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Autoantibodies from Vasectomized Guinea Pigs Inhibit Fertilization in vitro

Abstract. *Immunoglobulin G and Fab antibodies were isolated from the serum of vasectomized guinea pigs, and the effects of the antibodies on fertilization in vitro were investigated. These antibodies had profound inhibitory effects on (i) sperm-to-sperm adhesion, (ii) the acrosome reaction, (iii) sperm-zona binding, and (iv) sperm-ovum fusion. This finding may explain certain cases of infertility after vasovasostomy in men.*

Because vasectomy is a widely accepted option of fertility control, it is imperative to assess whether vasectomy results in long-term detrimental effects and whether production of antibodies to sperm attendant to vasectomy (*1*) may prevent reversal of fertility control through vasovasostomy.

Davis (*2*) reported a high incidence of infertility in vasovasostomized men. The hypothesis that some cases of infertility have an immunological basis is plausible because vasectomy results in a high incidence of circulating autoantibody directed against sperm (*1*), and infertility after vasovasostomy is correlated with sperm agglutinins in serum (*3*) and semen (*4*). Infertility in vasovasostomized rhesus monkeys is also correlated with a high titer of circulating antibody to sperm (*5*). This general appraisal led us to investigate whether autoantibody to sperm produced after vasectomy has biological effects on fertilization. We used the guinea pig as an experimental model for the following reasons. First, guinea pigs are widely used to study sperm autoimmunity (*6*), including its genetic basis (*7*). Second, conventionally prepared autoantibody to guinea pig sperm inhibits the sperm acrosome reaction (*8*) and fertilization in vitro (*9*). Finally, owing to the extraordinarily large size of the acrosome, we can readily distinguish acrosome-intact from acrosome-reacted spermatozoa by using phase contrast microscopy (*10*); this enables us to determine whether the observed effects of antibodies are due to interference with the acrosome reaction itself or with events preceding or following the acrosome reaction.

Bivalent immunoglobulin G (V-IgG) and univalent Fab fragments (V-Fab) were prepared (*11*) from the serum of strain 13 guinea pigs that had been bilaterally vasectomized 9 to 13 months earlier and that showed high levels of antibodies to surface antigens of guinea pig spermatozoa and spermatids (*7*). Animals with sham vasectomies served as the source of control reagents (SV-IgG and SV-Fab, respectively). All reagents were dialyzed against and stored in potassium-containing minimal capacitation medium (K-MCM) (*12*) at 10 mg/ml. We designed experiments to assess the effects of V-IgG and Fab on (i) sperm capacitation and (ii) sperm-ovum interactions in vitro.

Mammalian spermatozoa must reside for a time in the female reproductive tract before they become competent to fertilize ova (*13, 14*). Austin (*13*) termed this process capacitation. It is possible to capacitate spermatozoa from the guinea pig cauda epididymis by incubating them in defined media, such as K-MCM (*8*). Initially, the majority of spermatozoa are in "rouleaux," in which eight to ten cells adhere in orderly stacked arrays (*10, 15*); the rest are single cells ($\leq$5 percent) or doublets (10 to 20 percent), in which two cells are attached like those in a rouleau. Within 5 minutes in K-MCM, sperm rouleaux spontaneously agglutinate in a head-to-head fashion; within 1 hour, more than 95 percent of the entire motile sperm population joins into large clusters of spermatozoa (*8*).

In V-IgG (5 mg/ml), agglutination of rouleaux was largely inhibited, over 80 percent of the spermatozoa continuing to swim as individual rouleaux, singlets, or doublets; SV-IgG had no inhibitory effect (Fig. 1A). It is not clear why V-IgG did not augment agglutination of rouleaux, but similar observations have been made with a conventionally prepared autoantibody to guinea pig sperm (*8*). Additionally, an antibody directed against discoidin, a cell adhesion molecule in the slime mold, does not agglutinate target cells in adhesion assays (*16*).

