HISTOLOGICAL STUDY OF THE PATHOLOGY OF OOGENESIS IN MICE WITH THE SMOKY MUTATION

E. L. Ignat'eva, A. M. Malashenko, and Z. K. Blandova*

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Mutants with light colored hair have been discovered in the C57BL/6Y (B6) line. Their mutation, known as smoky (smk), is caused by a recessive autosomal gene, which has not been localized. The smk gene has been shown not to be linked with sex or genes of chromosomes 1, 4, 7, and 17. It produces light colored hair more than the p or ln genes, and is characterized by definite instability, which is expressed as somatic mosaicism (the appearance of black stripes), which can formally be classified as the result of reversion of the smk gene. Instability depends on the genotype of the lines: 20.5% of mosaics were found in the B6 line, and 7% in the WR/Y line, into which this gene was introduced by a series of back-crosses. Probably the mutation of the smk gene is due to insertion of a region of sequences of virus DNA, by analogy with other unstable mouse genes (p^{un}, pe, Mi, W, etc.) [6]. The smk gene determines pathology of the female reproductive system while leaving fertility of males relatively normal. Under these circumstances two-thirds of the population of B6-smk/smk females are sterile on account of atresia of the vagina, due to which during sexual maturation they develop mucocolpos and mucometrium, with corresponding hypertrophy of the genital passage. In addition, in B6-smk/smk females with an open vagina one-third of individuals also are sterile (Table 1). Thus only one-quarter of individuals among B6-smk/smk females are fertile, and besides, with a low fertility coefficient (1.3 ± 0.1 offspring/month). Maintenance of the mutation on the basis of the WR line leads to sterility of all smk/smk females. Autopsy on sterile or potentially sterile smk/smk females aged from 1 week to 5 months revealed agenesis of the ovaries in some of them in the form of a rudimentary formation in the periovarian capsule, not always visible macroscopically: in five of 18 B6-smk/smk and nine of 13 WR-smk/smk females. At the infantile age agenesis of the ovaries is combined with aplasia of the uterus, which consists of a thin band. When an ovary is present in this group of mutants hypogonadism is observed and is most marked at the age of puberty and, moreover, independently of the state of the vagina, and is manifested by reduction in size of the ovaries of the sterile mutants at the age of puberty at least by half compared with the size of the ovaries of phenotypically normal females in the same phase of the estrous cycle.

To elucidate the character and causes of this hypogonadism, a histological study was made of the ovaries of smk/smk mutants at different times during the postnatal period.

EXPERIMENTAL METHOD

Inbred female C57BL/6Y-smk and WR/Y-smk mice aged 1, 2, and 4 weeks and 2, 4, and 5 months were used. The animals were kept under conventional conditions in the Department of Genetics of the Research Laboratory of experimental Biological Models, in T2 cages (from VELAZ) and were fed on granulated combined food of the PK-120-3 formula. Phenotypically normal females of the original line were used as controls for the smk/smk females. Before removal of the ovaries from the mature mutants with an open vagina, and also ln control females, vaginal smears were studied, and the animals were killed in the metestrus I phase. The ovaries together with the oviducts were fixed in Bouin's fluid and subjected to standard histological processing with embedding in paraffin wax. Serial sections 7 μ thick were stained with Ehrlich's hematoxylin and counterstained

*Deceased.

