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Histological Profiles of Nuclear Degeneration of Oocytes in Mouse Ovaries and Possible Roles of Follicle Stimulating Hormone for Nuclear Degeneration of Oocytes in the Atretic Follicles

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Abstract: The pattern of nuclear degeneration of oocytes and the effect of follicle stimulating hormone (FSH) on the profile of nuclear degeneration of oocytes in the follicles at the stage of Type 4, 5, 6 or 7 was studied. Mouse ovaries were examined by serial histological sections. Nuclear degeneration of oocytes was found among follicles at Type 4–7 stage of development and the pattern of nuclear degeneration was strongly correlated with the developmental stage of follicule or oocyte. Nuclear degeneration of oocytes was observed in 52.2% of Type 4–5 (preantral) follicles and 39.2% of Type 6–7 (antral) follicles. In the Type 4–5 follicles, degeneration, such as pycnotic changes and disappearance of chromosomes, was dominant. In Type 6–7 follicles, however, about 80% of oocytes induced pseudo-maturation division during the degenerating processes. Pseudo-maturation was observed in the oocytes more than 51 μm in diameter. The administration of FSH significantly increased the number of Type 6–7 follicles, but not Type 4–5 follicles, and decreased the number of both Type 4–5 and Type 6–7 follicles with degenerating oocytes. A large number of macrophages were identified immunohistochemically in the interstitial tissue around the follicles. Macrophages make a cluster around the atretic follicles, but were not identified inside of the follicles containing degenerating oocytes. These results suggest that the pattern of oocyte degeneration depends on the developmental stage of follicles and oocytes, and that FSH accelerates the process of degeneration of oocytes, and that macrophages are involved in the process of removal of degenerating oocytes.

Key words: Atresia, Follicle, Oocytes, Pseudo-maturation, Pycnosis, FSH.

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In oocytes of atretic follicles, degeneration of nuclei and pseudo-maturation division, such as germinal vesicle breakdown, chromosomes at metaphase, or expulsion of a polar body are observed [1]. These degenerative changes in the nucleus of oocytes are followed by fragmentation and disappearance of chromosomes and then finally degenerated oocytes are removed from follicles by phagocytic activity of granulosa cells [1–4]. Although morphology of the degenerating oocytes in mouse ovaries has been reported, the "pattern of degeneration" in relation to the size of oocytes and different stages of follicular development has not yet been clarified.

The induction of superovulation with pregnant mare's serum gonadotropin (PMSG) in mice is a well established and widely used procedure. Whether this effect of PMSG results from recruitment of follicles from the non-growing pool or from a decrease in the rate of atresia is apparently specific to the species. In immature mice, administration of PMSG decreased the number of large atretic follicles and therefore it has been suggested that the action of gonadotropin is prevention of the atretic process [5, 6]. On the other hand, in the ovary of the cyclic hamster PMSG not only decreases follicular atresia but also recruits "reserve" follicles [7]. But the morphological profiles of the action of follicle stimulating hormone (FSH)/PMSG in follicular recruitment and atresia remain

to be determined.

In higher organisms, cell death can be classified into two different categories: necrosis, which occurs as a result of massive tissue damage; and apoptosis, which is a process of active cellular self destruction [8]. Several authors have speculated whether compounds released during necrosis and apoptosis activate or attract macrophages or induce phagocytosis in neighbouring cells [9]. Potentially stimulating proteins and enzymes would certainly be released in degenerating cells, because degenerated cells are frequently digested by macrophages [9]. This evidence leads to the idea that macrophages are involved in processes in the degeneration of oocytes.

The purpose of this paper was (1) to determine whether a pattern of oocyte degeneration is dependent on type of the follicle or size of the oocyte, (2) to clarifiy the effect of FSH on the degeneration of oocytes, and (3) to identify distribution or density of macrophages around the follicles including degenerating oocytes.

Materials and Methods

Animals: ICR mice 25–27 days of age were used in the present study. They were given a commercial diet (Oriental Kobo MF, Oriental Co., Tokyo) *ad libitum* and maintained at 22–24°C in a 13L:11D photoperiod. The middle of the dark period was set at midnight.

Light microscopy: Ten of the animals were divided into two groups and injected intraperitoneally (ip) with saline or 2.5 IU of FSH (human, Sigma, St. Louis, Mo.). They were sacrificed at 48 hr after the saline or FSH injection. The ovaries were fixed in Bouin's solution for 24 hr, dehydrated in a graded series of ethanol, embedded in paraffin, and sectioned serially at 4 μm in the horizontal plane. Serial sections were deparaffinized, and stained with hematoxylin-eosin or colloidal iron. A classification modified from that of Pedersen and Peters [10] was used to describe stages of follicle development. For each stage of follicular development (i.e. type of follicle) the pattern of degeneration was determined.

