Morphological Observations of Abnormal Follicles in Mature Ovaries of the Norway Rat, *Rattus norvegicus*[1)]

By

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(*With one Plate and three Textfigures*)

It has been generally known that the great number of ova are produced in the mammalian ovary at the early stage, but the majority of them undergo degeneration, only a few attaining maturation, developing into healthy functional ova which are discharged at ovulation. Lane('38) reported that in immature rats the total number of follicles observed in one hundred ovaries was 28522. According to Sobotta and Burckhard('10) and Arai('20), the number of eggs discharged at ovulation was from four to seventeen in one ovary of the rat. The literature regarding atretic follicles containing abnormal ova and polyovular follicles, which are to be disintegrated during the maturation course is very extensive in mammals covering many classes, as a glance at Hartman's thorough review of these subjects('26) shows. Though such atypical follicles are of common occurrence in mammalian ovaries, however, in view of attention given towards the rat and mouse, the present author is struck with the scarcity of references to these forms. The present paper deals with morphological observations of these abnormal follicles and their seasonal variation in number studied in mature ovaries of the wild Norway rat, *Rattus norvegicus* (Berkenhaut), presenting the data obtained from one hundred and twenty ovaries of 60 individuals which were collected in the vicinity of Sapporo during from April of 1942 to March of 1943. The ovaries were fixed in Allen-Bouin's solution and cut into 12 micra thick.

1) Contribution No. 195 from the Zoological Institute, Faculty of Science, Hokkaido Imperial University, Sapporo.

The large part of sections were stained with Delafield's haematoxylin and partly with iron-haematoxylin after Heidenhain.

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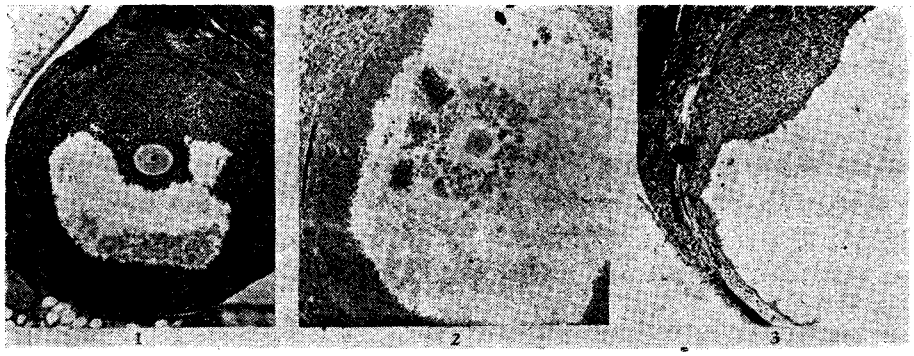
Observations

1. The normal follicle and the general course of maturation

The young immature follicles showing various stages of the growth are found in deeper parts of the ovary embedded in the connective tissue. They are variable in size according to the stage of growth. In larger ones several rows of granulosa cells are found and the antrum has been formed. The mature follicles attaining 410.90 μ to 502.32 μ in diameter, on the other hand, are found occupying a position near the periphery of the ovary (Fig. 1). The ovum contained in such a follicle, called by the name of the Graafian follicle, has a diameter ranging from 65.8 mm to 71.4 mm. It is surrounded by the zona pellucida and the latter again by the corona radiata. Surrounding the corona there are considerable layers of the granulosa cells which enclose a huge cavity filled up with the fluid, liquor folliculi.

The changes in the nucleus of the ovum advancing towards the first maturation division seem to proceed thoughout a very short duration. With the disappearance of the nuclear membrane, the chromosomes arrange on the equatorial plate and there is formed the first polar spindle in the ovum. After the division of chromosomes, the outer pole of the spindle which upheaves on the egg surface gets extruded as the first polar body. After the expulsion of the first polar body, the chromosomes of the ovum organize very rapidly, and soon they are drawn into a new spindle, which is the second polar spindle. As thus noted, the ovum undergoes the first maturation division, prior to ovulation, and still farther advances in its maturation course up to the metaphase stage of the second division. The maturation process in the ovum stops at this stage, until insemination occurs following ovulation.

The Graafian follicle just before its rupture is very remarkable in feature. The ovum lies practically free from the granulosa cells and also from the cells of the corona radiata, suspended in a naked state in the liquor folliculi (Figs. 2–3). Insemination occurs in the upper portion of the oviduct. A single spermatozoon enters the egg, only the head of the former penetrating the egg. Shortly after the penetration of the spermatozoon the course of the second maturation division of the egg advances, and thus the second polar body is formed. The detailed descriptions regarding maturation and fertilization may be referable to another paper of the author.



Photomicrographs taken with the aid of Leitz-Makam.

