BIOLOGY OF REPRODUCTION 19, 597–605 (1978)

Morphometric Analysis of Follicular Development in the Rat

ANNE N. HIRSHFIELD[1] and A. REES MIDGLEY, JR.[2]

Reproductive Endocrinology Program,
Division of Biological Science,
and
Department of Pathology,
The University of Michigan,
Ann Arbor, Michigan 48109

ABSTRACT

Ovarian follicular development was examined in the cycling rat using histological techniques. The number and size distribution of follicles was determined for each day of the estrous cycle. While the number of follicles in the early stages of growth did not appear to vary dramatically throughout the estrous cycle, the final stages of growth and differentiation showed great variation throughout the cycle. No follicles of 390-500 μm were seen on proestrus, but many appeared the next day suggesting that there was a discontinuous movement of follicles into the largest size range. Follicular atresia was most marked in follicles 200-400 μm in diameter suggesting that there may be a critical stage during follicular development when, unless rescued, most follicles become atretic.

INTRODUCTION

While it has been clearly demonstrated that FSH and LH are each required for follicular development to progress beyond the earliest stages (Moore and Greenwald, 1974), hormonal events during the estrous cycle of the murine rodent appear to have little effect on the pattern of early follicular growth and development. Studies have indicated that primordial follicles enter the pool of growing follicles at a relatively constant rate regardless of the day of the estrous cycle (Pedersen, 1970) and that the total number of small and medium sized growing follicles within the ovary does not fluctuate throughout the estrous cycle (Mandl and Zuckerman, 1952). The rate of growth of granulosa cells appears to be affected only slightly by the cycle, since follicles of a given size show similar amounts of incorporation of tritiated thymidine on all days of the cycle (Pedersen, 1970). However, the number and size of the largest follicles vary throughout the estrous cycle (Mandl and Zuckerman, 1952; Pedersen, 1970; Peppler and Greenwald, 1970), suggesting that in contrast to earlier stages, the final stages of follicular development may be regulated during the cycle.

The following study was undertaken to examine follicular growth in the cycling rat. The object of this work was to identify those stages of follicular development which are markedly affected by known cyclic variations in hormone concentrations.

MATERIALS AND METHODS

Rats weighing approximately 250-280 g (Holtzman Co., Madison, WI) were caged in pairs and kept at 25°C with intervals of 14 h light (0500-1900 h) and 10 h darkness. Purina rat chow and tap water were available *ad libitum*. Vaginal smears were taken daily and only those rats displaying at least 3 unambiguous, consecutive, 5 day cycles were used. In our colony, approximately 80% of the rats displayed 5 day cycles. The timing of events in the estrous cycle in relation to vaginal smears was similar to that described earlier in this laboratory by Gay et al. (1970). The first day of vaginal cornification, accompanied in all cases by uterine ballooning and an LH surge between 1600 and 2000 h, was designated proestrus. On the second day of cornification, estrus, ova were found in the oviducts of all rats.

Classification of Follicles

Several studies of follicular growth in other species have employed the number of layers of granulosa cells to distinguish different size classes

Accepted April 6, 1978
Received May 4, 1977

[1]Present address: Department of Anatomy, The University of Maryland, Baltimore, MD 21201

[2]Kindly address reprint requests to: A. Rees Midgley, Jr. Reproductive Endocrinology Program, Department of Pathology, The University of Michigan, Ann Arbor, Michigan 48109

of follicles. In the hamster, for example, it has been reported that rings of granulosa cells remain discrete and up to 5 layers can be discerned clearly (Moore and Greenwald, 1974). In our studies of the rat, however, follicles displayed such indistinct layers of granulosa cells that it was impossible to distinguish even a 2 versus 3 layer stage of follicular development. Follicular diameter was chosen as the parameter by which to classify follicles for the following reasons: 1) it was a property which could be measured rapidly and accurately; 2) it was a measure which did not impose arbitrary class limits on the continuum of follicular growth and 3) as will be shown, it very closely correlated with granulosa cell number, an important index of follicular growth and development.

For purposes of classification, 3 rats were killed at 1200 h on each day of the 5 day estrous cycle, using an overdose of ether. The ovaries were removed and fixed for 24 h in Bouin's fluid at room temperature. The tissues were dehydrated and embedded in Paraplast (Scientific Products), using standard histological procedures (Preece, 1972). Complete serial sections of 5 μm thickness were prepared for each ovary. The sections were mounted and stained with Harris' alum hematoxylin and eosin. At least 50 random sections of each ovary were examined closely at a magnification of 100 diopters and all follicles displaying the nucleolus of the oocyte were measured using a calibrated occular micrometer. The maximum diameter and a diameter at right angles to it were used to obtain a mean diameter for each such follicle. Follicles showing evidence of atresia (see below) were excluded from this portion of the study. The diameter of the oocyte and presence or absence of an antrum were noted for each follicle. Follicles with a mean diameter between 25 and 390 μm will be referred to as small and medium. Follicles with a diameter greater than 390 μm will be termed "pre-Graafian." This name was chosen since not all of these follicles are destined to ovulate, so they are not strictly "Graafian" or "preovulatory." These pre-Graafian follicles have been further subdivided into small (390-500 μm) and large (greater than 500 μm) classes.

