韓國家畜繁殖學會誌 20(4) : 385~393(1996)
Korean J. Animal Reprod.

Follicle Cell Death during Ovarian Atresia in the Rat

Sang-Young Chun, Hyuk-Bang Kwon and Aaron J. W. Hsueh*
Hormone Research Center, Department of Biology, College of Natural Sciences,
Chonnam National University, Kwangju, 500-757, Republic of Korea;

Rat난소폐쇄에서의 난포의 사망기전

전상영 · 권혁방 · A. J. W. Hsueh*
전남대학교 자연과학대학 생물학과 호르몬 연구센터

요 약

다세포 생물에서 몸의 효율적 생존을 위한 각 기관의 homeostasis는 세포 증식과 사망에 의해 조절된다. 따라서, apoptosis라 명명된 세포사망은 정교한 기전에 의한 능동적이고 자발적인 사망기전으로써 몸의 정상적 유지를 위한 필수적인 현상이다. 발생기 세포나 신경세포 또는 흉선세포 분화 동안 과다한 세포의 제거가 apoptosis의 대표적인 예이며, 각종 호르몬에 의해 그 기능이 조절되는 난소세포에서도 apoptosis가 활발히 일어난다.

Rat난소에는 태어날 때 수십 만개의 난포를 지니고 있는데, 이 중 단지 1% 만이 배란에 사용되어질 뿐이고 나머지는 모두 사망하게 된다. 이러한 난포사망은 난소의 적절한 세포 수를 유지하기 위한 필수적 과정이며, 인위적으로 apoptosis를 억제하는 유전자인 bcl-2를 과다 발현시키면 난소암이 발생하는 연구결과가 이를 입증해주고 있다. 이처럼 중요한 난포 사망기전은 apoptosis라는 개념이 정립되면서 최근 들어 점차 그 연구가 활발해지고 있다. Apoptosis의 특징 중 뚜렷한 점은 DNA가 일정한 간격으로 (180~200 bp)잘려지는 DNA fragmentation현상으로, 이를 이용하여 DNA 3′-end 부위에 방사선동위원소를 label한 후 이를 전기영동으로 분리하면 apoptosis를 손쉽게 측정할 수 있다.

난소의 기능은 시상하부호르몬인 LH와 FSH 뿐만 아니라 난소에서 분비되는 각종 난소국부호르몬들에 의해 조절된다. 특정한 발육단계의 난포는 특정한 호르몬에 의해 그 기능을 조절 받는데, 이러한 난소기능 조절기작은 매우 복잡한 경로를 지니고 있다. 이러한 복잡한 기작으로 인해 초기 연구에서처럼 생체 내에서 밝히려는 연구 시도는 어려움에 부딪치게 되었다. 생체내 실험은 난소가 다양한 발육단계의 난포를 동시에 지니고 있어 특정한 발육단계의 난포 사망기전을 연구하기 어렵다. 또한 난포는 생체 내에서 다양한 호르몬을 동시에 분비하기 때문에 특정한 난소국부호르몬이 사망기전에 미치는 영향을 조사하기 힘든 점이 있다. 최근 들어 난포체외배양이 다양하게 개발되면서, 이러한 어려운 점을 극복할 수 있게 되었다.

본 논문은 각 발육단계의 난포를 절단해 체외배양하면서, apoptosis DNA 절단 현상을 이용하여 각종 난소국부 호르몬들이 난포발육단계별로 사망기전에 미치는 영향을 요약해 보았다. 난포는 발육하면서 점차 복잡한 호르몬 경로를 생존을 위해 필요로 한다. Preovulatory난포생존에 필요한 난소국부호르몬들은 early antral 단계의 난포에서는 그 미치는 영향이 감소되다가 preantral단계의 난포에서는 영향을

* 미국 스탠포드의과대학 산부인과 번식생물학교실(Division of Reproductive Biology, Department of Gynecology and Obstetrics, Stanford University School of Medicine, Stanford, CA 94305-5317, USA)

전혀 미치지 못했다. 단지 예외는 cGMP처리로써, 세포내 cGMP수준을 일정하게 유지시켜주는 것이 난포 발육단계에 무관하게 생존에 중요한 인자로, 장래 연구는 난포 세포내의 cGMP수준을 조절하는 기작을 규명하는데 있을 것이다.