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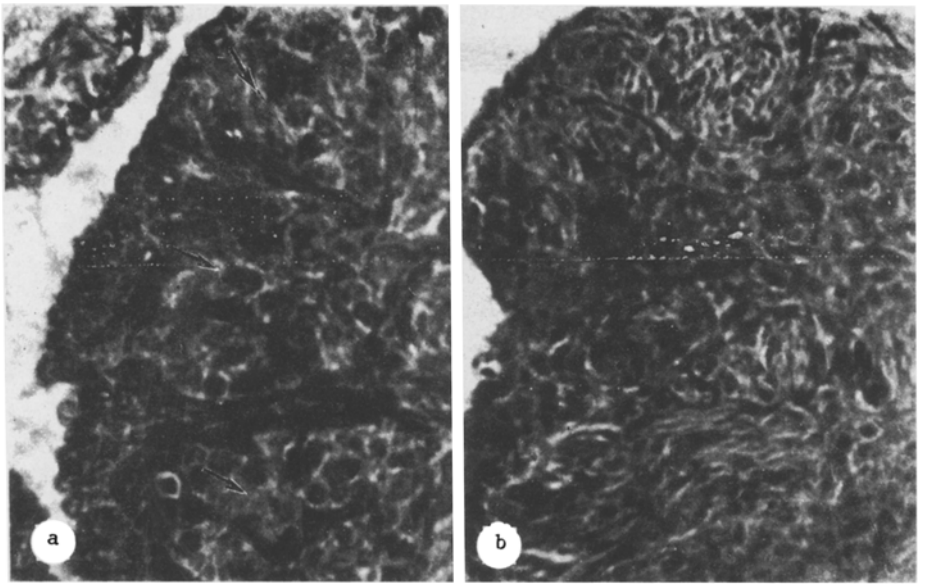

Fig. 1. Pathology of ovaries of mice with the smoky mutation. a) Fragment of ovary of WR-smk/smk mutant aged 1 week; arrows indicate sterile ovarian bands. 807×. b) Rudiment of ovary of B6-smk/smk mutant aged 2 months. 504×.

