

# Age-Specific Features of Estrous Cycles and Folliculogenesis in GC Female Rats Selected by Catatonic Reactivity

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A decrease in the total count of follicles in the diestrus and proestrus and higher incidence of permanent estruses were found in 3-month-old females of the catatonic GC rat strain in comparison with Wistar females (control). At the age of 6-12 months, GC females had lower incidence of long estrus and diestrus. The estrous cycle of GC females was shorter than of Wistar rats. With aging (at the age of 18 months), the counts of growing and maturing follicles significantly decreased. Higher counts of growing single-layer and bilayer follicles were found in 18-month-old GC females. Differences in the length of estrus and diestrus and in the incidence of abnormal phases in the two strains also disappeared at this age. Selection by high catatonic reactivity was associated with a decrease in body weight.

**Key Words:** *folliculogenesis; GC strain; estrous cycle; catatonia*

GC (genetic catatonia) rats were derived from the Wistar outbred stock by selection by the duration of cataleptic reaction. Selection of this strain was undertaken in order to create a model of human psychopathology and was based on the hypothesis according to which low threshold liability to catatonic reactions could underlie the development of schizophrenia [5]. The studies showed that GC rats by their characteristics could be regarded as a model of schizophrenia and/or depression, and most likely of their common biological core [2].

It has been persuasively proven that selection-associated changes in normal and pathological behavior are associated with changes in the neurohumoral systems, including shifts in the parameters characterizing sexual function [11]. It is known that estrogens modulate activities of the majority of neurotransmitter systems, whose functions largely determine the psy-

chosomatic status [10]. The impact of sex hormones for the course of schizophrenia [3,4,6,7] and depression [8,12] was demonstrated. On the other hand, there are in fact no models of psychopathologies created with consideration for gender differences. The relationship between depression-like behavior of females and phases of the estrous cycle was recently demonstrated [13].

Hence, studies of the reproductive function in catatonic GC females seem to be important. We studied the rhythm of the estrous cycle and its dynamics in the ontogenesis and the pattern of folliculogenesis in two age groups.

## MATERIALS AND METHODS

Experiments were carried out on GC ( $n=40$ ) and Wistar ( $n=46$ ; control) females, obtained by 9 matings of GC and 9 matings of Wistar rats, respectively, weaned at the age of 1 month. Outbred GC and Wistar rat populations were maintained in a vivarium of Institute of Cytology and Genetics. The animals were kept in 60×40×20 cm cages, 4-6 rats per cage, under standard

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conditions at natural light with free access to water and food.

The structure and duration of the estrous cycle were studied by analysis of vaginal smears over 3-4 weeks in rats of different age (3, 6, 9, 12, and 18 months) [1].

Folliculogenesis was studied in two age groups. Three-month-old diestrus, proestrus, and estrus females and 18-month-old diestrus females (because the overwhelming majority of females at this age were in diestrus) were decapitated, the ovaries were removed, cleansed from adjacent tissues, and fixed in Lilly solution. At each stage of the estrous cycle, the ovaries of 8-12 females of each strain were examined. After standard processing, the ovaries were embedded in paraffin and serial sections (8  $\mu$ ) were stained with hematoxylin and eosin. Follicles at different stages of maturation were counted in serial sections of the ovaries as described elsewhere [9] according to this method, the follicles were differentiated into 9 types by the development and count of the granular cell layers. The following types of follicles were distinguished: growing (oocyte enveloped by well-developed granular cells, 1-4 layers and more: 1L, 2L, 3L, >4L, respectively), maturing cells (emergence of follicular cavity, EC), well-developed cavity (DC), and mature Graafian vesicle (GV).

The animals were weighed at birth and at the age of 20 days and 3, 6, 9, 12, and 18 months.

The results were statistically processed using Statistica 6.0 software. The differences between the means were evaluated using Student's *t* test, differences by the incidence by Fisher's *F* test, and were considered significant at  $p < 0.05$ .

## RESULTS

In all the studied age groups GC females had lower body weight than Wistar females (Table 1). The differ-

ence at birth was 15.7%. By the end of nursing period (20 days) the difference increased to 20%. Later, adult GC females progressively lagged behind the controls and by the age of 18 months the difference between the strains reached 35.6% ( $p < 0.001$ ).

Analysis of estrous cycle characteristics showed that the duration of the cycle in GC females before the age of 12 months was more stable and was 4-4.5 days, while in Wistar rats the cycle duration varied from 4.5 to 6.9 days (Table 2). Variability of the duration of the estrous cycle in females of these strains was primarily determined by differences in the duration of diestrus and estrus, while the duration of proestrus and metestrus was always 1 day.

