Species-Related Differences in the Mechanism of Apoptosis During Structural Luteolysis

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Abstract. Luteolysis is defined as the loss of function and subsequent involution of the luteal structure. The luteolytic process is usually subdivided, whereby the decline in progesterone is described as functional luteolysis and the structural involution is described as structural luteolysis. After the corpus luteum ceases to produce progesterone, it decreases in size, experiences a loss of cellular integrity, and then disappears from the ovary as a result of apoptosis of luteal cells. However, the control mechanisms responsible for initiating and mediating apoptosis during structural luteolysis seem more complex than originally envisioned. Furthermore, efforts to elucidate the apoptotic mechanisms have been complicated by the fact that different mammalian species have different mechanisms for controlling luteal function. Therefore, it is of interest to know whether different mammalian species have different apoptotic mechanisms. The goal of this review was to focus on species-related differences in the mechanism of apoptosis during structural luteolysis in rodents, cattle and humans, the species that are used most for luteolysis research.

Key words: Apoptosis, Corpus luteum, Luteolysis, Species-Related Differences

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It is generally believed that structural luteolysis involves apoptosis of luteal cells in a number of animal species. However, the control mechanisms responsible for initiating and mediating apoptosis during structural luteolysis are complex. Elucidation of the apoptotic mechanisms is complicated by the fact that different animal species have different mechanisms for controlling luteal function. The purpose of this review is to summarize what is known regarding the mechanisms of apoptosis during structural luteolysis in rodents, cattle and humans, the species that are used most for research on luteolysis. The present review focuses on species-related differences in the mechanisms of apoptosis during structural luteolysis.

Rodents

Since rats are the most analyzed rodent species, we provided an overview of the data for rats and cited partial data for mice as required. There is a great difference in the mechanisms of apoptosis between cycling rats and pregnant rats. Normally cycling rats and pregnant rats are discussed separately below.

Normally cycling rats

In cycling rats, a preovulatory prolactin (PRL) surge occurs concomitantly with a luteinizing hormone (LH) surge at ovulation, which is observed between 2100 h on the day of proestrus and 0400 h on the day of estrus. Functional luteolysis begins within 2–3 days after ovulation unless the female rat mates or experiences uterine cervical stimulation. Structural luteolysis occurs after functional luteolysis, and corpora lutea disappear from the ovary within 3–4 cycles.

Factors initiating apoptosis: Gel electrophoretic DNA fragmentation (DNA ladder formation) and an increase in apoptotic luteal cells are observed in the corpus luteum between 2100 h on the day of proestrus and 0400 h on the day of estrus in cycling rats [4, 5]. Treatment with bromocriptine, which is used to suppress the PRL surge, blocks this change in apoptosis whereas PRL injection offsets the effect of bromocriptine treatment [4, 5]. Accumulation of apoptotic cells and macrophages occurs observed in the corpus luteum from 1600 h on the day of proestrus to 1100 h on the day of estrus in cycling rats, and both increases are blocked by PRL suppression with bromocriptine [6]. These reports clearly show that the preovulatory PRL surge is a trigger for luteal cell apoptosis in cycling rats. Furthermore, expression of monocyte chemoattractant protein-1 (MCP-1) is found in the non-apoptotic luteal cells of the regressing corpus luteum together with an increased number of macrophages, suggesting that macrophages are chemotacted by MCP-1 toward the regressing corpus luteum and that they eliminate apoptotic luteal cells by phagocytosis [7, 8]. Since RU486, which has been used to suppress the action of progesterone, does not block the accumulation of macrophages in the regressing corpus luteum, the preovulatory PRL surge, but not progesterone, induces MCP-1 expression in non-apoptotic luteal cells and contributes to macrophage infiltration into the corpus luteum [9].

Factors mediating apoptosis:

(a) Fas-ligand and Fas

Fas antigen, a cell-surface protein that modulates apoptosis, has been reported to be constitutively expressed in luteal cells [10, 11]. The corpus luteum of normally cycling mice at proestrus disappeared when they were injected with anti-Fas monoclonal antibodies, which have apoptosis-inducing activities [11]. Incubation of corpora lutea obtained at proestrus with PRL induced apoptosis of luteal cells with the increased Fas-ligand expression [12]. Interestingly, CD3-positive T lymphocytes and Fas-ligand-positive cells were co-localized in the region where apoptosis convergently occurred [12]. Furthermore, Fas-ligand was only expressed by CD3-positive T lymphocytes when corpora lutea were incubated with PRL [13]. Removal of T lymphocytes from the isolated luteal cell fraction inhibited PRL-induced apoptosis, suggesting that T lymphocytes expressing Fas-ligand are required for PRL-induced luteal cell apoptosis [13]. That is, PRL stimulates Fas-ligand expression of T lymphocytes, which then acts on luteal cells expressing Fas and induces luteal cell apoptosis. In addition, PRL-induced apoptosis is blocked by addition of progesterone, and progesterone decreases Fas expression, but not Fas-ligand expression [14]. These findings suggest that progesterone acts as an important factor that changes the sensitivity of the corpus luteum to PRL. This mechanism supports the finding that the apoptotic effect of PRL is apparent only during the period from proestrus to estrus when serum progesterone levels are low.
(b) Caspase-3