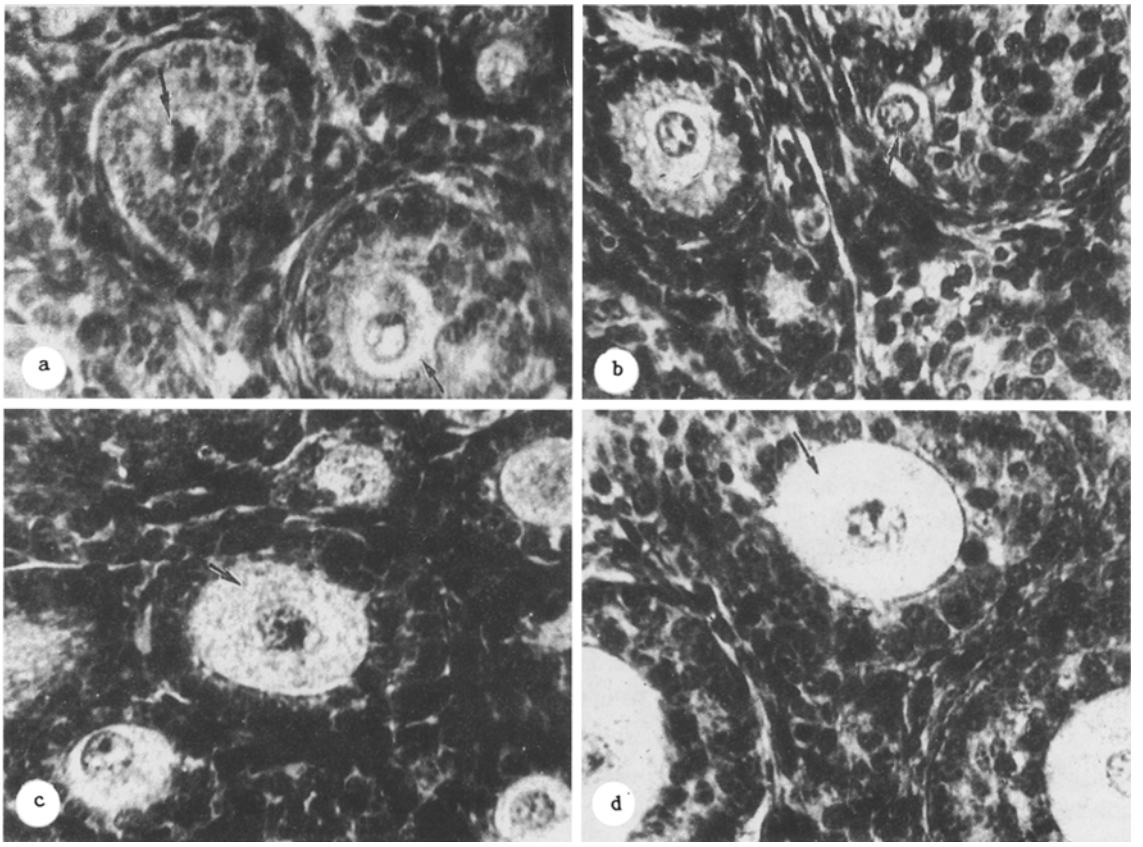

Fig. 2. Pathology of oogenesis of mice of B6-smk line. a) Fragment of ovary of B6-smk/smk mutant aged 4 weeks; arrows indicate growing follicles at stage 3b with atypical oocytes (lysis of oocyte in follicle on left). 807×; b) fragment of ovary of B6-smk/smk mutant aged 4 months; atypical oocyte of growing follicle at stage 4 (arrow). 807×; c, d) Fragments of postnatal ovary of female of control group with normal phenotype, arrows indicate normal oocytes of growing follicles at stages 3b and 4. 807×.

TABLE 1. Distribution of Sterile Female Mice in Population of B6-smk/smk Mutants

Group of animals	Number of females	Females			
		with atresia of vagina	with open vagina	sterile, with open vagina	fertile
Weaned young (1 month old)	68	42	26	—	—
Mature females of breeding nucleus	—	—	64	24	40

TABLE 2. Distribution of Atypical Oocytes by Stages of Follicle Formation in Ovary of B6-smk/smk Mutant Aged 4 Weeks

Stage of follicle formation (granulosa cells)	B6-smk/smk mutant		Normal phenotype	
	total number of oocytes	number of atypical oocytes	total number of oocytes	number of atypical oocytes
2 (to 10)	56	—	2081	—
3a (10—20)	122	101	29	0
3b (21—60)	124	98	35	0
4 (61—100)	1	1	67	0
5a (101—200)	2	0	93	0
5b—6—7 (more than 200)	0	0	98	0
Total number of oocytes in ovary	306	200	2403	0
Volume of ovary, mm^3	0,523		1,017	

with eosin. In cases of agenesis of the ovary the oviduct was fixed together with the periovarian capsule and adipose tissue adjacent to it. In histological sections of the ovaries the stages of oogenesis and follicle formation were assessed in accordance with the classification in [5], elaborated for mice, which is based on counting the number of granulosa cells in a median section through a follicle, passing through the nucleus and nucleolus in the oocyte. The nucleocytoplasmic ratio was determined by measuring the diameters of the oocyte and its nucleus and calculating their area, as well as the area occupied by the ooplasm, by the formula: $\pi r^2/(\pi R^2 - \pi r^2)$, where r denotes the mean radius of the nucleus, and R the mean radius of the oocyte. The volume of the ovaries was calculated by the formula $^4/_3\pi R^3$, where R is the mean radius of the ovary.

EXPERIMENTAL RESULTS

The histological study of the ovaries of smk/smk mutants of both lines revealed variants of pathological features which differed in their degree. For instance, at the age of 1-2 weeks ovaries were observed with oocytes at only the earliest stages of follicle formation (stages 2 and 3), whereas in ovaries of the control group of the same age we observed more advanced changes (stages 4 and 5). Serial sections through whole ovaries of immature mutants showed extensive zones with empty ovarian bands, i.e., completely without sex cells (Fig. 1a).

During sexual maturation and later, a marked deficiency of oocytes and follicles was observed in the ovaries of the mutants, mainly on account of primordial forms (stage 2). In addition, in B6-smk/smk females mass delay of development of oocytes in the initial stage of dictyotene was observed, despite continuing autonomous growth of their follicles. The phase of great growth of the oocytes [3] is evidently absent in the oogenesis of these mutants, and during the transition of the follicles to stage 3a, and thereafter 3b and 4, when under normal conditions the nucleocytoplasmic ratio in the oocytes falls to its minimal level due to intensive growth of the ooplasm, which is absent in the mutants (Tables 2 and 3; Fig. 2). The nucleus of these atypical oocytes, which were absent in the ovaries of the control animals, were not increased in size and preserved the interphase structure characteristic of the stage of the primordial follicle (stage 2): subsequent scattering of the chromosomes did not take place in the nucleoplasm and the nuclear membrane did not become folded [1]. The nonviability of the atypical oocytes was confirmed by their observed lysis in the growing follicles (Fig. 2a). This last phenomenon differs from the usual physiological atresia of the follicles with characteristic necrosis of the oocytes which have passed through the phase of great growth. The main mass of follicles of mature B6-smk/smk mutants were in the stage of average solid follicles (stages 3b and 4) with atypical

