

Follicular Growth and Atresia in Mammalian Ovaries: Regulation by Survival and Death of Granulosa Cells

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Abstract. The mammalian ovary is an extremely dynamic organ in which a large majority of follicles are effectively eliminated throughout their reproductive life. Due to the numerous efforts of researchers, mechanisms regulating follicular growth and atresia in mammalian ovaries have been clarified, not only their systemic regulation by hormones (gonadotropins) but also their intraovarian regulation by gonadal steroids, growth factors, cytokines and intracellular proteins. Granulosa cells in particular have been demonstrated to play a major role in deciding the fate of follicles, serving molecules that are essential for follicular growth and maintenance as well as killing themselves by an apoptotic process that results in follicular atresia. In this review, we discuss the factors that govern follicular growth and atresia, with a special focus on their regulation by granulosa cells. First, ovarian folliculogenesis in adult life is outlined. Then, we explain about the regulation of follicular growth and atresia by granulosa cells, in which hormones, growth factors and cytokines, death ligand-receptor system and B cell lymphoma/leukemia 2 (BCL2) family members (mitochondria-mediated apoptosis) are further discussed.

Key words: Apoptosis, Follicular atresia, Follicular growth, Granulosa cell, Mammalian ovary

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Ovarian follicle development is a process regulated by various endocrine, paracrine and autocrine factors that act coordinately to select follicles for ovulation. The vast majority of follicles (more than 99% of all follicles) fail to reach the preovulatory stage, but instead undergo the degenerative process called atresia. After the endocrine mechanisms by a hypothalamo–pituitary–ovarian axis were elucidated, granulosa cells have been the focus of interest in numerous studies that examined the mechanisms of follicular growth and atresia. From *in vivo* and *in vitro* experiments, factors secreted from granulosa cells, including gonadal steroids, growth factors, and cytokines, were shown to be essential for the survival of granulosa cells and their eventual follicular growth. Moreover, granulosa cells were observed as initial cell populations that underwent apoptosis in atretic follicles earlier than oocytes and theca cells, suggesting their role as the initiator of follicular atresia. Factors so far associated with apoptosis expressed in granulosa cells were shown to be crucial for the precise regulation of follicular growth and atresia. Here, we provide an overview of ovarian folliculogenesis in adult life and then discuss the intraovarian factors that govern follicular growth and atresia, with special emphasis on the precise mechanisms of granulosa cell survival and death.

Ovarian Folliculogenesis in Adult Life

In mammals, ovarian folliculogenesis originates from the formation of primordial follicles (oocytes surrounded by a single layer of flattened granulosa cells) that occur before or immediately after birth [1]. After puberty, a number of primordial follicles start growing to ovulate in each estrus cycle throughout the female reproductive life span. Primordial follicles are activated to grow into primary follicles (those with a single layer of cuboidal granulosa cells) and subsequently into secondary follicles (those with stratified granulosa cells without an antrum). Thecal cells begin to emerge and form a layer around the granulosa layers after the formation of secondary follicles [2]. Antral follicles with mature thecal cells and a vascular network within the thecal layer are then formed, and further developed antral follicles are finally ovulated. Thus, a follicle is composed of three distinct compartments: an oocyte and two kinds of somatic cells, granulosa cells and theca cells. A granulosa cell is further classified into a cumulus granulosa and a mural granulosa, and a theca cell is further classified into an internal and external theca. Though atresia can occur at any time during folliculogenesis, the majority of follicles become atretic during the antral stage [3]; most follicles at the antral stage undergo atresia, and few are selected for ovulation.

In many species including domestic animals, preantral follicular development does not strictly require stimulation by the pituitary gonadotropins, while follicles become gonadotropin-dependent at the antral stage [1, 4]. The pituitary gonadotropins, i.e., follicle-

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stimulating hormone (FSH) and luteinizing hormone (LH), whose secretion is stimulated by hypothalamus-derived gonadotropin-releasing hormone (GnRH), provide the primary mechanisms that control follicular selection and dominance via feedback loops with the hypothalamo–pituitary–ovarian axis. FSH is the main hormone controlling follicular growth, resulting in the secretion of estradiol and inhibin from a large dominant follicle(s) [5]. Granulosa cells produce inhibin, while theca cells produce androgens that are used by the granulosa cells to synthesize estradiol-17 β (estradiol). Estradiol and inhibin act on the hypothalamo–pituitary system and decrease FSH secretion (known as ovarian negative feedback) to suppress the further growth of subordinate follicles. By that time, the dominant follicle(s) acquires LH receptors on granulosa cells and transfers dependency to LH for further development, finally resulting in ovulation induced by an LH surge.

Regulation of Follicular Growth and Atresia by Granulosa Cells

Factors secreted from granulosa cells such as estradiol and insulin-like growth factor (IGF) are revealed to be essential for follicular growth/development. If these key survival-promoting factors are depleted, granulosa cells not only lose their appropriate functions but also undergo cell death. The granulosa layer is aligned along the follicular basal lamina, and no apoptotic cells are observed in growing healthy follicles (Fig. 1A). Apoptotic granulosa cells start appearing and gradually increase their numbers in early atretic follicles (Fig. 1B, C). Finally, the majority of granulosa cells undergo apoptosis in progressed atretic follicles, with a severely disrupted granulosa layer (Fig. 1D), and those follicles will then be eliminated. Morphologically, apoptosis is induced in granulosa cells located in the inner surface of the granulosa layer, but not in cumulus cells, oocytes or inner or extra theca layers in the early stages of atresia [6], indicating an initiating role of granulosa cell apoptosis in follicular atresia [7, 8] (Fig. 1B). The deprivation of key survival-promoting factors or stimulation by death ligands is the main cause of apoptosis, both of which contribute to granulosa cell apoptosis [9]. These two apoptotic stimulations induce different intracellular signaling pathways, both of which result in the common features of the apoptosis, i.e., the activation of caspase-3 (CASP3) and subsequent internucleosomal DNA fragmentation. Recent studies have reported many apoptotic-signaling molecules at work in granulosa cells and have revealed that they affect each other during the progression of apoptosis.

In this section, we discuss how hormones/growth factors/cytokines, a death ligand-receptor system and B cell lymphoma/leukemia 2 (BCL2) family members (mitochondria-mediated apoptosis) in granulosa cells regulate follicular growth and atresia. Schematic models of growing granulosa cells (granulosa cells in healthy follicles) and those undergoing apoptosis (granulosa cells in atretic follicles) are shown in Figs. 2 and 3, respectively.