Ⅰ. INTRODUCTION

The homeostasis of an organism is controlled by regulating the rates of proliferation, differentiation and death of constituent cells[1]. It is now clear that apoptosis is the physiological process by which cells die. Apoptosis occurs during embryonic development particularly in complex organs where a subpopulation of cells are selectively removed. For example, many germ cells die within the developing gonads during neonatal life[2]. In the adult, apoptosis can be detected particularly in tissues undergoing reversible expansion in hormone-dependent cells of the breast, prostate[3], and ovary[4] after trophic hormone removal.

The mammalian ovary provides a valuable model to study hormonal regulation of apoptosis because constant physiological removal of somatic and germ cells is characteristics of ovarian life cycle. At birth, mammalian ovaries are endowed with a fixed number of non-growing follicles that will be gradually recruited into growing pool during reproductive life. Once follicles start growth they are either selected for ovulation or, for the majority of them, removed by apoptosis[5]. Thus, removal of excess ovarian cells by apoptosis is necessary for normal development of ovary. Despite the important role of follicle atresia in the maintenance of normal follicle development, studies on the hormonal control of follicle cell demise during follicle growth have not been possible until recent development of apoptosis detection methods and *in vitro* follicle culture models[6], [7]. The present studies mainly summarize research performed recently in our laboratory using *in vivo* and *in vitro* follicle culture models.

Ⅱ. FOLLICLE ATRESIA DURING OVARIAN DEVELOPMENT

The major difficulty in investigating follicle atresia is probably due to the fact that incipient atresia is recognized only in retrospect, after the follicle has undergone distinct morphological changes. Hence, most of the early studies on atresia have been done in retrospect based on morphological and histochemical changes associated with atresia. Subsequently, these morphological studies have been coupled with endocrine and physiological parameters[8]. In order to circumvent the retrospect nature of studies on atresia, investigators have developed several models of induced atresia in attempt to obtain a population of follicles undergoing atresia synchronously.

Atresia can be induced in large antral follicles in the cyclic animals by preventing the preovulatory surge of gonadotropins with timed injections of pentobarbital on the day of proestrus[9], [10]. This model has permitted the study of the sequence of biochemical events that precede morphological signs of atresia including a decline in gonadotropin receptors and a shift from estrogen to progesterone production[11], [12]. A somewhat similar approach has been used to create a synchronous population of antral follicles, which are then induced to become atretic at a discrete point in their development. In this model, abrupt deprivation of gonadotropins has been induced by hypophysectomy on the morning of the day of proestrus or by treatment with

antibodies against pregnant mare's serum gonadotropin (PMSG) in PMSG-treated immature rats[13, 14, 15].

Studies utilizing models of experimentally induced atresia have indicated that gonadotropins rescue healthy follicles, and that estrogens protect follicles against atresia whereas androgens promote atresia[15]. However, the evaluation of atresia in these models is based on morphological signs which are subjective and time-consuming and, above all, it provides only semi-quantitative analysis. Recent demonstration of apoptotic cell death during follicle atresia[5, 16] has provided a method to circumvent these difficulties and to investigate hormonal control of follicle cell death.

Ⅲ. *IN VIVO* STUDIES ON FOLLICLE CELL DEMISE

Our laboratory has recently used *in vivo* apoptosis models to study hormonal regulation of follicle apoptosis. Treatment of immature hypophysectomized rats with estrogen results in the growth of a cohort of early antral follicles[17, 18]. Estrogen removal for two days causes a marked increase in apoptotic DNA fragmentation in granulosa cells[17]. The increased apoptosis can be blocked by treatment with FSH or estrogen[18]. The anti-apoptosis effect of estrogen is abolished by the addition of testosterone. These data suggest that gonadotropins and sex steroids play an important role in the regulation of ovarian apoptotic cell death with estrogens preventing apoptosis and androgens antagonizing the effect of estrogens. In addition, treatment with a GnRH agonist has been shown to increase follicle apoptosis directly, with or without co-treatment with FSH[17]. Indeed, the highest expression of GnRH receptor mRNA was recently demonstrated in atretic follicles[19], implying atretogenic feature of GnRH.