To obtain an estimate of the number of granulosa cells contained within selected cross sections of follicles (those containing the nucleolus of the oocyte), the number of cells in photomicrographs of representative sections of follicles was counted. In the case of larger follicles, for which direct count would have been impractical, an estimate of the cell number was obtained by counting all cells contained within a small area of the photomicrograph and relating this number to the ratio of the weight of this portion of the photomicrograph to the weight of the area containing the total granulosa cell mass:

$$\frac{\text{Weight of area counted}}{\text{total weight}} = \frac{\text{No. of cells counted}}{\text{estimated cell number}}$$

This procedure was validated by estimating cell number and counting all cells in a sample of follicles.

Atresia

A follicle was considered to be undergoing atresia whenever 2 or more pyknotic granulosa cells could be found in a single section or whenever the oocyte showed obvious signs of degeneration, such as fragmentation, loss of the nuclear membrane or thinning of the cumulus oophorus.

Distribution of Small and Medium Follicles

Two different methods were used to examine the size distribution of small and medium follicles (25-390 μm): 1) The number of follicles in 1 ovary from a metestrous rat was estimated by examining every fifth section. Nonatretic follicles containing the nucleolus of the oocyte in the plane of section were measured. These follicles were then classified arbitrarily into 9 size classes and the number of follicles observed in each size class was multiplied by 5 to give an estimate of the total number of nonatretic follicles in the entire ovary. 2) The actual number of nonatretic follicles in this ovary and another ovary from a different metestrous rat was obtained by examining every serial section of the ovaries and counting and measuring all nonatretic follicles greater than 25 μm in diameter.

Distribution of Pre-Graafian Follicles

Fifteen ovaries (1 from each of 3 rats killed on each of the 5 days of the cycle) were scanned at low magnification for follicles larger than 390 μm in diameter. These follicles were measured and examined closely at 250 diopters for evidence of atresia.

Labeling Index

To obtain a measure of the proportion of granulosa cells synthesizing DNA at selected points in time, the labeling index was determined. For this purpose, 1 animal from each day of the cycle was lightly anesthetized with ether and injected i.p. with 1 ml distilled water containing [^{3}H] thymidine (4 μCi/g body weight; specific activity 45 Ci/mmole, Nuclear Dynamics, CA). One h after injection, the rats were killed with an overdose of ether. The ovaries were fixed for 24 h

at room temperature in Bouin's fluid. Following dehydration and paraffin embedding, serial sections of 5 μm thickness were prepared for each ovary. Sections of the entire ovary were then mounted, heavily stained with Harris' alum hematoxylin (15 min) and counterstained with "Navy Manual Alcoholic Eosin" (Preece, 1972) (30 min). Autoradiograms were prepared essentially as described by Rajaniemi and Midgley (1975). Slides were coated with nuclear emulsion (Kodak NTB-2), exposed at −20° C for 3 days and developed in D170 for 6 min at 18° C. These procedures reduced the hematoxylin and eosin stain to reasonable amounts. Representative follicles were photographed at 250 diopters of magnification with the cells in focus. Although the silver grains were not in the same plane of focus, labeled and unlabeled cells were easily distinguished and counted in the photographs. In most cases the grain density over labeled nuclei was so high that it was not possible to count individual grains even when they were in the focal plane.

RESULTS

Relationship Between Mean Follicular Diameter and Granulosa Cell Number

The relationship between follicular diameter, oocyte diameter, incidence of antral spaces and the number of cells in the largest cross section of follicles is shown in Fig. 1. Follicles less than 25 μm in diameter consisted of an oocyte which had not begun to grow (less than 10 μm in diameter) and which was surrounded by an incomplete ring of granulosa cells. Mature oocytes (about 70 μm in diameter) were found in some follicles less than 150 μm in diameter and in all follicles greater than 150 μm. Antrum formation, beginning as isolated fluid filled spaces in the granulosa layer, was seen in some follicles as small as 75 μm in diameter, while other follicles as large as 400 μm in size showed no evidence of antral fluid.

