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*Review*

# Role of apoptosis-related factors in follicular atresia

**Basak Gumus, Sevtap Kilic and Ebru Zulfikaroglu***

Zekai Tahir Burak Women's Health Care Education and Research Hospital, Department of Obstetrics and Gynecology, Ankara, Turkey.

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**Follicular atresia is the term used for the fate of follicles which undergo degenerative changes before rupturing during ovulation. Recent studies suggest that granulosa cell apoptosis play a major role in follicular atresia. The factors which lead the cell to apoptosis and which protect the cell death, still remain complicated and more studies are needed to elucidate the whole process. Here in this review, we aimed to simplify the factors and mechanisms taking place in granulosa cell apoptosis, to make the process more understandable.**

**Key words:** Granulosa cell, apoptosis, follicular atresia.

## INTRODUCTİON

When a female human embryo is 8-week-old, she has 600,000 germ cells in her gonads and this number increases to 6 to 7 million at 20 weeks of gestation. But with the increasing rate of atresia, the number declines progressively and 1 million oocytes are present in the newborn, whereas 300,000 oocytes remain in puberty, of which approximately 400 will ovulate during the fertile lifespan (Oktem and Oktay, 2008; Gougeon, 1996; Matova and Cooley, 2001).

'Follicular atresia' term is used to define antral follicles undergoing degenerative changes before rupturing during ovulation. It is initiated within the granulosa cell layer and subsequently in the theca cells (Morita et al., 1999; Hsueh et al., 1994). In mammals, the basic mechanism of follicular atresia is apoptosis (Depalo et al., 2003). Apoptosis is a way which multicellular organisms use to eliminate unwanted cells in response to developmental signals or toxic stimuli (Quirck et al., 2004). It is regulated at the level of transcription or translation (Manabe et al., 2008). Major morphological characteristics of apoptosis are the internucleosomal DNA fragmentation, cell shrinkage, plasma membrane blebbing, and the apoptotic body formation (Schwartzman and Cidlowski, 1993).

Granulosa cells possess endogenous pathways to trigger apoptosis, that are inhibited in the presence of survival factors (Quirck et al., 2004). To date, many apoptosis-related factors have been implicated in follicular atresia, including death ligands and receptors, Bcl-2 family proteins, Nodal, caspases, growth factors, gonadotropins, and calcium. In this report, we will overview these factors one by one.

*Corresponding author. E-mail: zebru33@gmail.com. Tel: +90 (312) 2158652. Fax: +90 (312) 3124931.

### Proapoptotic regulators in the cell death receptor-ligand system

Death receptors constitute a subgroup within the tumor necrosis factor (TNF) receptor family. They are located on the cell surface, anchored to the cell membrane, are trimerized and have cytoplasmic death domains (DDs) which are necessary to induce apoptosis (Park et al., 2005; Ashkenazi and Dixit, 1998; Wallach et al., 1999). Fas receptor, TNF receptor, and TNF related apoptosis inducing ligand receptors (TRAILr) are the members of TNF receptor family that are found to have roles in follicular atresia in mammalian ovaries (Park et al., 2005).

In most of the cases, the cell death receptor-mediated apoptosis takes place as follows:

1) The cell death ligand binds to the extracellular domain of cell death recepor (Fas ligand-Fas L for Fas receptor [CD 95, APO-1, TNFR sf 6], TNF α for TNF α receptors, and TNF α related apoptosis inducing ligand [TRAIL] for TRAIL receptors).

2) The intracellular DD of the receptor becomes activated and binds to the DD of the adaptor protein through homophilic interactions (Fas associated death domain [FADD] for Fas-Fas L system, TNF receptor associated death domain [TRADD] for TNF α-TNF receptor system)
3) Procaspase 8 (also named FLICE) is an initiator caspase and binds to FADD with the death effector domain (DED) through homophilic interactions. The complex formed by procaspase 8 and FADD is called death inducing complex (DISC).
4) Dimerization of procaspase 8 induces auto-proteolytic cleavage and procaspase 8 becomes activated (Medema et al., 1997; Boldin et al., 1995; Chinnaiyan et al., 1995; Muzio et al., 1996; Nagata, 1997; Scaffidi et al., 1998).
5) Activated caspase 8 subsequently activates downstream caspases either directly (in type 1 cells) or via mitochondrial perturbation (in type 2 cells) (Matsui et al., 2003).

In type 1 apoptotic cells, caspase 8 directly activates the effector enzyme caspase 3, active form of which activates endogenous endonucleases and causes apop-tosis. In type 2 apoptotic cells, activation of procaspase 8 leads to the release of cytochrome c from the mitochon-drion. Cytochrome c binds to the apoptosis activating factor (Apaf 1) and causes activation of procaspase 9 (the complex formed by cytochrome c, Apaf 1, and cas-pase 9 is called apoptosome). Activated caspase 9 cleaves procaspase 3 and causes its activation, leading to activa-tion of endonucleases, and apoptosis is the result of the pathway (Ashkenazi and Dixit, 1998; Nagata, 1997; Matsui et al., 2003).

**Fas-Fas L system**

Fas and Fas L are the best characterized apoptotic signalling machinery in the granulosa cells of many species, including humans. In human females, Fas is expressed in granulosa cells of atretic antral follicles and its level increases as atresia progresses (Quirk et al., 1995; Kondo et al., 1996).

When Fas L binds to the extracellular domain of Fas, the intracellular DD of the receptor interacts with FADD through its DD. FADD and procaspase 8 interact through their DEDs and procaspase 8 becomes activated. This leads to activation of a caspase system and eventually apoptosis is induced as shown in the Figure 1 (Inoue et al., 2006).

**TNF α-TNF receptor system**

TNF is produced by granulosa cells and the oocyte, and it is another important regulator of follicular development and atresia (Jiang et al., 2003). When it binds to the TNF receptor 1, it stimulates apoptosis via its DD and when it binds to TNF receptor 2 which lacks DD, it acts as a survival factor (Matsuda-Minehata et al., 2006).

Increased mRNA expression of TNF receptor asssociated DD (TRADD) which transmits the death signal from death receptor 4 and/or 5 to intracellular apoptosis inducing pathways in granulosa cells, was demonstrated only in atretic follicles, showing that the TRAIL receptor system induces apoptosis in granulosa cells during atresia in porcine ovaries (Wada et al., 2002).

**Caspases**

Caspases are a family of intracellular cysteine proteases which have roles in both initial and final stages of apoptosis in almost all types of vertebrate cells (Johnson and Bridgham, 2002). As discussed earlier, procaspase 3 activates endogenous endonucleases and causes apoptosis, while procaspase 9 is activated via cytochrome c and Apaf 1, and the complex named apoptosome formed by these three, activates procaspase 3 in type 2 cells. In humans and mice, it showed that antibody raised against the activated form of procaspase 3 reacted strongly with the granulosa cells of degenerating antral follicles. The studies in caspase 3 deficient mice showed that caspase 3 is required for granulosa cell apoptosis and therefore necessary for the process of follicular atresia (Matikainen et al., 2001). Caspase 9 deficient mice were found to contain numerous developing follicles that failed to complete the process of atresia due to the failure of granulosa cell apoptosis (Johnson and Bridgham, 2002).