Fig. 1. Normal Graafian follicle. ×75.
Fig. 2. The Graafian follicle just before rupture. ×75.
Fig. 3. The follicle in preovulatory stage. The egg is found suspenped in naked state in the liquor folliculi. ×75.

2. Accounts on the atretic follicle

The atretic follicles that are destined to undergo degeneration without growing into the functional follicle are morphologically investigated here. The widely encountered feature of atresia is that the usual signs of degeneration are to be found in the egg nucleus (Figs. 4–5). The nuclear elements are found scattered in small masses in the ooplasm. Some ova contain no nuclear element at all. In most of cases the ovum is quite irregular in shape. There are observed several instances in which fragments of destroyed eggs are contained in the follicles (Fig. 6). In the extreme case the follicles contain no egg element. There are also encountered

not a few examples which form the maturation spindle carrying one polar body in the young follicle with no antrum (Fig. 7). In every of the above cases, the evidence seems to be suggestive of the fact that in the course of degeneration the disintegration takes place earlier in the egg body than in the granulosa cells, contrary to the views emphasized by Asami ('20), Branca('25), Ereud & Vedder('38) and Pliske('40). In this respect, the events represented by this study are agreeable to those reported by Engle('27) in the mouse.

For convenience of description, the atretic follicles herein considered are divided into two general types according to size. Type I refers to the small follicle, ranging from 130.20 μ to 184.24 μ in diameter, with a few layers of granulosa cells, but in which the antrum folliculi has not yet been formed. The follicles referring to this type are further subdivided into the following three kinds: a) the follicles in which the spindle has not been found, b) those which show the irregular first polar spindle, and c) those which undergo the second maturation division carrying the first polar body. The numbers of follicles in these three kinds which were counted in 120 ovaries under examination are 2218, 269 and 51 respectively.

The follicles belonging to type II show a diameter ranging from 211.96 μ to 303.94 μ and have an antrum. Three kinds of follicles are also recognizable as in the former type, as follows: a) the follicles showing no maturation spindle, b) those having the first polar spindle, and c) those undergoing the second maturation division. The count of these follicles in 120 ovaries shows that the follicles under examination were found to be 286, 378 and 21 in number respectively.

3. Accounts on the polyovular follicle

The review of the extensive literature regarding polyovular follicles presented by Hartman('26) emphasizes the common occurrence of such follicles in the majority of mammals, including the marsupials at one end and man at the other. The large number of reports for man may be an expression of the interest in relation to the problem of twinning. Upon reviewing the list given by Hartman's paper('26), however, the absence of the rat from it, is very surprising, The present author's study with the Norway rat

reveals that the polyovular follicle is not less common in the rat than in the other mammals.

The total number of polyovular follicles obtained by counting in 120 ovaries from 60 individuals was found to be 375. Of them there were 316 follicles (84%) which were biovular; triovular follicles were 47 (13%); 9 follicles (2%) contained four ova, and 3 follicles (1%) included five ova. Thus the occurrence of the polyovular follicle is rather frequent in the rat. In the mouse, on the other hand, its occurrence seems to be rather rare since, according to Engle('27), only 18 polyovular follicles were reported from the study of one hundred ovaries.

Biovular follicles, 316 in total number, occurred in ovaries of fifty individuals. They averaged 5.26 per individual. Fifty-nine per cent of these biovular follicles were of small size and the antrum had not yet been formed. Forty-one per cent of them were large in size having the antrum. In the majority of cases two egg cells, being in contact or not, were enclosed by a common zona pellucida (Figs. 8 and 14). Some ones contained two ova separated by several numbers of granulosa cells and enclosed by an individual zona pellucida (Fig. 9). A single follicle showed ova of unequal size, enclosed by an individual zona pellucida and suspended in liquor folliculi, assuming the feature of the stage just before ovulation (Fig. 10). One of these ova possessed the second maturation spindle carrying the first polar body. There were also other ten examples which showed the first maturation spindle in the ovum.

Triovular follicles, 47 in number, were found in ovaries of twenty-two individuals. The ratio in number of the small follicles without the antrum and the large follicles with it was seen to be nearly 1 : 1. It is noteworthy that in every of triovular follicles herein concerned three egg cells were surrounded by a common zona pellucida (Fig. 11). The size of the egg cell was generally small in all cases coming under examination.

Follicles containing four egg bodies were observed in ovaries of eight individuals and the total number obtained was nine. Of them only two follicles had progressed in development to the stage of formation of the antrum (Fig. 12). The egg bodies of this series were surrounded by a common zona pellucida.

Three cases of five-ova-containing follicles occurred in ovaries from three individuals. They had no antrum and were found under a common membrane (Fig. 13). A similar type of follicle was recorded in the mouse ovary by Takewaki('37).

