Follicular atresia in the infant human ovary

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Summary. The pattern of follicular atresia was studied in nine ovaries from children between the ages 3 months and 8 years. Atretic follicles were found among follicles at all stages of development. The percentage of follicles with signs of atresia became larger as the size of the follicles increased. Only 2% of small follicles (Type 3b) showed signs of atresia, while all follicles >1 mm in diameter (Type 8) were atretic. In follicles of Type 5 and larger, four stages of atresia, which represent consecutive stages of a single atretic process, were defined. The beginning of atresia was characterized by the presence of pyknotic granulosa cells. As atresia progressed, the granulosa layer disappeared, the oocyte became necrotic, the follicle collapsed and the theca cells became hypertrophied.

The oocyte can degenerate in several ways: it can be penetrated by cells, the nucleus can become pyknotic or it may complete meiotic prophase. It is suggested that the last event is only possible after the oocyte has reached its full size and has completed RNA synthesis.

Introduction

During the infant period many follicles start to grow in the human ovary but none of them reaches preovulatory size (Sauramo, 1954; Kraus & Neubecker, 1962; Lintern-Moore *et al.*, 1974). At different stages during growth they become atretic and disappear.

In previous studies (Stevens, 1903; Shaw, 1925; Allen *et al.*, 1930; Watzka, 1957; Baker, 1963; Valdes-Dapena, 1967), the morphology of atretic follicles in the infant human ovary has been described, but the 'pattern of atresia', i.e. the sequence of changes within a follicle that becomes atretic, and the proportions of atretic follicles found within each follicle type was not studied.

The purpose of this paper was (1) to define morphologically the stages a follicle passes through as it becomes atretic, (2) to determine whether a uniform pattern of atresia occurs among different types of follicles, and (3) whether atresia attacks with the same frequency at all stages of follicle development.

Materials and Methods

Ovaries were obtained at autopsy from nine children aged 3 months to 8 years, who died either from misadventure or acute disease. The ovaries were fixed in Bouin's solution and embedded in paraffin wax. At least 100 serial sections at 7 μ m were obtained and stained with Harris' haematoxylin and eosin or Heidenhain's Azan. Microscopic examination was done under a Zeiss projection microscope which made the counting of follicles and cells in these large sections possible.

A classification modified from that of Lintern-Moore *et al.* (1974) was used to describe stages of follicle development. A distinction was made between follicles without signs of atresia and atretic follicles. The stages of atresia were defined, and for each stage of follicular development (i.e. type of follicle) the proportion of atretic follicles was determined (Table 1).

Since the number of developing follicles, especially of the large ones (Types 6–8), in any one ovary was small, the follicles at each stage of development from the 18 ovaries were considered together and used to compile the tables.

Results

All the ovaries contained follicles of various sizes that were not atretic as well as those in different stages of atresia. Atretic follicles were of Type 3b and larger.

Follicles of Types 3b and 4

These follicles consisted of an oocyte, in the resting stage of prophase (diplotene), surrounded by one or two layers of granulosa cells (Pl. 1, Fig. 1). In Type 3b follicles the zona pellucida was not completely formed, while Type 4 follicles had a complete and intact zona pellucida.

Few follicles with signs of atresia were seen in this group: 2% of Type 3b and 13% of Type 4 (Table 1). Necrosis of the oocyte (eosinophilia of the cytoplasm, contraction of chromatin material, wrinkling of nuclear membrane) was the only sign of atresia seen in Type 3b follicles. In contrast, three signs of atresia were noted in Type 4 follicles: (1) presence of cells within the oocyte; (2) necrotic oocytes; and (3) presence of pyknotic nuclei among the granulosa cells. The most frequent sign of atresia was the presence of cells inside the oocyte (Pl. 1, Fig. 2), and cells have been seen penetrating the zona pellucida (Pl. 1, Fig. 3), although the granulosa cells of such follicles were not always pyknotic. The penetrating cells were indistinguishable from granulosa cells and lay within the cytoplasm. In advanced stages of atresia more than fifteen cells were found inside the zona pellucida and only a small rest of the oocyte remained. A necrotic oocyte was often the only sign of atresia in follicles of Type 4. Pyknotic granulosa cell nuclei were found in follicles without any other sign of atresia as well as in follicles with the signs of atresia as described above.