Ⅳ. *IN VITRO* APOPTOSIS MODELS

Studies on the hormonal regulation of follicle cell demise *in vivo* are difficult due to the presence of a heterogenous population of follicles in the ovary at any given time. Moreover, it is not possible to study the effect of individual intraovarian hormones on follicle cell death *in vivo*. In order to circumvent these difficulties, we have developed an *in vitro* follicle culture system to examine the hormonal regulation of apoptosis in a homogenous population of follicles.

1. Preovulatory follicle apoptosis

Preovulatory follicles (900 mm in diameter), obtained from immature rat ovaries primed for two days with PMSG, undergo spontaneous apoptosis when they were cultured in serum-free conditions (5% CO_2, 95% O_2) for 24 h. Using this model, we have found that treatment with LH /hCG or FSH suppresses apoptosis, underscoring the role of gonadotropins as follicle survival factors[7].

In addition to gonadotropins, it is well known that intraovarian hormones including growth factors, cytokines, and activins /inhibins are modulators of folliculogenesis and may be critical in determining the fate of follicles. Indeed, treatment with IGF-I, EGF, and bFGF suppresses the apoptosis of preovulatory follicles in a dose-dependent manner[7, 20], implying that these growth factors are necessary for follicle survival similar to gonadotropins. Interestingly, the suppressive effects of gonadotropins on follicle apoptosis are partially reversed by the addition of IGFBP-3, probably due to the sequestration of endogenous IGF-I[7], suggesting the mediatory role of IGF-I in gonadotropin action. Moreover,

treatment with growth hormone (GH) also suppresses apoptosis which is partially reversed by the co-treatment with IGFBP-3[21], implying the role of endogeneously produced IGF-I in GH action.

In cultured preovulatory follicles, Il-1βsuppresses apoptosis in a dose-dependent manner, and the addition of Il-1 receptor antagonist eliminates its effect, confirming receptor mediation[22]. Of interest, the suppressive effect of gonadotropins on follicle apoptosis is also partially blocked by the addition of Il-1 receptor antagonist, indicating the mediatory role of endogenous Il-1β. Because Il-1β has been shown to increase nitric oxide (NO) production in a number of tissues including the ovary[23], [24], we have also investigated the role of NO and its second messenger cGMP in the suppression of follicle cell apoptosis. Indeed, Il-1β increases NO production by cultured preovulatory follicles. Furthermore, an inhibitor of NO synthase, L-NMA (NG-monomethyl-L-arginine), causes a reversal of both Il-1β stimulation of NO production and suppression of apoptosis, suggesting a mediatory role of NO in these Il-1β effects. Similarly, sodium nitroprusside (SNP), an NO generator, and an analog of cGMP, the second messenger for NO, also suppress follicle apoptosis. Based mainly on studies using preovulatory follicle cultures, redundant pathways regulate the survival of follicles. Gonadotropins are the main survival factors by activating through the cAMP-dependent pathway to produce intraovarian hormones such as IGF-I, estrogens and IL-1. These factors, in turn, promote follicle survival through the activation of tyrosine phosphorylation, nuclear estrogen receptors, and the cGMP-dependent pathways, respectively.

2. Early antral follicle apoptosis

It is known that the majority of follicles undergo degeneration at the early antral follicle stage[25], suggesting that the penultimate stage of follicle development is most vulnerable to apoptosis. We have thus used an *in vitro* culture model of early antral follicles (350 μm in diameter) obtained from estrogen-treated immature rat ovaries to study the effect of endocrine and intraovarian factors on follicle apoptosis during this critical stage of follicle development.

FSH treatment suppresses apoptosis of early antral follicles as it does in preovulatory follicles[26], demonstrating the role of FSH as a follicle survival factor. In contrast to their suppressive effect on apoptosis of preovulatory follicles, treatment with LH /hCG, however, suppresses apoptosis in early antral follicles only marginally. These findings reflect the fact that LH /hCG receptors are restricted to theca cells in small antral follicles whereas only preovulatory follicles possess LH /hCG receptors in both the granulosa and theca cells[27].

Unlike in preovulatory follicles, intraovarian growth factors including IGF-I, EGF, and bFGF only have a minimal effect on the suppression of early antral follicle apoptosis. Binding studies demonstrate that the number of EGF receptors in preovulatory follicles is much higher than that of early antral follicles26, explaining the higher potency of EGF on the suppression of apoptosis in preovulatory follicles compared with early antral follicles. Thus, it is likely that follicles become more responsive to these intraovarian growth factors possibly through the acquisition of increasing numbers of receptors during follicular development. In contrast, GH is unable to suppress apoptosis of early antral follicles although it markedly increases IGF-I mRNA levels in these follicles[26]. Our findings suggest that local IGF-I induced by GH is sufficient to suppress apoptosis in preovulatory, but not in early antral follicles.