**Nodal**

Nodal is a member of transforming growth factor β (TGF β) family whose members act through cell surface serine / threonine kinase receptor complexes. It was shown that Nodal is a critical regulator of early vertebrate development and involved in the induction of dorsal mesoderm, anterior paterning, and formation of left-right asymmetry (Iannaccone et al., 1992; Brennan et al., 2002; Eimon and Harland, 2002). Proapoptotic and growth inhibitory effects of Nodal in ovarian granulosa cells have also been reported (Wang et al., 2006). Like the other members of the TGF β family, Nodal has type 1 (ALK 4 and ALK 7) and type 2 receptors (Activin type 2 receptors ActR2A and ActR2B) (Oktem and Oktay, 2008). Nodal exerts its function by binding to and bringing together on the cell surface type 1 and 2 receptors to form a ternary ligand-receptor complex (Massague, 1998). Then, type 2 receptor phosphorylates type 1 receptor, which activates Smads, the intracellular signalling members of TGF β family, by phosphorylation. Smads 2 and Smad 3 are the ones that respond to Nodal. The phosphorylated Smads are released from the receptors and form complexes with common partner Smad and Smad 4 and translocate into the nucleus to regulate the transcription of target genes (Wang and Tsang, 2007). Nodal and its type 1 receptor ALK 7, are expressed in a cell type specific and follicular stage-dependent manner during folliculogenesis. Nodal immunoreactivity is the strongest in the preantral follicles

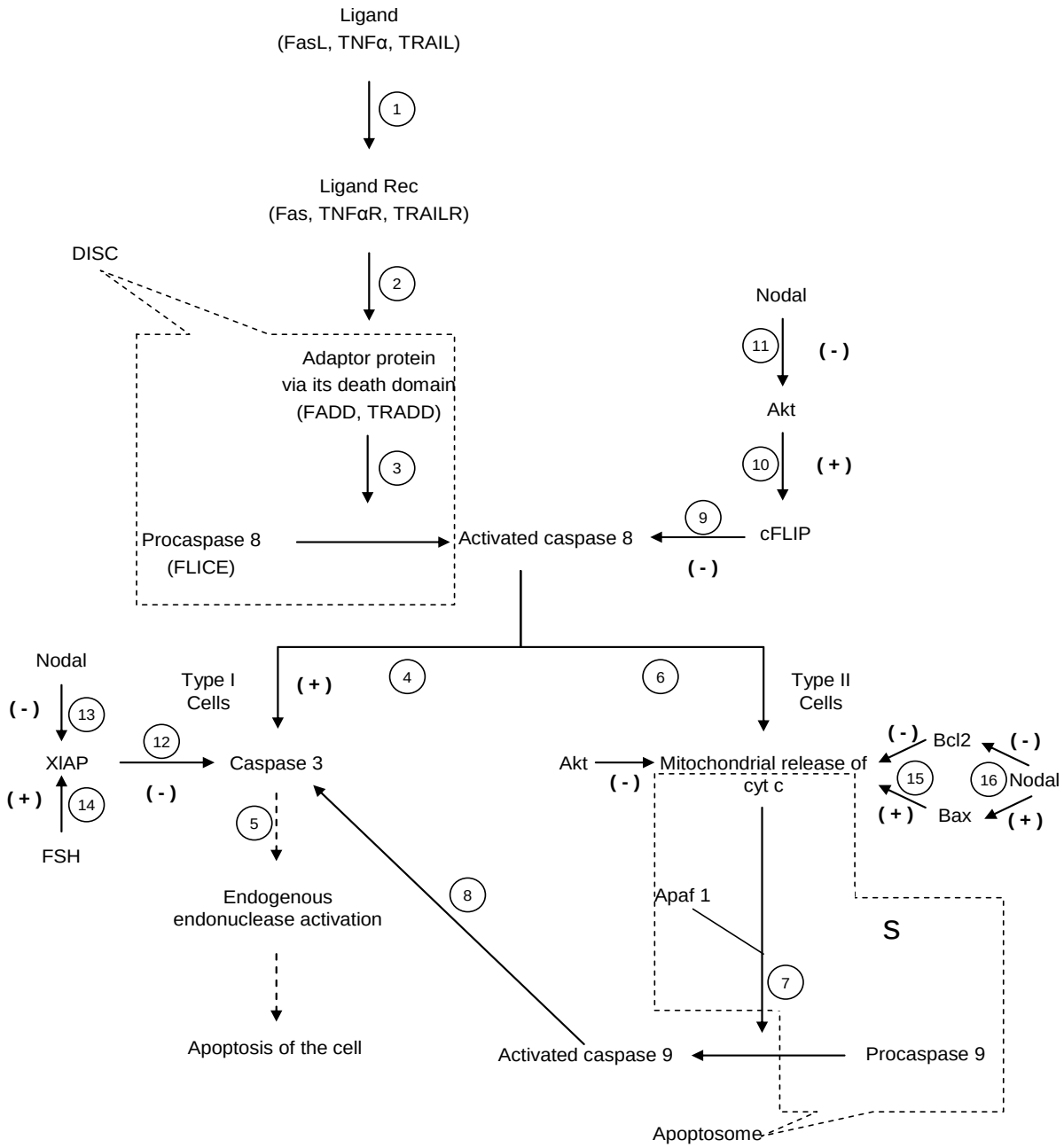


**Figure 1.** (1) Ligand binds to its receptor on the cell membrane, (2) intracellular death domain of the receptor binds to death domain of adaptor protein, (3) procaspase 8 binds to adaptor protein and becomes activated procaspase 8 and FADD together called death inducing signalling complex (DISC), (4 and 5) type I cells activated caspase 8 activates caspase 3 which activates endogenous endonucleases andcauses apoptosis of the cell, (6 and 7) type II cells activated caspase 8 causes mitochondrial release of cyt c which together with apoptosis activationg factor 1 (Apaf 1) activates procaspase 9 (cytochrome c (cyt c) Apaf 1 and procaspase 9 together are called apoptosome), (8) activated caspase 9 activates caspase 3 and leads to apoptosis, (9) cFLIP inhibits activation of caspase 8, (10 and 11) Akt, which is inhibited by Nodal improves cFLIP effect, (12, 13 and 14) X linked inhibitor of apoptosis is protein (XIAP) which is inhibited by Nodal and activated by FSH, inhibits caspase 3, (15) mitochondrial release of cyt c is inhibited by Bcl2 and activated by Bax protein, and (16) Nodal inhibits Bcl2 and improves Bax protein effection mitochondrial release of cyt c.

when compared with the later stages of development, whereas ALK 7 was mainly detected in the interstitial cells at the preantral stage. Nodal was found to be expressed in theca cells, but ALK 7 was only present in granulosa cells. During the development through the penultimate stage, the granulosa cells have the capability of under-

going apoptosis due to the presence of ALK 7, but fail to do so due to low levels of its ligand (Wang et al., 2006). When the gonadotropin support decreases the antral follicle destined for atresia begins to express increased levels of Nodal and also shows colocalization of both the ligand and its receptor in the granulosa cells. This allows us to accept that increased granulosa cell Nodal expression may be a physiological signal for induction of atresia (Wang and Tsang, 2007). It was also shown in studies that Nodal or ALK 7 activation downregulates the X linked inhibitor of apoptosis protein (XIAP) which acts as a direct inhibitor of caspases 3, 7, and 9 (Wang et al., 2006; Asselin et al., 2001; Deveraux et al., 1998).

Overexpression of Nodal and ALK 7 activation can significantly decrease the ratio of acitvated Akt protein which is an important antiapoptotic factor in granulosa cells (Wang et al., 2006; Asselin et al., 2001). Inactivation of Akt increases the mitochondrial release of Smac-Omi and cytochrome c, thus leading to activation of caspases and eventually granulosa cell apoptosis (Wang and Tsang, 2007).

