisms and genes of cellular suicide *Science* **267** 1445–1449

Sugino N, Shimamura K, Tamura H, Ono M, Nakamura Y, Ogino K and Kato H (1996): Progesterone inhibits superoxide radical production by mononuclear phagocytes in pseudopregnant rats *Endocrinology* **137** 749–754

Tilly JL (1993): Ovarian follicular atresia: a model to study the mechanisms of physiological cell death *Endocrine Journal (Endocrine)* **1** 67–72

Tilly JL (1994): Use of the terminal transferase DNA labeling reaction for the biochemical and *in situ* analysis of apoptosis. In *Cell Biology: A Laboratory Handbook* pp 330–337 Ed. JE Celis. Academic Press, San Diego

Tilly JL and Ratts VS (1996): Biological and clinical importance of ovarian cell death *Contemporary Obstetrics and Gynecology* **41** 59–86

Tilly JL and Tilly KI (1995): Inhibitors of

oxidative stress mimic the ability of follicle-stimulating hormone to suppress apoptosis in cultured rat ovarian follicles *Endocrinology* **136** 242–252

Tilly JL, Kowalski KI, Johnson AL and Hsueh AJW (1991): Involvement of apoptosis in ovarian follicular atresia and postovulatory regression. *Endocrinology* **129** 2799–2801

Tilly JL, Billig H, Kowalski KI and Hsueh AJW (1992a): Epidermal growth factor and basic fibroblast growth factor suppress the spontaneous onset of apoptosis in cultured rat ovarian granulosa cells and follicles by a tyrosine kinase-dependent mechanism *Molecular Endocrinology* **6** 1942–1950

Tilly JL, Kowalski KI, Schomberg DW and Hsueh AJW (1992b): Apoptosis in atretic ovarian follicles is associated with selective decreases in mess-enger ribonucleic acid transcripts for gonadotropin receptors and cyto-chrome P450 aromatase *Endocrinology* **131** 1670–1676

Tilly JL, Tilly KI, Kenton ML and Johnson AL (1995a): Expression of mem-bers of the *bcl-2* gene family in the immature rat ovary: equine chorionic gonadotropin-mediated inhibition of apoptosis is associated with decreased *bax* and constitutive *bcl-2* and $bcl\text{-}x_{long}$ messenger ribonucleic acid levels *Endocrinology* **136** 232–241

Tilly JL, Flaws JA, DeSanti A, Kugu K, Rubin JS and Hirshfield AN (1995b): Role of intrafollicular growth factors in maturation and atresia of rat ovarian follicles *Biology of Reproduction (Supplement 1)* **52** 159

Tilly KI, Banerjee S, Banerjee PP and Tilly JL (1995c): Expression of the p53 and Wilms' tumor suppressor genes in the rat ovary: gonadotropin repres-sion *in vivo* and immunohistochemical localization of nuclear p53 protein to apoptotic granulosa cells of atretic follicles *Endocrinology* **136** 1394–1402

Tilly JL, Kugu K, Ratts VS, Piquette GN, Tilly KI, Aberdeen GW, Pepe J and Albrecht ED (1996): Apoptosis and cell death gene expression in the human and baboon ovary *Journal of the Society for Gynecologic Investigation Supplement* **3** 70A

Trbovich AM and Tilly JL (1995): Specific cleavage of U1 small nuclear ribonucleoprotein (SNRP) during ovarian granulosa cell apoptosis and fol-licular atresia *Proceedings from the 77th Annual Meeting of the Endocrine Society*, 74

Tsafriri A and Braw RH (1984): Experimental approaches to atresia in mammals *Oxford Reviews of Reproductive Biology* **6** 226–265

Walker PR and Sikorska M (1994): Endonuclease activities, chromatin structure, and DNA degradation in apoptosis *Biochemistry and Cell Biology* **72** 615–623

Wiesen JF and Midgley AR, Jr (1993): Changes in expression of connexin 43 gap junction messenger ribonucleic acid and protein during ovarian fol-licular growth *Endocrinology* **133** 741–746

Williams JR, Little JB and Shipley WU (1974): Association of mammalian cell death with specific endonucleolytic degradation of DNA *Nature* **252** 754–756

White MK, Cinti C. A (2004): morphologic approach to detect apoptosis based on electron microscopy. Methods Mol Biol.;285:105–11.

Wyllie AH (1995): The genetic regulation of apoptosis *Current Opinion in Genetics and Development* **5** 97–104

Zeleznik AJ, Ihrig L and Bassett SG (1989): Developmental expression of a Ca^{++}/Mg^{++}-dependent endonuclease activity in rat granulosa and luteal cells *Endocrinology* **125** 2218–2220

Yuan Song YU, Hong Shu SUI, Zheng Bin HAN, Wei LI, Ming Jiu LUO and Jing He TAN (2004): Apoptosis in granulosa cells during follicular atresia: relationship with steroids and insulin-like growth factors. *Cell Research* **14,** 341

الملخص

يتميز موت الخلايا المبرمج عموما بخصائص مورفولوجية متميزة وآليات بيوكيميائية تعتمد على الطاقة . ويعتبر موت الخلايا المبرمج عنصرا حيويا من عمليات مختلفة تشمل التداول العادي للخلية ، التنمية السليمة ، أداء الجهاز المناعي ، والضمور المعتمد على الهرمونات ، النمو الجنيني وموت الخلايا الناجم عن المواد الكيميائية . موت الخلايا المبرمج الغير مناسب (إما صغير جدا أو كبير جدا) هو أحد العوامل العديدة من الظروف الإنسانية بما فيها الأمراض التنكسية العصبية، والأضرار الناجمة عن فقر الدم، واضطرابات المناعة الذاتية وأنواع عديدة من السرطان. الآليات المسؤولة عن نضوب الخلية الجرثومية من المبيض، إما مباشرة خلال فترة ما حول الولادة أو غير مباشر عن طريق الرتق الجريبي خلال فترة ما بعد الولادة، تعتمد على تفعيل آليات موت الخلايا المبرمج .ويتم إنجاز ذلك من خلال تفعيل مسار 'عام ' للانتحار الخلوي ينطوي على التعبير عن الجينات المختصة. والهدف من هذا الاستعراض هو تقديم لمحة عامة عن المعرفة الحالية لموت الخلايا المبرمج بما في ذلك الشكل، الكيمياء الحيوية ، ودوره في الصحة والمرض وطرق الكشف ومناقشة الأشكال البديلة المتمثلة في موت الخلايا المبرمج. أيضاً يتم استكشاف هوية المؤثرات الهرمونية والخلايا المسؤولة عن تنسيق قرارات الحياة والموت التي تتخذها خلايا المبيض خلال النمو، فضلا عن الآثار البيولوجية والسريرية لموت الخلايا الموجهة بالجينات في المبيض.