During the development of the estrous cycle (at 3 months) GC rats demonstrated shorter diestrus and a greater percent of permanent estruses in the cycle in comparison with Wistar rats. The percent of animals with permanent estrus was higher among GC females (Table 2). Adult GC females (6, 9, 12 months) exhibited lower percent of abnormal estruses and diestruses in the cycle with lower incidence in the group. The estrous cycle of GC females was shorter, the duration of estrus and diestrus at the age of 6 and 12 months was shorter than in Wistar rats. In old females (at 18 months), an opposite picture was observed: the duration of the cycle in GC females was significantly longer than in Wistar rats. The differences in the duration of estrus and diestrus and in the incidence of abnormal cycle phases in the two strain disappeared (Table 2).

Study of folliculogenesis in 3-month-old animals showed differences between the strains (Fig. 1). A trend to lesser counts of growing and maturing follicles was observed during the diestrus stage in the ovaries of GC rats compared to Wistar females (Fig. 1, *a*). In GC females, the percent of growing follicles (1L, 2L, 3L, and >4L) was lower by 22.5% and per-

**TABLE 1.** Dynamics of Body Weight Gain in GC and Wistar Females

Age	GC		Wistar		Difference, %
	<i>n</i>	body weight, g	<i>n</i>	body weight, g	
Newborns	40	5.4 $\pm$ 0.1***	46	6.40 $\pm$ 0.05	-15.7
20 days	38	28.7 $\pm$ 0.6***	46	35.9 $\pm$ 0.7	-20.0
3 months	47	204.0 $\pm$ 3.3***	35	264.5 $\pm$ 4.0	-22.9
6 months	18	210.5 $\pm$ 3.7***	18	301.6 $\pm$ 5.5	-30.2
12 months	17	228.2 $\pm$ 5.0***	17	342.9 $\pm$ 6.3	-33.5
18 months	16	243.1 $\pm$ 3.9***	11	377.2 $\pm$ 7.1	-35.6

**Note.** \*\*\* $p < 0.001$  compared to the control (Wistar; Student's *t* test).

cent of maturing follicles (EC, DC, GV) was lower by 36.2% ( $p<0.05$ ). The percent of three-layer follicles and maturing follicles of two classes (EC and DC) was lower in GC. More active maturation of follicles during the proestrus stage led to an increase in the percent of mature GV (Fig. 1, *b*). More intense recruiting and transformation of follicles during this stage were paralleled by a significant decrease in the percent of single-layer follicles in GC and Wistar rats in comparison with that in diestrus. In addition, lower total count of follicles during the proestrus was found in GC compared to Wistar rats. For growing and maturing follicles this value was 42.5% ( $p<0.05$ ) and 39.9% ( $p<0.05$ ), respectively. Significant differences were found for the four follicle classes: lower percent of 2L, 3L, >4L, and EC follicles were found in GC compared to Wistar females. The estrus phase was characterized by ovulation and more active morphophysiological preparation to the next cycle (Fig. 1, *c*). During that period the number of growing follicles increased greatly in GC females in comparison with the proestrus:  $123.5\pm13.9$  vs.  $61.6\pm9.0$  ( $p<0.01$ ). The number of growing follicles in Wistar females remained virtually unchanged ( $98.3\pm8.9$  during estrus and  $107.0\pm9.0$  during proestrus). The percent of 1L and EC follicles during estrus was greater in GC than in Wistar females. With aging (18 months), the percent of growing and maturing follicles decreased significantly (Fig. 1, *d*). The number of growing 1L and 2L follicles during this period was high in GC rats compared to Wistar rats.

Hence, selection by high catatonic reactivity was associated with a significant body weight decrease and changes in the morphophysiology of the ovaries and estrous cycle in GC females.