Treatment of spermatozoa with V-Fab (5 mg/ml) immediately dispersed all sperm rouleaux to single cells (approximately 25 to 30 percent) or to doublets (70 to 75 percent) (Fig. 1B). The V-Fab effect was half-maximal at 1 mg/ml and absent at 0.1 mg/ml (data not shown). Control SV-Fab did not perturb normal rouleau agglutination. The V-IgG and V-Fab results indicate that serum from vasectomized guinea pigs reacts with surface determinants involved in homotypic sperm adhesion.

Under ordinary conditions in vitro, spermatozoa that have undergone acrosome reactions detach from stacked or agglutinated configurations and swim individually with "activated" motility (*10, 17*). Spermatozoa suspended in K-MCM containing V-IgG (5 mg/ml) did not un-

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Table 1. Effects of IgG and Fab antibodies on sperm-zona binding and sperm-ovum fusion. Spermatozoa were induced to undergo acrosome reactions by treating them with lysolecithin (*18*). Guinea pig ova matured in vitro (*22*) were freed from cumulus cells by treatment with 0.2 percent hyaluronidase (to obtain zona-intact ova) or from both cumulus cells and zonae pellucidae by treatment with 0.2 percent hyaluronidase plus 0.2 percent pronase (to obtain zona-free ova) (*10*). Ova were thoroughly rinsed and inseminated in K-MCM medium containing 0.3 percent bovine serum albumin. The concentration of spermatozoa in insemination media was 4×10^5 to 6×10^5 per milliliter. Sperm attachment to the zona was assessed 20 to 30 minutes after insemination; sperm penetration into ova (sperm-ovum fusion) was examined 3 to 4 hours after insemination; sperm heads decondensing in the vitelli were counted (*10*, *22*).

Reagent in insemination medium (5 mg/ml)	Zona-intact ova inseminated		Zona-free ova inseminated			
	Ova (No.)	Mean number of spermatozoa attached to each zona	Ova (No.)	Ova penetrated by spermatozoa		Mean number of swollen sperm heads in each penetrated ovum
				Number	Percent	
V-IgG	52	0*	27	2	7	2
SV-IgG	32	15	20	15	75	7
V-Fab	28	12	21	18	86	9
SV-Fab	26	21	16	15	93	8
Pure K-MCM	23	25	10	9	90	6

*Even after prolonged incubation times no spermatozoa attached to the zona and no ova were fertilized.

dergo acrosome reactions even after long incubation times and remained unagglutinated (Fig. 1C). Total inhibition occurred at antibody concentrations as low as 0.1 mg/ml, but further dilutions were increasingly permissive (data not shown). This experimental protocol did not distinguish between inhibition of the acrosome reaction per se and inhibition of capacitation that would result in inhibition of the acrosome reaction. Cauda epididymal spermatozoa were therefore capacitated by incubating spermatozoa in Ca^{2+}-free Tyrode's medium supplemented with lysolecithin (100 μg/ml) (*18*). After 1 hour in this medium, addition of Ca^{2+} resulted in the acrosome reaction of 80 to 95 percent of the spermatozoa within 10 minutes (Fig. 1D). Exposure of spermatozoa to V-IgG (5 mg/ml) at the end of the 1-hour incubation period completely prevented the