## Bcl-2 family proteins

Bcl 2 family proteins regulate apoptosis of granulosa cells bidirectionally. Some act as promoters of apoptosis like Bax, Bid, Bak, Bim, Mtd/Bok, Diva/Boo, etc., and some act as inhibitors like Bcl-2, Bcl-$X_L$, Mcl-1 (Datta et al., 1999; Kim and Tilly, 2004; Hsu and Hsueh, 2000).

One of the most studied members of the Bcl-2 family proteins is Bax, which is proapoptotic as previously mentioned. Its role has been emphasized by many studies. It has been shown that Bax deficient mice have abnormal follicles with an excessive number of granulosa cells (Perez et al., 1999). In humans and other species it was found that the atretic follicles and the granulosa cells going to apoptosis had increased levels of Bax expression at the mRNA and protein levels (Kim and Tilly, 2004). Moreover, Bax protein was abundantly expressed in granulosa cells of early atretic follicles, while it was found to be scarce in amount or undetectable in healthy follicles (Kugu et al., 1998). It was also shown that apoptosis could be induced by microinjection of recombinant Bax protein in oocytes (Kim and Tilly, 2004).

Interaction between pro- and antiapoptotic members of Bcl-2 family proteins in the mitochondrion determines whether pathways of apoptosis will be activated or not (Zinkel et al., 2006). The exact mechanism of apoptosis inhibition is not well-known, but the antiapoptotic members of Bcl-2 family are supposed to inhibit the mitochondrial release of some apoptotic molecules like cytochrome c.

Many studies have shown the importance of Bcl-2 in follicular development and growth. The number of follicles was shown to decrease in Bcl-2 deficient mice (Ratts et al., 1995) and overexpression of Bcl-2 was correlated with decreased follicular apoptosis and atresia, and increased susceptibility for germ cell tumorigenesis (Hsu et al., 1996; Morita and Tilly, 1999).

## Antiapoptotic regulators in the cell

The antiapoptotic members of Bcl-2 family was discussed in the Bcl-2 family proteins.

## cFLIP

Cellular FLICE-like inhibitory protein (cFLIP), also named as CASH, Casper, CLARP, FLAME, I-FLICE, MRIT or usurpin (Goltsev et al., 1997; Han et al., 1997; Hu et al., 1997; Inohara et al., 1997; Irmler et al., 1997; Shu et al., 1997; Srinivasula et al., 1997), is a homlog of procaspase 8 (FLICE) and is one of the intracellular proteins interfering with the apoptotic effects of death ligands. It was discovered in several viruses as viral FLIP (vFLIP) which contains two DEDs that interact with FADD to avoid the host's apoptotic response (Thome et al., 1997). In mammalian cells, homologue of vFLIP was discovered and named as cFLIP (Irmler et al., 1997). cFLIP was defined to have two forms, short and long ones, $cFLIP_S$ and $cFLIP_L$, respectively. $cFLIP_S$ is similar to vFLIP in structure and has two DEDs, whereas $cFLIP_L$ contains an additional pseudoenzymatic domain which is similar to the enzymatic domain of procaspase 8, but lacks enzymatic activity. Therefore, $cFLIP_L$ blocks the death ligand inducible apoptosis by competing with procaspase 8 and interfering the activation of caspase 8 (Thome and Tschopp, 2001).

The researches in this subject showed that granulosa cells of healthy follicles had highly expressed $cFLIP_L$ mRNA and proteins, whereas the atretic ones had decreased levels (Goto et al., 2004). The mRNA levels of $cFLIP_S$ in granulosa cells are low and showed no changes among the stages of follicular development (Matsuda-Minehata et al., 2005, 2006, 2007).

cFLIP acts as a survival promoting factor in granulosa cells and not only inhibits Fas-signalling, but also can inhibit TNF α and TRAIL signalling (Manabe et al., 2008; Cheng et al., 2007; Manabe et al., 2003).

## Phosphotidyl inositide-3 kinase and Akt pathway

The phosphotidyl inositide-3 kinase ($PI_3K$)/Akt pathway has an important role in regulating granulosa cell apoptosis (Asselin et al., 2001). Activation of $PI_3K$ results in activation of broad spectrum of downstream kinases one of which is Akt (Matsuda-Minehata et al., 2006). Akt is a serine/threonine phosphokinase and is an important antiapoptotic factor. Bcl-2 associated death promoter (Bad), caspase 9, and forkhead transcription factors (FOXO) are some of the targets of Akt and they are proapoptotic (Datta et al., 1999).

When growth factor receptors are stimulated by their ligands, first activation of $PI_3K$ occurs, leading to activation of downstream kinases including Akt. Growth factor-

activated Akt, phosphorylates and thereby regulates proteins that function to maintain the basic needs of the cell like transportation and oxidation of glucose (Cross et al., 1995; Kohn et al., 1996), or attenuates apoptotic pathways by its substrates Bad and procaspase 9.

Akt causes upregulation of the expression of cFLIP which results in inhibition of apoptosis (Suhara et al., 2001; Panka et al., 2001), and also suppresses the mitochondrial release of death proteins like Smac, Omi or cytochrome c (Wang and Tsang, 2007).

## Growth factors

Insulin like growth factors (IGFs) play an important role in follicular development and the granulosa cell apoptosis. There are two forms of it, namely, IGF 1 and IGF 2. Researches have shown us that mice lacking IGF 1 are sterile and have arrested follicular development at the preantral and early antral stages leading to ovulation failure (Baker et al., 1996; Zhou et al., 1997).

IGF 1 was shown to activate $PI_3K$/Akt pathway by phosphorylation in rat and bovine granulosa cells (Matsuda-Minehata et al., 2006), and thereby prevented apoptosis. Although, IGF 1 has an essential role in ovarian follicular development in many species, and IGF 2 is more abundant in humans (Geisthovel et al., 1989; Zhou and Bondy, 1993; Thierry van Dessel et al., 1996).

IGF is believed to play its role in follicular development by stimulating proliferation, increasing responsiveness to gonadotropins mainly FSH, and by the way increasing estradiol secretion (Monniaux and Pisselet, 1992; Campbell et al., 1995; Glister et al., 2001). It was also shown that follicle-stimulating hormone (FSH) receptor expression was reduced in preantral IGF 1 null follicles and mostly restored to wild type levels after two weeks of exogeneous IGF 1 supplementation. FSH receptor expression and aromatase activity found to be decreased in IGF deficient mice, and IGF 1 administration restored normal FSH expression (Zhou et al., 1997).

Progression through the cell cycle is necessary for IGF to prevent apoptosis (Quirck et al., 2004). IGF, basic fibroblast growth factor (bFGF), and EGF were shown to decrease Fas L induced apoptosis of cultured bovine granulosa cells (Quirk et al., 2000), and this protective effect of IGF 1 is mediated through the $PI_3K$/Akt pathway (Hu et al., 2004). When the granulosa cells were treated with $PI_3K$ inhibitor LY294002, the protective effect of IGF 1 against Fas L induced apoptosis was shown to be blocked (Quirck et al., 2004).

IGF is found to be bound to IGF binding proteins (IGFBP), which have six subgroups, in the body fluids. Although, circulating IGFBPs prolong the half life of IGF, they mostly inhibit its functions (Ui et al., 1989; Bicsak et al., 1990; Adashi et al., 1992). IGFBPs were shown to be expressed in the ovary (Adashi et al., 1985; Giudice, 1992). IGFBP 4 and IGFBP 5 are produced by rat granulosa cells and FSH treatment decreases their secretion (Erickson et al., 1992a, b; Adashi et al., 1990). Dominant and subordinate follicles differ in their IGFBP contents (Monget et al., 1996). Their presence is controlled at the level of synthesis (Armstrong et al., 1998) by the gonadotropins and by the presence of proteases which breakdown low molecular weight IGFBP in the healthy antral follicles of cows (Rivera and Fortune, 2001, 2003a, b), humans (Conover et al., 2001), and mice (Conover et al., 2002).