Concerning the origin of polyovular follicles, Hartman('26) enumerated three possible modes of formation: 1) by division of polynuclear ova; 2) by concrescence of previously separated follicles; 3) by persistent union of ova in the egg tube. In the great majority of cases encountered in this study, egg cells were surrounded by a common zona pellucida. And further, there were present a few follicles in which the egg showed the mitotic division, as seen in Fig. 14. In view of these facts, the present author is of opinion that polyovular follicles arise by separation of the original definitive ovum, so far as the data obtained in this study are concerned.

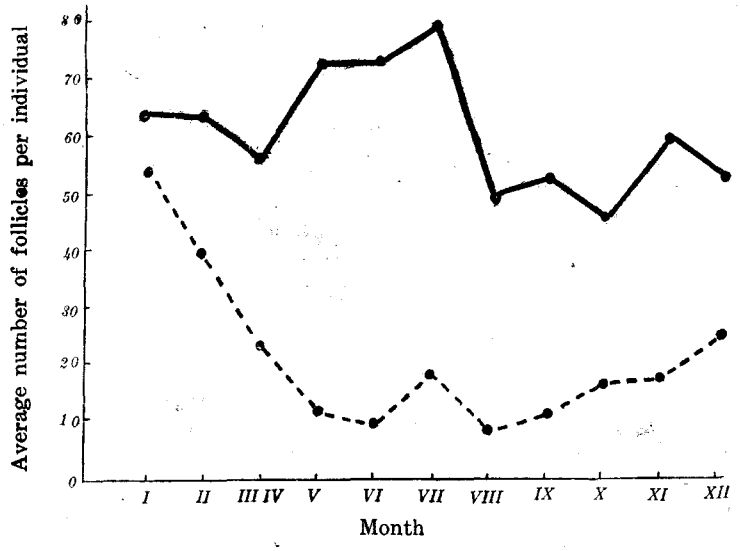


Chart 1. Graph showing average numbers of both atretic and normal follicles calculated per individual by month. Solid line denotes the number of aberrant follicles and the dotted line that of normally maturating follicles.

Most of polyovular follicles observed in this study represent obvious evidences of atresia, all tracing the course of degeneration. Thus the evidence at hand is in favour of the explanation that the

majority of polyovular follicles are destroyed through atresia, as described by Hartman('26). On the other hand, however, another picture was also furnished which is suggestive of the possibility that occasionally such a follicle may reach maturity, though the evidence is not fully conclusive.

4. Seasonal variation in number of aberrant follicles

The data herein presented were based on observations upon the ovaries from sixty individuals collected through the year, the material obtained from five rats per month coming under examination. The total number of the aberrant follicles, both atretic and polyovular, counted in the present material was found to be 3597; namely the atretic follicles were 3222 and polyovular ones 375. The average numbers of these aberrant follicles calculated in one individual per month are shown in Table 1. The data in this table are graphically expressed in Chart 1.

TABLE 1.

Average numbers of aberrant follicles and normally maturating follicles, calculated per individual by month.

Month	I	II	III-IV	V	VI	VII	VIII	IX	X	XI	XII
Aberrant follicle	62.33	62.00	55.50	73.66	73.50	79.16	48.33	52.00	46.50	58.66	52.16
Normal mature follicle	53.66	38.33	23.00	11.50	8.00	17.16	7.66	10.83	16.00	17.00	26.66

It is suggested from these data that the number of aberrant follicles represents no appreciable seasonal wave in the rat ovary, since a slight variation there occurred is that which is not beyond the limit of the individual variation. In other words, it may be possible to say that the occurrence of the degenerating follicles in ovaries shows no significant variation by season through the year. As compared with the case of the normaly maturating follicle (for details on this respect, see Makino, Sigemoro and Kobayashi '43), the condition is very interesting and of significance in connection with the reproductive cycle of this wild rat.

Summary

In 120 ovaries from a series of sixty mature wild rat, *Rattus norvegicus*, the occurrence and morphology of both atretic and polyovular follicles were observed. The atretic follicles of smaller size, ranging from 130.20 μ to 184.24 μ in diameter, are very abundant in number, being 2537 in total. The larger ones having a diameter ranging from 211.96 μ to 303.94 μ are 685 in total number. The total number of the polyovular follicles obtained is 375. Of them there are 316 follicles which are biovular; triovular follicles are 47; the number of follicles containing four ova are nine and those containing five ova are three. Almost all of these atypical follicles are those which contain obvious evidences of atretic degeneration, either in atretic follicles or in polyovular ones.

The evidence presented indicates the fact that the occurrence of the degenerating follicles in the ovaries represents no significant variation by season through the year.