Caspase-3-deficient mice have provided a lot of information so far. Time-dependent increases in the level of caspase-3 and number of apoptotic cells were observed in wild-type mice, but not in caspase-3-deficient mice, when corpora lutea were incubated in the absence of serum and growth factors [15]. Interestingly, the ovaries of the caspase-3-deficient mice retained many corpora lutea, but their serum progesterone levels declined, providing evidence that caspase-3 is required for apoptosis to proceed normally but is not a direct mediator of the decrease in steroidogenesis associated with luteolysis [15]. Furthermore, injection of Fas-activating antibodies failed to induce apoptosis in the corpus luteum of caspase-3-deficient mice, whereas apoptosis was found with an 8-fold increase in caspase-8 activity in wild-type mice [16]. Thus, the apoptotic pathway, Fas-ligand and Fas to caspase-8 to caspase-3, is present and functioning in the corpus luteum during structural luteolysis in mice.

(c) Cytokines

Cytokines are involved in the apoptotic mechanism related to the Fas and Fas-ligand system. It has been shown that when luteal cells are incubated with tumor necrosis factor-α (TNF-α) or interferon-γ (IFN-γ) alone, Fas increases by 2-fold at most and that subsequent addition of Fas-activating antibodies fails to induce luteal cell apoptosis. However, when luteal cells are preincubated with both TNF-α and IFN-γ, Fas increases by 8-fold, and subsequent addition of Fas antibodies induces luteal cell apoptosis [17]. Furthermore, Fas and TNF-α are localized in both the normal and regressing corpus luteum, whereas IFN-γ is localized only in the regressing corpus luteum [17]. This data suggests that the presence of both TNF-α and IFN-γ plays an important role in inducing luteal cell apoptosis in the regressing corpus luteum. On the other hand, an interesting finding of this previous report was that apoptosis is not induced in the functional corpus luteum despite the increase in Fas and Fas-ligand [17]. The authors of this report suggest that this may have been due to the presence of a soluble form of Fas, which binds to Fas-ligand and prevents apoptosis induction [17].

The apoptotic mechanisms of normal cycling rats are summarized in Fig. 1. After corpora lutea form after ovulation, progesterone is metabolized by activation of a progesterone metabolizing enzyme, 20α-hydroxysteroid dehydrogenase (20α-HSD), and serum progesterone levels decline in the absence of coitus [18]. Fas antigens are induced in luteal cells by a decline in the serum progesterone level. The preovulatory PRL surge at proestrus induces Fas-ligand expression in the T lymphocytes of the corpus luteum, which acts on luteal cells expressing Fas and induces luteal cell apoptosis by activating the Fas pathway. The apoptotic pathway induced by the Fas and Fas-ligand system is mediated through caspase-8 and caspase-3. The preovulatory PRL surge also induces macrophage chemoattractant protein-1 (MCP-1) expression in non-apoptotic luteal cells; MCP-1 chemoattracts macrophages, which then eliminate apoptotic luteal cells by phagocytosis. Activated macrophages and T lymphocytes also produce TNF-α and IFN-γ, and these cytokines cooperatively increase Fas expression in luteal cells.

![Fig. 1. Possible mechanism for luteal cell apoptosis in normal cycling rats.](image-url)
Pregnant rats

