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MORPHOLOGICAL AND FUNCTIONAL CHARACTERISTICS OF THE FEMALE REPRODUCTIVE SYSTEM IN MICE OF THE MUTANT B10-hr^{rhY} LINE

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KEY WORDS: mutation; hairless; sterility; ovary; homozygotes; transplantation.

A spontaneous autosomal recessive mutation causing loss of hair has been discovered at the Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR. The mutation is allelic with the hairless (hr) mutation in chromosome 14, but differs in its effect from known alleles of that locus. Phenotypically the mutants are similar to the rhino mutation, and the gene is designated by the symbol hr^{rhY} (hairless rhino—Yurlovo). The mutants are characterized by defects of the hair cover and of skin structure, by a defect of the immune system, and by sterility of the females [1]. The mutation has now been maintained on a genetic basis in line C57BL/10SnY (abbreviated to B10), by crossing heterozygous females with male hr^{rhY}/hr^{rhY} homozygotes. The heterozygous females have normal fertility, but ability of the males to reproduce is depressed, and by the age of 4-6 months they lose their fertility.

The aim of this investigation was to study the causes of sterility in females, for this is a characteristic that is unknown for other alleles of the hr gene which have been described, and it was also aimed to develop an effective method of breeding these mutants.

EXPERIMENTAL METHOD

Mice of inbred line B10 and mutant line B10-hr^{rhY} were used and bred under conventional conditions in the Department of Genetics, Research Laboratory of Experimental Biological Models. The animals were kept in T2 cages (VELAZ) and were fed on the granulated combined feed PK-120-3.

For cytological investigation of the estrous cycle in the mutants and mice of the isogenic line aged 2-6 months, morning vaginal smears were obtained daily for 2 weeks during the summer. For histological processing the ovaries of females whose vaginal smears had previously

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TABLE 1. Comparative Investigation of Estrous Cycle of Mutant hr^{rhY}/hr^{rhY} and Normal B10 Females

Line of mice	Duration of estrous cycle, days	Fraction of cycle accounted for diestrous phase, %	Average volume of ovaries, mm^3
B10	4,6±0,5 (n=5)	24,3±1,5 (n=5)	14,8±1,6 (n=8)
B10—hr^{rhY}	6,0±0,3 (n=8)	32,9±1,5 (n=8)	8,9±1,4 (n=8)
p	<0,05	<0,05	<0,01

been examined were removed from the peritoneal cavity together with the oviduct and part of the uterine cornu. After measurement of the parameters of the ovary, the material was fixed with Bouin's fluid, dehydrated, and embedded in paraffin wax. Serial sections 7 μ thick were stained with Ehrlich's hematoxylin and counterstained with eosin. The ovaries were transplanted by the method described by Stevens [4]: one half of an ovary of a mutant donor of the same age was transplanted as a free graft into 5-week-old recipients after bilateral ovariectomy, on each side, into the residual ovarian bursa, and 3 weeks after the operation, the recipients were mated with males.

EXPERIMENTAL RESULTS

Hairless females, homozygous with respect to the hr^{rhY} gene were sterile despite periodic estrus. The estrous cycle of these females at the age of 2-6 months (Table 1) differed significantly from the cycle in females of the original B10 control line in its longer duration: the average duration of the cycle in the mutants was 6.0 ± 0.3 days, compared with 4.6 ± 0.5 days ($p < 0.05$) in the control mice. The diestrous phase accounted for a larger than normal fraction of the total for the original line. In some cases, however, especially in 2-month-old mutants, protracted estrus up to 2 days in duration was observed.

The uterus of the hairless mutant females was anemic at all phases of the estrous cycle. Histological sections of the endometrium of a 2-month-old mutant during estrus revealed weaker activity of the uterine epithelium than the ordinary estrous reaction in the control: only occasionally was the epithelium stratified, with slight secretion of mucus. Examination of serial sections through the oviducts of estrous and metestrous hairless females revealed no ovulated oocytes whatever, whereas in the control mice of the isogenic line, 2-4 oocytes were found in each oviduct during these same phases of the cycle.