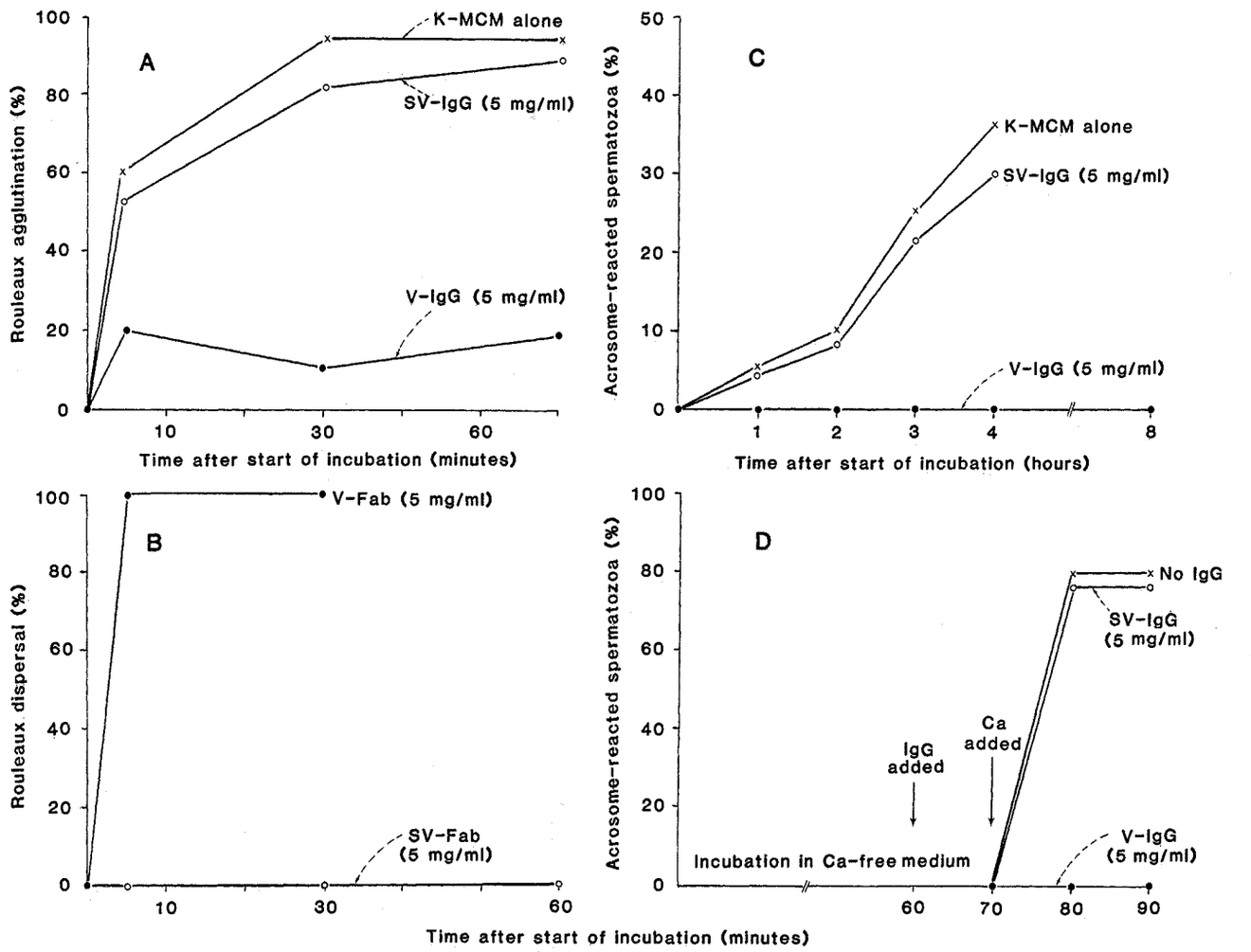

Fig. 1. (A) Effects of V-IgG and SV-IgG on spermatozoa in K-MCM; V-IgG inhibits agglutination of rouleaux, in contrast to SV-IgG. (B) Effects of V-Fab and SV-Fab on spermatozoa in K-MCM; V-Fab completely disperses rouleaux into singlets or doublets, in contrast to SV-FAB. (C) Effects of V-IgG and SV-IgG on the acrosome reaction of spermatozoa in K-MCM; spermatozoa incubated with V-IgG do not undergo acrosome reactions, whereas SV-IgG has no inhibitory effect. (D) Effects of V-IgG and SV-IgG on the acrosome reaction of capacitated spermatozoa: spermatozoa were capacitated by incubating them for 1 hour in C^{2+}-free Tyrode's solution supplemented with lysolecithin (100 μg/ml); exposure of spermatozoa to V-IgG prior to Ca^{2+} addition completely inhibits the acrosome reaction in contrast to SV-IgG. Each point in figure represents the mean of four experiments using four different males.

acrosome reaction when Ca^{2+} was added (Fig. 1D). Unlike the bivalent V-IgG, V-Fab was not inhibitory (data not shown); SV-IgG (5 mg/ml) was not inhibitory (Fig. 1D).