Figure 1 shows that there is a very strong positive exponential correlation between granulosa cell number and diameter of follicles of all sizes in the rat. As the diameter of a follicle increased by a given amount, the area occupied by the granulosa cells increased by the square of that amount. These data were examined by linear regression analysis to determine the closeness of the correlation and to derive an equation for a line which could be used to estimate cell number from follicular diameter. Regression analysis of the logarithmically transformed variables revealed a linear relationship and a correlation coefficient of

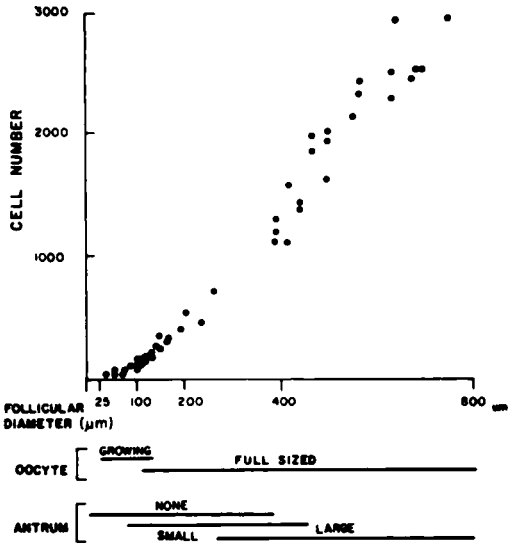

FIG. 1. Correlation of follicular diameter, number of granulosa cells, size of oocyte and degree of antrum formation in follicles of adult cycling rats. The data were obtained from single counts of cells in a total of 46 follicles from single ovaries from 15 rats.

0.99. The resulting equation for the line was:

$$\log \text{cell number} = -4.0717 + (1.8674)(\log \text{follicular diameter})$$

This equation could be used to obtain accurate estimates of cell number from follicular diameter.

In the largest follicles (>500 μm in diameter) in which a significant portion of the cross sectional area is occupied by an antral space, the relationship between follicular diameter and cell number became more linear. Thus, as the diameter of these antral follicles increased, the cell number became proportional to the perimeter of the follicle rather than to the area. However, as follicle size increased, there was also a slight increase in density of granulosa cells per unit area with increasing follicular size. This may be due to a decrease in size of each granulosa cell, a closer "packing" of granulosa cells or a combination of the two.

Size Distribution of Small and Medium Follicles

To determine if accurate estimates of the number of small and medium follicles could be obtained by a sampling method, 1 ovary of a rat killed at metestrus was examined by both procedures described in the methods section. Table 1 shows the actual number of nonatretic follicles in each size class in this ovary (obtained by examining each section) contrasted with two estimates, both obtained by examining every fifth section, one beginning with section number one and the next with section number two. The discrepancy between the two estimates was so great that this method was

TABLE 1. The accuracy of estimating the number of follicles in an ovary by sampling every fifth section. The number of follicles in each of 9 sies was determined by examining every single section of the ovary (actual) or by a sampling technique--examining every fifth section of the ovary and multiplying the number of follicles counted by 5. Estimate 1 was obtained by using this sampling technique and beginning with section number 1. Estimate 2 was obtained using the same technique and beginning with section number 2.

	Size distribution of follicles in one ovary									
	Size (μm)									
	25-40	41-80	81-100	101-130	131-195	196-260	261-325	326-390	>390	Sum
Actual	47	41	60	80	53	20	13	4	5	323
Estimate 1	30	45	70	70	70	15	25	0	10	335
Estimate 2	70	35	70	35	30	25	20	0	0	285

discarded because it was so inaccurate.

All nonatretic follicles larger than 25 μm in diameter were counted and measured in every serial section from 1 ovary from each of 2 rats killed on metestrus. One ovary contained a total of 280 follicles, while the other contained 294 follicles. Figure 2 shows the size distribution of follicles in these 2 ovaries. The great variation in the distribution pattern of follicles between the ovaries of rats killed on the same day indicated that a large sample size would be needed to determine whether or not there was a difference in distribution of small and medium follicles among different days of the cycle.

Size Distribution of Pre-Graafian Follicles

A large sample size was not necessary to reveal the striking differences observed in the size distribution of follicles greater than or equal to 390 μm in diameter throughout the estrous cycle. The number of follicles in this size range varied from 4-10 per ovary. The average size of these follicles increased from estrus to proestrus, as can be seen in Fig. 3. Follicle distribution on each of the 5 days of the estrous cycle is represented by analysis of 3 ovaries from each of 3 different rats. The average

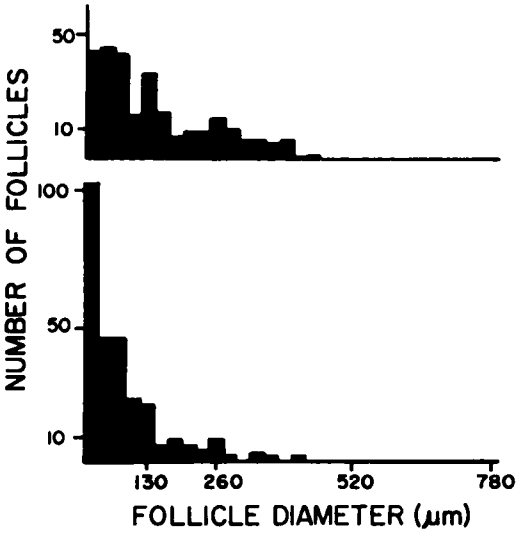

FIG. 2. The size distribution of all follicles greater than 20 μm in diameter, in 1 ovary of each of 2 different rats killed on metestrus.