Serial histological sections through the ovaries of the hairless mutants in different phases of the estrous cycle (in proestrus, estrus, and metestrus) showed a relatively normal state of oogenesis and of folliculogenesis, starting from the stage of primordial follicles and ending with the stage of large antral follicles with karyospherical nuclei of their oocytes (stage 7 according to the classification of Pedersen and Peters [3]). However, during further growth of the follicle, reinitiation of meiosis, which is usual in the preovulatory stage, was not observed in oocytes of the mutants, although the presence of reinitiated oocytes in the atretic follicles of these animals was evidence of the potential ability of their oocytes to undergo maturation divisions. No fresh corpora haemorrhagica, which could be a sign of recent ovulation, likewise were found in late estrus or in metestrus in females of the original line. Complete absence of corpora lutea in ovaries of the mutants also was characteristic (for example, in the control from 5 to 15 corpora lutea were counted in 2-month-old females. This last finding is evidently reflected also in the average volume of the ovaries, which was much less in the mutants.

On the basis of the results of this histomorphological investigation, it was postulated that the cause of sterility of the hairless B10-hr^{rhY} females lay outside the gonads, and it was the result of insufficient production or activity of the pituitary luteinizing hormone, responsible for ovulation, which led to an anovulatory cycle. However, the possibility of disturbance of reception of this hormone by the follicular cells in the ovaries of the mutants cannot be ruled out. In turn, the absence of ovulation and, correspondingly, of corpora lutea in these mice was responsible for the deficiency of gestagens and estrogens and, consequently, for the weak (sometimes protracted) estrous response of the uterus.

TABLE 2. Results of Transplantation of Ovaries from Sterile hr^{rhY}/hr^{rhY} Females into Fertile Recipients

Expt. No.	Line and genotype of recipient	Number of recipients	Genotype of mating partner	Number of offspring		Average size of litter on weaning
				total	homozygotes	
1	B10 - hr^{rhY} +/+ or +/hr^{rhY}	4	+/hr^{rhY}	52	25 (48.8%)	4.7±0,5
2	F1(B10×129) +/+	4	+/hr^{rhY}	66	35 (53.0%)	5.1±0,8

To test the hypothesis that the cause of sterility of the hairless females lies outside gonads, experiments were carried out with transplantation of their ovaries into isogenic B10 females. The recipients were mated with males heterozygous for the hr^{rhY} gene. Half of the progeny was homozygous for the hr^{rhY} gene, i.e., hairless, whereas the other half had a normal hair cover and was heterozygous (+/hr^{rhY}). The same results were obtained on transplantation of the ovaries from homozygous mutants into (B10/Sn × 129/J)F_1 hybrid recipients with a similar subsequent mating (Table 2). The fertility of the females with transplanted ovaries was normal, and characteristic in the first experiment on the B10 line.

Ovaries transplanted from mutant females against the hormonal background of nonmutant recipients were thus shown to possess reproductive function and, consequently, the cause of sterility of hairless B10-hr^{rhY} females was proved to lie outside the gonads, possibly connected with steroid-dependent neuroendocrine disturbances [2]. Moreover, the positive results of experiments involving transplantation of ovaries into sterile mutants are of considerable practical importance, for they suggest the optimal method of maintaining a valuable mutant line of mice which are incapable of natural reproduction.

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SYNTHESIS AND CONTENT OF DNA IN EPIDERMAL CELL NUCLEI OF MOUSE SKIN DURING DIFFERENTIATION AND SPECIALIZATION

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The keratinocytes of mammals have distinct morphological criteria of the successive stages of their multiplication, differentiation, and specialization and they provide a convenient model with which to study the mechanisms regulating proliferation and differentiation. It was shown previously on other model systems that during development of renewing cell populations the cells the mitotic cycle for differentiation not only in the G_1, but also in the G_2 phase and they form fractions of specialized cells with a double DNA content and with enhanced functional activity [4, 5]. This phenomenon, which is one form of hyperreplication of DNA [3], was particularly well marked in cases when extremal conditions of development of populations have necessitated a more rapid rate of cell differentiation and specialization [2]. It therefore seemed interesting to investigate a cell population in which, even under ordinary conditions,

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