In the guinea pig, the acrosome-reacted spermatozoon attaches to the zona pellucida of the ovum (*19*). This is followed by zona penetration, fusion with the ovum, and swelling of the sperm head in the vitellus (*20*). We placed acrosome-reacted spermatozoa in K-MCM containing antibody. Fifteen minutes later, we introduced either zona-intact or zona-free guinea pig ova. Zona attachment of spermatozoa was completely inhibited by V-IgG, but not by the SV-IgG control; V-Fab showed a 57 percent inhibition (Table 1).

Bivalent V-IgG, but not V-Fab, effectively blocks sperm-ovum fusion (Table 1). The different effects of these reagents indicate that cross-linking or some modulation of surface autoantigens on the spermatozoon prevents fusion with the ovum. The lack of inhibition by the control SV-IgG rules out a significant effect through the Fc portion of the V-IgG molecule.

The effects of autoantibodies on agglutination and on the acrosome reaction of spermatozoa may be important when considering a possible immunological basis of male infertility. Exposure of spermatozoa within the male reproductive tract to antibodies would result in binding of antibody to the plasma membrane of the sperm head. This would result in the prevention of the acrosome reaction which is an essential preliminary to both sperm penetration through the zona pellucida and fusion with the ovum (*21*). It has been shown that V-IgG can gain access to spermatozoa in the male reproductive tract and can be detected in the seminal plasma of vasectomized men (*4*). It remains to be determined whether spermatozoa treated in vitro with V-IgG can subsequently fertilize ova in vivo after artificial insemination.

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Glucose Transfer from Male to Female Schistosomes

Abstract. *The rate of glucose assimilation by male and female* Schistosoma mansoni *was significantly greater in copulating than separated flukes, especially in copulating females. In the three medically important schistosome species, glycogen content was greater in unpaired males than in copulating males, suggesting that the female depletes glycogen stored in the male. Transfer of [^{14}C]glucose from the male to the copulating female was demonstrated over a period of minutes. A considerable portion of the glucose utilized by the female during her life may be supplied by the male.*

Schistosomiasis afflicts more than 200 million people worldwide. It is caused by permanently copulating pairs of male and female blood flukes living in afferent tributaries of the portal veins (*Schistosoma mansoni* and *S. japonicum*) and the vesicular venous plexus of the bladder and colon (*S. haematobium*) of man and animals (*1*). Tissue reactions resulting from the deposition of eggs are primarily responsible for the disease state (*2*).

Male and female schistosomes are interdependent. In experiments in which the male is separated from the female, the female does not grow to adult size

Table 1. Comparison of hexose assimilation in copulating and separated male and female *Schistosoma mansoni*. Each value is the mean (± the standard deviation) for 5 to 12 schistosomes. Ages of schistosomes were 68 days (D-glucose), 101 days (3-*O*-methylglucose), and 103 days (2-deoxy-D-glucose).

^{14}C-Labeled hexose	Time exposed to isotopic medium (min)	Tissue uptake index (*7*)			
		Unpaired males	Paired males	Unpaired females	Paired females
D-Glucose	3.5	175.6 ± 12.3	188.2 ± 28.0	109.1* ± 40.1	208.1 ± 53.5
3-*O*-Methyl-glucose	4.0	41.9† ± 6.7	59.9 ± 4.8	24.4 ± 16.5	71.7 ± 4.4
2-Deoxy-D-glucose	3.0	154.4† ± 22.0	213.9 ± 33.5	109.8* ± 61.5	170.2 ± 49.4
	4.0	181.3† ± 51.7	368.3 ± 98.1	126.8* ± 48.9	252.7 ± 47.1
	5.0	307.4 ± 17.6	352.9 ± 81.1	171.6* ± 47.3	277.5 ± 37.3

*Significantly different from corresponding value for paired females ($P < .01$, Student's *t*-test). †Significantly different from corresponding value for paired males ($P < .01$).

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