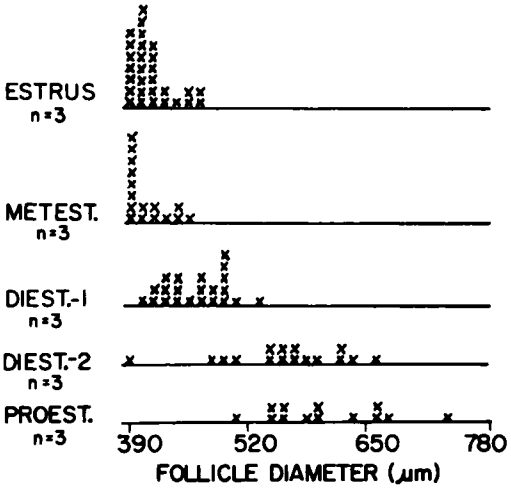

FIG. 3. The size distribution of the pre-Graafian follicles throughout the estrous cycle.

diameter of the nonatretic follicles increased from 402 μm at estrus to a high of 611 μm at proestrus. On proestrus, no follicles of the 390-500 μm size range were observed. However, on the following days, estrus and metestrus, a cohort of follicles in this particular range reappeared.

Labeling Index

The labeling index of individual follicles was calculated to determine if the rate of granulosa cell proliferation was different in various sized follicles and if this rate of proliferation changed throughout the estrous cycle. The labeling index plotted against follicular diameter is shown in Fig. 4. The labeling index increased as the follicular diameter increased for follicles less than 300 μm in diameter; a greater proportion of cells in larger follicles were actively synthesizing DNA than in smaller follicles. The labeling index was not constant for a given size class of follicles and varied greatly from one follicle to another. The relationship between labeling index and follicular diameter was reversed for follicles greater than 300 μm in diameter. As follicles in this size range grew larger, the proportion of granulosa cells incorporating [^{3}H] thymidine decreased. This decline in the labeling index in follicles of larger size appeared to be associated with the differentiation of the peripheral granulosa cells since they ceased to incorporate thymidine and became more columnar in appearance (Fig. 5). The zone of proliferating cells became increasingly restricted to those cells bordering the antral cavity and forming the cumulus oophorus. The decline in labeling index does not necessarily indicate that the mitotic rate of proliferating cells was decreasing, but may only reflect a reduction in the number of proliferating cells.

Atresia

Pyknosis of granulosa cells, our minimum criterion for atresia, was never observed in follicles

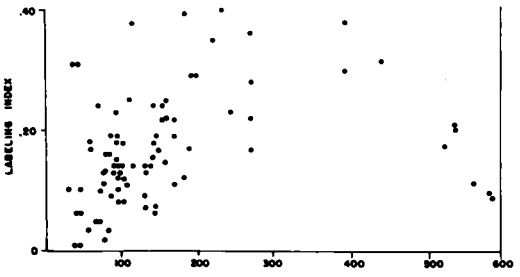

FIG. 4. The relationship between the labeling index and the follicular diameter. Each dot represents 1 follicle.

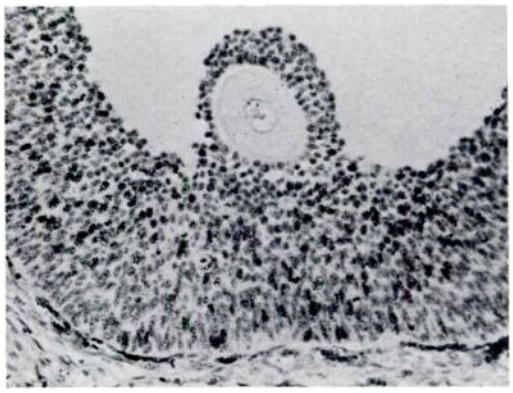

FIG. 5. A section of a large follicle. Silver grains are restricted to nuclei of those cells close to the antrum. Granulosa cells bordering the basement membrane are columnar in appearance and have ceased to incorporate thymidine.

without antra and, while occasional small follicles contained oocytes which appeared shrunken or distorted, it was often unclear as to whether or not this was an artifact of the fixation process. Several follicles contained granulosa layers that had a lace-like appearance, as if the cells were only loosely held together. There was no evidence of pyknosis in the granulosa cells of these follicles nor in the oocytes (Fig. 6).