1. Hormones, growth factors and cytokines

Estradiol

Estradiol is not only one of the main systemic regulators of

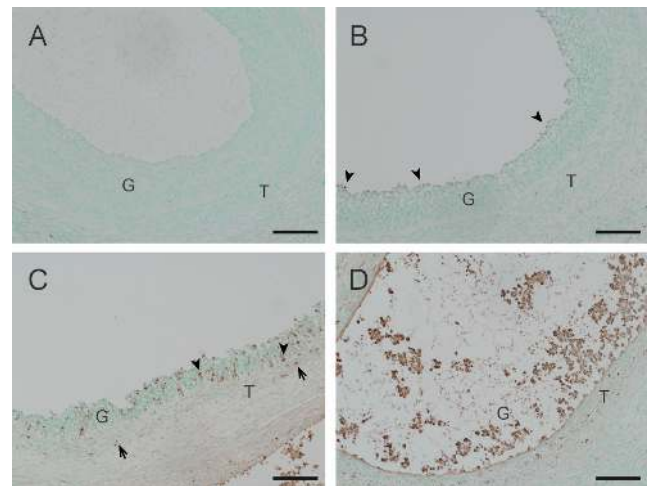


Fig. 1. Apoptosis during follicular atresia in porcine antral follicles. Apoptotic cells were detected by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining. No apoptotic cells were found in healthy follicles (A). With the progression of follicular atresia, apoptotic cells increase and the granulosa layer degenerates (B, C and D). Apoptotic cells are found in the inner granulosa layers of early atretic follicles (arrowheads) (B). Apoptosis extends across the granulosa layer (arrowheads) and is also detected in theca cells (arrows) (C). In progressed atretic follicles, a highly disrupted granulosa layer with strongly stained apoptotic granulosa cells was observed (D). G, granulosa layer; T, theca layer. Original magnification $\times 200$. Scale bar = 100 μm .

ovarian follicles by the hypothalamo–pituitary–ovarian axis but is also essential for the local stimulation of granulosa cell survival. Granulosa cells are the main source of estradiol and also one of the targets of estradiol, as they express estrogen receptors (ER α and ER β) in cattle, sheep, pigs, mice, rats and humans [10–13]. Estradiol has various actions on granulosa cells, such as promoting folliculogenesis, increasing the expression of gonadotropin receptors and inhibiting cell apoptosis [10, 14, 15]. *Cyp19* (aromatase) null mice, which are unable to produce estradiol, were infertile, exhibiting many abnormal antral follicles with uneven granulosa cell layers [16]. Similarly, mice with double knockout of ER α and ER β were found to be infertile, and adult ovaries lacking sufficiently large antral follicles and granulosa cell disruption were observed [17]. ER β is expressed in granulosa cells of growing follicles, whereas ER α is predominantly expressed in theca and interstitial cells in mice [18], suggesting the significance of ER β rather than ER α in granulosa cells. However, ER α may also have redundant effects on granulosa cells, since ER β single knockout mice were subfertile in contrast to ER α single knockout mice, which were infertile [17]. In bovine ovaries, granulosa cells were dominant cells that express ER α and ER β [19], indicating the varied roles of estrogen receptors among species. In any case, estradiol is essential for normal folliculogenesis, whose target is likely to be granulosa cells.

Estradiol implantation prevented granulosa cell apoptosis caused by hypophysectomy in the antral follicles of rat ovaries [20]. In the granulosa cell culture of ovine and porcine large antral follicles, estradiol protected cells from oxidative (H₂O₂) stress-induced

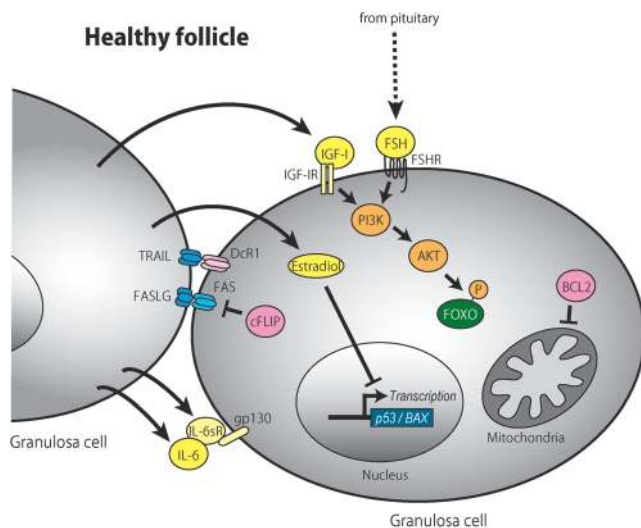


Fig. 2. Schematic model of growing granulosa cells (granulosa cells in healthy follicles). FSH secreted from the pituitary is essential for granulosa cell survival. IGF-I, estradiol, IL-6 and IL-6sR are expressed from granulosa cells, acting as the survival factors of granulosa cells in a paracrine/autocrine manner. IGF-I and FSH activate the intracellular PI3K/AKT pathway and phosphorylate FOXO transcription factors, which retain FOXO within the cytoplasm. Estradiol inhibits the transcription of proapoptotic genes, *p53* and *Bax*. Death ligands-receptors (FASLG-FAS and TRAIL-DcR1) are expressed on the cellular membranes. An intracellular molecule, cFLIP, inhibits the FASLG-FAS death signal. The TRAIL signal is inhibited by its decoy receptor, DcR1. BCL2 inhibits mitochondria-mediated apoptosis.

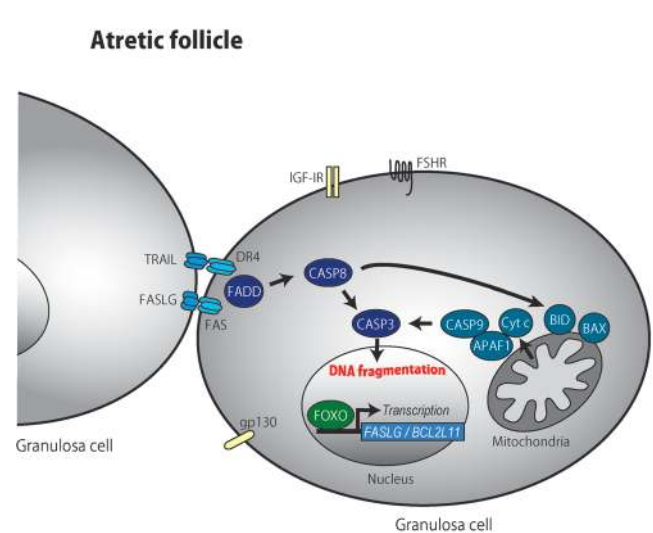


Fig. 3. Schematic model of granulosa cells undergoing apoptosis (granulosa cells in atretic follicles). Cell survival signals such as FSH, IGF, estradiol and IL-6 decline. As a result, FOXO is dephosphorylated and transferred into the nucleus, where it transactivates proapoptotic factors, *FASLG* and *BCL2L1*. Death ligands-receptors (FASLG-FAS and TRAIL-DR4) expressed on the cellular membrane increase. Subsequent activation of intracellular signaling (FADD, CASP8 and CASP3) induces DNA fragmentation. CASP8 also stimulates the mitochondrial apoptotic pathway by inducing BID, BAX, Cyt c release, APAF1 and CASP9.

apoptosis [21, 22]. A disruption of *Cyp19* increased follicular atresia and granulosa cell apoptosis with age, which was suggested to be due to an upregulation of the proapoptotic genes, *p53* and *Bcl2* homologous antagonist/killer (*Bax*), in granulosa cells [16, 23]. Such *in vivo* and *in vitro* studies suggest the inhibitory effect of estradiol on granulosa cell apoptosis and subsequent follicular atresia.

