

## EXPERIMENTAL BIOLOGY

### INTRAUTERINE DEVELOPMENT OF RATS TREATED WITH HOMOLOGOUS PLACENTAL CYTOTOXIC SERUM

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The pathogenic effect of antiplacental cytotoxic serum in rats is associated with a severe disturbance of placentation and is manifested by death of the fetus and resorption of the amnion. The harmful action of the antiserum is most marked if it is given on the 8th day after conception.

An important role in the pathogenesis of late toxemias of pregnancy is played by immunization of the mother by placental tissue antigens [1, 4]. The author has shown that such immunization occurs in 69.1% of pregnant women with toxemia [2]. As the result of prolonged circulation in the maternal blood, placental antibodies may be one of the causes of the various morphological injuries to placental tissue observed in late toxemias, which disturb the intrauterine development of the fetus.

In addition, residual phenomena of sensitization with placental tissue may lead to a disturbance of placentation and may complicate the course of subsequent pregnancies.

The object of the present investigation was to confirm this hypothesis experimentally by studying the effects of various doses of homologous placental cytotoxic serum on the course and outcome of pregnancy in albino rats.

#### EXPERIMENTAL METHOD

Placental cytotoxic serum was obtained by immunizing rabbits with a homogenate of a 19-day placenta preliminarily washed to remove blood.

The immunization cycle consisted of 4 injections of 2 ml homogenate (100 mg protein), with an interval of one week between injections. For the first intramuscular injection the homogenate was mixed with an equal volume of Freund's adjuvant. Subsequently the homogenate was injected intraperitoneally.

Experiments were carried out on noninbred female albino rats. Placental antiserum with a titer of 1:100 was injected intraperitoneally into the animals in doses of 1, 0.5, and 0.2 ml/100 g body weight on the 4th, 6th, 8th, 10th, and 12th days of pregnancy.

Pregnancy was considered to have started on the day when spermatozoa were found in the morning vaginal smear. Control animals received injections of normal rabbit serum by the same method, in the same doses, and at the same times of pregnancy. The results of the experiment were ascertained at laparotomy and opening of the uterine cornua on the 19th day of pregnancy (the number of corpora lutea, the number of dead and living fetuses, and the number of resorbed amnions were counted).

#### EXPERIMENTAL RESULTS

Following injection of serum at all the times of pregnancy specified above, the mean number of corpora lutea and the mean number of implanted embryos did not differ significantly from the corresponding numbers

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TABLE 1. Outcome of Pregnancy for Fetuses Depending on Time of Injection of Placental Cytotoxic Antiserum (1 ml/100 g body weight)

Day of injection after conception	No. of animals	Living fetuses				Fetuses dying after implantation			
		abs.		M $\pm$ m, %		dead		resorbed	
		abs.	t	M $\pm$ m, %	t	abs.	M $\pm$ m, %	abs.	M $\pm$ m, %
4 th Control	4 3	35 27		87,5 $\pm$ 5,2 96,4 $\pm$ 3,5	1,4	1 1	2,5 $\pm$ 2,5 3,6 $\pm$ 3,5	4 —	10,0 $\pm$ 4,7 —
6 th Control	5 3	39 24		79,6 $\pm$ 5,7 92,3 $\pm$ 5,2	1,6	1 —	2,0 $\pm$ 2,0 —	9 2	18,4 $\pm$ 5,5 7,7 $\pm$ 5,2
8 th Control	6 4	6 35		10,5 $\pm$ 4,0 92,1 $\pm$ 4,4	1,36	3 —	5,3 $\pm$ 2,9 —	48 3	84,2 $\pm$ 4,8 7,9 $\pm$ 4,4
10 th Control	7 3	19 22		29,7 $\pm$ 5,7 88,0 $\pm$ 6,5	6,8	4 —	6,3 $\pm$ 2,5 —	41 3	64,0 $\pm$ 6,0 12,0 $\pm$ 6,5
12 th Control	5 4	27 34		54,7 $\pm$ 7,2 94,4 $\pm$ 3,8	4,8	7	17,6 $\pm$ 5,5 —	13 2	27,7 $\pm$ 6,5 5,6 $\pm$ 3,8

