A QUANTITATIVE STUDY OF OOCYTES IN YOUNG AND OLD NULLIPAROUS LABORATORY RATS

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SUMMARY

The population of oocytes in the ovaries of the rat decreases slowly and continuously throughout middle and old age. The proportion of oocytes diagnosed as ‘atretic’ declines as the total number of oocytes diminishes.

Reproductive capacity declines before the ‘stock’ of oocytes is exhausted.

The age at which reproductive capacity begins to decline, and its relationship to the life-span of the animal, varies greatly between different species of mammals [see Eckstein & Zuckerman, 1955]. In women the decline is associated with the disappearance of oocytes, and the suggestion has been made that the two phenomena may be causally associated in all species in which they occur [see Zuckerman, 1956a]. An analysis of the breeding records of a colony of rats used for a series of ovarian studies showed that the number of females giving birth to successive litters declines progressively from about 360 days [Ingram, Mandl & Zuckerman, 1958]. At first sight this fall in fertility might be regarded as reflecting a parallel decline in the size of the population of oocytes. Unfortunately, however, there are no data which would permit of such a conclusion being drawn. A direct relationship between age and the number of oocytes in rats belonging to the Birmingham Strain has already been established, but the data refer only to animals aged 360 days or less [Mandl & Zuckerman, 1951]. The estimates of the total number of oocytes in older rats are limited to four specimens aged 385, 454, 559 and 947 days [Arai, 1920], while the numerous investigations of senile rat ovaries made by Wolfe, Burack & Wright [1940], Wolfe [1943] and Wolfe & Wright [1943] were restricted to Graafian follicles (which constitute less than 2% of the total population of oocytes).

In view of the lack of information about numbers of oocytes in old rats, two new investigations were undertaken, of which the first is reported here. The second deals with possible differences in the rate of decrease of oocyte numbers between nulliparous and multiparous senile rats of the same strain [Shelton, 1959].

Animals

Sixty-eight virgin females aged 49–1036 days belonging to the Birmingham Strain were used. Twenty-one animals, which will be referred to as senile, were over 400 days old. The remaining animals were less than 360 days old.

MATERIALS AND METHODS
Autopsy

The animals were killed by means of chloroform vapour. The ovaries were dissected free from adjacent tissue and immediately fixed in Bouin's alcoholic fluid. Serial sections were cut at 7µ and stained with Weigert's iron haematoxylin and counterstained with 'chromotrop 2R'.

Such pathological changes in the reproductive tract and such mammary tumours which were visible to the naked eye were recorded. No systematic study, however, was made of the thoracic cavity and abnormalities such as enlargement of the kidney, discoloration of the liver, etc., were not specifically looked for.

Estimation of number of oocytes

The oocytes were counted and classified into the following six stages of follicular growth:

- stage 1: one layer of rounded or flattened granulosa cells;
- stage 2: one layer of cuboidal granulosa cells;
- stage 3: two layers of cuboidal granulosa cells;
- stage 4: three layers of cuboidal granulosa cells;
- stage 5: four or more layers of cuboidal granulosa cells but no antrum;
- stage 6: antrum present.

Oocytes in stages 3–6 were traced in every fifth section. In fifty-four of the sixty-eight pairs of ovaries (animals aged 60–1036 days), every tenth section was scanned in order to estimate the number of oocytes in stages 1 and 2. Of these fifty-four pairs of ovaries, thirty-three were available from previous studies [Mandl & Zuckerman, 1951]. In the remaining fourteen pairs of ovaries from animals aged 49–198 days (controls in experiment reported by Mandl [1959a]) every twentieth section was examined. There was good agreement between the more recent counts and those made 7–8 years ago.

Oocytes in the ovaries of the twenty-one senile animals were recorded as 'normal' or 'atretic'. The classification, which was made consistently by only one observer in the present study (M.S.), was based on the criteria used before [see Zuckerman, 1956b].

Analysis of breeding records

The study of the relationship between age and fertility, recently reported by Ingram et al. [1958], has been extended so as to include more recent findings. The breeding records for the Birmingham colony of rats were used in order to determine the age of breeding females at the time when they gave birth to their last litter. The analysis refers only to females which had given birth to four or more litters, and which then failed to become pregnant although housed with a male for at least 3 months after giving birth to their last litter.

The number of litters produced before infertility set in, and the number of young in the last litter, have also been recorded.
**RESULTS**

All forty-nine animals aged 500 days or less appeared to be in good health. Only eight of the nineteen animals aged 500 days or more (all aged less than 800 days) appeared healthy. A variety of pathological changes were noted in the remaining eleven animals (four aged less than 700 days; seven aged 700–1036 days), the most common being tumours of the mammary glands, hypertrophy of the pituitary and abscesses in the reproductive tract [see Mandl, 1959b].