We have demonstrated that Il-1β also suppresses apoptosis in early antral follicles[26], but with a 3-fold higher dose as compared to preovulatory follicles. Because Il-1β believed to exert its action through theca cell receptors[28], the lower potency of Il-1β to suppress apoptosis in early antral follicles may be due to the presence of less differentiated theca cells in these follicles. In contrast, a NO generator (SNP) and an analog of its second messenger, cGMP, could suppress apoptosis of early antral follicles as effective as that of preovulatory follicles. These results indicate an important role of the NO/cGMP pathway in the suppression of follicle apoptosis independent of the stage of follicle growth.

Activin subunits have been found in the granulosa cells of growing follicles, and their expression increases with follicle size[29, 30]. It thus becomes apparent that locally produced activins may play autocrine and paracrine roles in folliculogenesis. The actions of activin have been shown to be suppressed by the activin-binding protein, follistatin, produced by granulosa cells of growing follicles in the rat ovary[31]. Because a recent study indicates that activin, in the presence of FSH, causes primary follicles to develop into large preovulatory follicles *in vitro*[32], we have examined the possible role of activin in follicle apoptosis. Activin causes a dose-dependent suppression of apoptotic DNA fragmentation, reaching a maximal suppression of 40%, in early antral follicles. Furthermore, co-incubation of activin with follistatin abolishes the anti-apoptotic effect of activin, implying that the action of activin is specific. Thus, activin is one of the follicle survival factors in early antral follicles.

We have recently studied the action of TNF-α on follicle apoptosis using *in vitro* culture of early antral follicles. The suppressive effect of FSH on follicle apoptosis is reversed by co-treatment with TNF-α in a dose-dependent manner, indicating that TNF-α is one of the intraovarian death mediators[33]. Because the apoptotic effect of TNF-α is coupled to the sphingomyelin signaling pathway with ceramide as a second messenger in various systems[34], we have tested the atretogenic action of ceramide. Ceramide analogue mimics the effect of TNF-α, and is able to completely abolish the suppressive action of FSH in follicle apoptosis whereas its isomer has been shown to be ineffective, verifying the specificity of ceramide action[33]. These studies suggest a potential role for TNF-a as an intraovarian regulator of follicle atresia by acting through the ceramide signaling pathway.

In vivo and *in vitro* studies on apoptosis in early antral follicles suggest several pathways causing follicle cell death. Because GnRH is known to increase intracellular levels of Ca^{++} and phosphatidylinositol turnover in rat granulosa cells[35, 36], induction of follicle apoptosis by GnRH may involve the activation of the protein kinase C pathway. In contrast to the anti-atretogenic action of estrogens, androgens cause follicle cell death through the activation of nuclear androgen receptors. Lastly, TNF-α enhances follicle apoptosis by acting through the ceramide signaling pathway.

3. Preantral follicle apoptosis

We have also studied hormonal regulation of apoptosis of preantral follicles (150 mm in diameter) obtained from 12-day-old rat ovaries. Preantral follicles are known to be relatively impervious to atresia under physiological conditions[25]. Neither gonadotropins nor intraovarian regulators suppress apoptosis in preantral follicles. We have, however, found that a cGMP analog is capable of suppressing preantral follicle apoptosis[37].

V. CONCLUDING REMARKS

Based on studies using *in vivo* and *in vitro* models of apoptosis, it becomes clear that the regulation of follicle apoptosis involves multifactorial regulatory mechanisms which are dependent upon the stage of follicular development as shown in Fig. 1. In highly differentiated preovulatory follicles, several hormones are equally effective at suppressing apoptosis. In early antral follicles, however, intraovarian hormones such as IGF-I, EGF, bFGF and Il-1 are less effective survival factors, and none of these factors are effective in preantral follicles. Thus, it is likely that follicles become more responsive to intraovarian hormones during follicle maturation, ensuring follicle survival.