Immunohistochemistry: Ovaries from mice at 48 hr after FSH administration were fixed with 10% formalin for 24 hr, dehydrated in a graded series of ethanol, embedded in paraffin, and sectioned serially at 4 μm in the horizontal plane. Serial sections were deparaffinized, incubated in phosphate buffered saline (PBS) and blocked endo-peroxidase with 0.3% H_2O_2/methanol for 30 min at room temperature. After rinsing with PBS, the sections were incubated with normal goat serum for 20 min at room temperature. The medium was removed, replaced with anti-macrophage monoclonal antibody (Oncogene Science Inc., CA) and incubated overnight at 4°C. The sections were then processed by the avidin-biotin immunoperoxidase procedure (Vector Lab., Burlingame, CA) with 0.5% diaminobenzidine and 0.03% H_2O_2 as the substrate. The sections were then rinsed, dehydrated and protected with cover slips for microscopic examination.

Results

Nuclear degeneration of oocytes in the follicle in stages 1 through 3 was not identified clearly under the light microscope, and it was impossible to identify the stage of development of follicles in the advanced stage of atresia. Therefore, the pattern of nuclear degeneration of oocytes was determined in Type 4–5 (preantral) or Type 6–7 (antral) follicles.

Profiles of degenerating oocytes related to the stage of follicular development: In most Type 4–7 follicles, the oocyte had a centrally located nucleus at the resting stage of the prophase (Fig. 1); pycnotic nuclei were absent, the zona pellucida was intact, mitotic figures were seen in the granulosa layer, and the follicular fluid was "clean", i.e. without cell debris or leukocytes, and the theca interna consisted of a few concentric layers of elongated cells with a rich bed of capillaries. The number of follicles recognized as Type 4, 5, 6 or 7 was 335.0+12.8 per ovary, and about 29.0% of these follicles included oocytes with a degenerated nucleus (Fig. 2). The percentage of follicles with a degenerated nucleus was smaller in Types 6–7 than in Types 4–5. Most of the oocytes with degenerated nuclei in Type 4–5 follicles showed pycnosis or disappearance of chromosomes in the serial sections of the oocytes (Fig. 3). In Type 6–7 follicles, pseudo-maturation division (progression to diakinesis, 1st and 2nd metaphase, 1st anaphase), activation and pycnotic changes were observed (Fig. 4). The dominant stage of the pseudo-maturation division was the first or second metaphase. About 3% of oocytes did not possess chromosomes even though the serial sections were examined.

After FSH treatment, the number of Type 4–5 follicles was not changed, but that of Type 6–7 follicles and the total number of Type 4–7 follicles increased (Fig. 2). The number of oocytes with a degenerating nucleus decreased significantly after the administration of FSH in Type 4–5 and 6–7 follicles.

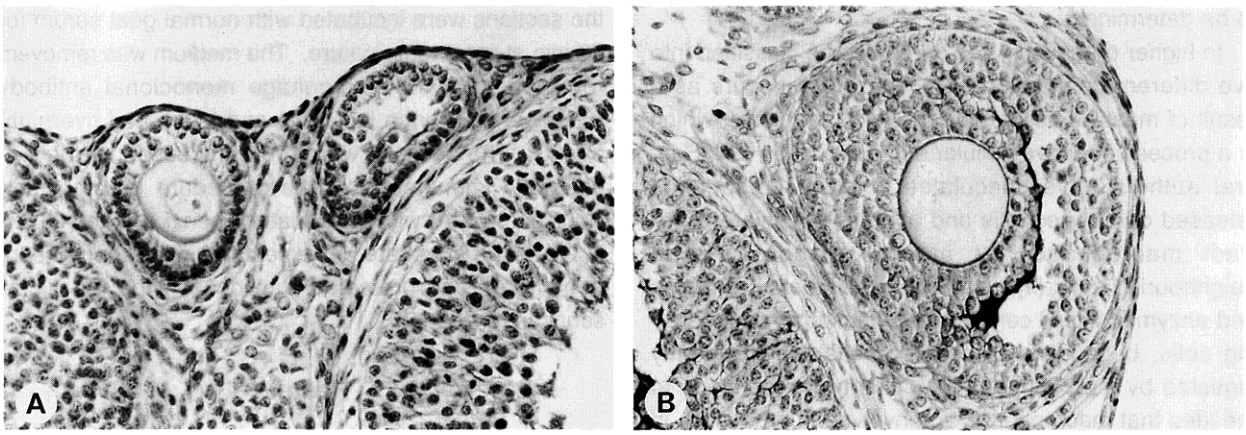

Fig. 1. Intact oocytes with germinal vesicle in Type 5 (A) and Type 6 (B) follicles. × 200.