Growth differentiation factor 9 (GDF 9) is another growth factor found to be important in granulosa cell apoptosis. It exerts its function by activating the $IP_3K$/Akt pathway. It was observed that when the intracellular GDF 9 decreased by intraoocyte injection of its inhibitor GDF 9 antisense morpholino, caspase 3 activation increased, leading to granulosa cell apoptosis (Craig et al., 2007).

EGF, TGFα, and bFGF, as well as their receptors, have been found in the ovary (Hsu and Hammond, 1987; Khan-Dawood, 1987; Kudlow et al., 1987) and shown to inhibit spontaneous onset of apoptotic DNA cleavage in cultured granulosa cells (Tilly et al., 1992a, b). EGF suppression of granulosa cell apoptosis is mediated by the stimulation of progesterone production and the regulation of intracellular free calcium concentration (Luciano et al., 1994).

## Gonadotropins

Follicular development of primordial to secondary follicles does not need gonadotropin support. FSH is required for the follicular growth from the time of postantrum formation till ovulation (Craig et al., 2007). Decrease of circulating gonadotropins by hypophysectomy or blockage of LH/FSH surge were shown to cause massive atresia of preovulatory follicles on the day of proestrus (Ingram, 1953; Braw and Tsafriri, 1980). FSH treatment of hypophysectomized immature rats, decreased granulosa cell apoptosis *in vivo* (Billig et al., 1994). Prevention of apoptotic cell death in early antral and preovulatory follicles by FSH or hCG treatment was also shown in cultured follicles (Chun et al., 1994; Eisenhauer et al., 1995a, b).

Gonadotropins can induce the expression of survival molecules like Bcl-2, FLIP, and XIAP (Kim and Tilly, 2004; Hsu and Hsueh, 2000; Perez et al., 1999; Kugu et al., 1998; Hsu et al., 1996; Craig et al., 2007; Krysko et al., 2008). In mid- to late-follicular stages, FSH increases XIAP expression and activates $PI_3K$/Akt pathway, leading to suppression of the release of mitochondrial death proteins (Wang et al., 2003). In mid- to late-follicular stages when the FSH levels decrease, colocalization of Nodal and its receptor ALK 7 in granulosa cells takes place, and triggering of the downsteram events including Smad 2 activation, Akt inhibition, and XIAP downregulation take place (Wang and Tsang, 2007).

A preovulatory follicle undergoes the final stage of maturation if it is stimulated by the luteinizing hormone

(LH) surge. Studies have shown that granulosa cells of rodents and primates withdraw from the cell cycle after the LH surge (Robker and Richards, 1998a, b; Chaffin et al., 2001), and become resistant to apoptosis (Quirck et al., 2004; Porter et al., 2000).

Progesterone receptor (PR) is a potential mediator of the changes in granulosa cell proliferation and survival induced by the LH surge. Before the LH surge, PR expression is very low in preovulatory follicles, but begins to increase in the granulosa cells after the LH surge in cows (Cassar et al., 2002; Jo et al., 2002), and in primates (Chaffin et al., 1999). When PR antagonist RU 486 is used, to test the antiapoptotic effect of PR, it was observed that granulosa cells reentered the cell cycle, and regained susceptibility for the Fas L induced apoptosis (Quirck et al., 2004).

Gonadotropin withdrawal may also cause increased p53 protein expression which is an antiproliferative transcription factor that regulates the expression of several genes involved in mitosis and apoptosis. Overexpression of p53 results in granulosa cell apoptosis (Wang et al., 2006; Kim et al., 1999; Hughes and Gorospe, 1991; Wang et al., 2002; Mussche and D'Herde, 2001; Orisaka et al., 2006; Zwain and Amato, 2001).

## Estradiol

Estradiol is an important intraovarian growth, differentiation, and survival factor (Rosenfeld et al., 2001). It stimulates the proliferation of granulosa cells and prevents apoptosis (Quirck et al., 2004). Treatment of immature hypophysectomized rats with diethylstilbestrol (DES) caused stimulation of development of large numbers of healthy, multilayered preantral follicles (Richards, 1980). Subsequent removal of DES resulted in apoptosis in the granulosa cell layer of antral and preantral follicles. No increase in DNA breakdown was detected in the primordial or primary follicles (Billig et al., 1993).

Estradiol enhances the ability of FSH to induce expression of LH receptors by which follicular growth and differentiation was shown to be promoted in cattle (Oktem and Oktay, 2008). Estradiol was also shown to increase IGF 1 in porcine granulosa cells (Hsu CJ, Hammond, 1987). But in the bovine granulosa cells, it was shown that treatment with anti IGF receptor antibody which effectively blocked the protective effect of IGF 1 against apoptosis, did not prevent the protective effect of estradiol (Quirck et al., 2004). In the research, estradiol treatment was found to be effective in preventing Fas L induced apoptosis, and this protective effect occurs if only progression through the cell cycle takes place.

Cell cycle progression is mediated by a family of cyclin dependent kinases (cdk) which are activated by binding with specific cyclin proteins. Estradiol also increases expression of cyclin D2 in rat granulosa cells (Robker and Richards, 1998a, b).

## Progesterone

Progesterone was shown to prevent apoptosis in immature rat granulosa cells, although these cells did not contain nuclear progesterone receptor (PR) (McMurray et al., 2000; Tibbetts et al., 1999). Researches have shown us that PR is expressed by granulosa cells just prior to the ovulation (Natraj and Richards, 1993; Park and Mayo, 1991). So, we can assume that it inhibits apoptosis of the granulosa cells isolated during the periovulatory period (Svensson et al., 2000).

Recent evidence demonstrate that progesterone controls apoptosis by maintaining low basal intracellular calcium ion levels via membrane initiated events (Peluso et al., 2001a, b; Peluso, 2003). The exact mechanism of how the progesterone maintains low intracellular calcium ion levels in the granulosa cells is not known but cGMP dependent protein kinase (protein kinase G) plays an important role in other cells like the cardiac muscle cell, smooth muscle cell or endothelial cells. Protein kinase G, stimulates $Ca^{+2}$ influx by closing calcium channels and blocks inositol triphospate ($IP_3$) receptor mediated $Ca^{+2}$ release from cellular stores in these cell types (Carvajal et al., 2000). Prevention of apoptosis by protein kinase G activators and attenuation of antiapoptotic action of progesterone by protein kinase G antagonists are the pharmacological evidence for us to assume that progesterone exerts its antiapoptotic effects via protein kinase G pathway (Peluso, 2003; Hubbard and Greenwald, 1981; Hubbard, 1980).

## Calcium

Calcium is a signalling agent involved in cell growth and differentiation. Transient intracellular $Ca^{+2}$ rise was shown to induce apoptosis in quail granulosa explants (D'Herde and Leybaert, 1997, 1998). Bcl-2 was demonstrated to suppress apoptosis by a mechanism that is linked to intracellular $Ca^{+2}$ to compartmantalization (McConkey, 1996). Abnormal $Ca^{+2}$ elevations cause fragmentation of the DNA and other indicators of apoptosis due to the loss of the balance between anti- and proapoptotic proteins (Fissore et al., 2002; Gordo et al., 2002). DNA fragmentation promotes cytochrome c release from the respiratory chain into the cytosol and caspase cascade becomes activated (Kluck et al., 1997; Vander Heiden and Thompson, 1999).

