Antiserum Inhibition of Rabbit Spermatozoal Adherence to Ova[1] (35839)

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Inhibition of fertilization has been reported after isoimmunization of females with spermatozoa, semen, and testis in mice (1), rabbits (2, 3), and cattle (4). Treatment of semen with antisera against these antigenic materials also resulted in fertilization failure in rabbits (5–7) and cattle (6) after artificial insemination. An interference with transport of spermatozoa has been suggested as the cause of the female infertility in isoimmunized mice (1) and rabbits (8), and in rabbits inseminated with antibody-treated semen (9). Other evidence (10) suggested that effects on transport and motility of spermatozoa alone were not responsible for the immuno-infertility in rabbits, but that there appeared to be an effect on the attachment to and penetration of ova by spermatozoa. Under *in vitro* conditions rabbit spermatozoa will rapidly and in large numbers, adhere to ova. The present study describes the effects of different normal and immune sera, rabbit oviductal fluid and culture media from incubations of female reproductive tissues on the adherence of rabbit spermatozoa to rabbit and rat ova *in vitro*.

Materials and Methods. Antisera were prepared in guinea pigs, rabbits, and one goat by weekly intramuscular and subcutaneous injections of antigen preparations mixed with an equal volume of complete Freund's adjuvant for a total of 4 to 6 weeks. Normal sera were obtained prior to immunization and the antisera from bleedings 7 to 12 days after the last injection. For testing, pools of serum samples from at least two or more animals were used except for serum from the single goat. Univalent antibody preparations were obtained by papain digestion of gamma globulin fractions of serum samples (11). Phosphate buffered saline [(PBS), pH 7.2] extracts of frozen-thawed epididymal spermatozoa of the rabbit were subjected to hydrolysis by adding 5 mg of enzyme to 25 mg of extract protein and incubating overnight at 37°. These materials were mixed with adjuvant and used to immunize guinea pigs. Oviductal fluids were collected by implanting collecting flasks (12) into control and semen-immunized rabbits. Pooled samples from two rabbits in each group were used. Culture media were obtained by incubating the reproductive tract mucosal tissues from two control and three systemically immunized rabbits in TC 199 medium in culture tubes. The tubes were placed on a rotor drum and incubated at 37° for 18 to 24 hr. Tissue amounts ranged from 100 to 200 mg/ml of media. After incubation the samples were centrifuged and the supernatants were dialyzed against distilled water, lyophilized, and reconstituted with PBS to obtain a tenfold concentration. Spermatozoal agglutination in gelatin (13) and spermatozoal immobilization (14) were used to determine antibody titers in the serum and oviductal fluid samples. The serum and fluid samples were heated at 56° for 30 min before use.

Sperm adherence test. A pool of freshly collected semen from two to four male rabbits was diluted with Tyrode's solution to contain approximately 10×10^6 spermatozoa/ml. Equal volumes of serum, oviduct fluid, or culture media and the spermatozoal suspension were mixed together and incubated at 37° for 15 to 20 min. The samples were then centrifuged (1000 rpm, 5 min), the supernatant discarded and the spermatozoa resuspended to the original volume with Tyrode's solution.

Ova were collected from the oviducts of

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superovulated mature rabbits and immature (26–32 days old) rats from 14 to 18 hr after injection of 100 and 30 IU, respectively, of human chorionic gonadotropin (HCG). The rabbits had received daily injections of 0.5 mg of follicle-stimulating hormone (Sigma) for 3 days; and the rats, 30 IU of pregnant mare serum, 54 to 60 hr before HCG treatment. The ova were collected in a 0.1% solution of hyaluronidase in either Ringer's or Tyrode's solution to aid in dispersal of the cumulus cells. After the ova were free of cells, they were washed twice in the salt solutions by transfer with a small glass pipette. Drops (approx 20 μl) containing 4 to 10 ova, were placed in the wells of depression slides. An equal volume of spermatozoal suspension was added to each drop and the sample was covered with heavy mineral oil. The slides were placed into an air incubator at 37° for 30 min. After incubation, the samples were examined under a phase interference microscope (Nachet, France) to assess the degree of both spermatozoal adhesion to ova and the spermatozoal agglutination. Each treatment sample was tested at least once with rat ova except for Tyrode's solution and normal serum which were run with every test. In general, only one or two samples of each type of test media were used with rabbit ova, except for the guinea pig antisera against RBC, serum and spleen cells which were not tested with rabbit ova.

Results. There were no apparent differences in results due to species of ovum used so the results are combined (Tables I and II). Based on a comparison with spermatozoa suspended in Tyrode's solution, adherence of spermatozoa to ova was not affected by treatment with normal sera (Figs. 1 and 2). Antisera against materials that contained spermatozoa, *i.e.*, semen, epididymal spermatozoa and testis, however, completely inhibited spermatozoa from attaching to ova (Fig. 3). These sera also caused considerable agglutination of sperm cells in the incubation slides and possessed relatively high titers of spermatozoal agglutinins and immobilizins.