Insulin-like growth factor (IGF)

IGF has been well characterized as the local growth factor system essential for folliculogenesis. Granulosa cells express both IGF-I and its receptor, type-I IGF receptor (IGF-IR), in cattle, sheep, pigs, mice, rats and humans [24–30]. In mice, *Igf1* transcripts in granulosa cells were low during the primary follicular stage and increased to a maximum in the late preantral and early antral stages but were low in apoptotic follicles [27]. Mice lacking IGF-I are infertile, since their follicular development is arrested at the small antral stage, so no mature large antral follicles are produced [31, 32]. The expression of FSH receptor in granulosa cells was deficient in mice lacking IGF-I [32], while IGF-I enhanced FSH-induced aromatase production and LH receptor expression in cultured murine granulosa cells [33]. In cattle, sheep and pigs, the addition of IGF-I also increased the responsiveness to FSH in a granulosa cell culture [34–36]. Thus, IGF promoted an increased responsiveness of the dominant follicle to gonadotropins at the time of follicle selection, which should act as the main mechanism of IGF-I to ensure follicular survival. Another common function

of IGF-I is to increase the estradiol secretion of granulosa cells [35, 36]. It was also reported that estradiol or FSH promotes the synthesis of IGF-I [37, 38], indicating that these three factors essential for follicular growth stimulate the expression among each other and amplify their survival effect. In addition, IGF promotes the proliferation and suppresses the apoptosis of granulosa cells, an issue to be discussed in detail in the next section.

Apoptosis by depletion of cell survival factors

Estradiol, as mentioned above, IGF, FSH, epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), interleukin-1 β (IL-1 β) and interleukin-6 (IL-6) have been characterized as prosurvival factors of granulosa cells [39–45]. Isolated granulosa cells cultured with serum-free medium undergo apoptosis, showing that in the absence of survival factors, endogenous apoptosis pathways within the granulosa cells become activated and lead to follicular atresia. Addition of anti-apoptotic molecules rescues granulosa cells from a spontaneous onset of apoptosis [39, 40, 42–44].

Phosphatidylinositol 3-kinase (PI3K)-AKT signaling is the central anti-apoptotic intracellular signal transduction pathway that is initiated by hormones or growth factors. The PI3K-AKT pathway exerts its anti-apoptotic efficacy in part by phosphorylating the forkhead box O (FOXO) subfamily of forkhead transcription factors. When survival-promoting factors are depleted, FOXOs are dephosphorylated and translocate to the nucleus, resulting in enhancement of the transcription of proapoptotic factors [46–49]. In granulosa cells, both IGF-I and FSH activate the PI3K-AKT

pathway [50, 51] and regulate transcription of the FOXO1 gene and phosphorylation of the FOXO1 protein that impacts the proliferation, differentiation and survival of granulosa cells [52–54]. Another FOXO transcription factor, FOXO3, is also indicated to act on follicular atresia in pig ovaries; the expression of FOXO3 in granulosa cells increased during follicular atresia, and an overexpression of FOXO3 induced granulosa cell apoptosis that is likely to be caused by the upregulation of proapoptotic factors such as FAS ligand (FASLG) and BCL2-like 11 (BCL2L11) [55]. The addition of FSH significantly decreased *FASLG* mRNA levels and attenuated apoptosis in porcine granulosa cells [56]. This response may be mediated by FOXO transcription factors, i.e., FSH inactivated FOXO and after which the transcription of FASLG decreased.

Treatment with EGF, bFGF or IL-1 β also inhibited the spontaneous initiation of apoptosis in rat granulosa cells [39, 40, 43]. The inclusion of a tyrosine kinase inhibitor completely blocked the ability of EGF and bFGF to suppress apoptosis in granulosa cells [39], indicating that the PI3K-AKT-FOXO pathway may be involved in these processes. IL-6 was shown to induce the PI3K-AKT pathway and then inhibit apoptosis in several types of cells [57, 58]. Transcripts of *IL-6* and its receptor subunit, *gp130*, in granulosa cells and proteins of IL-6 and IL-6 soluble receptor (IL-6sR) in follicular fluid decreased during atresia [45, 59], suggesting that the intrafollicular IL-6 system plays a significant role in porcine follicular growth and atresia.

2. Death ligand-receptor system

FAS ligand (FASLG) and FAS system

One main death ligand-receptor system, FASLG and FAS (CD95), is the common apoptosis inducer of granulosa cells across species, as both FASLG and FAS are expressed in granulosa cells, and apoptosis is inducible by FASLG-FAS stimulation *in vitro* [9]. Treatment of female mice with the FAS-activating antibody promoted granulosa cell apoptosis and follicular atresia [60, 61]. In human females, FAS is expressed in the granulosa cells of antral follicles at the early stage of atresia, and its expression increases as atresia progresses [62]. FASLG and FAS are expressed in bovine granulosa cells, being higher in atretic subordinate follicles relative to healthy dominant follicles on day 5 of the estrous cycle [63, 64]. Moreover, the killing of granulosa cells by FASLG was greater in subordinate compared with healthy dominant follicles isolated on day 5 [63, 65]. In porcine granulosa cells of antral follicles, both FASLG and FAS are expressed, their expressions being higher in atretic antral follicles than in healthy ones [56, 66], and stimulation by anti-FAS antibody induced cell death in a porcine granulosa cell line [67].

Though their levels are relatively low, both FASLG and FAS are clearly expressed in granulosa cells of healthy preantral and antral follicles [66]. In addition, when granulosa cells are cultured with serum *in vitro*, a single stimulation by FASLG is insufficient to kill them, and additional treatment by interferon-gamma (IFN- γ) or cycloheximide (an inhibitor of protein biosynthesis) is required [63, 65, 68–70]. These findings suggest that the factor(s) that blocks the FASLG-FAS-mediated apoptotic signal is at work in granulosa cells, which is essential for maintaining granulosa cells

and preserving the follicles healthy. In contrast to the expression patterns of FASLG and FAS, that of cellular FADD-like interleukin-1 β -converting enzyme (FLICE)-inhibitory protein (cFLIP), an intracellular inhibitory factor of FAS-signaling, decreases during atresia in the granulosa cells of pig ovaries [71]. cFLIP inhibited FAS-mediated apoptosis, and the suppression of cFLIP by small interfering RNA induced cell death in a porcine granulosa cell line [67]. It is presumed that cFLIP blocks the intracellular cascade of FAS-signaling and subsequent apoptosis in the granulosa cells of healthy follicles. Other intracellular molecules that mediate the FASLG-FAS death signal, such as FAS-associated death domain (FADD) and caspase-8 (CASP8), are also involved in porcine granulosa cell apoptosis [72, 73], suggesting that FASLG-FAS stimulation and subsequent intracellular signaling are among the main factors causing follicular atresia.

