Review

Novel trends in follicular development, atresia and corpus luteum regression: a role for apoptosis

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Abstract

During ovarian follicular development in humans, only a limited number of follicles mature and ovulate. The vast majority of follicles stop developing after the formation of an antrum and then undergo atresia. The few that are selected to become ovulatory follicles are transformed into corpora lutea following ovulation. The lifespan of the corpus luteum is also limited. In each oestrus/menstrual cycle, corpora lutea regress and are eliminated by a progress called luteolysis. During atresia and luteolysis, granulosa and lutein cells undergo apoptosis. It is believed that there are many signal transduction pathways that control apoptosis in order to suppress full maturation of too many follicles and to protect the dominant follicle from the apoptotic process prior the ovulation. Such interplay between different factors, some of them produced in the ovary, may modulate apoptosis of corpus luteum cells, in order to preserve the function of the corpus luteum during pregnancy or to eliminate the old corpora lutea of the previous cycle. The present review reports a number of factors that regulate follicular atresia and corpus luteum regression, via apoptotic pathways. Elucidation of apoptotic mechanisms may lead to prevention of female infertility or other pathological conditions.

Keywords: *apoptosis, corpus luteum, follicles, ovary*

Follicular development

The major function of the female gonad is the differentiation and release of the mature oocyte for fertilization and successful pregnancy in order to produce offspring. Additionally, the ovary produces steroids that allow the development of female secondary sexual characteristics and support pregnancy.

Therefore, in the human, the ovaries have the role of producing gametes, the oocytes, as well as sex hormones, oestrogens and progesterone. These two functions are exerted cyclically between puberty and the menopause, and result from the evolution of a morphological unit, the ovarian follicle, situated within the cortical stroma. In mammalian ovaries the individual follicles consist of an innermost oocyte, surrounded by granulosa cells, and outer layers of thecal cells (McGee and Hsueh, 2000). The fate of each follicle is controlled by endocrine as well as paracrine factors (Gougeon, 1996). The oocyte undergoes a progressive series of morphological modifications as it grows and proceeds through the different stages of development in order to achieve meiotic and embryonic developmental competence. Several factors determine the ultimate competence of the oocyte. The complexity of the interrelation of the events that control oocyte growth and ultimate acquisition of developmental competence

is under continuous investigation (Fair, 2003).

Follicles correspond to different stages of the evolution from primordial follicles up to the rupture of the mature follicle (ovulation). The follicles develop through primordial, primary, and secondary stages before acquiring an antral cavity. At the antral stage, most follicles undergo atretic degeneration, whereas a few, under the cyclic gonadotrophin stimulation that occurs after puberty, reach the pre-ovulatory stage.

Once primordial follicles are formed, the first meiotic division is arrested at the diplotene stage. In humans, the follicle may remain at this stage of development for 40–50 years up to the moment at which a signal initiates oocyte and follicle growth. All remaining oogonia that are not surrounded by somatic cells are expelled from the ovary. During prepubertal life continuous initiation of follicle growth leads to further depletion of the store of gametes available.

More than 99.5% of the ovarian follicles present at birth never reach ovulation, and so the most common fate of follicles is to undergo atresia. In recent years, evidence has accumulated that atresia is mediated by a highly organized type of cell death called apoptosis or programmed cell death (Hsueh *et al.,* 1994; Billig *et al.,* 1996; Kaipia and Hsueh, 1997; Markstrom *et al.,* 2002). The reduction of the large number of growing follicles to a single ovulatory follicle is achieved primarily by cell death of granulosa cells (Hsueh *et al.,* 1994; Reynaud and Driancourt, 2000).

Apoptosis

Apoptosis is a physiological cell death process. It serves as a defence mechanism to remove unwanted and potentially dangerous cells, such as self-reactive lymphocytes or cells that have been infected by virus and tumour cells (Wyllie, 1992). In addition to the beneficial effects, the inappropriate activation of apoptosis may contribute to the pathogenesis of many diseases such as cancer, neurodegenerative disorders, autoimmune diseases, acquired immunodeficiency syndrome and resistance to chemotherapy (Barr and Tomei, 1994; Kerr and Winterford, 1994; Vinatier *et al.,* 1996). The main characteristic of apoptosis is to eliminate cells without inducing local inflammatory response, liable to damage adjacent cells.

