# THE ANTIBIOTIC PROPERTIES OF THE EJACULATES OF MAMMALS AND MAN

## V. N. Kulikova

Dept. of Embryology (Chief - Prof. B. P. Tokin), Leningrad State University

(Received April 19, 1956. Presented by Prof. P. S. Kupalov, Active Member of the Academy of Medical Sciences, USSR)

The purpose of our work was to examine the secreta of the accessory genital glands of mammals and man for bactericidal and fungicidal properties.

## EXPERIMENTAL METHODS

We used the sperm of chinchilla rabbits in the experiments. A series of experiments was conducted using human ejaculate. Bacillus subtilis, Micrococcus lysodeikticus, Staphylococcus aureus, Vibrio fluorescens, Torula utilis yeast and fungi from the Actinomycetaceae family, Actinomyces alba and Actinomyces griseus were used to determine bactericidal and fungicidal properties.

Rabbit sperm was received in an artificial vagina constructed according to V. K. Milovanov [1]. Human sperm was provided by the clinic of the Otto Gynecologico-Obstetrical Institute. The rabbit sperm was used in the experiment 10-15 minutes after it had been received; the human sperm, on the average, was used within 4 hours. Ejaculate with active spermatozoa was used to set up the experiment. Two experimental methods were used.

In the first method(I), the rabbit ejaculate was sterilized by being passed through a Seitz filter with an asbestos pad. First, however, the experimental fluid was diluted with 5 parts of water, since the amount obtained from two ejaculations (1-2 cm³) was not enough for the rate of filtration. An emulsion of bacteria or fungi was added to the filtrate in a proportion of 2:1. In the experiments with bacteria, the emulsion contained 100,000 cells per 1 cm³ — with fungi, 10,000 cells per 1 cm³. Either a fluid favorable to microorganisms (meat-peptone bouillon or must) or sterilized water was used as the control. The experimental and control test tubes were placed in an incubator at a temperature of 37° (for the bacteria) or 27° (for the fungi). Immediately, and then in 1, 5 and 24 hours, the culture medium from the experimental and control test tubes was transferred to a solid medium in sectors by a platinum loop. The experiment was repeated 8 times. The results were determined after 24 hours (for bacteria) or after 48 hours (for fungi).

To verify the results obtained from the experiments with diluted rabbit ejaculate, we set up another series of experiments in which human sperm was examined by another method (II). 1.5-2 cm of a Petri dish was filled with a meat-peptone agar and then seeded with a one-day bacteria culture of 1,000 colonies transferred on to the dish by a pipette (with this method, no experiments with fungi were conducted). Then, under sterile conditions, a heated, sterile glass "ring" (cylinder), 0.6 cm in diameter was lightly fixed to the agar. 0.5 cm of the undiluted sperm or ejaculate, which had previously been stripped of spermatazoa by centrifugation in order to exclude the possibility of bactericidal action from the substances secreted by the spermatazoa during their activity, was dropped into the ring. As the control, meat-peptone, bouillon was used, or an empty ring was fixed in order to find whether the ring temperature influenced the bacteria. The experimental and control dishes containing the cultures were placed in an incubator at 37°. The results were calculated after 24 hours.

### EXPERIMENTAL RESULTS

The secreta of the accessory genital glands of the rabbit are destructive to V. fluorescens. A. alba and A. griseus (see table). Even after an hour's exposure, the growth of the Actinomycetes colonies was noticeably weaker than in the control. An average of about 100 colonies grew up in the control; in the experiment, about 10. The cultures showed no growth whatsoever after 5-24 hours. From this one can conclude that the secreta of rabbit accessory sex glands have strong fungicidal properties with respect to the Actinomycetes used.

Action on Different Microorganisms of the Accessory Genital Gland Secreta of the Rabbit

Microorganism	Number of rabbits	Number of experiment		Bactericidal and fungicidal properties discovered		No bactericidal or fungicidal proper ties discovered	
		1	11	1	l II	1	п
B. subtills	7	35	10	2	-	33	10
M. lysodeikticus	7	46	10	_		46	10
S. aureus	7	42	2	-	. –	42	2
V. fluorescens	6	27	2	18	2	.9	_
T. utilis	3	17		_		17	
A. alba	3	27	-	27	_		
A. griseus	3	27	-	27	_		

Note: I) experiments set up according to the first method; II) experiments set up according to the second method.

In the experiments with the action of the fluid portion of the rabbit ejaculate on <u>V. fluorescens</u>, it was discovered that not only the ejaculate from different males, but even different batches from the same male act in different ways upon this microorganism. The feeding and maintenance conditions of the experimental animals and the experimental processes were identical. Eighteen out of 27 experiments conducted by the first method had a bactericidal effect. Only 2 experiments were done with the second method; in both cases bactericidal action resulted.

The secreta of the accessory genital glands of male rabbits were found to have no bactericidal properties with respect to M. lysodeikticus, B. subtilis, S. aureus, or fungicidal properties with respect to T. utilis.

S. aureus and M. lysodeikticus were used to study the bactericidal properties of human ejaculate. The ejaculate of 20 men was examined — in 11 cases it was normal; spermatozoa quantity was low (up to 10 specimens per visual field) in 4 cases, and azoospermia was found in 5 cases. The amount of experimental fluid (1-3 cm<sup>3</sup>) was not always sufficient to permit experiments with both kinds of bacteria. Twenty experiments were done with Staphylococcus aureus, in 10 of these a sterile zone 0.2-0.5 cm in size was established around the ring affixed to the agar. S. aureus did not grow inside the ring in any case, including the one experiment in which azoospermatic ejaculate was used. Twelve experiments were done with M. lysodeikticus — in two a bactericidal effect was discovered.

The human ejaculate was not sterilized. In the course of culturing the media, different bacteria of a common aerial microflora and other specific bacteria were discovered in it. Special tests established that these were not antagonistic to S. aureus and M. lysodeikticus. Consequently, their presence had no influence on the results of the experiments.

Since the experiments were done in vitro, one cannot state that the experimental fluid would have the same properties in the body. Moreover, the microorganisms were chosen arbitrarily.

## SUMMARY

Fungicidal and bactericidal properties of the secreta of the accessory genital glands in mammals and man were studied.

It was found that the secreta of the accessory genital glands in rabbits possess strong fungicidal properties with respect to Actinomycetes (A. alba and A. griseus) and to V. fluorescens.

The growth of colonies in the experiments was ten times less than that in the control.

Human ejaculate possesses bactericidal properties with regard to Staphylococcus aureus.

## LITERATURE CITED

[1] V. K. Milovanov, Artificial Insemination of Agricultural Animals, Moscow 1938.

<sup>\*</sup> In Russian.