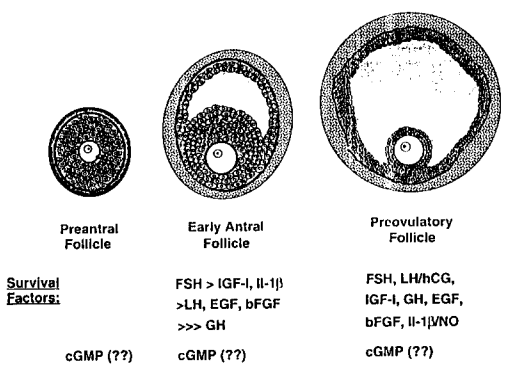

Fig. 1. Stage-dependent hormonal regulation of apoptosis during follicular development. The redundant pathways regulate the survival of preovulatory and early antral follicles. In preovulatory follicles, gonadotropins, growth hormone (GH), several growth factors (IGF-I, EGF, and bFGF) and a cytokine (Il-1β) appear to be equally effective to suppress apoptosis. In contrast, FSH is a major survival factor for early antral follicles whereas growth factors and Il-1β are less effective. In preantral follicles, none of these factors are effective. Treatment with cGMP rescues follicles regardless of follicle size.

Interestingly, cGMP is a survival factor at all stages of follicle growth including preantral, early antral, and preovulatory follicles. A hypothetical model for the putative factors that control cGMP levels is depicted in Figure 2. Receptor-mediated action of Il-1, as shown in the present studies, and other unknown factors may increase cGMP levels through the activation of nitric oxide synthase (NOS) that stimulates ni-

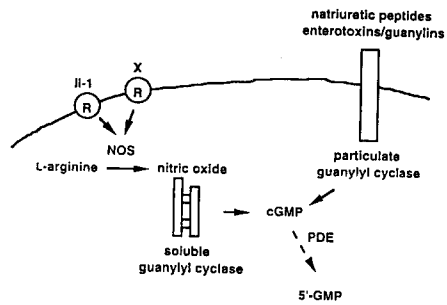

Fig. 2. A hypothetical model for the regulation of intracellular levels of cGMP in granulosa cells of ovarian follicles required to suppress apoptosis. Interleukin-1 (Il-1) and other unknown factors (X) may increase the production of nitric oxide from L-arginine by stimulating nitric oxide synthase (NOS). Nitric oxide, in turn, increases the activity of a soluble guanylyl cyclase, leading to the formation of cGMP. Through an independent pathway, natriuretic peptides including atrial, brain, and C-type natriuretic peptides or enterotoxins/guanylins bind and activate particulate guanylyl cyclase receptors that increase the formation of cGMP. Intracellular levels of cGMP may also be regulated by cGMP-specific phosphodiesterase (PDE) that converts cGMP to inactive 5′-GMP.

tric oxide (NO) production[38]. Alternatively, the natriuretic peptide family and heat-stable enterotoxins/guanylins activate membrane forms of guanylyl cyclase receptors[39], resulting in the enhanced production of cGMP in granulosa cells. Finally, intracellular levels of cGMP may also be regulated by cGMP-specific phosphodiesterase (PDE) that converts cGMP to inactive 5′-GMP[40].

Future analysis of the hormonal regulation of ovarian follicle apoptosis should provide a better understanding of the molecular process underlying ovarian cell demise as well as improved treatment protocols for the management of pathological conditions involving excessive follicle cell degeneration such as premature ovarian failure and polycystic ovarian syndrome.