Some reports suggest that prosurvival hormones, growth factors or cytokines affect FASLG-FAS-mediated granulosa cell apoptosis. In cultured bovine granulosa cells, FASLG-induced apoptosis was inhibited by the addition of IGF, bEGF or EGF [68]. Treatment with estradiol protected cultured bovine granulosa cells from FASLG-induced apoptosis [74]. The addition of FSH significantly decreased *FASLG* and *FAS* mRNA levels in granulosa cells isolated from porcine antral follicles and could attenuate apoptosis [56]. Though the precise mechanism is unclear, the addition of IL-6 upregulated cFLIP expression in a human granulosa tumor cell line [59].

Tumor necrosis factor-alpha (TNF- α)

Tumor necrosis factor-alpha (TNF- α) and TNF receptor (TNFR) are also among the death ligands and death receptors, respectively. The characteristic of TNF- α is that it can induce both cell death and cell proliferation, exerting its effects by binding to either TNFR1 or 2 [75]. The binding of TNF- α to TNFR1 works as a death ligand-receptor, while binding to TNFR2 upregulates survival/anti-apoptotic gene expressions. TNF- α is reported to localize within adult ovaries, i.e., oocytes, granulosa cells and theca cells [76–78], while information on ovarian TNF- α receptors is very limited. TNF- α deficient mice exhibit an increased proliferation of granulosa cells and a decreased apoptosis of oocytes [79]. TNFR1 knockout mice have exhibited early senescence and poor fertility, whereas TNFR2 knockout has no effect on female fertility [80]. Thus, it is difficult to define the role of TNF- α in follicular growth and atresia based on the phenotypes of knockout mice. Primary cultured bovine granulosa cells underwent apoptosis after treatment with TNF- α and IFN- γ , but not by treatment with TNF- α alone [65]. Addition of recombinant TNF- α to primary cultured porcine granulosa cells significantly increased Ki-67-positive cells (proliferating cells) as well as terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL)-positive cells (apoptotic cells), indicating that TNF- α could induce both cell proliferation and cell death in granulosa cells [81]. TNF- α likely induces follicular atresia, since its mRNA expression is higher in bovine granulosa cells of subordinate follicles than in those of dominant follicles [78]. Meanwhile, TNF- α seems to have an effect on cell survival based on the expression pattern in porcine granulosa cells; the expression levels of both TNF- α and TNFR2 decrease during atresia [77]. Based on research to date, it is dif-

difficult to judge whether TNF- α promotes the survival or apoptosis of granulosa cells and the growth or atresia of follicles, which may be due to its quite complex activities in numerous cells and organs.

TNF- α -related apoptosis-inducing ligand (TRAIL)

As for the remaining death ligand-receptors, the TNF- α -related apoptosis-inducing ligand (TRAIL) and its receptors (Death receptor 4 (DR4), Death receptor 5 (DR5), Decoy receptor 1 (DcR1) and Decoy receptor 2 (DcR2)), few studies have been reported in regard to follicular growth and atresia. Adult human ovaries expressed TRAIL, DR5, DcR1 and DcR2 in the granulosa cells and oocytes of small primary/secondary follicles as well as in granulosa and theca cells of antral follicles. In a human granulosa cell tumor-derived cell line, TRAIL efficiently induced apoptosis, which was blocked by a caspase inhibitor [82]. The expression level of TRAIL increases during atresia, whereas that of its receptor, DR4, shows no change in the granulosa cells of pig antral follicles [83]. DcR1, the decoy receptor that inhibits the death signal triggered by TRAIL, was strongly expressed in the granulosa cells of healthy antral follicles, and a DcR1 inhibitor initiated TRAIL-induced apoptosis in a porcine granulosa cell culture [84, 85]. Thus, TRAIL and its receptors are suggested to have a role in granulosa cell apoptosis.

3. BCL2 family members (mitochondria-mediated apoptosis)

Death ligand-death receptor signaling undergoes different pathways following the activation of CASP8, whether passing through the mitochondrial pathway or not, before the subsequent activation of CASP3 [86]. Deprivation of a survival-promoting signal(s) is also known to stimulate the mitochondrial pathway. The BCL2 family proteins, which include both anti-apoptotic (BCL2, B cell lymphoma/leukemia X (BCLX), etc.) and proapoptotic proteins (BCL2 interacting domain (BID), BCL2L11, BAX, BCL2 homologous antagonist/killer (BAK), etc.), are key regulators of apoptosis whose main action site is the mitochondrial membrane: the former inhibits while the latter initiates the release of cytochrome c (Cyt c) from mitochondria. Members of the BCL2 protein family play significant roles in follicular growth/atresia by regulating germ cell apoptosis as well as somatic cell apoptosis. BCL2-deficient mice exhibit a decrease in the number of oocytes and primordial follicles [87], whereas overexpression of BCL2 increases oocyte tumorigenesis and decreases granulosa cell apoptosis of large antral follicles, which lead to enhanced folliculogenesis [88]. BAX is expressed in both oocytes and granulosa cells, and *Bax*-deficient mice exhibit excessive numbers of abnormal follicles [89]. BAX expression is strong in the granulosa cells of atretic follicles compared with that of healthy follicles in human ovaries [90]. In porcine granulosa cells, the expressions of BAX and BID are higher in atretic follicles than in healthy follicles [91].

After the release of Cyt c from mitochondria, apoptosis-activating factor 1 (APAF1) and caspase-9 (CASP9) mediate the apoptotic signal that results in the activation of CASP3 [86]. APAF1 and CASP9 are expressed in granulosa cells and are demonstrated to cause follicular atresia in mice and pigs [92, 93]. Thus, the mitochondrial pathway is suggested to mediate the apoptotic signal in granulosa

cells, which should be critical in the execution of apoptosis.

Some members of the BCL2 family proteins are regulated by other apoptosis-related factors in granulosa cells. The expression of BAX is increased in granulosa cells in both *Cyp19* (aromatase)-deficient mice and IGF-I-deficient mice [23, 94]. Bovine granulosa cells treated with IGF-I but not FSH exhibited increased mRNA expression of *BAX* [95]. In isolated monkey granulosa cells, gonadotropin depletion stimulated both *BAX* and *CASP3* expressions and induced apoptosis [96]. In human granulosa tumor cells, BCL2L11 was upregulated by FOXO3 overexpression [55].