Table 1. The classification of follicles in the infant human ovary (modified from that of Lintern-Moore *et al.*, 1974) and the proportions of each type showing signs of atresia

Follicle type	No. of granulosa cells/largest cross-section	Follicular diameter (µm)	Atretic follicles/total no. of follicles	
			No.	%
Resting follicle				
2	1-10	26-40	—	—
3a	8-14	26-40	—	—
Growing follicle				
3b	15-25	40-50	7/323	2
4	26-100	50-100	114/890	13
5	101-300	100-200	86/152	57
6	301-500	200-500	17/32	53
7	501-1000	500-1000	15/17	88
8	>1000	1000-6000	17/17	100

Follicles of Type 5 and larger

In non-atretic follicles (Pl. 1, Fig. 4), the oocyte had a centrally located nucleus in the resting stage of prophase; the zona pellucida was intact; mitotic figures were seen in the granulosa layer; pyknotic

EXPLANATION OF PLATE 1

Fig. 1. A Type 4 non-atretic follicle. The nucleus is in the resting stage of prophase, the zona pellucida is intact, and there are no pyknotic granulosa cells. H & E, ×650.

Fig. 2. A Type 4 follicle with cells inside the oocyte. H & E, ×650.

Fig. 3. Part of a follicle with a cell penetrating the zona pellucida (arrow). H & E, ×1000.

Fig. 4. A Type 7 non-atretic follicle. The oocyte has a centrally located nucleus in the resting stage of prophase. Pyknotic granulosa cells are absent. There are no leucocytes or cell debris in the follicular fluid. H & E, ×160.

Fig. 5. A Type 7 follicle in Stage I of atresia. The follicular fluid contains cell debris (arrows). H & E, ×40.

Fig. 6. An oocyte of a Type 7 follicle being penetrated by cells. H & E, ×400.

PLATE I

(Facing p. 56)

PLATE 2

nuclei were absent; a follicular cavity was present in all follicles of Type 6 and larger; the follicle fluid was 'clean', i.e. without cell debris or leucocytes; and the theca interna consisted of a few concentric layers of elongated cells with a rich bed of capillaries.

Considerably more atretic follicles were seen in these larger follicles than among Type 3b and 4 follicles (Table 1). Four stages of atresia were defined. Only the follicles in Stages I and II of atresia were used to calculate the percentage of follicles with signs of atresia, as the follicle type could not be recognized when Stages III and IV were reached.

Stage I (Pl. 1, Fig. 5). The oocyte of these follicles had a centrally located nucleus in diplotene and 40% of them had cells inside the zona pellucida (Pl. 1, Fig. 6). 1–10% of the nuclei in the granulosa layer were pyknotic, but mitotic figures could also still be seen. An antrum was always present and the follicular fluid contained some cell debris but no leucocytes. The theca interna was similar to that of the non-atretic follicles.

Stage II (Pl. 2, Fig. 7). The nucleus of the oocyte when present was located at the periphery of the cytoplasm and often maturation division had started in follicles of Type 6 and larger. Few of the oocytes were pyknotic. Cells inside the zona pellucida were seen in 10% of the follicles. 5–20% of the granulosa cell nuclei were pyknotic and no mitotic figures were seen. The granulosa layer was uneven in thickness and in places cells had disappeared, leaving the basement membrane naked (Pl. 2, Fig. 8). Follicular fluid was always present and contained cell debris and leucocytes. Fibroblasts had invaded the theca interna.

Stage III (Pl. 2, Fig. 9). The oocyte in these follicles was necrotic and the nucleus had disappeared, although in some a polar body had formed. No cells were seen inside the oocyte. Granulosa cells were only present around the oocyte. Mitotic figures were not seen and about 5% of the remaining granulosa cells were pyknotic. In the follicular fluid only occasional leucocytes were found. Invasion of the fibroblasts into the cavity was seen in some places. The theca interna was hypertrophied and contained many fibroblasts and collagen fibres. Follicles began to collapse at this stage.

Stage IV (Pl. 2, Fig. 10). Fragments of the oocyte could sometimes be recognized, but in most follicles the oocyte had disappeared. In the early Stage IV atresia follicular fluid was still present and contained freely floating, often necrotic, granulosa cells. No leucocytes were seen. As atresia progressed the follicles either collapsed, leaving an irregular lumen, or the cavity became filled with cells and fibres of connective tissue, particularly near the basement membrane. Cells of the theca interna were hypertrophied and arranged in irregular short columns radiating outward from the margin of the contracting antrum. They were separated by fibroblasts forming radial bands between the columns.