Ⅵ. REFERENCES

1. Thompson, C. B. 1995. Apoptosis in the pathogenesis and treatment of disease. Science, 267, 1456-62.
2. Ioannou, J. M. 1964. Oogenesis in the guinea pig. J. Embryol. Exp. Morphol., 12, 673-91.
3. Collins, M. L. and Rivas, A. L. 1993. The control of apoptosis in mammalian cells. TIBS., 18, 307-9.
4. Hsueh, A. J. W., Eisenhauer, K. M., Chun, S-Y., Hsu, S-Y. and Billig, H. 1996. Gonadal cell apoptosis. Recent Prog. Hormone Res., 52, 453-58.
5. Hsueh, A. J. W., Billig, H. and Tsafriri, A. 1994. Ovarian follicle atresia: a hormonally controlled apoptotic process. Endocrine. Rev., 15, 1-18.
6. Tilly, J. T. and Hsueh, A. J. W. 1993. A microscale autoradiographic method for the qualitative and quantitative analysis of apoptotic DNA fragmentation. J. Cell. Physiol., 154, 519-26.
7. Chun, S-Y., Billig, H., Tilly, J. L., Furuta, I., Tsafriri, A. and Hsueh, A. J. W. 1994. Gonadotropin suppression of apoptosis in cultured preovulatory follicles: mediatory role of endogenous insulin-like growth factor I. Endocrinology, 135, 1845-53.
8. Tsafriri, A. and Braw, R. H. 1984. Experimental approaches to atresia in mammals. Oxf. Rev. Reprod., 6, 226-65.
9. Braw, R. H. and Tsafriri, A. 1980. Follicles explanted from pentobarbitione-treated rats provide a model for atresia. J. Reprod. Fertil., 59, 267-72.
10. Teerranova, P. F. 1980. Effect of phenobarbital-induced ovulatory delay on the follicular population and serum levels of steroids and gonadotropins in the hamster: a model for atresia. Biol. Reprod., 23, 93-9.
11. Uilenbroek, J. Th. J., Woutersen, P. J. A. and van der Schoot, P. 1980. Atresia of preovulatory follicles: gonadotropin binding and steroidogenic activity. Biol. Reprod., 23, 219-29.
12. Terranova, P. F. 1981. Steroidogenesis in experimentally induced atretic follicles of the hamster: a shift from estradiol to progesterone synthesis. Endocrinology, 108, 1885-90.
13. Braw, R. H., Bar-Ami, S. and Tsafriri, A. 1981. Effect of hypophysectomy on atresia of rat preovulatory follicles. Biol. Reprod., 25, 989-96.
14. Hubbard, C. J. and Greenwald, G. S. 1983. *In vitro* effects of luteinizing hormone on induced atretic Graafian Follicles in the hamster. Biol. Reprod., 28, 849-59.
15. Bagnell, C. A., Mills, T. M., Costoff, A. and Mahesh, V. B. 1982. A model for the study of androgen effects on follicular atresia and ovulation. Biol. Reprod., 27, 903-14.
16. Hughes, F. M. and Gorospe, W. C. 1991. Biochemical identification of apoptosis (pro-

grammed cell death) in granulosa cells: evidence for potential mechanism underlying follicular atresia. Endocrinology, 129, 2415-22.