**Estimation of number of oocytes**

When plotted, the total number of oocytes was found to decrease with age, the relationship being curvilinear. In an earlier study, based on animals aged less than 360 days [Mandl & Zuckerman, 1951], the curve was transformed into a straight line by calculating a linear regression according to the formula \( \log y = \log a + b \log x \), where \( y \) = number of oocytes and \( x \) = age in days. In the present study, no attempt was made to subject the figures to statistical analyses, since they derived from a heterogeneous group consisting of (i) young animals, all of whom were healthy, and

![Graph showing total number of oocytes (groups 1–6) in rats killed at different ages. □, Animals showing macroscopic pathological changes; •, animals in which macroscopic pathological changes were not observed.](image-url)
(ii) old animals more than half of whom showed various pathological changes. The data are therefore presented only graphically, counts referring to animals showing pathological changes being marked by means of different symbols (see Figs. 1–3).

While the proportion of the total number of oocytes in stage 1 (surrounded by a single layer of flattened or rounded granulosa cells) remain approximately constant (mean for sixty-eight animals 82·5%; range 64·6–94·7%), the numbers in each of the six stages of development decrease with age. The total number of oocytes, and the numbers of oocytes in stages 3–6 in animals killed at different ages, are shown in Figs. 1 and 2 respectively.

Fig. 2. Number of oocytes at different stages of development (groups 3–6) in rats killed at various ages. For explanation, see Fig. 1.
**Atresia**

The percentage of oocytes diagnosed as 'atretic' in the senile animals tended to decline with age and hence with the total population of oocytes (Fig. 3). There appeared to be no correlation between the condition of the animal and the proportion of oocytes diagnosed as 'atretic'. On the other hand, since no count of 900 oocytes or less was derived from an animal free from pathological abnormalities, it is not possible to say whether the presence of such abnormalities tends to be associated with a decrease in the number of 'atretic' cells.

![Graph showing percentage of oocytes diagnosed as 'normal' in ovaries containing varying populations of oocytes.](image)

**Breeding performance of senile rats belonging to the Birmingham colony**

The breeding records for a total of 178 females were used. The majority of these were included in the study reported by Ingram *et al.* [1958]. The analysis is here presented in a slightly different form, with the emphasis laid on the age at which infertility sets in, rather than on the effect of age upon litter-size.

The results of the present analysis are summarized in Fig. 4. The mode of the number of litters born before infertility sets in was seven. When their last litters were born, 73% of the mothers were 480 days old or less. Only 16% of the animals continued breeding after reaching the age of 555 days. The number of young per litter in the last litter varied between 1 and 14.

Fig. 4 also shows the mean estimated total number of oocytes in rats aged 255–675 days. The results suggest that cessation of breeding in this species is not primarily due to the exhaustion of oocytes in the ovary.

**DISCUSSION**

The population of oocytes in the ovary of the rat is known to decrease rapidly during the first few weeks after birth, and then to decline more gradually [Arai, 1920; Mandl & Zuckerman, 1951]. The present study has shown that the slow reduction in
the number of oocytes continues steadily throughout middle and old age, the ovaries still containing a stock of variable size at the time of death. Thus the ovary of the rat, unlike that of certain inbred strains of mice [Jones & Krohn, personal communication] and that of women, does not become depleted of oocytes at an age which is less than the life-span.

Fig. 4. (A) Number of litters produced by breeding animals of the Birmingham colony before the onset of infertility (mode 7). (B) Age of breeding animals at the time of birth of the last litter (———). Mean estimated number of oocytes in virgin animals aged 255-675 days (— — —).

The longevity of rats belonging to the Birmingham Strain has not been accurately established. An approximate estimate would be that only about 60-70% of the female rats (both nulliparous and multiparous) live beyond the age of about 2 years. A high proportion of the surviving animals develop one or more pathological changes, and nulliparous rats aged more than 800 days free from any abnormality have not as yet been encountered. The age at which pathological changes associated with old age become manifest is somewhat greater in the multiparous rats; on the other hand, the numbers of oocytes in the ovaries of old nulliparous and multiparous rats is about the same [Shelton, 1959].

It is also worth noting that the proportion of oocytes diagnosed as ‘atretic’ declines as the total population of oocytes decreases. Given that the subjective criteria of diagnosis of 'atresia' are not affected by the numbers of oocytes counted,
it would appear that the fewer oocytes available, the smaller the proportion that is ‘wasted’ by undergoing atresia.

The present study also shows that numbers of oocytes in all stages of development decrease with age. Although there is considerable variability between individual animals in the number of Graafian follicles (stage 6), it appears that the number of these also decreases with age. Wolfe [1943] also reported that the number of normal follicles over 500\(\mu\) in diameter decreases with age (mean numbers in rats aged 20–24 months and over 25 months is given as 11-2 and 5-8 respectively). This does not necessarily imply, however, that the number of Graafian follicles shed at any one ovulation decreases with age. Not all Graafian follicles present at one time are necessarily destined to ovulate or degenerate at the next ensuing oestrus. The numerous Graafian follicles present in young rats vary in size between 250\(\mu\) in diameter when the antrum begins to appear, and 650\(\mu\) shortly before ovulation, only a fraction of the total number reaching the latter size [Mandl & Zuckerman, 1952]. In contrast, most of the Graafian follicles in old animals are above 450\(\mu\) in diameter [Mandl, 1959b]. In view of the fact that the rate of atresia for the total population of oocytes decreases with age, it is possible that it does so to the same or even a greater extent for the final stages of follicular development.

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REFERENCES