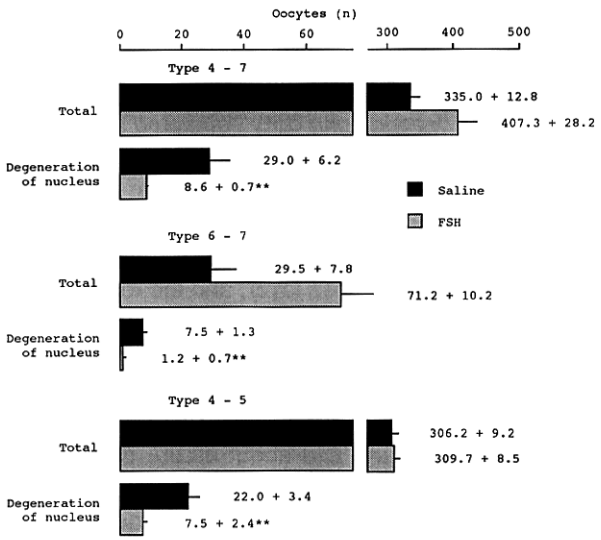

Fig. 2. The classification of follicles and the proportion of oocytes containing intact germinal vesicles and a degenerated nucleus in the mouse ovary, and the effect of FSH on the number of oocytes with a degenerated nucleus.

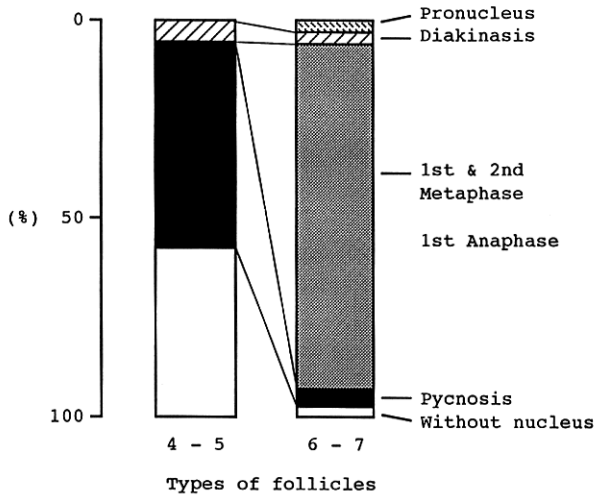

Fig. 3. The proportion of oocytes showing each type of sign of degeneration in relation to the types of follicles in mouse ovary.

Profiles of degenerating oocytes related to the stage of oocyte development: To clarify the relationship between oocyte size and degenerative changes, degenerating oocytes in Type 4–7 follicles were classified according to the diameter of the oocytes (Fig. 5). Most of the oocytes ranging from 61 to 70 μm in diameter showed pseudo-maturation in the first metaphase, the first anaphase or the second metaphase. The number of oocytes with pycnotic changes was small. A very small number of oocytes (3.5%) were activated and possessed pronucleus-like structures. None of the oocytes with a diameter less than 50 μm had meiosis-like changes such as chromosomes in the metaphase or a polar body. Pycnotic changes and the disappearance of chromosomes are dominant in oocytes with a diameter less than 60 μm.

Distribution of macrophages: Fig. 6 shows cells stained with macrophage antibody. Macrophages were identified in the structually interstitial tissue, especially around the follicles (Fig. 6A). The intensity of immunostaining of the macrophage was greatly reduced when the anti-macrophage monoclonal antibody was