The great preponderance of atretic follicles fell into the 200-400 μm size category. The first sign of atresia was a small number of pyknotic granulosa cells scattered throughout an otherwise "normal" follicle containing an intact oocyte. Follicles with larger numbers of pyknotic cells were easily recognized at low power by a distinct change in appearance of those granulosa cells adjacent to the basement membrane (Fig. 7). These cells were arrayed in a single symmetric ring giving the

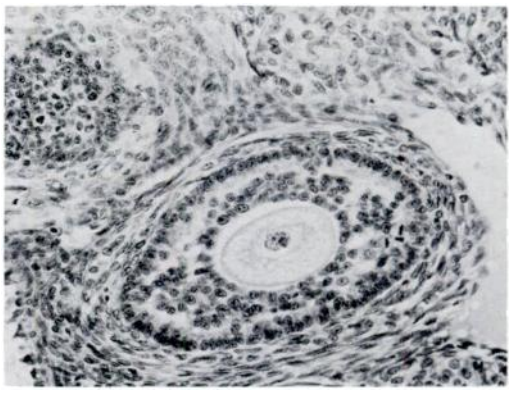

FIG. 6. A medium-sized follicle with a lace-like granulosa layer. There are no pyknotic granulosa cells evident in this follicle, suggesting that it is not atretic.

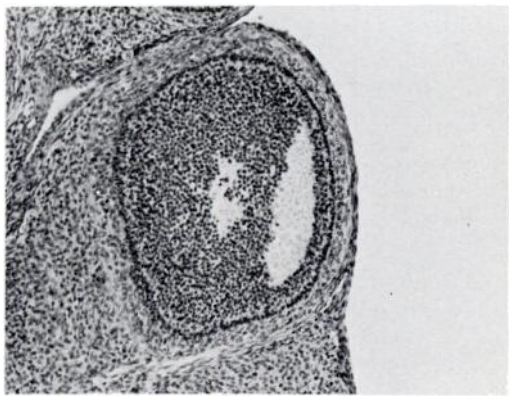

FIG. 7. A follicle in an early stage of atresia. The plane of section is not through the oocyte. The "string of beads" (clustering of granulosa cells along the basement membrane) is clearly visible and serves as a distinct marker for atretic follicles.

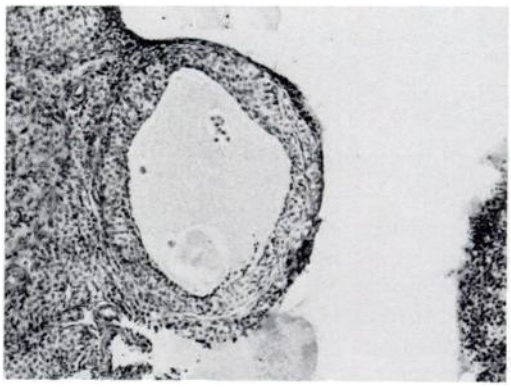

FIG. 9. A follicle in an advanced stage of atresia. A granulosa layer is no longer distinguishable and macrophages are visible in the antrum. The oocyte has divided several times and is seen as a ball of cells floating freely in the antral cavity.

impression of a string of beads along the outermost border of the granulosa cell layer. Although some granulosa cells were pyknotic in all such follicles, the nuclei of these outermost cells did not appear pyknotic.

Although no attempts were made to classify and characterize various stages of atresia as has been done for the mouse (Byskov, 1974), more advanced stages could be easily identified. Thus, some follicles contained a great number of pyknotic granulosa cells, cell debris in the antrum and degenerative changes in the oocyte such as loss of the nuclear membrane, meiotic division or dissolution of the egg cytoplasm. This was accompanied by a thinning of the granulosa cell layer and of the cumulus oophorus (Fig. 8).

Some follicles, presumably in the most advanced stages of atresia, were characterized by absence of a granulosa cell layer and hypertrophy of the remaining cells (Fig. 9). Usually, fragments of the oocyte or a ball of cells resulting from parthenogenetic division of the oocyte could be seen floating in the follicular cavity.

Early atresia in large follicles (greater than 400 μm) was accompanied by a cessation of DNA synthesis in the granulosa cells (Fig. 10). Follicles containing as few as 2 or 3 pyknotic granulosa cells showed little incorporation of [^{3}H] thymidine. On the other hand, early atresia in smaller follicles was not always characterized by cessation of cell proliferation. Some follicles showed obvious morphological signs of atresia as well as heavily labeled granulosa cells (Fig. 11).

The following examination of the size

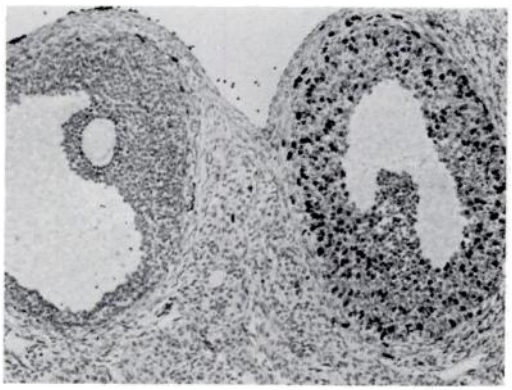

FIG. 8. Two large follicles. The one on the right is healthy and contains many heavily labeled granulosa cells. The one on the left is atretic (note the cellular debris in the antrum and pyknotic granulosa cells) and has ceased to incorporate thymidine into the granulosa cells.

