A METHOD OF OBTAINING IMMUNOBIOLOGICAL CONFLICT BETWEEN MOTHER AND FETUS IN RATS

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A model of immunobiological conflict between mother and fetus was obtained by immunizing female rats with homologous testicular antigens.

Since the discovery [6, 8, 9] of the antigenic properties of sperm, consideration has been given to the immunological hypothesis of sterility. It is only during the last 30 years that the use of sperm injections into women for contraceptive purposes have been investigated. Temporary sterility and a lowering of fertility have been observed [4] in women sensitized with human sperm. Circulation of antibodies against sperm, which are present in only 12% of women known to be pregnant [5], can be detected in 78% of patients with sterility of unknown origin.

Isoantigenic incompatibility between mother and fetus is an important cause of perinatal and fetal mortality. Hemolytic disease of the newborn is a disease encountered in obstetric practice. A model of hemolytic disease of the newborn has been obtained in experiments on rats and monkeys by isoimmunization of females with the blood of males of incompatible blood groups [1, 2]. After immunization of rabbits with the semen, testis, and seminal fluid, the rate of survival of embryos and fetuses was reduced compared with the results of injection of seminal fluid and physiological saline in the control group [7].

The object of the investigation described below was to obtain an experimental model of isoantigenic incompatibility between fetus and mother in rats by immunizing pregnant rats with homologous testicular tissue.

EXPERIMENTAL METHOD

A total of 100 female Wistar and noninbred rats weighing 150-280 g, with a normal estrous cycle, subdivided into 60 experimental and 40 control animals, was used. To rule out the possibility of primary sterility, females which had already had a normal pregnancy and given birth to viable young rats were selected for the experiments. Antigenic material for immunization and for the complement fixation test (CFT) consisted of the supernatant of homogenized testes of noninbred and Wistar rats. The testes of 20 animals killed at the same time were homogenized with physiological saline (1 part by weight of tissue to 4 volumes of physiological saline) for 10 min. The homogenate was kept in a sterile container at 4°C. The protein content in the supernatant was determined refractometrically after 24 h.

Subcutaneous injections of the supernatant fluid into the region of the anterior abdominal wall were given to the animals 3 times a week for 4 weeks in increasing doses: 1st injection 3 mg protein, 2nd injection 4 mg, 3rd 7 mg, 4th 9 mg, and all subsequent injections 13 mg protein each. Altogether each animal received 86 mg protein. After the end of immunization, all the females in a state of estrus were mated. The stages of the estrous cycle were assessed from fresh vaginal smears and smears stained by the May-Gruenwald method, the occurrence of copulation was judged from the presence of spermatozoa, and the initial stages of pregnancy were determined from the presence of a blood clot in the vagina, and the

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TABLE 1. Effect of Immunization with Homologous Testicular Tissue on the Course of Pregnancy and State of the Fetuses in Rats

State of mother and fetus	Experimental group		Control group		X^2
	abs.	%	abs.	%	
Death of mother at parturition	4	6.66	1	2.5	41.5
Intrauterine absorption of fetus	5	8.33	2	5.0	36.2
Stillbirth	8	13.3	1	2.5	47.4
Postnatal death on 1st day	7	11.6	–	–	64.4
Death of rats during 1st week of life	6	10.0	–	–	55.6
Secondary sterility	24	40.0	–	–	84.1
Death of mother from other causes	3	5.0	1	2.5	21.4
Normal birth of viable fetuses	3	5.0	35	87.5	
Total number of females	60	100.0	40	100.0	
Number of living, viable fetuses born	35	–	321		

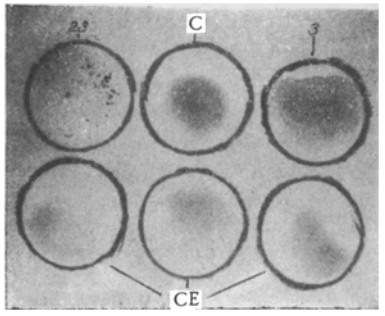

Fig. 1. Direct Coombs' test. CE) Erythrocyte control; C) control; 23, 3) serial numbers of experimental rats.

presence or absence of spermagglutination. The titer of antispermatozoal antibodies in the blood serum of the epxerimental and control females was determined by the CFT. The direct Coombs' test in the modification described in [3] was performed on all the newborn rats.

EXPERIMENTAL RESULTS

The results are given in Table 1.

In 21 of 60 cases the newborn rats had hemolytic disease, in a latent clinical form in 13, but with frank signs of anemia and a positive direct Coombs' test. The hemagglutination test became positive in 12 cases after 10-15 sec, and in only 1 case after 3 min (Fig. 1). In the control group the Coombs' test was negative in every case. Microspermagglutination in the vaginal mucus (Fig. 2) was observed in the experimental females which were sterile more than 40-60 days after the end of immunization. No such picture was observed in the smears of the vaginal contents of the control females.

The results of the CFT with the serum of the immunized females showed that 1 week after the end of immunization the titer of antibodies was 1:100, after which it increased or remained within the same limits

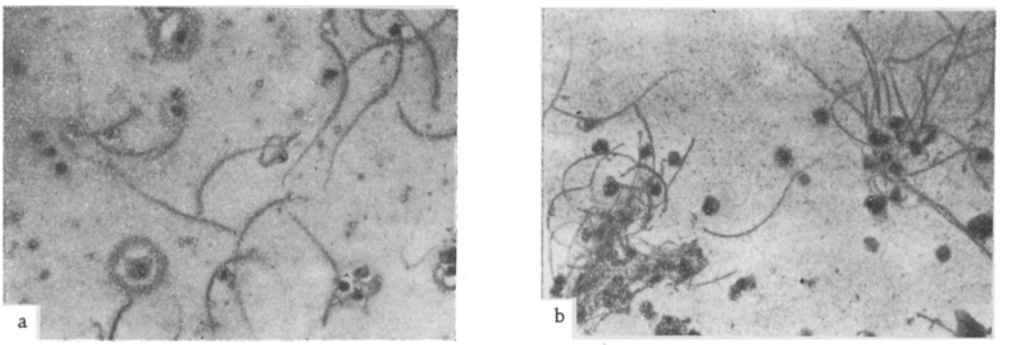

Fig. 2. Free-lying spermatozoa in vaginal smear of control rats (a) and spermagglutination in experimental rats (b). May–Grünwald method, 120 ×.

for 6 months. No antibodies were found in the control animals. The statistical significance of the difference between the results ($P < 0.001$) suggests that by immunizing the females with antigens of homologous testicular tissue, a model of immunobiological conflict between mother and fetus was obtained. In all probability antibodies against spermatozoa, formed in the female as a result of immunization, circulate in the blood stream and also penetrate into the cervical canal and vagina, where they immobilize the spermatozoa, thus causing sterility. Where immunization of the female did not prevent conception, these antibodies possibly penetrated as far as the fetus and reacted with its paternal component, thus leading to fetal and neonatal pathology.

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