TABLE I. Effect of Antisera on Adherence of Rabbit Spermatozoa to Ova.

Sperm treatment	Final dilution	Degree of sperm		Titer of sperm[a]	
		Adherence	Agglutination	Agglutinins	Immobilizins
Tyrode's solution	—	++	—	—	—
Normal sera (goat, guinea pig)					
untreated	1:2	++	+	2	<1
papain treated	1:4	++	—	<1	<1
Goat antisemen serum					
untreated	1:20	—	+++	12	11
papain treated	1:4	—	—	<1	<1
Guinea pig antisera					
serum	1:2	++	+	2	<1
RBC	1:2	++	±	1	<1
spleen cells	1:2	++	+	3	<1
seminal plasma	1:2	++	+++	10	<1
epididymal sperm	1:10	—	+++	10	11
semen	1:10	—	+++	11	10
testis	1:5	—	++	9	10
epididymal sperm extract treated					
pronase	1:4	—	—	<1	9
α-chymotrypsin	1:4	+	+	7	8
α-amylase	1:4	++	++	9	10

[a] Titers are given as reciprocals of greatest positive dilution to the powers of base 2.

TABLE II. Effect of Rabbit Antisera, Oviduct Fluid, and Culture Media on Adherence of Rabbit Spermatozoa to Ova.

Sperm treatment	Final dilution	Degree of sperm		Titer of sperm[a]	
		Adherence	Agglutination	Agglutinins	Immobilizins
Normal serum	1:2	++	±	<1	<1
Isoantisera against					
seminal plasma	1:2	++	+	5	<1
epididymal sperm	1:8	—	+++	9	11
semen	1:8	—	+++	10	10
Antisemen serum					
papain treated	1:2	—	—	<1	<1
absorbed with					
epididymal sperm	1:2	++	±	2	<1
ejaculated sperm	1:2	++	—	<1	<1
Oviduct fluid					
control	1:2	++	±	1	<1
antisemen	1:2	+	+	3	2
antisemen (10X)	1:2	—	+	6	5
Culture media of tissues[b]					
control cervix and vagina	1:2	++	+		
immune cervix and vagina	1:2	—	++		
immune uterus	1:2	+	++		

[a] Titers are given as reciprocals of greatest positive dilution to the powers of base 2.
[b] Titers not determined.

The possibility that the inhibition of adhesion was due to the agglutination of spermatozoa was discounted by the following three observations. Rabbit and goat antisera against semen were treated with papain to render univalent antibodies. This procedure, while completely abolishing the agglutinating and immobilizing activities, did not remove the antiadherent effect of the antisera (Fig. 4). Secondly, antiserum against epididymal spermatozoal extract treated with pronase was without agglutinins but possessed immobilizing and antiadherent activities. Treatment of the extracts with alpha amylase and alpha chymotrypsin had little, if any, effect on the factors measured. Thirdly, antiserum against seminal plasma of vasectomized rabbits caused considerable agglutination of spermatozoa but had no immobilizing nor antiadherent effects. These latter two observations also suggest the inhibition of spermatozoal adherence to ova is more closely associated with immobilizing antibody than with the agglutinating type. Motility of spermatozoa was apparently unaffected in the adherence tests as all the samples had 50% or greater ratings when examined.

Absorption of antisemen serum with an equal volume of packed, washed epididymal and ejaculated spermatozoa completely removed the antibody activities and antiadhesive effect. Substituting cauda epididymal spermatozoa in the adhesion tests gave results similar to those obtained with ejaculated spermatozoa after treatment with rabbit normal and immune sera.

To approximate the types of secretions spermatozoa encounter in the reproductive tract of isoimmune rabbits, oviduct fluid and culture medium of reproductive tract tissues were used in the spermatozoal attachment system. These materials also interfered with the adherence of spermatozoa to ova. Preincubation of ova in antiserum and in extracts of epididymal spermatozoa and whole semen did not appear to influence subsequent adherence of spermatozoa to these ova after two washings in Tyrode's solution.