As shown in Fig. 2A, the corpus luteum seems to undergo structural luteolysis after parturition because it gradually decreases in volume after parturition in pregnant rats. However, the involvement of luteal cell apoptosis in structural luteolysis after parturition is still controversial. Guo et al. [19] reported that only a few apoptotic cells were recognizable until day 1 after parturition, but more than 50% of cells were apoptotic in the corpus luteum of pregnancy by 3 days postpartum. On the other hand, there are several reports showing that the number of apoptotic cells remains low during postpartum [5, 20]. Gel electrophoretic DNA fragmentation analysis revealed no apoptosis in corpora lutea isolated either during the last 2 days of pregnancy (days 21 and 22 of pregnancy) or during the first the 4 days after parturition [21]. Taken together, it is generally agreed that apoptosis is not induced in the corpus luteum during pregnancy or very early postpartum. Another study showed that there is a 50% decrease in the corpus luteum volume and 30% decrease in the mean cross-sectional area of luteal cells from day 21 of pregnancy to day 2 postpartum; this raises the possibility that luteal cell apoptosis is not a main mechanism for structural luteolysis after parturition [5]. However, factors initiating or mediating apoptosis in the corpus luteum of pregnancy have been reported, such as the Fas and Fas-ligand and Bcl-2 and Bax systems [20, 22]. TNF-α may be also involved in apoptosis induction in the corpus luteum of pregnancy [23]. TNF-α expression is increased in the corpus luteum from day 22 of pregnancy to day 3 postpartum, and incubation of corpora lutea from day 16 of pregnancy with TNF-α induces apoptosis; this effect is inhibited by caspase-3 inhibitors.

Recently, Takiguchi et al. [20] raised an interesting hypothesis; they suggested that a caspase-3-independent mechanism for apoptosis is present in the corpus luteum of pregnancy after parturition based on the findings that changes in caspase-3 activities were inconsistent with those in a number of apoptotic cells. Furthermore, they showed that there is a difference in the occurrence of apoptosis between lactating and non-lactating rats after parturition [20] (Fig. 2B). In lactating rats, the number of apoptotic cells remains low throughout the postpartum period (until day 9), whereas non-lactating rats resume ovulatory cycles after parturition and experience transient increases in the number of apoptotic cells in the corpus luteum of pregnancy in accordance with the preovulatory PRL surge. The low occurrence of apoptosis in lactating rats may be due to the anti-apoptotic effects of progesterone, as reported by Goyeneche et al. [21, 24], because progesterone is produced from the newly formed corpus luteum after parturition as a result of the high serum PRL levels that are induced by lactation.

In conclusion, the involvement of luteal cell apoptosis in structural luteolysis after parturition is still controversial. The control mechanisms for apoptosis in the rat corpus luteum of pregnancy during structural luteolysis are complex.

Cattle

Several lines of evidence have shown that luteal cell apoptosis contributes to structural luteolysis in cattle. DNA ladder formation and an increase in degenerating luteal cells are observed in the corpus...
luteum during structural luteolysis, but not during the mid-luteal phase [25, 26]. DNA ladder formation is also found in the regressing corpus luteum 24 and 48 h after prostaglandin F2α (PGF2α) administration, indicating that apoptosis occurs during PGF2α induced structural luteolysis [25]. The corpus luteum undergoes functional luteolysis until 12 h after PGF2α administration and then structural luteolysis 12 h later in cattle [27]. An overview of the control mechanisms for initiating and mediating apoptosis during structural luteolysis is provided below.

**Fas and Fas-ligand system**

The Fas and Fas-ligand system seems to play important roles in the regulation of luteal cell apoptosis during structural luteolysis in cattle. Fas expression is higher in the corpus luteum during structural luteolysis (days 19–21) than during other luteal phases [28]. The increase in Fas expression during structural luteolysis may be due to TNF-α and IFN-γ produced by immune cells [29], which are actually increased in number in the corpus luteum during both spontaneous and PGF2α-induced luteolysis in cattle [30]. IFN-γ and TNF-α are derived from T lymphocytes and macrophages, respectively, in the bovine corpus luteum [30, 31]. When luteal cells are incubated with TNF-α or IFN-γ alone, Fas mRNA expression is increased by IFN-γ, but not by TNF-α, and is synergistically increased by both TNF-α and IFN-γ [28]. Furthermore, when luteal cells are preincubated with IFN-γ alone or both TNF-α and IFN-γ, subsequent addition of Fas-ligand induces luteal cell apoptosis [28].

Progesterone has been reported to have anti-apoptotic effects in cattle and rats. Incubation of luteal cells with a P450scc inhibitor or a specific progesterone antagonist induced apoptosis, and this effect was blocked by addition of progesterone [32]. Furthermore, incubation with a specific progesterone antagonist increased Fas mRNA expression, and subsequent addition of Fas-ligand induced apoptosis [33]. Thus, the decline in progesterone levels during the regression phase could be one of the important factors that induce luteal cell apoptosis via the Fas and Fas-ligand system.