Conclusion

From many studies, it is obvious that granulosa cells are essential in determining whether follicles continue growth or undergo atresia. As discussed above, secreted molecules, apoptotic signals from the cell surface and intracellular signaling molecules are themselves responsible for follicular fate but are also intricately interacting with each other within follicles and ovaries. Thus, the balance between those factors should determine the destiny of the follicles. It seems that the insufficiency of granulosa cells finally results in their death by apoptosis, and thus the apoptosis-related factor(s) in granulosa cells should be a promising target for industrial and clinical application. A number of known apoptosis-related factors were shown to contribute to a greater or lesser extent to granulosa cell survival/death. We need to ascertain the central factor(s) among those already known if we hope to put our knowledge into practice for treating disorders of the ovarian follicles or improving the low rate of gestation in domestic animals and humans. *In vivo* research that applies the above factor(s) to domestic animals or to those disease models would help to solve these problems.

References

1. Edson MA, Nagaraja AK, Matzuk MM. The mammalian ovary from genesis to revelation. *Endocr Rev* 2009; **30**: 624–712. [Medline] [CrossRef]
2. Young JM, McNeilly AS. Theca: the forgotten cell of the ovarian follicle. *Reproduction* 2010; **140**: 489–504. [Medline] [CrossRef]
3. Hirshfield AN. Development of follicles in the mammalian ovary. *Int Rev Cytol* 1991; **124**: 43–101. [Medline]
4. Webb R, Garnsworthy PC, Campbell BK, Hunter MG. Intra-ovarian regulation of follicular development and oocyte competence in farm animals. *Theriogenology* 2007; **68**: S22–S29. [Medline] [CrossRef]
5. Hunter MG, Robinson RS, Mann GE, Webb R. Endocrine and paracrine control of follicular development and ovulation rate in farm species. *Anim Reprod Sci* 2004; **82–83**: 461–477. [Medline] [CrossRef]
6. Inoue N, Matsuda F, Goto Y, Manabe N. Role of cell-death ligand-receptor system of granulosa cells in selective follicular atresia in porcine ovary. *J Reprod Dev* 2011; **57**: 169–175. [Medline] [CrossRef]
7. Tilly JL, Kowalski KI, Johnson AL, Hsueh AJW. Involvement of apoptosis in ovarian follicular atresia and postovulatory regression. *Endocrinology* 1991; **129**: 2799–2801. [Medline] [CrossRef]
8. Jiang JY, Cheung CKM, Wang YF, Tsang BK. Regulation of cell death and cell survival gene expression during ovarian follicular development and atresia. *Front Biosci* 2003; **8**: d222–d237. [Medline] [CrossRef]
9. Matsuda-Minehata F, Inoue N, Goto Y, Manabe N. The regulation of ovarian granulosa cell death by pro- and anti-apoptotic molecules. *J Reprod Dev* 2006; **52**: 695–705. [Medline] [CrossRef]
10. Rosenfeld CS, Wagner JS, Roberts RM, Lubahn DB. Intraovarian actions of oestrogen. *Reproduction* 2001; **122**: 215–226. [Medline] [CrossRef]
11. Berisha B, Pfaffl MW, Schams D. Expression of estrogen and progesterone recep-