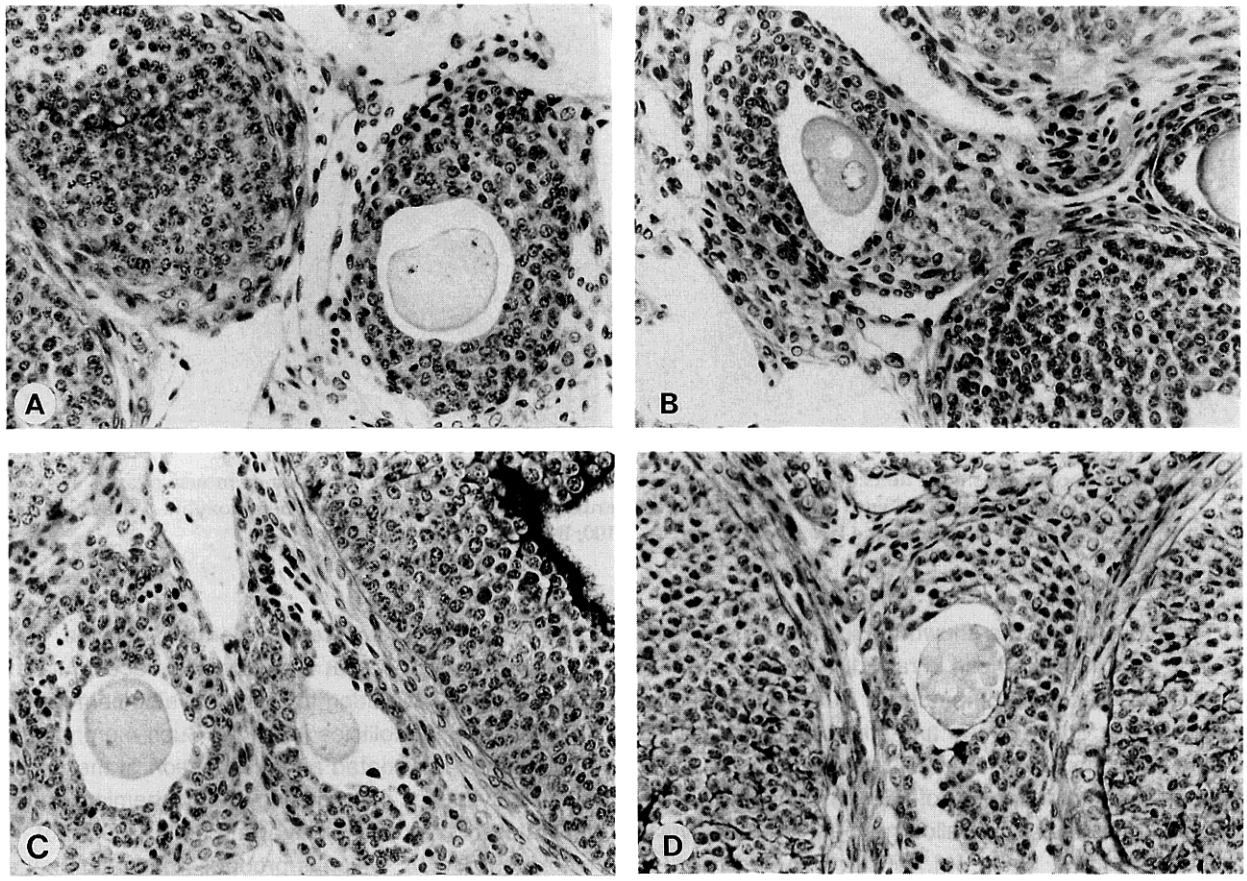

Fig. 4. Histological profile of nuclear degeneration of oocytes. (A) pseudo-maturation, (B) pronucleus (activation), (C) pycnosis, (D) fragmentation. × 200.

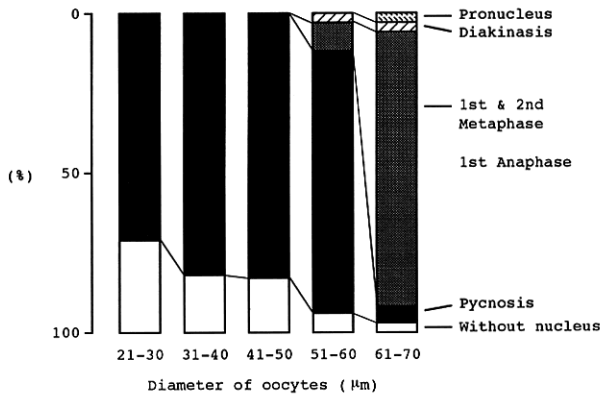

Fig. 5. The proportion of each type showing signs of degeneration in relation to the diameter of the oocyte cytoplasm in mouse ovary.

replaced by normal mouse ascites. Strong positive staining and a large number of macrophages were observed around the follicles with degenerating oocytes (Fig. 6, A and B).

Discussion

It is well known that pycnosis and pseudo-maturation are observed in the atretic follicles [1]. In this study, we clarified that the pattern of nuclear degeneration of oocytes was strongly correlated with the stage of follicular and oocyte development. In the oocytes more than 51 μm in diameter in Type 6–7 follicles, degenerating oocytes showed pseudo-maturation division. In Type 4–5 follicles, pycnotic changes (condensation of chromosomes) and disappearance of chromosomes were dominant.