## CONCLUSION

There are many researches ongoing to elucidate the exact mechanism of granulosa cell apoptosis *in vivo* and factors that cause it. Solving this problem is crucial for us to prevent premature ovarian failure, and to increase the success rates in *in vitro* fertilization trials. The mediators

which are found to be increased in the atretic granulosa cells, like Fas, Nodal, and activated procaspase 3 may be targeted by the new researches; and the inhibiton of these in the selected patient population of suffering poor ovarian capacity may lead to more follicular development, enabling us to obtain more oocytes in the *in vitro* fertilization programmes. The antiapoptotic factors like XlAP, Akt protein, IGF 1 and 2, and cFLIP can also be used for prevention of apoptosis in the same patient group. On the other hand, germ cell tumorigenesis which is increased by Bcl-2 overexpression, can be inhibited by producing inhibitors of Bcl-2, or recombinant proapoptotic molecules.

**REFERENCES**


Adashi EY, Resnick CE, D'Ercole AJ, Svoboda ME, Van Wyk JJ (1985). Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. Endocr. Rev., 6: 400-420.

Adashi EY, Resnick CE, Hernandez ER, Hurwitz A, Rosenfeld RG (1990). Follicle-stimulating hormone inhibits the constitutive release of insulin-like growth factor binding proteins by cultured rat ovarian granulosa cells. Endoc., 126: 1305-1307.

Adashi EY, Resnick CE, Ricciarelli E et al. (1992). Granulosa cell-derived insulin-like growth factor (IGF) binding proteins. J. Clin. Invest., 90: 1593-1599.

Armstrong DG, Baxter G, Gutierrez CG, Hogg CO, Glazyrin AL, Campbell BK, Bramley TA, Webb R (1998). Insulin-like growth factor binding protein-2 and -4 messenger ribonucleic acid expression in bovine ovarian follicles: Effect of gonadotropins and developmental status. Endoc., 139: 2146-2154.

Ashkenazi A, Dixit VM (1998). Death receptors: signalling and modulation. Science, 281: 1305-1308.

Asselin E, Wang Y, Tsang BK (2001). X-linked inhibitor of apoptosis protein activates the phosphatidylinositol 3-kinase/Akt pathway in rat granulosa cells during follicular development. Endocrinology, 142: 2451-2457.

Baker J, Hardy MP, Zhou J, Bondy C, Lupu F, Bellve AR et al. (1996). Effects of an Igf1 gene null mutation on mouse reproduction. Mol. Endocrinol., 10: 903-918.

Bicsak TA, Shimonaka M, Malkowski M, Ling N (1990). Insulin-like growth factor binding protein inhibition of granulosa cell function: effect on cAMP, DNA synthesis and comparison with the effect of an IGF-I antibody. Endoc, 126: 2184-2189.

Billig H, Furuta I, Hsueh AJW (1993). Estrogens inhibit and androgens enhance ovarian granulosa cell apoptosis. Endoc., 133: 2204-2212.

Billig H, Furuta I, Hsueh AJW (1994). Gonadotropin releasing hormone (GnRH) directly induces apoptotic cell death in the rat ovary: biochemical and in situ detection of DNA fragmentation in granulosa cells. Endoc., 134: 245-252.

Boldin MP, Mett IL, Varfolomeev EE, Chumakov I, Shemer-Avni Y, Camonis JH, Wallach D (1995). Self-association of the ''death domains'' of the p55 tumor necrosis factor (TNF) receptor and Fas /APO1 prompts signaling for TNF and Fas /APO1 effects. J. Biol. Chem., 270: 387-391.

Braw RH, Tsafriri A (1980). Follicles explanted from pentobarbitone-treated rats provide a model for atresia. J. Reprod. Fertil., 59: 259-265.

Brennan J, Norris DP, Robertson EJ (2002). Nodal activity in the node governs left–right asymmetry. Genes Dev., 16: 2339-2344.

Campbell BK, Scaramuzzi RJ, Webb R (1995). Control of antral follicle development and selection in sheep and cattle. J. Reprod. Fertil., 49: 335-350.

Carvajal JA, Germain AM, Huidobro-Toro JP, Weiner CP (2000). Molecular mechanism of cGMP-mediated smooth muscle relaxation. J. Cell. Physiol., 184: 409-420.

Cassar CA, Dow MPD, Pursley JR, Smith GW (2002). Effect of the preovulatory LH surge on bovine follicular progesterone receptor mRNA expression. Domest. Anim. Endoc., 22: 179-187.

Chaffin CL, Schwinof KM, Stouffer RL (2001). Gonadotropin and steroid control of granulosa cell proliferation during the periovulatory interval in Rhesus monkeys. Biol. Reprod., 65: 755-762.

Chaffin CL, Stouffer RL, Duffy DM (1999). Gonadotropin and steroid regulation of steroid receptor and aryl hydrocarbon receptor messenger ribonucleic acid in macaque granulosa cells during the periovulatory interval. Endoc., 140: 4753-4760.

Cheng Y, Maeda A, Goto Y, Matsuda-Minehata F, Manabe N (2007). Molecular cloning of porcine (Sus scrofa) tumor necrosis factor receptor 2. J. Reprod. Dev., 53: 1291-1297.

Chinnaiyan AM, O'Rourke K, Tewari M, Dixit VM (1995). FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. Cell, 81: 505-512.

Chun SY, Billig H, Tilly JL, Furuta I, Tsafriri A, Hsueh AJW (1994). Gonadotropin suppression of apoptosis in cultured preovulatory follicles: mediatory role of endogenous IGF-I. Endoc., 135: 1845-1853.

Conover CA, Faessen GF, Ilg KE, Chandrasekher YA, Christiansen M, Overgaard MT, Oxvig C, Giudice LC (2001). Pregnancy-associated plasma protein-A is the insulin-like growth factor binding protein-4 protease secreted by human ovarian granulosa cells and is a marker of dominant follicle selection and the corpus luteum. Endoc., 142: 2155-2158.

Craig J, Orisaka M, Wang H, Orisaka S, Thompson W, Zhu C et al. (2007). Gonadotropin and intra-ovarian signals regulating follicle development and atresia: the delicate balance between life and death. Front. Biosci., 12: 3628-3639.

Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA (1995). Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature, 378: 785-789.

D'Herde K, Leybaert L (1997). Intracellular free calcium related to apoptotic cell death in quail granulosa cell sheets kept in serum -free culture. Cell. Death. Differ., 4: 59-65.

D'Herde K, Leybaert L (1998). Apoptotic granulosa cells have moderately increased intracellular free calcium during phosphatidylserine exposure and a normal resting calcium level during DNA fragmentation. Apoptosis, 3: 337-343.

Datta SR, Brunet A, Greenberg ME (1999). Cellular survival: a play in three Akts. Genes Dev., 13: 2905-2927.

Depalo R, Nappi L, Loverro G, Bettocchi S, Caruso ML, Valentini AM et al (2003). Evidence of apoptosis in human primordial and primary follicles. Hum. Reprod., 18: 2678-2682.

Deveraux QL, Roy N, Stennicke HR, Van Arsdale T, Zhou Q, Srinivasula SM, Alnemri ES, Salvesen GS, Reed JC (1998). IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. EMBO J., 17: 2215-2223.

Eimon PM, Harland RM (2002). Effects of heterodimerization and proteolytic processing on Derriere and Nodal activity: implications for mesoderm induction in Xenopus. Development, 129: 3089-3103.

Eisenhauer K, Chun SY, Billig H, Hsueh AJW (1995a). Growth hormone suppression of apoptosis in preovulatory follicles and partial neutralization by insulin-like growth factor binding protein (IGFBP). Biol. Reprod., 53: 13-20.