17. Billig, H., Furuta, I. and Hsueh, A. J. W. 1994. Gonadotropin-releasing hormone directly induces apoptotic cell death in the rat ovary: biochemical and *in situ* detection of deoxy-ribonucleic acid fragmentation in granulosa cells. Endocrinology, 134, 245-52.
18. Billig, H., Furuta, I. and Hsueh, A. J. W. 1994. Estrogens inhibit and androgens enhance ovarian granulosa cell apoptosis. Endocrinology, 133, 2204-12.
19. Whitelaw, P. F., Eidne, K. A., Seller, R., Smyth, C. D. and Hillier, S. G. 1995. Gonadotropin-releasing hormone receptor messenger ribonucleic acid expression in rat ovary. Endocrinology, 136, 172-79.
20. Tilly, J. L., Billig, H., Kowalski, K. I. and Hsueh, A. J. W. 1992. Epidermal growth factor and basic fibroblast growth factor suppress the spontaneous onset of apoptosis in cultured rat granulosa cells and follicles by a tyrosine kinase-dependent mechanism. Mol. Endocrinol., 6, 1942-50.
21. Eisenhauer, K. M., Chun, S-Y., Billig, H. and Hsueh, A. J. W. 1995. Growth hormone suppression of ovarian follicle apoptosis and partial neutralization by insulin-like growth factor binding protein (IGFBP). Biol. Reprod., 53, 13-20.
22. Chun, S-Y., Eisenhauer, K. M., Kubo, M. and Hsueh, A. J. W. 1995. Interleukin-1b suppresses apoptosis in rat ovarian follicles by increasing nitric oxide production. Endocrinology, 136, 3120-7.
23. Ellman, C., Corbett, J. A., Misko, T. P., McDaniel, M. and Beckerman, K. P. 1993. Nitric oxide mediates interleukin-1-induced cellular cytotoxicity in the rat ovary: a potential role for nitric oxide in the ovulatory process. J. Clin. Invest., 92, 3053-56.
24. Ben-Shlomo, I., Adashi, E. Y. and Payne, D. W. 1994. The morphogenic /cytotoxic and prostaglandin-stimulating activities of interleukin-1b in the rat ovary are nitric oxide independent. J. Clin. Invest., 94, 1463-9.
25. Hirshfield, A. N. 1988. Size-frequency analysis of atresia in cycling rats. Biol. Reprod., 38, 1181-8.
26. Chun, S-Y., Eisenhauer, K. M., Minami, S., Billig, H., Perlas, E. and Hsueh, A. J. W. 1996. Hormonal regulation of apoptosis in early antral follicles: follicle-stimulating hormone as a major survival factor. Endocrinology, 137, 1447-56.
27. Camp, T. A., Rahal, J. O. and Mayo, K. E. 1991. Cellular localization and hormonal regulation of follicle-stimulating hormone and lutienizing hormone receptor messenger RNAs in the rat ovary. Mol. Endocrinol., 5, 1405-17.
28. Hurwitz, A., Ricciarelli, E., Botero, L., Katz, E., McAllister, J. M., Garcia, J. E., Loukides, J., Rohan, R. M., Adashi, E. Y. and Hernandez, E. R. 1991. The human intraovarian interleukin-1 Il-1 system: highly-compartmentalized and hormonally-dependent regulation of the genes uncoding Il-1, its receptor, and its receptor antagonist. J. Clin. Invest., 89, 1746-54.
29. Mayo, K. E. 1994. Inhibin and activin: molecular aspects of regulation and function. Trends. Endocrinol. Metab., 5, 407-15.
30. Meunier, H,. Roberts, V. J., Sawchenko, P. E., Cajander, S. B., Hsueh, A. J. W. and Vale, W. W. 1989. Periovulatory changes in the expression of inhibin a-, bA-, and bB-subunits in hormonally induced immature female rats. Mol. Endocrinol., 3, 2062-9.
31. Nakatani, A., Shimasaki, S., DePaolo, L. V.,

Erickson, G. F. and Ling, N. 1991. Cyclic changes in follistatin messenger ribonucleic acid and its protein in the rat ovary during the estrous cycle. Endocrinology, 129, 603-11.

32. Li, R., Phillips, D. M. and Mather, J. P. 1995. Activin promotes ovarian follicle development *in vitro*. Endocrinology, 136, 849-56.
33. Kaipia, A., Chun, S-Y., Eisenhauer, K. M. and Hsueh, A. J. W. 1996. Tumor necrosis factor-a and its second messenger, ceramide, stimulate apoptosis in cultured ovarian follicles. Endocrinology, 137, November; in press.
34. Jarvis, W. D., Kolesnik, R. N., Fornari, F. A., Traylor R. S., Gewirtz, D. A. and Grant, S. 1993. Induction of apoptotic DNA damage and cell death by activation of the sphingomylin pathway. Proc. Natl. Acad. Sci. USA., 91, 73-7.
35. Naor, Z. and Yavin, E. 1982. Gonadotropin-releasing hormone stimulates phospholipid labeling in cultured granulosa cells. Endocrinology, 111, 1615-9.
36. Wang, J., Steele, G. L., Baimgridge, K. G., Rodway, M. R. and Leung, P. C. 1992. Intracellular calcium and the signaling mechanism of luteinizing hormone-releasing hormone in rat granulosa cells. Am. J. Obstet. Gynecol., 167, 541-7.
37. Minami, S., Chun, S-Y., Eisenhauer, K. M. and Hsueh, A. J. W. 1996. Suppression of apoptosis in cultured preantral follicles following activation of the cGMP but not the cAMP pathway: gonadotropins are not survival factors during early follicle development. Presented at the 10th International Congress of Endocrinology, July 12-14, San Francisco, USA
38. Drewett, J. G. and Garbers, D. L. 1994. The family of guanylyl cyclase receptors and their ligands. Endocrine Rev., 15, 135-62.
39. Garbers, D. L. and Lowe, D. G. 1994. Guanylyl cyclase receptors. J. Biol. Chem., 269, 30741-4.
40. Conti, M., Nemoz, G., Sette, C. and Vicini, E. 1995. recent progress in understanding the hormonal regulation of phosphodiesterases. Endocrine Rev., 16, 370-89.