Discussion

All ovaries contained follicles at different stages of development, that were healthy as well as atretic. They were 'actively growing' ovaries as seen in normal children (Peters *et al.*, 1975).

The present findings show that follicular atresia in the infant human ovary may occur at any time during follicular development. It confirms the previous observations of Stevens (1903), Allen *et al.* (1930) and Baker (1963). The percentage of follicles with signs of atresia in the human ovary becomes larger as the size of the follicles increases. All the follicles became atretic by the time they

EXPLANATION OF PLATE 2

Fig. 7. Part of a Type 8 follicle in Stage II of atresia. The follicular fluid contains much cell debris and leucocytes. H & E, $\times 60$.

Fig. 8. Stage II of atresia in a Type 8 follicle showing the absence of granulosa cells in places. H & E, $\times 230$.

Fig. 9. Follicle in Stage III of atresia. The granulosa cells are present only around the necrotic oocyte. The follicular fluid contains cell debris. The theca interna is hypertrophied, and the follicle is beginning to collapse. H & E, $\times 60$.

Fig. 10. Follicle in Stage IV of atresia. The oocyte and nearly all granulosa cells have disappeared. In the area of the basement membrane collagen fibres are accumulated—forming the 'glassy membrane' (arrows). The theca interna cells are hypertrophied and are separated by fibroblasts. Azan, $\times 90$.

reached Type 8. A similar observation was made in the rhesus monkey (Vermande-van Eck, 1956), although the proportion of atretic follicles among larger follicles in the monkey was smaller than that found in the human ovary. This difference could be due to the fact that different criteria for atresia were used; Vermande-van Eck (1956) considered only changes in the oocyte whereas in our study morphological changes in all components of the follicle were evaluated to define atresia. In the adult human ovary the percentage of atretic follicles >1 mm in diameter (here Type 8) is reported to vary from 50% during most of the menstrual cycle up to 75% in the postovulatory phase (Block, 1951). However, it was the advanced stages of atresia, comparable to Stages III and IV, which were considered by Block. In our study, early stages of atresia (Stages I and II) were used in the calculations, because the follicle type could be determined accurately only at these stages by the number of granulosa cells and follicular diameter. In the more advanced stages of atresia the reduced number of granulosa cells and the shrinking of the follicle obscured the follicle type in which the atresia began.

In follicles of Types 3b and 4 it was difficult to follow consecutive stages of atresia because of the low percentage of atretic follicles, which might have been due either to a low incidence of atresia or to their rapid elimination.

Cells in mitosis were found in follicles of Type 5 and larger, even in the follicles with 10% pyknotic granulosa cell nuclei. This occurs also in other mammals (Pincus & Enzmann, 1937; Dalmane, 1967; Byskov, 1974). The pattern of atresia in the follicles of Type 5 and larger described in the infant human ovary resembles closely that reported for follicles in the adult rhesus monkey (*Macaca mulatta*) (Koering, 1969). In the ovary of the immature mouse, progressive stages of atresia have been demonstrated by cell kinetic study (Byskov, 1974), and a relationship was shown between the number of pyknotic cells in a follicle and its stage of atresia. The proportions of pyknotic granulosa cells in the different stages of atresia as well as the presence of leucocytes and occurrence of mitotic figures in the infant human ovary were comparable to those described for the mouse. It therefore seems likely that in the human the four stages of atresia represent consecutive stages of a single process.

Three phenomena were observed within the oocyte during the process of atresia: (1) an engulfing of the apparently healthy oocyte by penetrating cells; (2) a necrosis of the oocyte; and (3) presence of the maturation division.

Penetration of cells, probably granulosa cells, into the oocyte occurs during atresia of all follicle types. The function of these cells remains uncertain. Stevens (1903) suggested that they assist in the removal of the oocyte by phagocytic activity. The finding of follicles with more than fifteen cells inside the oocyte of which only a small rest remained suggests that cytoplasmic material has been removed by these cells. Zamboni *et al.* (1972) in a study of the fine morphology of the human oocyte *in vitro* described the presence of cells within the perivitelline space of healthy and degenerating oocytes and they regarded them as granulosa cells. They reported, however, that these cells did not display any apparent activity directed at digesting cytoplasmic material. They suggested further that oocyte degeneration did not mediate a stimulus for granulosa cells to invade through the zona pellucida. The fate of the cells within the oocyte during progressive stages of atresia remains obscure. The largest incidence of oocytes with cells was observed during Stage I of atresia. It is possible that the cells might leave the oocyte again while the oocyte undergoes maturation division or becomes necrotic (Stages II and III of atresia).