Eisenhauer K, Chun SY, Minami S, Hsueh AJW (1995b). Hormonal regulation of apoptosis is dependent on the stage of differeantiation: FSH, LH and IGF-I as survival factors for early antral follicles. Biol. Reprod. 52 (Suppl. 1): 369.

Erickson GF, Nakatani A, Ling N, Shimasaki S (1992a). Localization of insulin-like growth factor-binding protein-5 messenger ribonucleic acid in rat ovaries during the estrous cycle. Endoc., 130: 1867-1878.

Erickson GF, Nakatani A, Ling N, Shimasaki S (1992b). Cyclic changes in insulin-like growth factor-binding protein-4 messenger ribonucleic acid in the rat ovary. Endoc., 130: 625-636.

Fissore RA, Kurokawa M, Knott J, Zhang M, Smyth J (2002). Mechanisms underlying oocyte activation and postovulatory ageing. Reprod., 124: 745-754.

Geisthovel F, Moretti-Rojas I, Asch RH, Rojas FJ (1989). Expression of insulin-like growth factor-II (IGF-II) messenger ribonucleic acid (mRNA), but not IGF-I mRNA, in human preovulatory granulosa cells. Hum. Reprod., 4: 899-902.

Giudice L (1992). Insulin-like growth factors and ovarian follicular development. Endocr. Rev., 13: 641-669.

Glister C, Tannetta DS, Groome NP, Knight PG (2001). Interactions between follicle-timulating hormone and growth factors in modulating secretion of steroids and inhibin-related peptides by nonluteinized bovine granulosa cells. Biol. Reprod., 65: 1020-1028.
Goltsev YV, Kovalenko AV, Arnold E, Varfolomeev EE, Brodianskii VM, Wallach D (1997). CASH, a novel caspase homologue with death effector domains. J. Biol. Chem., 272: 19641-19644.
Gordo AC, Rodrigues P, Kurokawa M, Jellerette T, Exley GE, Warner C, Fissore R (2002). Intracellular calcium oscillations signal apoptosis rather than activation in in vitro aged mouse eggs. Biol. Reprod., 66: 1828-1837.
Goto Y, Matsuda-Minehata F, Inoue N, Matsui T, Maeda A, Manabe N (2004). Porcine (Sus scrofa) cellular FLICE-like inhibitory protein (cFLIP): molecular cloning and comparison with the human and murine cFLIP. J. Reprod. Fertil., 50: 549-555.
Gougeon A (1996). Regulation of ovarian follicular development in primates: facts and hypotheses. Endocr. Rev., 17: 121-155.
Han DK, Chaudhary PM, Wright ME, Friedman C, Trask BJ, Riedel RT, Baskin DG, Schwartz SM, Hood L. (1997). MRIT, a novel death effector domain-containing protein, interacts with caspases and BclXL and initiates cell death. Proc. Notl. Acad. Sci. USA, 94: 11333-11338.
Hsu CJ, Hammond JM (1987). Gonadotropins and estradiol stimulate immunoreactive insulin-like growth factor-I production by porcine granulosa cells in vitro. Endoc., 120: 198-207.
Hsu SY, Hsueh AJ (2000). Tissue-specific Bcl-2 protein partners in apoptosis: an ovarian paradigm. Physiol. Rev., 80: 593-614
Hsu SY, Lai RJ, Finegold M, Hsueh AJ (1996). Targeted overexpression of Bcl-2 in ovaries of transgenic mice leads to decreased follicle apoptosis, enhanced folliculogenesis, and increased germ cell tumorigenesis. Endocrinology, 137: 4837-4843.
Hsueh AJ, Billig H, Tsafriri A (1994). Ovarian follicle atresia: a hormonally controlled apoptotic process. Endocr. Rev., 15: 707–724.
Hu C-L, Cowan RG, Harman RM, Quirk SM (2004). Cell cycle progression and activation of Akt kinase are required for IGF-1-mediated suppression of apoptosis in granulosa cells. Mol. Endoc., 18: 326-338.
Hu S, Vincenz C, Ni J, Gentz R, Dixit VM (1997). I-FLICE, a novel inhibitor of tumor necrosis factor receptor 1 and CD 95 induced apoptosis. J. Biol. Chem., 272: 17255-17257.
Hubbard CJ (1980). Ovarian cAMP and cGMP fluctuations in the hamster during the oestrous cycle. J. Reprod. Fertil., 59: 351-355.
Hubbard CJ, Greenwald GS (1981). Changes in DNA, cyclic nucleotides and steroids during induced follicular atresia in the hamster. J. Reprod. Fertil., 63: 455-461.
Hughes FM Jr, Gorospe WC (1991). Biochemical identification of apoptosis (programmed cell death) in granulosa cells: evidence for a
potential mechanism underlying follicular atresia. Endoc., 129: 2415-2422.
Iannaccone PM, Zhou X, Khokha M, Boucher D, Kuehn MR (1992). Insertional mutation of a gene involved in growth regulation of the early mouse embryo. Dev. Dyn., 194: 198-208.
Ingram DL (1953). The effect of hypophysectomy on the number of oocytes in the adult albino rat. J. Endoc., 9: 307-311.
Inohara N, Koseki T, Hu Y, Chen S, Nunez G (1997). CLARP, a death effector domain-containing protein interacts with caspase 8 and regulates apoptosis. Proc. Natl. Acad. Sci. USA, 94: 10717-10722.
Inoue N, Maeda A, Matsuda-Minehata F, Fukuta K, Manabe N (2006). Expression and localization of Fas ligand and Fas during atresia in porcine ovarian follicles. J. Reprod. Dev., 52: 723-730.
Irmler M, Thome M, Hahne M, Schneider P, Hofmann K, Steiner V, Bodmer JL, Schroter M, Burns K, Mattmann C, Rimoldi D, French LE, Tschopp J (1997). Inhibition of death receptor signals by cellular FLIP. Nature, 388: 190-195.
Jiang JY, Cheung CK, Wang Y, Tsang BK (2003). Regulation of cell death and cell survival gene expression during ovarian follicular development and atresia. Front. Biosci., 8: 222-237.
Jo M, Komar CM, Fortune JE (2002). Gonadotropin surge induces two separate increases in messenger RNA for progesterone receptor in bovine preovulatory follicles. Biol. Reprod., 67: 1981-1988.
Johnson AL, Bridgham JT (2002). Caspase-mediated apoptosis in the vertebrate ovary. Reproduction, 124: 19-27.
Khan-Dawood FS (1987). Human corpus luteum: immunocytochemical localization of epidermal growth factor. Fertil Steril., 47: 916-919.
Kim JM, Yoon YD, Tsang BK (1999). Involvement of the Fas/Fas ligand system in p53-mediated granulosa cell apoptosis during follicular development and atresia. Endoc., 140: 2307-2317.
Kim MR, Tilly JL (2004). Current concepts in Bcl-2 family member regulation of female germ cell development and survival. Biochim. Biophys. Acta, 1644: 205–210.
Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD (1997). The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. Science, 275: 1132-1136.
Kohn AD, Summers SA, Birnbaum MJ, Roth RA (1996). Expression of a constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. J. Biol. Chem., 271: 31372-31378.
Kondo H, Maruo T, Peng X, Mochizuki M (1996). Immunological evidence for the expression of the Fas antigen in the infant and adult human ovary during follicular regression and atresia. J. Clin. Endocrinol. Metab., 81: 2702-2710.
Krysko DV, Diez-Fraile A, Criel G, Svistunov AA, Vandenabeele P, D'Herde K (2008). Life and death of female gametes during oogenesis and folliculogenesis. Apoptosis,13: 1065-1087.
Kudlow JE, Kobrin MS, Purchio AF et al. (1987). Ovarian transforming growth factor-alpha gene expression: immunohistochemical localization to the theca-interstitial cells. Endoc., 121: 1577-1579.
Kugu K, Ratts VS, Piquette GN, Tilly KI, Tao XJ, Martimbeau S et al. (1998). Analysis of apoptosis and expression of bcl-2 gene family members in the human and baboon ovary. Cell Death Differ., 5: 67-76.
Luciano AM, Pappalardo A, Ray C, Peluso JJ (1994). Epidermal growth factor inhibits large granulosa cell apoptosis by stimulating progesterone synthesis and regulating the distribution of intracellular free calcium. Biol. Reprod., 51: 646-654.
Manabe N, Inoue N, Miyano T, Sakamaki K, Sugimoto M, Myamoto H (2003). Ovarian follicle selection in mammalian ovaries: regulatory mechanisms of granulosa cell apoptosis during follicular atresia. The Ovary, 2: 369-385.
Manabe N, Matsuda-Minehata F, Goto Y, Maeda A, Cheng Y, Nakagawa S, Inoue N, Wongpanit K, Jin H, Gonda H, Li J (2008). Role of cell death ligand and receptor system on regulation of follicular atresia in pig ovaries. Reprod. Dom. Anim., 43(Suppl. 2): 268-272.
Massague J (1998). TGF-beta signal transduction. Ann. Rev. Biochem., 67: 753-791.
Matikainen T, Perez GI, Zheng TS, Kluzak TR, Rueda BR, Flavell RA et al. (2001). Caspase-3 gene knockout defines cell lineage specificity for programmed cell death signaling in the ovary. Endocrinology, 142: 2468-2480.
Matova N, Cooley L (2001). Comparative aspects of animal oogenesis. Dev. Biol., 231: 291-320.
Matsuda-Minehata F, Goto Y, Inoue N, Manabe N (2005).Changes in expression of anti-apoptotic protein, cFLIP, in granulosa cell during follicular atresia in porcine ovaries. Mol. Reprod. Dev., 72: 145-151.
Matsuda-Minehata F, Goto Y, Inoue N, Sakamaki K, P Chedrese PJ, Manabe N (2007). Anti-apoptotic activity of porcine cFLIP in ovarian granulosa cell lines. Mol. Reprod. Dev., 74: 1165-1170.
Matsuda-Minehata F, Inoue N, Goto Y, Manabe N (2006). The regulation of ovarian granulosa cell death by pro- and antiapoptotic molecules. J. Reprod. Dev., 52: 695-705.
Matsuda-Minehata F, Inoue N, Goto Y, Manabe N (2006). The regulation of ovarian granulosa cell death by pro- and anti-apoptotic molecules. J. Reprod. Dev., 52: 695-705.
Matsui T, Manabe N, Goto Y, Inoue N, Nishihara S, Miyamoto H(2003). Expression and activity of Apaf 1 and caspase-9 in granulosa cells during follicular atresia in pig ovaries. Reproduction, 126: 113-120.
McConkey DJ (1996). The role of calcium in the regulation of apoptosis. Scanning. Microsc., 10: 777-793.; discussion 793-774.
Medema JP, Scaffidi C, Kischkel FC, Shevchenko A, Mann M, Krammer PH, Peter ME (1997). FLICE is activated by association with the CD95 death-inducing signaling complex(DISC). EMBO J., 16: 2794-2804.
Monget, Besnard PN, Huet C, Pisselet C, Monniaux D (1996). Insulin-like growth factor-binding proteins and ovarian folliculogenesis. Horm.