- tors in the bovine ovary during estrous cycle and pregnancy. *Endocrine* 2002; **17**: 207–214. [Medline] [CrossRef]
12. LaVoie HA, DeSimone DC, Gillio-Meina C, Hui YY. Cloning and characterization of porcine ovarian estrogen receptor beta isoforms. *Biol Reprod* 2002; **66**: 616–623. [Medline] [CrossRef]
 13. Juengel JL, Heath DA, Quirke LD, McNatty KP. Oestrogen receptor alpha and beta, androgen receptor and progesterone receptor mRNA and protein localisation within the developing ovary and in small growing follicles of sheep. *Reproduction* 2006; **131**: 81–92. [Medline] [CrossRef]
 14. Richards JS. Maturation of ovarian follicles—actions and interactions of pituitary and ovarian hormones on follicular cell-differentiation. *Physiol Rev* 1980; **60**: 51–89. [Medline]
 15. Robker RL, Richards JS. Hormone-induced proliferation and differentiation of granulosa cells: A coordinated balance of the cell cycle regulators cyclin D2 and p27(Kip1). *Mol Endocrinol* 1998; **12**: 924–940. [Medline] [CrossRef]
 16. Britt KL, Drummond AE, Cox VA, Dyson M, Wreford NG, Jones MEE, Simpson ER, Findlay JK. An age-related ovarian phenotype in mice with targeted disruption of the Cyp 19 (aromatase) gene. *Endocrinology* 2000; **141**: 2614–2623. [Medline] [CrossRef]
 17. Dupont S, Krust A, Gansmuller A, Dierich A, Chambon P, Mark M. Effect of single and compound knockouts of estrogen receptors alpha (ER alpha) and beta (ER beta) on mouse reproductive phenotypes. *Development* 2000; **127**: 4277–4291. [Medline]
 18. Britt KL, Findlay JK. Regulation of the phenotype of ovarian somatic cells by estrogen. *Mol Cell Endocrinol* 2003; **202**: 11–17. [Medline] [CrossRef]
 19. Salvetti NR, Acosta JC, Gimeno EJ, Muller LA, Mazzini RA, Taboada AF, Ortega HH. Estrogen receptors alpha and beta and progesterone receptors in normal bovine ovarian follicles and cystic ovarian disease. *Vet Pathol* 2007; **44**: 373–378. [Medline] [CrossRef]
 20. Billig H, Furuta I, Hsueh AJW. Estrogens inhibit and androgens enhance ovarian granulosa-cell apoptosis. *Endocrinology* 1993; **133**: 2204–2212. [Medline] [CrossRef]
 21. Murdoch WJ. Inhibition by oestradiol of oxidative stress-induced apoptosis in pig ovarian tissues. *J Reprod Fertil* 1998; **114**: 127–130. [Medline] [CrossRef]
 22. Lund SA, Murdoch J, Van Kirk EA, Murdoch WJ. Mitogenic and antioxidant mechanisms of estradiol action in preovulatory ovine follicles: Relevance to luteal function. *Biol Reprod* 1999; **61**: 388–392. [Medline] [CrossRef]
 23. Toda K, Takeda K, Okada T, Akira S, Saibara T, Kaname T, Yamamura K, Onishi S, Shizuta Y. Targeted disruption of the aromatase P450 gene (Cyp19) in mice and their ovarian and uterine responses to 17 beta-oestradiol. *J Endocrinol* 2001; **170**: 99–111. [Medline] [CrossRef]
 24. Zhou J, Chin E, Bondy C. Cellular-pattern of insulin-like growth factor-I (IGF-I) and IGF-I receptor gene-expression in the developing and mature ovarian follicle. *Endocrinology* 1991; **129**: 3281–3288. [Medline] [CrossRef]
 25. Yuan W, Lucy MC, Smith MF. Messenger ribonucleic acid for insulin-like growth factors-I and -II, insulin-like growth factor-binding protein-2, gonadotropin receptors, and steroidogenic enzymes in porcine follicles. *Biol Reprod* 1996; **55**: 1045–1054. [Medline] [CrossRef]
 26. Adashi EY, Resnick CE, Payne DW, Rosenfeld RG, Matsumoto T, Hunter MK, Gargosky SE, Zhou J, Bondy CA. The mouse intraovarian insulin-like growth factor I system: Departures from the rat paradigm. *Endocrinology* 1997; **138**: 3881–3890. [Medline] [CrossRef]
 27. Wandji SA, Wood TL, Crawford J, Levison SW, Hammond JM. Expression of mouse ovarian insulin growth factor system components during follicular development and atresia. *Endocrinology* 1998; **139**: 5205–5214. [Medline] [CrossRef]
 28. Armstrong DG, Gutierrez CG, Baxter G, Glazyrin AL, Mann GE, Woad KJ, Hogg CO, Webb R. Expression of mRNA encoding IGF-I, IGF-II and type I IGF receptor in bovine ovarian follicles. *J Endocrinol* 2000; **165**: 101–113. [Medline] [CrossRef]
 29. Hastie PM, Haresign W. Expression of mRNAs encoding insulin-like growth factor (IGF) ligands, IGF receptors and IGF binding proteins during follicular growth and atresia in the ovine ovary throughout the oestrous cycle. *Anim Reprod Sci* 2006; **92**: 284–299. [Medline] [CrossRef]
 30. Liu J, Koenigsfeld AT, Cantley TC, Boyd CK, Kobayashi Y, Lucy MC. Growth and the initiation of steroidogenesis in porcine follicles are associated with unique patterns of gene expression for individual components of the ovarian insulin-like growth factor system. *Biol Reprod* 2000; **63**: 942–952. [Medline] [CrossRef]
 31. Baker J, Hardy MP, Zhou J, Bondy C, Lupu F, Bellve AR, Efstratiadis A. Effects of an Igf1 gene null mutation on mouse reproduction. *Mol Endocrinol* 1996; **10**: 903–918. [Medline] [CrossRef]
 32. Zhou J, Kumar TR, Matzuk MM, Bondy C. Insulin-like growth factor I regulates gonadotropin responsiveness in the murine ovary. *Mol Endocrinol* 1997; **11**: 1924–1933. [Medline] [CrossRef]
 33. Adashi EY, Resnick CE, Dercole AJ, Svoboda ME, Vanwyk JJ. Insulin-like growth-factors as intraovarian regulators of granulosa-cell growth and function. *Endocr Rev* 1985; **6**: 400–420. [Medline] [CrossRef]
 34. Maruo T, Hayashi M, Matsuo H, Ueda Y, Morikawa H, Mochizuki M. Comparison of the facilitative roles of insulin and insulin-like growth factor-I in the functional-differentiation of granulosa-cells – in vitro studies with the porcine model. *Acta Endocrinol (Copenh)* 1988; **117**: 230–240. [Medline]
 35. Monniaux D, Pisselet C. Control of proliferation and differentiation of ovine granulosa-cells by insulin-like growth factor-I and follicle-stimulating-hormone *in vitro*. *Biol Reprod* 1992; **46**: 109–119. [Medline] [CrossRef]
 36. Glister C, Tannetta DS, Groome NP, Knight PG. Interactions between follicle-stimulating hormone and growth factors in modulating secretion of steroids and inhibin-related peptides by nonluteinized bovine granulosa cells. *Biol Reprod* 2001; **65**: 1020–1028. [Medline] [CrossRef]
 37. Hsu CJ, Hammond JM. Gonadotropins and estradiol stimulate immunoreactive insulin-like growth factor-I production by porcine granulosa-cells *in vitro*. *Endocrinology* 1987; **120**: 198–207. [Medline] [CrossRef]
 38. Khalid M, Haresign W, Luck MR. Secretion of IGF-I by ovine granulosa cells: effects of growth hormone and follicle stimulating hormone. *Anim Reprod Sci* 2000; **58**: 261–272. [Medline] [CrossRef]
 39. Tilly JL, Billig H, Kowalski KI, Hsueh AJW. 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* 1992; **6**: 1942–1950. [Medline] [CrossRef]
 40. Chun SY, Eisenhauer KM, Kubo M, Hsueh AJW. Interleukin-1-beta suppresses apoptosis in rat ovarian follicles by increasing nitric-oxide production. *Endocrinology* 1995; **136**: 3120–3127. [Medline] [CrossRef]
 41. Hsu SY, Hsueh AJW. Hormonal regulation of apoptosis—An ovarian perspective. *Trends Endocrinol Metab* 1997; **8**: 207–213. [Medline] [CrossRef]
 42. Guthrie HD, Garrett WM, Cooper BS. Follicle-stimulating hormone and insulin-like growth factor-I attenuate apoptosis in cultured porcine granulosa cells. *Biol Reprod* 1998; **58**: 390–396. [Medline] [CrossRef]
 43. Lynch K, Fernandez G, Pappalardo A, Peluso JJ. Basic fibroblast growth factor inhibits apoptosis of spontaneously immortalized granulosa cells by regulating intracellular free calcium levels through a protein kinase C delta-dependent pathway. *Endocrinology* 2000; **141**: 4209–4217. [Medline] [CrossRef]
 44. Mao J, Smith MF, Rucker EB, Wu GM, McCauley TC, Cantley TC, Prather RS, Diodion BA, Day BN. Effect of epidermal growth factor and insulin-like growth factor I on porcine preantral follicular growth, antrum formation, and stimulation of granulosa cell proliferation and suppression of apoptosis *in vitro*. *J Anim Sci* 2004; **82**: 1967–1975. [Medline]
 45. Maeda A, Inoue N, Matsuda-Minehata F, Goto Y, Cheng Y, Manabe N. The role of interleukin-6 in the regulation of granulosa cell apoptosis during follicular atresia in pig ovaries. *J Reprod Dev* 2007; **53**: 481–490. [Medline] [CrossRef]
 46. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME. Akt promotes cell survival by phosphorylating and inhibiting a forkhead transcription factor. *Cell* 1999; **96**: 857–868. [Medline] [CrossRef]
 47. Dijkers PF, Birkenkamp KU, Lam EWF, Thomas NSB, Lammers JWJ, Koenderman L, Coffey PJ. FKHR-L1 can act as a critical effector of cell death induced by cytokine withdrawal: protein kinase B-enhanced cell survival through maintenance of mitochondrial integrity. *J Cell Biol* 2002; **156**: 531–542. [Medline] [CrossRef]
 48. Stahl M, Dijkers PF, Kops G, Lens SMA, Coffey PJ, Burgering BMT, Medema RH. The forkhead transcription factor FoxO regulates transcription of p27(Kip1) and bim in response to IL-2. *J Immunol* 2002; **168**: 5024–5031. [Medline]
 49. Accili D, Arden KC. FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. *Cell* 2004; **117**: 421–426. [Medline] [CrossRef]
 50. Sun GW, Kobayashi H, Suzuki M, Kanayama N, Terao T. Follicle-stimulating hormone and insulin-like growth factor I synergistically induce up-regulation of cartilage link protein (Crtl1) via activation of phosphatidylinositol-dependent Kinase/Akt in rat granulosa cells. *Endocrinology* 2003; **144**: 793–801. [Medline] [CrossRef]
 51. Hu CL, Cowan RG, Harman RM, Quirk SM. Cell cycle progression and activation of Akt kinase are required for insulin-like growth factor I-mediated suppression of apoptosis in granulosa cells. *Mol Endocrinol* 2004; **18**: 326–338. [Medline] [CrossRef]
 52. Richards JS, Sharma SC, Falender AE, Lo YH. Expression of FKHR, FKHL1, and AFX genes in the rodent ovary: Evidence for regulation by IGF-1, estrogen, and the gonadotropins. *Mol Endocrinol* 2002; **16**: 580–599. [Medline] [CrossRef]
 53. Cunningham MA, Zhu Q, Unterman TG, Hammond JM. Follicle-stimulating hormone promotes nuclear exclusion of the forkhead transcription factor FoxO1a via phosphatidylinositol 3-kinase in porcine granulosa cells. *Endocrinology* 2003; **144**:

- 5585–5594. [Medline] [CrossRef]
54. Park Y, Maizels ET, Feiger ZJ, Alam H, Peters CA, Woodruff TK, Unterman TG, Lee EJ, Jameson JL, Hunzicker-Dunn M. Induction of cyclin D2 in rat granulosa cells requires FSH-dependent relief from FOXO1 repression coupled with positive signals from Smad. *J Biol Chem* 2005; **280**: 9135–9148. [Medline] [CrossRef]
 55. Matsuda F, Inoue N, Maeda A, Cheng YA, Sai T, Gonda H, Goto Y, Sakamaki K, Manabe N. Expression and function of apoptosis initiator FOXO3 in granulosa cells during follicular atresia in pig ovaries. *J Reprod Dev* 2011; **57**: 151–158. [Medline] [CrossRef]
 56. Lin P, Rui R. Effects of follicular size and FSH on granulosa cell apoptosis and atresia in porcine antral follicles. *Mol Reprod Dev* 2010; **77**: 670–678. [Medline] [CrossRef]
 57. Kuo ML, Chuang SE, Lin MT, Yang SY. The involvement of PI3-K/Akt-dependent up-regulation of Mcl-1 in the prevention of apoptosis of Hep3B cells by interleukin-6. *Oncogene* 2001; **20**: 677–685. [Medline] [CrossRef]
 58. Wei LH, Kuo ML, Chen CA, Chou CH, Cheng WF, Chang MC, Su JL, Hsieh CY. The anti-apoptotic role of interleukin-6 in human cervical cancer is mediated by up-regulation of Mcl-1 through a PI3-K/Akt pathway. *Oncogene* 2001; **20**: 5799–5809. [Medline] [CrossRef]
 59. Maeda A, Goto Y, Matsuda-Minehata F, Cheng Y, Inoue N, Manabe N. Changes in expression of interleukin-6 receptors in granulosa cells during follicular atresia in pig ovaries. *J Reprod Dev* 2007; **53**: 727–736. [Medline] [CrossRef]
 60. Hakuno N, Koji T, Yano T, Kobayashi N, Tsutsumi O, Taketani Y, Nakane PK. Fas/APO-1/CD95 system as a mediator of granulosa cell apoptosis in ovarian follicle atresia. *Endocrinology* 1996; **137**: 1938–1948. [Medline] [CrossRef]
 61. Sakamaki K, Yoshida H, Nishimura Y, Nishikawa SI, Manabe N, Yonehara S. Involvement of Fas antigen in ovarian follicular atresia and luteolysis. *Mol Reprod Dev* 1997; **47**: 11–18. [Medline] [CrossRef]
 62. Kondo H, Maruo T, Peng XJ, Mochizuki M. 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* 1996; **81**: 2702–2710. [Medline] [CrossRef]
 63. Porter DA, Vickers SL, Cowan RG, Huber SC, Quirk SM. Expression and function of fas antigen vary in bovine granulosa and theca cells during ovarian follicular development and atresia. *Biol Reprod* 2000; **62**: 62–66. [Medline] [CrossRef]
 64. Porter DA, Harman RM, Cowan RG, Quirk SM. Relationship of Fas ligand expression and atresia during bovine follicle development. *Reproduction* 2001; **121**: 561–566. [Medline] [CrossRef]
 65. Vickers SL, Cowan RG, Harman RM, Porter DA, Quirk SM. Expression and activity of the Fas antigen in bovine ovarian follicle cells. *Biol Reprod* 2000; **62**: 54–61. [Medline] [CrossRef]
 66. Inoue N, Maeda A, Matsuda-Minehata F, Fukuta K, Manabe N. Expression and localization of Fas ligand and Fas during atresia in porcine ovarian follicles. *J Reprod Dev* 2006; **52**: 723–730. [Medline] [CrossRef]
 67. Matsuda-Minehata F, Goto Y, Inoue N, Sakamaki K, Chedrese PJ, Manabe N. Anti-apoptotic activity of porcine cFLIP in ovarian granulosa cell lines. *Mol Reprod Dev* 2007; **74**: 1165–1170. [Medline] [CrossRef]
 68. Quirk SM, Harman RM, Cowan RG. Regulation of Fas antigen (Fas, CD95)-mediated apoptosis of bovine granulosa cells by serum and growth factors. *Biol Reprod* 2000; **63**: 1278–1284. [Medline] [CrossRef]
 69. Quirk SM, Cowan RG, Joshi SG, Henrikson KP. Fas antigen-mediated apoptosis in human granulosa-luteal cells. *Biol Reprod* 1995; **52**: 279–287. [Medline] [CrossRef]
 70. Quirk SM, Porter DA, Huber SC, Cowan RG. Potentiation of Fas-mediated apoptosis of murine granulosa cells by interferon-gamma, tumor necrosis factor-alpha, and cycloheximide. *Endocrinology* 1998; **139**: 4860–4869. [Medline] [CrossRef]
 71. Matsuda-Minehata F, Goto Y, Inoue N, Manabe N. Changes in expression of anti-apoptotic protein, cFLIP, in granulosa cells during follicular atresia in porcine ovaries. *Mol Reprod Dev* 2005; **72**: 145–151. [Medline] [CrossRef]
 72. Inoue N, Matsuda-Minehata F, Goto Y, Sakamaki K, Manabe N. Molecular characteristics of porcine Fas-associated death domain (FADD) and procaspase-8. *J Reprod Dev* 2007; **53**: 427–436. [Medline] [CrossRef]
 73. Matsuda F, Inoue N, Goto Y, Maeda A, Cheng Y, Sakamaki K, Manabe N. cFLIP regulates death receptor-mediated apoptosis in an ovarian granulosa cell line by inhibiting procaspase-8 cleavage. *J Reprod Dev* 2008; **54**: 314–320. [Medline] [CrossRef]
 74. Quirk SM, Cowan RG, Harman RM. The susceptibility of granulosa cells to apoptosis is influenced by oestradiol and the cell cycle. *J Endocrinol* 2006; **189**: 441–453. [Medline] [CrossRef]
 75. Baud V, Karin M. Signal transduction by tumor necrosis factor and its relatives. *Trends Cell Biol* 2001; **11**: 372–377. [Medline] [CrossRef]
 76. Chen HL, Marcinkiewicz JL, Sanchotello M, Hunt JS, Terranova PF. Tumor-necrosis-factor- α gene-expression in mouse oocytes and follicular cells. *Biol Reprod* 1993; **48**: 707–714. [Medline] [CrossRef]