It was first reported that certain liver cells die with morphological features different from those of necrosis (Kerr *et al.,* 1972). The criteria by which apoptosis is characterized include a loss of cell volume (cytoplasmic condensation) accompanied by nuclear pyknosis resulting from margination of the chromatin and its redistribution against the nuclear envelope. In addition, many of the cytoplasmic organelles are maintained intact until the final stage of cell death, which is generally demarcated by the formation and release of plasma membrane-bound vesicles. One of the main characteristics of apoptotic cells is the loss of DNA integrity (Tilly, 1996).

Apoptosis is therefore an active form of cell death, dependent upon the internal machinery of the cell. In many cases, the cell's death is not inevitable, and so, when it is signalled, the cell needs to promote at least some of its 'suicidal' machinery (Vinatier *et al.,* 1996), including the so-called 'death genes'. Apoptosis-regulating genes have been found in every metazoan organism. These genes have proven to be exceptionally well conserved throughout evolution (Liu and Hengartner, 1999; Vaskivuo and Tapanainen, 2002). They include, among others, *p53*, *c-myc*, *bax*, *bcl-x short*, *c-fos* and *bad* (Vinatier *et al.,* 1996). Since in some cases the nucleus is not required for a cell to be able to undergo apoptosis (Jacobson *et al.,* 1994; Vinatier *et al.,* 1996), in many cells the protein components of the 'suicidal' programme are already expressed and are maintained in association with inhibitors. Blockage of inhibitor synthesis induces apoptosis. Once the inducing signals have been received, the cell expresses genes either promoting or inhibiting the cell death machinery. Expression of these regulator genes may be regulated by external factors such as cytokines (Canman *et al.,* 1995).

Apoptosis can be triggered by a wide variety of stimuli. Some can produce apoptosis in almost any cell, while most apoptosis-inducing factors show some selectivity in their targets (Rich *et al.,* 2000). The same inhibitors of mRNA and protein synthesis do not affect (Batisatatou and Greene, 1993) or even stimulate (Vaux and Weissman, 1993; Lewis *et al.,* 1995) apoptosis in others cell types. Apoptosis has to be under tight control by physiological mechanisms. Endocrine, paracrine and autocrine molecules can either induce apoptosis or rescue cells directly or through transcription of new genes (Vaskivuo and Tapanainen, 2002). To escape apoptosis, cells usually require survival factors. Failure to supply adequate concentrations of anti-apoptotic factors leads to activation of apoptosis (Conlon and Raff, 1999).

Apoptosis of germ cells

Primordial germ cells arise at about 3 weeks after fertilization. In the female fetus, the primordial germ cells are named oogonia upon arrival in the primitive gonad. Follicle formation in humans begins between week 16 and week 18 of fetal life. In the primordial follicles, the mesenchymal cells secrete an outer basement membrane and the same cells will give rise to granulosa cells in the growing follicle. Meanwhile, the mitotic activity of the oogonia ceases and the latter enter meiosis. Once the meiotic process is initiated, the oogonial germ cells are defined as primary oocytes. As a consequence of initiation of meiosis, multiplication is prohibited and the store of female gametes is set definitively at that stage of life (Smitz and Cortvrindt, 2002).

The maximum number of germ cells is present at about 4–5 months after conception, and decreases from 8.3×10^6 to $1–2.3 \times 10^6$ at birth by a process called 'attrition' (Baker, 1963; Reynaud and Driancourt, 2000). It has been shown that apoptosis is present in the human fetal ovaries from gestational week 13 onwards, when 10% of all oocytes are apoptotic (Vaskivuo *et al.,* 2001). From weeks 14–20, the rate of oocyte apoptosis is extremely high, ranging from 11 to 17%. Thereafter, the rate of cell death slightly decreases and, in the last trimester of pregnancy, the rate of apoptosis decreases considerably. Recent studies have identified that the number of apoptotic oocytes in fetuses at gestational weeks 23 (2.8%), 27 (5.7%), and 31 (3.8%) are considerably smaller than that of younger fetuses (Abir *et al.,* 2002), and at birth no apoptotic oocytes can be seen (Vaskivuo and Tapanainen, 2002). The extensive rate of cell death that is seen in oocytes during fetal