The maturation division has been found only in oocytes of follicles of Type 6 and larger. In these follicles the oocyte has reached its full size (Lintern-Moore *et al.*, 1974). It has been shown that RNA synthesis in the oocyte of the mature mouse ovary increases in the growing follicles (comparable here to Types 3b and 4), and reaches a peak in the oocytes of preantral follicles (Type 5). Thereafter the RNA synthesis decreases and is very low in antral follicles (Moore *et al.*, 1974). As maturation division occurs only in the follicles of Type 6 and larger, it is suggested that RNA synthesis and growth of the oocyte must be completed before the oocyte can progress beyond diplotene and complete meiotic prophase.

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References

- ALLEN, E., PRATT, J.P., NEWELL, Q.U. & BLAND, L.J. (1930) Human ova from large follicles; including a search for maturation divisions and observations on atresia. *Am. J. Anat.* **46**, 1-53.
- BAKER, T.G. (1963) A quantitative and cytological study of germ cells in human ovaries. *Proc. R. Soc. B*, **158**, 417-433.
- BLOCK, E. (1951) Quantitative morphological investigations of the follicular system in women. *Acta endocr., Copenh.* **8**, 33-54.
- BYSKOV, A.G.S. (1974) Cell kinetic studies of follicular atresia in the mouse ovary. *J. Reprod. Fert.* **37**, 277-285.
- DALMANE, A.P. (1967) Atresia of follicles in ovarium of mammals. *Archs Anat. histol. Embryol.* **53**, 73-84.
- KOERING, M.J. (1969) Cyclic changes in ovarian morphology during the menstrual cycle in *Macaca mulatta*. *Am. J. Anat.* **126**, 73-101.
- KRAUS, F.T. & NEUBECKER, R.D. (1962) Luteinization of the ovarian theca in infants and children. *Am. J. clin. Path.* **37**, 389-397.
- LINTERN-MOORE, S., PETERS, H., MOORE, G.P.M. & FABER, M. (1974) Follicular development in the infant human ovary. *J. Reprod. Fert.* **39**, 53-64.
- MOORE, G.P.M., LINTERN-MOORE, S., PETERS, H. & FABER, M. (1974) RNA synthesis in the mouse oocyte. *J. Cell Biol.* **60**, 416-422.
- PETERS, H., BYSKOV, A.G., HIMELSTEIN-BRAW, R. & FABER, M. (1975) Follicular growth: the basic event of the mouse and human ovary. *J. Reprod. Fert.* **45**, 559-566.
- PINCUS, G. & ENZMANN, E.V. (1937) The growth, maturation and atresia of ovarian eggs in the rabbit. *J. Morph.* **61**, 351-376.
- SAURAMO, H. (1954) Histology and function of the ovary from the embryonic period to the fertile age. *Acta obstet. gynec. scand.* **33**, Suppl. 2, 1-25.
- SHAW, W. (1925) The fate of the graafian follicle in the human ovary. *J. Obstet. Gynec. Br. Emp.* **32**, 679-689.
- STEVENS, T.G. (1903) The fate of the ovum and graafian follicle in pre-menstrual life. *Obstet. Trans.* **14**, 465-482.
- VALDES-DAPENA, M.A. (1967) The normal ovary of childhood. *Ann. N.Y. Acad. Sci.* **142**, 597-613.
- VERMANDE-VAN ECK, G.J. (1956) Neo-ovogenesis in the adult monkey. *Anat. Rec.* **125**, 207-224.
- WATZKA, M. (1957) Weibliche Genitalorgane. Das Ovarium. In *Handbuch der mikroskopischen Anatomie des Menschen*, Vol. 7, pp. 90-107. Eds. W. V. Möllendorf & W. Bargmann. Springer, Berlin.
- ZAMBONI, L., SMITH, D.M. & THOMPSON, R.S. (1972) Migration of follicle cells through the zona pellucida and their sequestration by human oocytes *in vitro*. *J. exp. Zool.* **181**, 319-340.

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