TABLE 2. Outcome of Pregnancy Depending on Dose of Placental Antiserum Injected on 8th Day of Pregnancy

Animals	Dose (in ml/100 g body weight)	No. of animals	Living fetuses				Fetuses dying after implantation			
			abs.		M $\pm$ m, %		dead		resorbed	
			abs.	t	M $\pm$ m, %	t	abs.	M $\pm$ m, %	abs.	M $\pm$ m, %
Experimental	1,0 0,5 0,2	6 3 3	6 6 9		10,5 $\pm$ 4,0 22,2 $\pm$ 8,0 32,2 $\pm$ 8,8	13,6 7,7 5,1	3 3 2	5,3 $\pm$ 2,9 11,1 $\pm$ 6,2 7,1 $\pm$ 4,9	3,1 1,8 1,4	84,2 $\pm$ 4,8 66,7 $\pm$ 9,2 60,7 $\pm$ 9,2
Control	1,0	4	33		9,2 $\pm$ 4,4		—	—	3	7,9 $\pm$ 4,4

in the control group. The number of corpora lutea in both groups of rats exceeded the number of implantation sites by 9.4-10%, corresponding to the physiological normal [3].

The outcome of pregnancy differed depending on the time of injection of the placental antiserum (Table 1). Although it had no appreciable effect when injected on the 4th and 6th days after conception, it exhibited a strong negative effect on the 8th day of pregnancy (10.5% of living fetuses). Later this effect was substantially weakened (the percentage of living fetuses was two-thirds that in the control group). At the time of autopsy dead fetuses were found only in the experimental rats, but their number was considerably higher if serum was injected on the 8th and subsequent days of pregnancy.

The harmful action of the antiserum on pregnancy was reflected most clearly in the number of resorbed embryos. In the control rats the frequency of resorption of the embryos varied from 5.6 to 12%. In the experimental animals the proportion of resorbed embryos on the 4th, 6th, 8th, 10th, and 12th day was 10, 18.4, 84.2, 64, and 27.7%, respectively. The differences for the 8th, 10th, and 12th days of pregnancy are statistically significant.

The harmful action of the cytotoxic antiserum thus began to show itself on the 6th day of pregnancy, and reached a maximum on the 8th day, and was still definitely present if the serum was injected on the 10th and 12th days. Consequently, the pathogenic effect of injection of placental antiserum coincided with the period of formation of placental tissue.

The most specific manifestation of this effect is the termination of development of pregnancy. Further progress of the pregnancy becomes impossible because of the severe disturbance of placentation. Death of the fetuses is a later result of this disturbance.

The results of experiments to examine the effect of different doses of the serum at the critical period of its action (8th day of pregnancy) are given in Table 2. To produce a severe form of disturbance of intrauterine development, manifested by resorption of the amnions in the early stages of ontogenesis, a dose of 0.2 ml serum/100 g body weight was sufficient provided that it was injected at the beginning of placentation. Increasing the dose of serum to 1 ml led to a particularly severe disturbance of placentation.

The results of these experiments described above indicate that immunologic injury to the placenta can take place, followed by an unfavorable outcome of pregnancy. The fact that severe forms of disturbance of these processes occur only at the period of initial formation of the placenta as an organ deserves special attention. After the completion of its formation the placenta becomes sufficiently stable to ensure the further progressive development of pregnancy and to withstand a single exposure to the action of cytotoxic placental antiserum.

#### LITERATURE CITED

1. P. G. Zhuchenko, *Akush. i Gin.*, No. 1, 64 (1964).
2. I. S. Klimets, *Vopr. Okhr. Mat.*, No. 8, 59 (1967).
3. P. G. Svetlov, in: *Pathophysiology of Intrauterine Development* [in Russian], Leningrad (1959), p. 114.
4. H. Wilkin, *Z. Geburtsh. Gynak.*, 161, 113 (1963).