**Bcl-2 family and reactive oxygen species**

The Bcl-2 and Bax system is also involved in luteal cell apoptosis. Increases in bax and caspase-3 mRNA expression and DNA ladder formation are observed in the regressing corpus luteum on day 21 of the reproductive cycle, whereas low bax and caspase-3 mRNA expression and no DNA ladder formation are observed in the corpus luteum on day 21 of pregnancy [34]. However, the Bcl-2 and Bax system did not seem to mediate the anti-apoptotic effect of progesterone in a culture system of bovine luteal cells [33].

Reactive oxygen species (ROS) are also involved in luteal cell apoptosis in cattle. ROS induce apoptosis with increased expression of Bax and caspase-3 [35]. In addition, apoptosis is induced by a glutathione peroxidase inhibitor in cultured bovine luteal cells [36]. Apoptosis induced by TNF-α is inhibited by addition of antioxidants, superoxide dismutase (SOD) and catalase, suggesting that TNF-α induced luteal cell apoptosis is mediated by ROS [37]. PGF2α induces apoptosis by producing ROS via activation of protein kinase C and increased intracellular Ca²⁺ [38].

**Nitric oxide**

PGF2α and TNF-α have been suggested to cause luteolysis in vivo by inducing production of nitric oxide (NO) in cattle [39, 40]. NO seems to serve as a mediator of PGF2α action in functional luteolysis of the bovine corpus luteum [39–42]. NO is produced by three types of cells in the corpus luteum, steroidogenic, endothelial and immune cells [40–43], and is generated from L-arginine by NO synthase (NOS). Both inducible and endothelial isoforms of NOS (iNOS and eNOS) have been demonstrated to be present in the bovine corpus luteum [39]. Infusion of an NOS inhibitor, L-NAME, inhibits plasma nitrite/nitrate and concomitantly stimulates progesterone secretion in vivo [44]. Furthermore, L-NAME has been demonstrated to block the luteolytic action of PGF2α analogue [39] and spontaneous luteolysis in cattle [44]. Moreover, NO donors inhibit progesterone secretion [41, 42, 46]. The above findings strongly suggest that NO is involved in functional luteolysis in cattle. Besides its role in functional luteolysis, there is increasing evidence that NO plays important roles in inducing apoptosis of bovine luteal cells (structural luteolysis). An NO donor (NONOate) has been demonstrated to stimulate Fas, bax and caspase-3 gene expression, intracellular Ca²⁺ mobilization and caspase-3 activities, resulting in cell death and DNA fragmentation in cultured bovine luteal cells [45]. Furthermore, since an NO donor
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(NONOate) inhibits progesterone synthesis in the bovine corpus luteum [41, 42, 46] and progesterone has been demonstrated to be a potent apoptosis inhibitor in bovine luteal cells [32, 33]. NO may induce apoptosis directly or indirectly through cessation of progesterone production and action in the corpus luteum.

On the other hand, Petroff et al. [37] reported that an NO synthase inhibitor did not inhibit cell death induced by TNF-α and IFN-γ in bovine mid-luteal cells in vitro. The sensitivity of bovine luteal cells to NO has been shown to increase dramatically from the early to late luteal phase [44, 46]. Thus, differences in the sensitivity of bovine luteal cells to NO action [44, 46] and in NOS activities [39] in the corpus luteum during the estrous cycle might cause the different reaction of luteal cells to cytokines and PGF2α.

In conclusion, it is generally agreed that the Fas and Fas-ligand system plays central roles in luteal cell apoptosis during structural luteolysis in cattle, and that the apoptotic pathway via the Fas and Fas-ligand system is modified by several factors, such as cytokines, TNF-α and IFN-γ and progesterone (Fig. 3). In addition, NO could be one of the most important inducers of apoptosis in the bovine corpus luteum. ROS and the Bcl-2 family may also be involved in apoptosis as mediators.

Humans

There is evidence showing that luteal cell apoptosis contributes to structural luteolysis in humans, which starts at the late stage of the late luteal phase during the menstrual cycle. DNA ladder formation and apoptotic cells are detected in the mid-luteal phase corpus luteum and apparently increase in the regressing corpus luteum, whereas they are not detected in the corpus luteum of pregnancy [47–49]. It is generally agreed that apoptotic cells remarkably increase in number in the corpus luteum during the late luteal phase [50, 51]. On the other hand, Fraser et al. [52] report that autophagocytosis may be a main mechanism for natural structural luteolysis rather than apoptosis in primates because electron microscopic analysis revealed that natural luteolysis was associated with luteal cell atrophy, condensation of cytoplasmic inclusions and organelles and accumulation of lipids, but was not associated with the ultrastructural criteria for apoptosis in marmosets. They also showed that there are some differences in the ultrastructural changes of luteal cells between natural luteolysis and GnRH antagonist- or PGF2α-induced luteolysis [52]. Although the extent to which luteal cell apoptosis contributes to structural luteolysis in humans has yet to be elucidated, an overview of possible factors mediating luteal cell apoptosis in humans is provided below.