 77. Nakayama M, Manabe N, Inoue N, Matsui T, Miyamoto H. Changes in the expression of tumor necrosis factor (TNF) alpha, TNF alpha receptor (TNFR) 2, and TNFR-Associated factor 2 in granulosa cells during atresia in pig ovaries. *Biol Reprod* 2003; **68**: 530–535. [Medline] [CrossRef]
 78. Evans ACO, Ireland J LH, Winn ME, Lonergan P, Smith GW, Coussens PM, Ireland JJ. Identification of genes involved in apoptosis and dominant follicle development during follicular waves in cattle. *Biol Reprod* 2004; **70**: 1475–1484. [Medline] [CrossRef]
 79. Cui LL, Yang GW, Pan J, Zhang C. Tumor necrosis factor alpha knockout increases fertility of mice. *Theriogenology* 2011; **75**: 867–876. [Medline] [CrossRef]
 80. Roby KF, Son DS, Terranova PF. Alterations of events related to ovarian function in tumor necrosis factor receptor type 1 knockout mice. *Biol Reprod* 1999; **61**: 1616–1621. [Medline] [CrossRef]
 81. Prange-Kiel J, Kreutzkamm C, Wehrenberg U, Rune GM. Role of tumor necrosis factor in preovulatory follicles of swine. *Biol Reprod* 2001; **65**: 928–935. [Medline] [CrossRef]
 82. Jääskeläinen M, Kyronlahti A, Anttonen M, Nishi Y, Yanase T, Secchiero P, Zauli G, Tapanainen JS, Heikinheimo M, Vaskivuo TE. TRAIL pathway components and their putative role in granulosa cell apoptosis in the human ovary. *Differentiation* 2009; **77**: 369–376. [Medline] [CrossRef]
 83. Inoue N, Manabe N, Matsui T, Maeda A, Nakagawa S, Wada S, Miyamoto H. Roles of tumor necrosis factor-related apoptosis-inducing ligand signaling pathway in granulosa cell apoptosis during atresia in pig ovaries. *J Reprod Dev* 2003; **49**: 313–321. [Medline] [CrossRef]
 84. Wada S, Manabe N, Inoue N, Nakayama M, Matsui T, Miyamoto H. TRAIL-decoy receptor-1 disappears in granulosa cells of atretic follicles in porcine ovaries. *J Reprod Dev* 2002; **48**: 167–173. [CrossRef]
 85. Wada S, Manabe N, Nakayama M, Inoue N, Matsui T, Miyamoto H. TRAIL-decoy receptor 1 plays inhibitory role in apoptosis of granulosa cells from pig ovarian follicles. *J Vet Med Sci* 2002; **64**: 435–439. [Medline] [CrossRef]
 86. Scaffidi C, Fulda S, Srinivasan A, Friesen C, Li F, Tomaselli KJ, Debatin KM, Krammer PH, Peter ME. Two CD95 (APO-1/Fas) signaling pathways. *EMBO J* 1998; **17**: 1675–1687. [Medline] [CrossRef]
 87. Ratts VS, Flaws JA, Kolp R, Sorenson CM, Tilly JL. Ablation of bcl-2 gene-expression decreases the numbers of oocytes and primordial follicles established in the postnatal female mouse gonad. *Endocrinology* 1995; **136**: 3665–3668. [Medline] [CrossRef]
 88. Hsu SY, Lai RJM, Finegold M, Hsueh AJW. Targeted overexpression of Bcl-2 in ovaries of transgenic mice leads to decreased follicle apoptosis, enhanced folliculogenesis, and increased germ cell tumorigenesis. *Endocrinology* 1996; **137**: 4837–4843. [Medline] [CrossRef]
 89. Perez GI, Robles R, Knudson CM, Flaws JA, Korsmeyer SJ, Tilly JL. Prolongation of ovarian lifespan into advanced chronological age by Bax-deficiency. *Nat Genet* 1999; **21**: 200–203. [Medline] [CrossRef]
 90. Kugu K, Ratts VS, Piquette GN, Tilly KI, Tao XJ, Martimbeau S, Aberdeen GW, Krajewski S, Reed JC, Pepe GJ, Albrecht ED, Tilly JL. Analysis of apoptosis and expression of bcl-2 gene family members in the human and baboon ovary. *Cell Death Differ* 1998; **5**: 67–76. [Medline] [CrossRef]
 91. Sai T, Goto Y, Yoshioka R, Maeda A, Matsuda F, Sugimoto M, Wongpanit K, Jin HZ, Li JY, Manabe N. Bid and Bax are involved in granulosa cell apoptosis during follicular atresia in porcine ovaries. *J Reprod Dev* 2011; **57**: 421–427. [Medline] [CrossRef]
 92. Robles R, Tao XJ, Trbovich AM, Maravei DV, Nahum R, Perez GI, Tilly KI, Tilly JL. Localization, regulation and possible consequences of apoptotic protease-activating factor-1 (Apaf-1) expression in granulosa cells of the mouse ovary. *Endocrinology* 1999; **140**: 2641–2644. [Medline] [CrossRef]
 93. Matsui T, Manabe N, Goto Y, Inoue N, Nishihara S, Miyamoto H. Expression and activity of Apaf1 and caspase-9 in granulosa cells during follicular atresia in pig ovaries. *Reproduction* 2003; **126**: 113–120. [Medline] [CrossRef]
 94. Kadakia R, Arraztoa JA, Bondy C, Zhou J. Granulosa cell proliferation is impaired in the Igf1 null ovary. *Growth Horm IGF Res* 2001; **11**: 220–224. [Medline] [CrossRef]
 95. Mani AM, Fenwick MA, Cheng ZR, Sharma MK, Singh D, Wathes DC. IGF1 induces up-regulation of steroidogenic and apoptotic regulatory genes via activation of phosphatidylinositol-dependent kinase/AKT in bovine granulosa cells. *Reproduction* 2010; **139**: 139–151. [Medline] [CrossRef]
 96. Uma J, Muraly P, Verma-Kumar S, Medhamurthy R. Determination of onset of apoptosis in granulosa cells of the preovulatory follicles in the bonnet monkey (*Macaca radiata*): Correlation with mitogen-activated protein kinase activities. *Biol Reprod* 2003; **69**: 1379–1387. [Medline] [CrossRef]