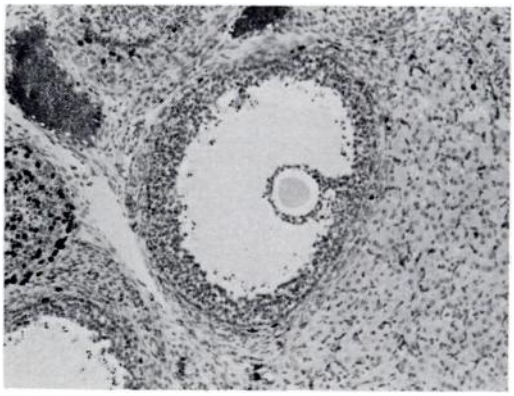

FIG. 10. Follicle in an advanced stage of atresia. The oocyte appears crumpled, no nucleus is visible and the cumulus oophorus is thin. Much debris fills the antrum. No silver grains are visible over the granulosa cells, while a few cells are heavily labeled.

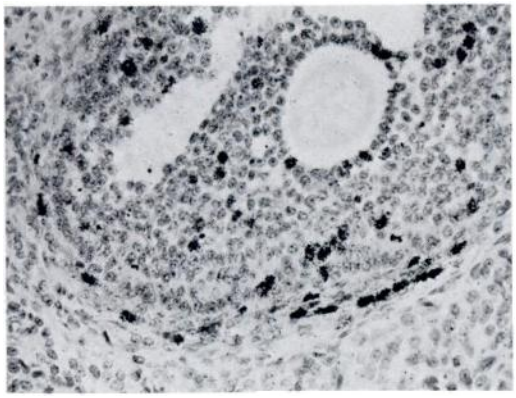

FIG. 11. An atretic follicle containing heavily labeled granulosa cells. There are several pyknotic cells visible in the granulosa layer.

distribution and occurrence of atretic follicles through the cycle will be limited to follicles containing pyknotic granulosa cells. All atretic follicles in more advanced stages of atresia were excluded from the study.

Figure 12 shows the distribution of all follicles larger than 170 μm in diameter, both healthy and in early stages of atresia, in 4 different ovaries. As can be seen, the greatest number of atretic follicles occurred in follicles approximately 200-400 μm in size in all the ovaries. However, some of the largest follicles also displayed signs of atresia. Although the ovaries contained highly variable numbers of developing follicles, Chi Squared analysis showed that an equal proportion of all follicles above 170 μm in size was atretic. Ovaries which contained the largest number of developing follicles (>170 μm in diameter), also contained the largest number of atretic follicles. Approximately 38% of all follicles in each ovary greater than 170 μm in diameter showed evidence of atresia (34.7% and 38.5% for each of the 2 proestrous ovaries, 44.6% for the ovary from diestrus-2 and 34.5% for metestrus). The ovary examined from the animal in metestrus in this figure appears to be exceptional since, in contrast to ovaries from other rats on this day (Fig. 3, 13), no pre-Graafian follicles were found. This rat had displayed 3, consecutive, 5 day cycles.

Figure 13 shows the size distribution of follicles 390 μm and greater in diameter in ovaries from rats killed on each day of the 5 day estrous cycle. Both healthy follicles and follicles in each stage of atresia are depicted. Very few atretic follicles larger than 500 μm were seen at 1200 h on estrus, suggesting that all follicles of ovulatory size had ovulated earlier that morning. This observation was further confirmed by examination of an additional 5 ovaries from rats killed on the day of estrus. Four of these ovaries contained no follicles larger than 500 μm, while 1 ovary contained 1 follicle of 520 μm in diameter in an early stage of atresia. Ovaries from rats killed on metestrus showed a great

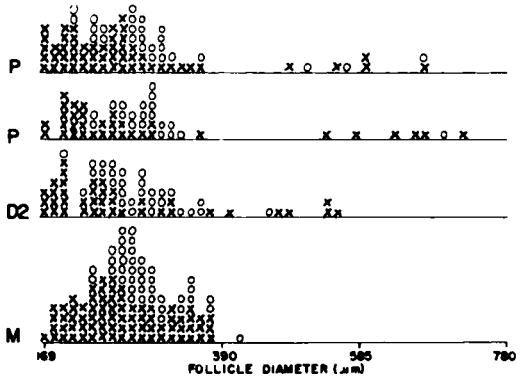

FIG. 12. The incidence of atresia in follicles greater than 169 μm in diameter. Two ovaries are from 2 different rats killed on proestrus (P), one is from a rat killed on diestrus-2 (D2) and one is from metestrus (M). Each X represents a normal follicle. Each O represents an atretic follicle.