Fas and Fas-ligand system

Fas antigen is expressed in human luteal cells [53–55], and an immunohistochemical study showed that Fas expression increases in the regressing corpus luteum [54]. When luteinized granulosa cells were preincubated with IFN-γ, subsequent addition of Fas-activating antibodies induced apoptosis [53]. These reports suggest involvement of the Fas and Fas-ligand system in luteal cell apoptosis during structural luteolysis in humans.
Bcl-2 family

Bcl-2 expression is high and Bax expression is low in the corpus luteum during the mid-luteal phase and early pregnancy, whereas Bcl-2 expression is low and Bax expression is high in the regressing corpus luteum [49]. The number of Bcl-2 positive cells, as determined by immunohistochemistry, decreases in the corpus luteum during the late luteal phase (day 26 to 28), while the number of Bax positive cells remains constant throughout the luteal phase [51]. These reports suggest that the Bcl-2 and Bax system is involved in luteal cell apoptosis during structural luteolysis in humans. In contrast with these reports, there is a report showing that the mRNA expression of bcl-2 and bax remains unchanged in the corpus luteum throughout the menstrual cycle and even in the corpus luteum rescued by human chorionic gonadotropin (HCG) injection [56, 57].

Cytokines

TNF-α may be a signal to initiate luteal cell apoptosis during structural luteolysis in humans. Immunohistochemical studies have shown that the number of TNF-α positive cells increases in the regressing corpus luteum [51] and that the immunointensity of TNF-α increases from the early luteal phase to the mid-luteal phase [58]. In fact, macrophages increase in number in the regressing corpus luteum [59]. It has also been demonstrated in vitro that TNF-α actually induces luteal cell apoptosis [60].

Caspase-3

Constant caspase-3 expression has been observed in luteal cells throughout the luteal phase by immunohistochemical study [51]. The enzyme activities of caspase-3 and caspase-8 are significantly higher in the corpus luteum during the late stage of the mid-luteal phase compared with the other luteal stages in monkeys [61]. A protein kinase C inhibitor, staurosporine, induced apoptosis with increases in both caspase-9 and caspase-3 activities in a culture system of human luteinized granulosa cells [62]. These findings suggest that caspases are involved in luteal cell apoptosis during structural luteolysis in primates.

Anti-apoptotic effects of HCG

Several lines of evidence show that HCG works as a survival factor. Extracellular adenosine triphosphate (ATP) induces apoptosis via mitochondrial depolarization in human granulosa cells in vitro, and HCG inhibits ATP-induced apoptosis [63]. Matsubara et al. [60] also reported that suppression of apoptosis by HCG is mediated not only by inhibition of the Fas pathway but also by the Bcl-2 and Bax system in luteinized granulosa cells [64]. In fact, HCG increases Bcl-2 expression and decreases Bax expression in the corpus luteum in vitro [49, 65]. In addition, there is a report indicating that HCG upregulates survivin in human granulosa cells, which is an inhibitor of apoptosis protein that directly interacts with several caspases to inhibit apoptosis [64].

In conclusion, it is likely that luteal cell apoptosis contributes to structural luteolysis in humans. The Fas and Fas-ligand and Bcl-2 and Bax systems are involved in the intracellular signaling pathway for apoptosis, and apoptosis is induced by caspase-3 activation. It is of special interest to note that HCG works as a survival factor by regulating apoptosis-related factors, such as Fas, the Bcl-2 and Bax system and survivin.
Summary

The present review reports the species-related differences in the mechanism for luteal cell apoptosis in rodents, cattle and humans. There is a common apoptotic pathway in these animal species, namely the Fas and Fas-ligand system followed by caspase-8 and caspase-3, and cytokines, such as TNF-α and IFN-γ produced by macrophages and T lymphocytes modify apoptotic activities by activating the Fas pathway. Interestingly, there seems to be several different mechanisms involved in trigger and modification of apoptosis between these animal species. In cycling rats, preovulatory PRL surges are a trigger, and progesterone works as a suppressor of apoptosis. In cattle, PGF2α is a trigger, and progesterone is a suppressor of apoptosis and NO works as a stimulator of apoptosis by activating the Fas pathway. In humans, HCG plays central roles in the regulation of apoptosis-related molecules including Fas, Bcl-2/Bax and survivin, suggesting that absence of HCG could be a signal for initiation of luteal cell apoptosis when pregnancy does not occur.

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