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Explanation of Plate XXX

All are photomicrographs taken with the aid of Leitz-Makam (Dr. S. Makino *Photo.*)

Fig. 4. A section of the ovary showing atretic follicles. The nuclear bodies found in the follicles undergo disintegration. ×100.

Fig. 5. The egg body containing the destroyed nucleus. ×300.

Fig. 6. The follicle containing egg fragments. ×300.

Fig. 7. The egg showing the second polar spindle at metaphase accompanied by the first polar body in the young follicle with no antrum. ×300.

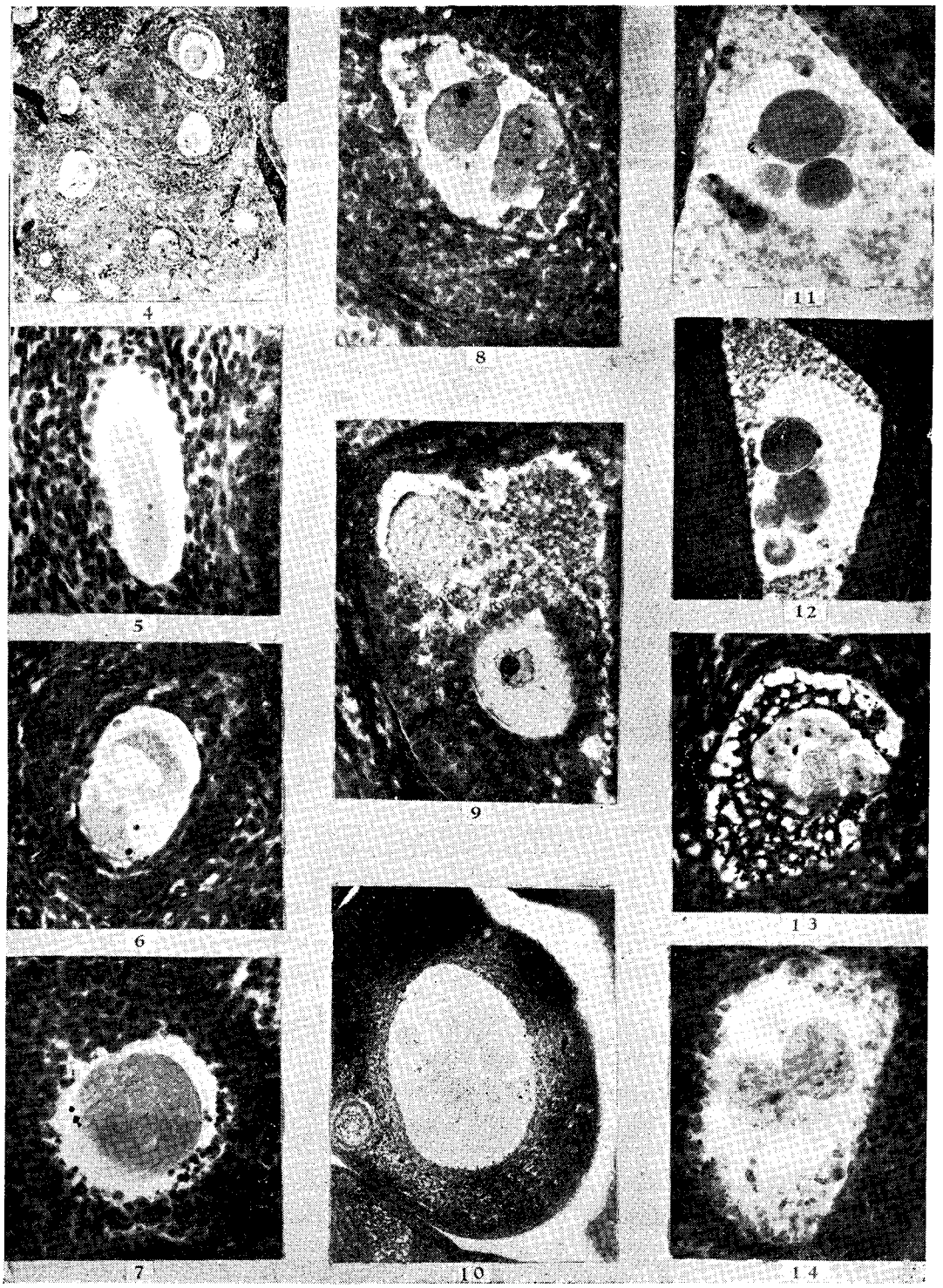
Figs. 8–10. Various types of the biovular follicles. In Fig. 10 the ova show the maturation spindles. 8–9; ×400. 10; ×100.

Fig. 11. Triovular follicle. ×300.

Fig. 12. Polyovular follicle containing four ova. ×300.

Fig. 13. Polyovular follicle containing five ova. ×300.

Fig. 14. Biovular follicle in which the egg body divides into two fragments. ×300



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