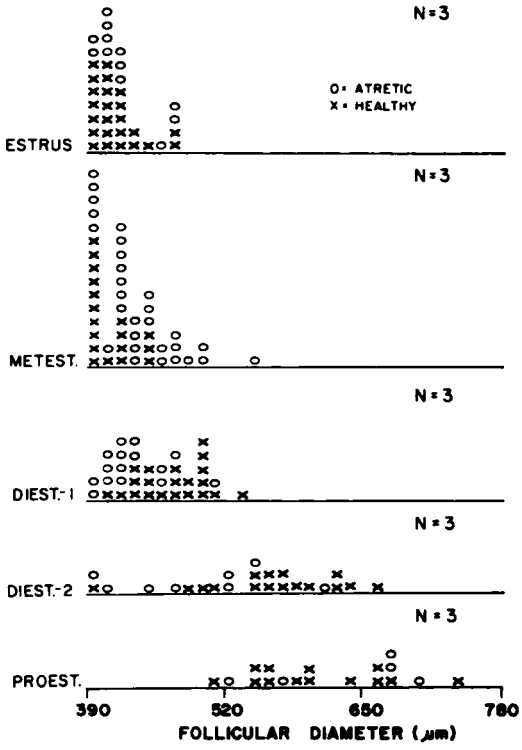

FIG. 13. Incidence of atresia in the pre-Graafian size range. All follicles, both normal (X) and atretic (O), are represented for 3 different animals killed on each day of the estrous cycle.

number of atretic follicles in the 400-500 μm size range.

DISCUSSION

Classification of Follicles

The classification of follicles in the mouse, proposed by Pedersen and Peters (1968), provides an excellent framework for studying follicular kinetics in that animal. It has been used in modified form for the classification of follicles in other species. However, caution should be used in applying this classification to the rat. While the size of a mature oocyte appears to be nearly constant for all mammals (about 70 μm), the size of mature follicles and the number of granulosa cells, is more closely related to body size and thus varies from species to species (Parkes, 1931). Therefore, rat follicles of a given stage of development would be expected to have a different number of granulosa cells than mouse follicles at a corresponding stage of development. For this reason, the classification proposed by Pedersen and Peters (which relies heavily on the number of granulosa cells in a follicle), can only roughly be applied to the rat.

In the present study, we have found that follicular diameter was both a convenient and appropriate measurement to use in describing the course of follicular development. It has the distinct advantage of being a more objective criterion than other methods of classification (such as those based on the number of layers of granulosa cells, or other morphological criteria), which require subjective judgements which may vary between investigators.

Changes in the Population of Follicles Throughout the Estrous Cycle

Follicular growth has been described as a continuum (Peters et al., 1975). Each day follicles begin to grow and do not cease to grow until they ovulate or undergo atresia. Thus, the number of follicles at any particular stage of growth is governed by: 1) the rate of entry of resting, primordial follicles into the pool of growing follicles; 2) the rate of growth of follicles and 3) the rate of loss of follicles through atresia. If the number of follicles entering the pool of growing follicles were constant, the rate of growth of follicles being related to the number of dividing cells and the rate of atresia were random or equal at all stages of development, then the number of follicles of all sizes would be constant. However, if any or all of the three processes varied through the estrous cycle, waves of follicles would be seen progressing through the growth stages and the number of follicles of a given size would be expected to be different on different days of the cycle.

The apparent nonrandomness of the spatial distribution of follicles within an ovary makes sampling difficult. As a consequence, it is not possible to draw firm conclusions regarding the size distribution of small and medium sized follicles at this time.

We believe that we have identified a critical point during follicular development where atresia plays an important role. From our findings, it appears that "selection" of follicles has occurred by the time of follicle reaches 390 μm in diameter. While follicles of all sizes below 390 μm are present on all days of the cycle, there is a discontinuous movement of follicles into the pre-Graafian size class (greater than 390 μm in diameter). Only small pre-Graafian follicles (390-500 μm) were seen on estrus and metestrus. The average size of pre-Graafian follicles increased throughout the remainder of the cycle and reached a maximum on proestrus. Few small pre-Graafian follicles were seen subsequent to metestrus. These observations suggest that the small pre-Graafian follicles seen on estrus or metestrus continue to grow and are those follicles destined to respond to the LH surge of the subsequent cycle by ovulating.

The pattern of distribution of follicles in this size range suggests that 3 days are required for development from 390 μm in size to the final preovulatory stage. Little growth appears to occur between estrus and metestrus. It is possible that the healthy follicles seen on estrus become atretic the following day, even though these follicles are growing rapidly on estrus (as estimated by incorporation of [^{3}H] thymidine). The large, nonatretic follicles seen on metestrus are presumably those that would go on to ovulate in response to the LH surge of the following proestrus, because they comprise the last major influx of follicles into the 390-500 μm size category.