The administration of FSH did not increase the total number of Type 4–5 follicles, but increased the number

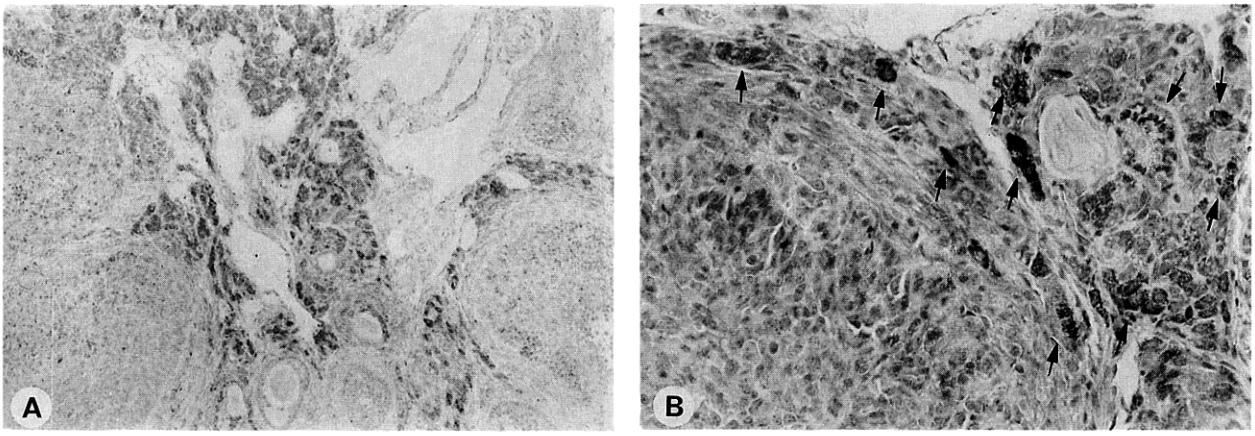

Fig. 6. Immunohistochemical detection of macrophages in a section of mouse ovary. Staining of mouse ovaries at 48 hrs after FSH administration with anti-macrophage antiserum. Nuclei were stained with hematoxylin. Arrows indicate the macrophages with strong positive staining. A, × 100; B, × 200.

of Type 6–7 follicles as shown in Fig. 1. These observations coincide with those in rats [11]. Moreover, FSH significantly decreased the number of follicles including degenerating oocytes. An anti-atretic effect of PMSG on the large mouse follicles has been reported [5, 6]. It was inferred that the surge of FSH rescues the Type 5 follicles from atresia, thus allowing them to reach ovulation in the next cycle [1]. Tsafriri and Braw [1] demonstrated an effect of PMSG in rescuing immature rat follicles of Types 5 and 6 from atresia. Estrogen treatment prevents follicular atresia in hypophysectomized immature female rats [12] and it is possible that FSH and PMSG exert their anti-atretic action through the stimulation of follicular estrogen production.

A small fraction of the FSH binding was on atretic follicles while the overwhelming majority of FSH-binding at all stages of the estrous cycle was on healthy follicles [13]. Byskov [13] suggested that PMSG induces the granulosa cells of atretic follicles to phagocytose the dying cells and thus rescues the follicles at an early stage of atresia. If PMSG/FSH rescues the Type 4–5 follicles including degenerating oocytes and stimulates the development of follicles, the number of Type 6–7 follicles with degenerating oocytes should increase. In this study, however, we clarify that the number of Type 4–5 and 6–7 follicles with degenerating oocytes after FSH administration was smaller than that of the control, and it was reported that PMSG/FSH does not stimulate the development of atretic follicles [1]. It is therefore possible that FSH hastens the degeneration of atretic follicles. It is also speculated that cytokines produced by macrophages influence the phagocytotic activity of granulosa cells to remove degenerating oocytes.

It was reported that macrophages accumulate in perifollicular sites during follicular development and demise of Graafian follicles [15–17]. Such morphological findings have prompted an investigation of the roles of specific cytokines in regulating the biochemical and differentiated function of ovarian cells. One of the cytokines, TNF-α, which is produced by macrophages, has been shown to be present in the mouse ovary [18]. In our previous study, a significant immunostaining of TNF-α was identified in oocytes of atretic follicles. Oocytes showing positive immunostaining of TNF-α had some characteristics of degeneration [18]. TNF-α has been shown to participate in the regulation of cellular growth [19–21], and exerts potent necrotic effects on transplantable tumors *in vivo* [22]. Strong immunoreactive TNF-α was detected in the shrunken oocytes in the atretic follicles, suggesting that TNF-α was expressed during the degenerative process and/or accumulated inside the oocytes [18]. In this study, a number of macrophages were observed around the follicles with degenerating oocytes, suggesting the possibility that macrophages secrete TNF-α which binds to oocytes to stimulate the process of degeneration.

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