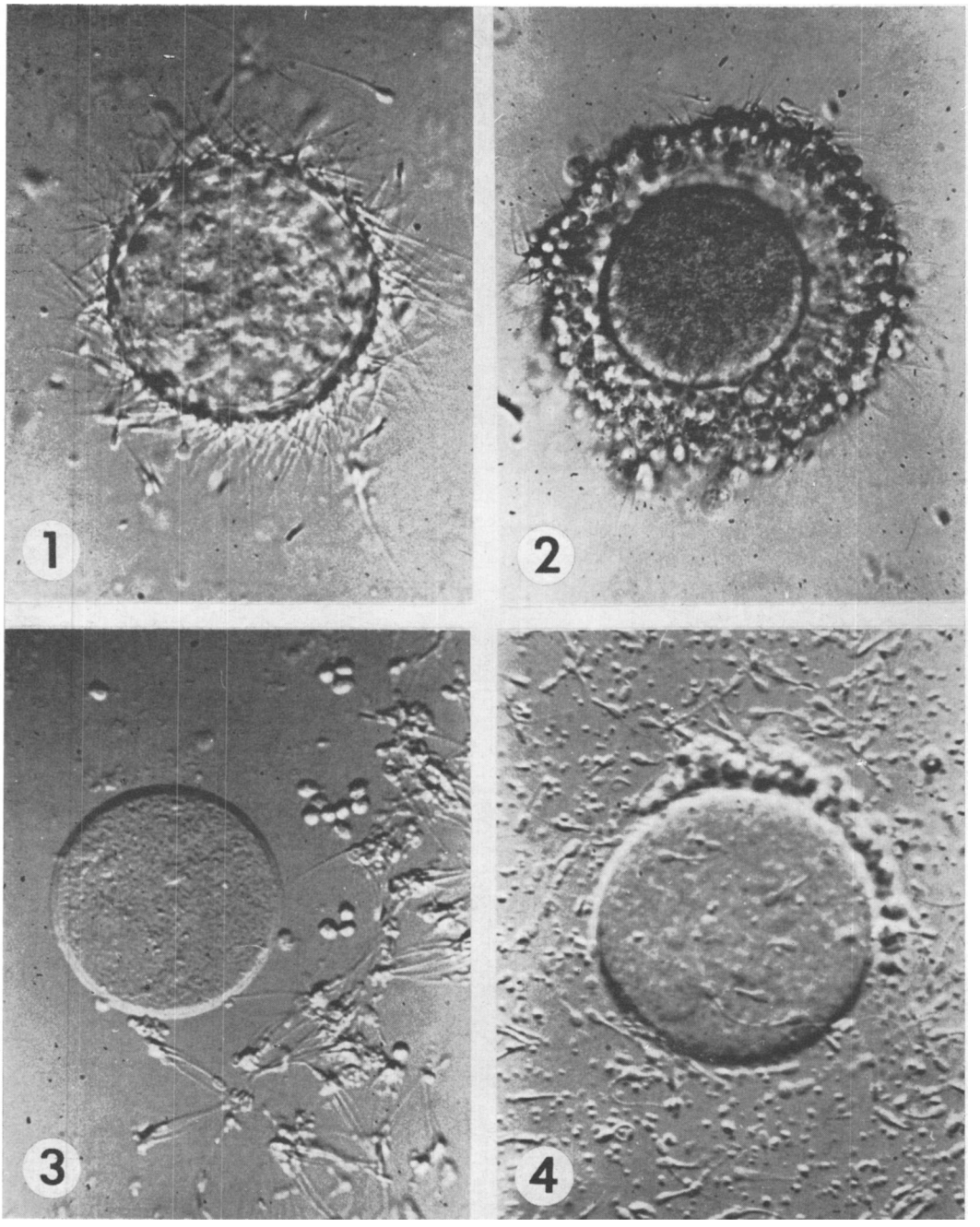

FIG. 1. Rat ovum with adhering spermatozoa that had been treated with guinea pig normal serum.

FIG. 2. Rabbit ovum with adhering spermatozoa that had been treated with rabbit normal serum.

FIG. 3. Rat ovum with spermatozoa that are nonadherent but agglutinated after treatment with antisemen serum.

FIG. 4. Rat ovum with spermatozoa that are both nonadhering and nonagglutinated after treating with antisemen serum digested with papain.

Discussion. The present results suggest that isoantibodies against spermatozoa may be preventing fertilization by inhibiting sperm cells from adhering to ova. Presumably spermatozoa must adhere or attach to the ovum and its surrounding cells after initial contact to enable their penetration of the zona pellucida. The finding that both oviductal fluid and culture media from tissues of semen-immunized rabbits have antiadherent activity suggests this may be a mechanism of preventing fertilization in the immune rabbit. Follicular fluid, reportedly, also contained antibodies against spermatozoa in heifers isoimmunized with semen (15). Conceivably, ova may be surrounded by antisperm antibodies entrapped in the cumulus mass which may also inhibit spermatozoal adherence and penetration. Peroxidase-labeled isoantisera against rabbit spermatozoa localizes in the acrosome of sperm cells (unpublished data) which is the point of attachment with ova. This suggests that the antiadherent effect of antibodies is the covering of antigenic sites on the acrosomal membrane that are essential for a spermatozoa–ovum interaction. Treatment of sea urchin spermatozoa with specific antiserum inhibited fertilization apparently by interfering with the adhesion of gametes (16).

In the rabbit it appears that isoimmunization with semen and treatment of spermatozoa with antisemen serum may be preventing fertilization by inhibition of spermatozoal attachment to ova as well as by decreased spermatozoal transport and motility (10–12).

Summary. Treatment with heterologous and isologous antisera against antigenic materials containing spermatozoa prevented adherence of spermatozoa to ova *in vitro*. These antisera caused agglutination of spermatozoa; however, removal of the agglutinating activity of antisera by papain treatment did not remove the antiadhesive effect. Inhibition of spermatozoal attachment to ova was caused also by oviductal secretions and concentrated media from culture of reproductive tissues from female rabbits isoimmunized with semen.

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