Res., 45: 211-217.
Monniaux D, Pisselet C (1992). Control of proliferation and differentiation of ovine granulosa cells by insulin-like growth factor-I and follicle-stimulating hormone in vitro. Biol. Reprod., 46: 109-119.
Morita Y, Perez GI, Maravei DV, Tilly KI, Tilly JL (1999). Targeted expression of Bcl-2 in mouse oocytes inhibits ovarian follicle atresia and prevents spontaneous and chemotherapy induced oocyte apoptosis in vitro. Mol. Endocrinol., 13: 841-850.
Morita Y, Tilly JL (1999). Oocyte apoptosis: like sand through an hourglass. Dev. Biol., 213: 1-17.
Mussche S, D'Herde K (2001). Contribution of progesterone, follicle stimulating hormone and glucocorticoids in survival of serum-free cultured granulosa cell explants. J. Endocr., 169: 321-331.
Muzio M, Chinnaiyan AM, Kischkel FC, O'Rourke K, Shevchenks A, Ni J, Scaffidi C, Bretz JD, Zhang M, Gentz R, Mann M, Krammer PH, Peter ME, Dixit VM (1996). FLICE, a novel FADD-homologous ICE/CED 3-like protease is recruited to the CD 95 (Fas/ Apo-1) death inducing signaling complex. Cell, 85: 817-827.
Nagata S (1997). Apoptosis by death factor. Cell, 88: 355-365.
Natraj U, Richards JS (1993). Hormonal regulation, localization, and functional activity of the progesterone receptor in granulosa cells of rat preovulatory follicles. Endoc., 133: 761-769.
Oktem O, Oktay K (2008).The ovary: anatomy and function througout human life. N.Y. Acad. Sci., 1127: 1-9.
Orisaka M, Orisaka S, Jiang JY, Craig J, Wang Y, Kotsuji F et al. (2006). Growth differentiation factor 9 is antiapoptotic during follicular development from preantral to early antral stage. Mol. Endocrinol., 20: 2456-2468.
Panka DJ, Mano T, Suhara T, Walsh K, Mier JW (2001). Phosphatidiyl inositol 3- kinase /Akt activity regulates c-FLIP expression in tumor cells. J. Biol. Chem., 276: 6893-6896.
Park KE, Mayo (1991). Transient expression of progesterone receptor messenger RNA in ovarian granulosa cells after the preovulatory luteinizing hormone surge. Mol. Endoc., 5: 967-978.
Park SM, Schickel R, Peter ME (2005). Nonapoptotic functions of FADD-binding death receptors and their signaling molecules. Curr. Opin. Cell. Biol., 17: 610-616.
Peluso JJ (2003). Progesterone as a regulator of granulosa cell viability. J. Steroid. Biochem. Mol. Biol., 85: 167-173.
Peluso JJ, Fernandez G, Pappalardo A, White BA (2001). Characterization of a putative membrane receptor for progesterone in rat granulosa cells. Biol. Reprod., 65: 94-101.
Peluso JJ, Fernandez G, Pappalardo A, White BA (2002). Membrane-initiated events account for progesterone's ability to regulate intracellular free calcium and inhibit rat granulosa cell mitosis. Biol. Reprod., 67: 379-385.
Perez GI, Robles R, Knudson CM, Flaws JA, Korsmeyer SJ, Tilly JL (1999). Prolongation of ovarian lifespan into advanced chronological age by Bax-deficiency. Nat. Genet., 21: 200-203.
Porter DA, Vickers SL, Cowan RG, Huber SC, Quirk SM (2000). Expression and function of Fas antigen vary in bovine granulosa and theca cells during ovarian follicular development and atresia. Biol. Reprod., 62: 62-66.
Quirck SM, Cowan RG, Harman RM, Hu C-L, Porter DA (2004). Ovarian follicular growth and atresia: The relationship between cell proliferation and survival. J. Anim. Sci., 82: E40-52.
Quirk SM, Cowan RG, Joshi SG, Henrikson KP (1995). Fas antigen-mediated apoptosis in human granulosa/luteal cells. Biol. Reprod., 52: 279-287.
Quirk SM, Harman RM, Cowan RG (2000). Regulation of Fas antigen (Fas, CD95)-mediated apoptosis of bovine granulosa cells by serum and growth factors. Biol. Reprod., 63: 1278-1284.
Ratts VS, Flaws JA, Kolp R, Sorenson CM, Tilly JL (1995). Ablation of bcl-2 gene expression decreases the numbers of oocytes and primordial follicles established in the post-natal female mouse gonad. Endocrinology, 136: 3665-3668.
Richards JS (1980). Maturation of ovarian follicles: Actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. Physiol. Rev., 60: 51-89.
Rivera GM, Fortune JE (2001). Development of codominant follicles in cattle is associated with a follicle-stimulating hormone- dependent insulin-like growth factor binding protein-4 protease. Biol. Reprod., 65: 112-118.
Rivera GM, Fortune JE (2003a). Proteolysis of insulin-like growth factor binding proteins-4 and -5 in bovine follicular fluid: implications for ovarian follicular selection and dominance. Endoc., 144: 2977-2987.
Rivera GM, Fortune JE (2003b). Selection of the dominant follicle and insulin-like growth factor (IGF)-binding proteins: evidence that pregnancy-associated plasma protein A contributes to proteolysis of IGF-binding protein 5 in bovine follicular fluid. Endoc., 144: 437-446.
Robker RL, Richards JS (1998a). Hormonal control of the cell cycle in ovarian cells: proliferation versus differentiation. Biol. Reprod., 59: 476-482.
Robker RL, Richards JS (1998b). Hormone-induced proliferation and differentiation of granulosa cells: a coordinated balance of the cell cycle regulators cyclinD2 and p27KIP1. Mol. Endocrinol., 12: 924-940.
Rosenfeld CS, Wagner JS, Roberts RM, Lubahn DB (2001). Intra-ovarian actions of oestrogen. Reprod. 122: 215-226.
McMurray RW, JG Wilson, L.Bigler, L.Xiang, A. Lagoo (2000). Progesterone inhibits glucocorticoid-induced murine thymocyte Apoptosis. Int. J. Immunopharmacol., 22: 955-965.
Scaffidi C, Fulda S, Srinivasan A, Friesen C, Li F, Tomaselli KJ, Debatin KM, Krammer PH, Peter ME (1998). Two CD 95 (Apo-1/ Fas) signaling pathways. EMBO J., 17: 1675-1687.
Schwartzman RA, Cidlowski JA (1993). Apoptosis: The biochemistry and molecular biology of programmed cell death. Endocr. Rev., 14: 133-151.
Shu HB, Halpin DR, Goeddel DV (1997). Casper is a FADD- and caspase- related inducer of apoptosis. Immunity, 6: 751-763.
Srinivasula SM, Ahmad M, Ottilie S, Bullrich F, Banks S, Wang Y, Fernandes-Alnemri T, Croce CM, Litwack G, Tomaselli KJ, Armstrong RC, Alnemri ES (1997) FLAME-1, a novel FADD-like antiapoptotic molecule that regulate Fas/ TNFR1-induced apoptosis. J. Biol. Chem., 272: 18542-18545.
Suhara T, Mano T, Oliveira BE, Walsh K (2001). Phosphatidyl inositol 3-kinase /Akt signaling controls endothelial cellsensitivity to Fas-mediated apoptosis via regulation of FLICE-inhibitory protein (FLIP). Circ. Res., 89: 13-19.
Svensson EC, Markström E, Andersson M, Billig H (2000). Progesterone receptor-mediated inhibition of apoptosis in granulosa cells isolated from rats treated with human chorionic gonadotropin. Biol. Reprod., 63: 1457-1464.
Thierry van Dessel HJ, Chandrasekher Y, Yap OW, Lee PD, Hintz RL, Faessen GH et al. (1996). Serum and follicular fluid levels of insulin-like growth factor I (IGF-I), IGF-II, and IGF binding protein-1 and -3 during the normal menstrual cycle. J. Clin. Endocrinol. Metab., 81: 1224-1231.
Thome M, Schneider P, Hofmann K, Fickenscher H, Meinl E, Neipel F, Mattmann C, Burns K, Bodmer JL, Schroter M, Scaffidi C, Krammer
PH, Peter ME, Tschopp J (1997). Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors. Nature, 386: 517-521.
Thome M, Tschopp J (2001). Regulation of lymphocyte proliferation and death by FLIP. Nat. Rev. Immunol., 1: 50-58.
Tibbetts TA, DeMayo F, Rich S, Conneely OM, O'Malley BW (1999). Progesterone receptors in the thymus are required for thymic involution during pregnancy and for normal fertility. Proc. Natl. Acad. Sci. USA, 96: 12021-12026.
Tilly JL, Billig H, Kowalski KI, Hsueh AJW (1992b). 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. Mol. Endocrinol., 6: 1942-1950.
Tilly JL, Kowalski KI, Schomberg DW, Hsueh AJW (1992a). Apoptosis in atretic ovarian follicles is associated with selective decreases in messenger RNA transcripts for gonadotropin receptors and cytochrome P450 aromatase. Endoc. ,131: 1670-1676.
Ui M, Shimonaka M, Shimasaki S, Ling N (1989). An insulin-like growth factor-binding protein in ovarian follicular fluid blocks follicle-stimulating hormone-stimulated steroid production by ovarian granulosa cells. Endoc., 125: 912-916.
Vander Heiden MG, Thompson CB (1999). Bcl-2 proteins: regulators of apoptosis or of mitochondrial homeostasis? Nat. Cell. Biol., 1: E209-E216.
Wada S, Manabe N, Nakayama M, Inou N, Matsui T, Miyamoto H (2002).