Our findings suggest that while the number of follicles in the early stages of growth do not appear to vary dramatically throughout the estrous cycle, the final stages of growth and differentiation show marked variation throughout the cycle. Our results concur with those of Mandl and Zuckerman (1952) who found that there was a cyclic variation in the number of follicles greater than 350 μm in diameter. Our findings also agree remarkably with those of Peppler and Greenwald (1970) who reported that while the number of follicles greater than 352 μm remained constant throughout the

estrous cycle, their average size increased from estrus to the following proestrus. In addition, they found that on Day 5 (proestrus), ovaries lacked follicles measuring 395-500 μm although follicles of this size were seen on other days. Although follicles of small and medium size (less than 390 μm) are present in great abundance on all days of the cycle, large preovulatory follicles are present in the ovary only on diestrus-2 and proestrus. These findings concur with observations of others that pharmacological doses of ovulating hormones are required to induce ovulation on diestrus and even pharmacological doses are ineffective in causing ovulation on metestrus and estrus (Peppler and Greenwald, 1970; Ying and Greep, 1971). If follicular growth were not a cyclic event and all follicles reached the ovulatory stage, the ovary would be capable of responding to LH by ovulating at all times. The absence of large preovulatory follicles on estrus, metestrus and diestrus-1 suggests that the ovary is capable of responding to the LH surge for only a limited period during the estrous cycle.

It may be hypothesized that follicular development normally terminates in atresia at 200-400 μm and, in order to ovulate, follicles must be "rescued" from the atretic processes. Atresia appears to be the "normal" termination of follicular growth; follicles that ovulate are the exception not the rule. It has been estimated that throughout the reproductive life span, 99.9% of all follicles become atretic and only 0.1% eventually ovulate (Ingram, 1962). Schwartz (1974) has suggested that the FSH surge of one cycle selects follicles which will ovulate during the subsequent cycle. We postulate that this FSH surge may act by preventing atresia in some follicles which have attained a size of 200-400 μm. Only the rescued follicles would then proceed through the final stages of growth and differentiation in preparation for ovulation the following estrus.

ACKNOWLEDGMENTS

This research was supported in part by a Program Project in Reproductive Endocrinology (HD-08333) and a grant from the Ford Foundation. We thank Dr. JoAnne S. Richards for her valuable suggestions and critical review of the manuscript.

REFERENCES

Byskov, A. G. (1974). Cell kinetic studies of follicular atresia in the mouse ovary. J. Reprod. Fert. 37, 277-285.

Gay, V. L., Midgley, A. R. and Niswender, G. D. (1970). Patterns of gonadotropin secretion associated with ovulation. Fed. Proc. 29, 1880-1887.

Ingram, D. L. (1962). Atresia. In: The Ovary. (S. Zuckerman, ed.). Academic Press, New York. pp. 247-273.

Mandl, A. M. and Zuckerman, S. (1952). Cyclical changes in the number of medium and large follicles in the adult rat ovary. J. Endocrinol. 8, 341-346.

Moore, P. J. and Greenwald, G. S. (1974). Effect of hypophysectomy and gonadotropin treatment on follicular development and ovulation in the hamster. Am. J. Anat. 139, 37-48.

Parkes, A. S. (1931). The reproductive processes of certain mammals. II. The Size of the Graafian follicle at ovulation. Proc. Roy. Soc. B. 109, 185-196.

Pedersen, T. (1970). Follicle kinetics in the ovary of the cyclic mouse. Acta. Endo. 64, 304-323.

Pedersen, T. and Peters, H. (1968). A proposal for classification of follicles in the mouse ovary. J. Reprod. Fert. 17, 555-557.

Peppler, R. and Greenwald, G. S. (1970). Effects of unilateral ovariectomy on ovulation and cycle length in 4- and 5-day cycling rats. Am. J. Anat. 127, 1-7.

Peters, H., Byskov, A. G., Himelstein-Braw, R. and Faber, M. (1975). Follicular growth: The basic event in the mouse and human ovary. J. Reprod. Fert. 45, 559-566.

Preece, A. (1972). A Manual for Histologic Technicians. Little, Brown and Co., Boston, MA. pp. 31-275.

Rajaniemi, H. J. and Midgley, A. R., Jr. (1975). Autoradiographic techniques for localizing peptide hormones in target tissue. In: Methods in Enzymology. Part B, Hormone Action. (B. W. O'Malley and J. G. Hardman, eds.). Academic Press, New York. pp. 145-167.

Schwartz, N. B. (1974). The role of FSH and LH and of their antibodies on follicle frowth and on ovulation. Biol. Reprod. 10, 236-272.

Ying, S. Y. and Greep, R. O. (1971). Responsiveness of follicles to gonadotropins during the estrous cycle in the rat. Endocrinology 89, 294-297.