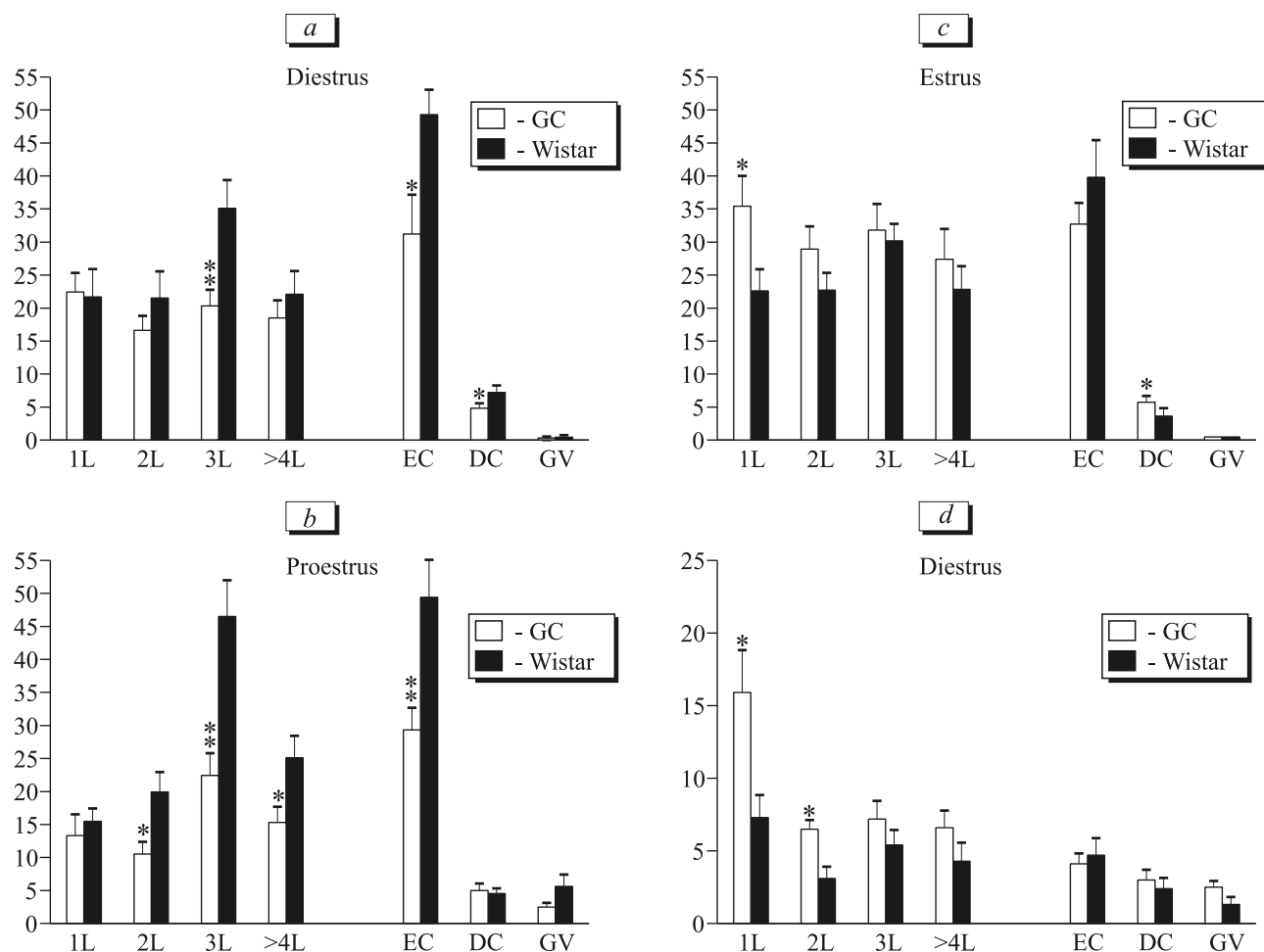
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**TABLE 2.** Characteristics of Estrous Cycles in GC and Wistar Rats of Different Age

Parameters	Age, months									
	3		6		9		12		18	
	GC (n=16)	W (n=15)	GC (n=18)	W (n=18)	GC (n=18)	W (n=18)	GC (n=17)	W (n=17)	GC (n=15)	W (n=15)
N	125	98	97	65	62	47	56	36	9	11
Duration of cycle, days										
diestrus	4.5±0.1	4.5±0.1	4.3±0.1***	6.9±0.4	4.00±0.01***	4.70±0.01	4.5±0.2***	5.3±0.3	9.4±0.8*	6.5±0.8
estrus	1.4±0.1*	1.7±0.1	1.7±0.2***	3.3±0.3	1.2±0.1	1.9±0.4	1.2±0.1***	2.2±0.3	5.7±0.6	6.6±1.2
Percent of PE in cycle	1.4±0.1	1.3±0.1	1.3±0.1***	1.8±0.1	1.0±0.0***	1.8±0.1	1.3±0.1**	2.0±0.2	1.1±0.1	1.4±0.1
Percent of AnD in cycle	7.0±2.2*	1.7±1.1	0.8±0.8*	16.9±4.4	3.9±2.9**	24.2±4.0	5.4±2.9*	26.0±8.7	2.8±2.8	3.7±3.7
Percent of PE in group	5.3±1.8	6.5±2.9	8.6±3.4*	45.1±8.4	0*	9.4±4.3	0*	16.7±7.3	81.8±6.3	82.5±8.1
Percent of AnD in group	43.8*	13.3	5.6**	50.0	11.1**	72.2	17.6*	47.1	6.7	10
Percent of group	37.5	33.3	33.3**	77.8	0*	22.2	0**	29.4	100	100

**Note.** N: number of analyzed estrous cycles; W: Wistar; PE: abnormal (permanent) estrus; AnD: abnormal diestrus. \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$  compared to the control (Wistar; Student's *t* test); \* $p<0.05$ , \*\* $p<0.01$  compared to the control (Wistar; Fisher's *F* test).



**Fig. 1.** Count of follicles of different types in GC and Wistar females at different stages of the estrous cycle at 3 (a-c) and 18 (d) months. 1L, 2L, 3L, >4L: growing follicles with 1, 2, 3, 4, and more layers of granular cells, respectively; maturing follicles: EC: emergence of cavity; DC: well-developed cavity; GV: Graafian vesicle. \* $p < 0.05$ , \*\* $p < 0.01$  compared to the control (Wistar; Student's  $t$  test).

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