TRAIL-decoy receptor 1 plays inhibitory role in apoptosis of granulosa cells from pig ovarian follicles. J. Vet. Med. Sci., 64: 435-439.
Wallach D, Varfolomeev EE, Malinin NL, Goltsev YV, Kovalenko AV, Boldin MP (1999). Tumor necrosis factor receptor and Fas signalling mechanisms. Annu. Rev. Immunol., 17: 331-367.
Wang H, Jiang JY, Zhu C, Peng C, Tsang BK (2006). Role and regulation of nodal/activin receptor-like kinase 7 signaling pathway in the control of ovarian follicular atresia. Mol. Endocr., 20: 2469-2482.
Wang H, Tsang BJ (2007). Nodal signalling and apoptosis. Reproduction, 133: 847-853.
Wang Y, Asselin E, Tsang BK (2002). Involvement of transforming growth factor alpha in the regulation of rat ovarian X linked inhibitor of apoptosis protein expression and follicular growth by follicle-stimulating hormone. Biol. Reprod., 66: 1672-1680.
Wang Y, Rippstein PU, Tsang BK (2003). Role and gonadotrophic regulation of X-linked inhibitor of apoptosis protein expression during rat ovarian follicular development in vitro. Biol. Reprod., 68: 610-619.
Zhou J, Bondy C (1993). Anatomy of the human ovarian insulin like growth factor system. Biol. Reprod., 48: 467-482.
Zhou J, Kumar TR, Matzuk MM, Bondy C (1997). Insulin-like growth factor I regulates gonadotropin responsiveness in the murine ovary. Mol. Endocrinol., 11: 1924-1933.
Zinkel S, Gross A, Yang E (2006). BCL2 family in DNA damage and cell cycle control. Cell Death Diff. 13: 1351-1359.
Zwain IH, Amato P (2001). cAMP-induced apoptosis in granulosa cells is associated with up-regulation of P53 and bax and downregulation of clusterin. Endocr. Res., 27: 233-249.