TABLE 3. Morphometry of Oocytes of Sterile B6-smk/smk Mutants ($M \pm m$)

State of follicle formation	Parameter	Atypical oocytes	Normal oocytes	p
	Diameter of oocytes	19,2±0,8	24,9±1,1	<0,001
	Diameter of nucleus in oocyte, μm	11,4±0,3	12,8±0,5	<0,05
3 a (n=10)	Nucleo-cytoplasmic ratio	0,62±0,09	0,37±0,02	<0,02
	Diameter in oocytes, μm	21,5±0,6	44,6±1,6	<0,001
3 b (n=10)	Diameter of nucleus in oocyte, μm	11,7±0,3	18,3±0,7	<0,001
	Nucleo-cytoplasmic ratio	0,48±0,07	0,20±0,01	<0,002
	Diameter in oocytes, μm	20,8±0,7	59,0±1,6	<0,001
4 (n=10)	Diameter in nucleus in oocyte, μm	11,6±0,4	22,3±0,7	<0,001
	Nucelo-cytoplasmic ratio	0,49±0,01	0,17±0,06	<0,001

oocytes, although single cases of the appearance of a large antral follicle with a normal oocyte could be observed (stages 5b-6-7, 1-3 follicles per ovary in the metestrus I phase). Corpora lutea also were rarely seen. Thus an acute deficiency of normally developing oocytes of the maturing follicles and of corpora lutea was found, and this may explain the dwarf size of the ovaries in the mutants.

Finally, rudimentary formations in the parovarian capsules with no sexual elements and with disorganized somatic cells, occasionally forming bands resembling the ovarian kind, were recorded in infantile and also sexually mature smk/smk mutants of both lines (Fig. 2b). In the case of a more highly developed somatogenesis of the ovaries and an acute deficiency of sex cells a tendency was found for actively invaginating epithelium to proliferate and also to invade the tissues surrounding the ovary. This recalls the pattern of development of a tubular adenoma in sterile female mice with the W^v mutation [5], in which the process of migration of primary sex cells and their colonization of the rudimentary gonad is disturbed, evidently because of disturbed metabolism [3]. The same cause may perhaps lie at the basis of sterility, at least of those smk/smk individuals in which only the rudiments of an ovary are observed, a situation which is commonest in the WR-smk line.

As regards B6-smk/smk mutants with atypical postnatal oogenesis, since this is essentially the arrest of nucleogenesis it can be postulated that in this case synthetic processes are affected at the transcription level, and their activity normally reaches a maximum at this stage of oogenesis because of the enormous consumption of ribosomal RNA [1, 2]. In turn, this last pathology may be the result of a disturbance of the preceding initial stages of prophase I of meiosis (synapsis, crossing over, chiasma formation). Pathological action of factors exogenous relative to postnatal oocytes, and in particular interaction of oocytes with follicle cells and, through them, with gonadotrophic hormones, seems unlikely, because growth of the oocytes ceases definitely after growth of their follicles whose autonomy is due to some degree, evidently, to the absence of a properly formed glassy membrane of the oocyte.

It is a fact of practical importance that the cyclic nature of estrus, as revealed by vaginal smears, although irregular (with a period of 6 to 11 days and with prolonged estrus to 4-6 days), was nevertheless found in smk/smk mutants even with ovarian agenesis. This confirms the existing view that a cyclic function can be transmitted to other steroid producers, such as the adrenals in the case of nonfunctioning ovaries. Consequently, the cyclic nature of estrus, i.e., its biphasic character, cannot be a sufficient criterion by itself for the diagnosis of ovarian function.

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