THE BLACK MINK (*MUSTELA VISON*)

A Natural Model of Immunologic Male Infertility*

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It has been established that >10% of married couples are infertile (1) and the cause of the infertile state is often obscure (2). There is increasing evidence that autoimmunity to sperm antigens may be an important mechanism of unexplained infertility.

A higher prevalence of antibodies to spermatozoa has been detected in the sera of couples with unexplained infertility as compared with fertile couples (3, 4). In many species, including guinea pigs, rabbits, rats, and mice, immunization with sperm or testicular antigens results in autoimmune response to sperm antigens and experimental allergic orchitis, an autoimmune disease of the testis (5–7). Orchitis can lead to aspermatogenesis and infertility. Testicular biopsy of some infertile men often reveals aspermatogenesis (8). Female animals immunized with sperm develop antisperm antibodies and may also become infertile (9–12). Autoantibodies to spermatozoa can block prefertilization and fertilization steps in vitro (13, 14). Vasectomy results in autoimmune response to sperm antigens (7, 15–18) and orchitis that resembles experiment allergic orchitis (19, 20). Over 70% of vasectomized patients who had vasovasostomy and resultant normal sperm count are infertile (21). In this regard, it is of interest that autoantibodies to sperm from vasectomized guinea pigs have recently been found to block fertilization in vitro (22). Finally, in infertile couples with antisperm antibodies, treatment with corticosteroids reduces antibody level and pregnancy may ensue (23, 24).

Research on any organ-specific autoimmune disease is facilitated by the study of a natural model of the disease. This helps to explore immunoregulatory aberrations in the diseased individuals, the physiologic and pathologic tolerance state of the individuals to the respective organ-specific autoantigens, and the genetic aspects of the disease. Side effects introduced by adjuvant can be ruled out. Moreover, the knowledge that a naturally occurring autoimmune disease of the testis exists provides clinical relevance and impetus for further investigation of orchitis.

In breeding mink for fine black fur, a colony of black mink was developed wherein ~20–30% of the males are infertile. Unequivocal evidence of orchitis and/or aspermatogenesis with testicular sperm antigen-antibody complexes, which was found in many of these animals, is documented in this paper. Infertile black mink thus provide a natural model of immunologic male infertility.

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Materials and Methods

Animals. Mink used in this study included 77 infertile and 9 fertile male black mink contributed by the Fur Breeders Agricultural Cooperative at Midvale, Utah, and by ranchers from Utah and Idaho. In addition, sera of the following fertile mink were studied for anti-sperm antibodies: 4 pastel males, 9 opal males, 7 violet males, 16 black females, 10 pastel females, 7 opal females, and 6 violet females.

Detection of Serum Anti-Sperm Antibodies by Indirect Immunofluorescence (IF).[1] Serum samples were obtained from anesthetized animals by cardiac puncture. Mink spermatozoa were obtained by trimming cauda epididymis in NaCl (0.1 M, pH 7, at 20°C), washed once in saline, smeared on the eight-hole teflon slides and then air dried (25). The sperm smears were then fixed in absolute methanol for 15 min, dried, and stored at −70°C. Sera, diluted serially (twofold) after 1:10, were incubated with the sperm smear for 30 min. After rinsing in phosphate-buffered saline (PBS), the slides were incubated for 30 min with fluorescein isothiocyanate (FITC)-conjugated antiserum IgG to mink IgG. After rinsing in PBS, the slides were coverslipped with Tris-buffered (pH 9.3) glycerol. The slides were studied with a Leitz fluorescence microscope (E. Leitz Inc., Rockleigh, N. J.) with a Phloem illuminator. The highest serum dilution that gave an unequivocal positive fluorescence was the antibody titer, expressed as $-\log_2$ tube dilutions (i.e., 1 = 1:10, 2 = 1:20, 3 = 1:40, 4 = 1:100, 5 = 1:200, 6 = 1:400, 7 = 1:800).

Clinical Criteria of Infertility. A mink was considered infertile after it failed to impregnate between 3 and 11 fertile females. In addition, ejaculated semen of infertile mink recovered from the vagina either contained no sperm or contained poorly motile or immotile spermatozoa.

Pathologic Studies. At autopsy, the position, size, and weight of each testis were determined. Three to five transverse sections through the testis, a section of the ductus efferentes, the caput, and the cauda epididymis (fixed in Zenker's fixative) were processed for histology. 5-mμ sections were stained with periodic acid Schiff's hematoxylin. In addition, thyroid, salivary gland, kidney, spleen, liver, adrenal gland, lung, brain, and spinal cord were fixed in buffered formalin and processed for histologic studies. Approximately 500 cross-sections of seminiferous tubules per mink were examined.

Direct IF Studies. One testis or parts of both testes, the corpus epididymis, and a section of the kidney were snap-frozen in liquid nitrogen. Frozen sections of these organs were processed for direct IF study as described previously (25). After fixation in ethanol-ether, the frozen sections were stained with FITC-conjugated goat antiserum IgG to mink IgG, mink IgM, mink IgA, or mink C3.

Quantitative Elution of Antibody from Testis Homogenates. A part of each of six mink testes known to contain diffuse peritubular deposits of immune complexes was decapsulated, weighed (1.5 g), finely minced, and washed repeatedly with PBS (pH 7). Each washing consisted of thorough suspension of the tissue in 75 ml of chilled PBS and centrifugation at 1,000 *g* at 4°C for 15 min. After nine washes, the tissue was divided into two aliquots: 1.2 g for extraction with 20 ml of citrate buffer (0.02 M, pH 3) and 0.8 g with 20 ml of PBS. After extraction for 90 min at room temperature, the tissue was centrifuged at 12,000 *g* for 30 min at 4°C. Bovine serum albumin (BSA) was added to each supernate to attain a final concentration of 10 mg/ml, and an equal volume of saturated ammonium sulfate, pH 3, was added to the supernate. The precipitate formed was dissolved in about 1 ml of PBS and dialysed extensively in PBS. The citrate buffer extract, the PBS extract, and the pooled serum of the six mink were studied for antibody to mink sperm by indirect IF and for mink immunoglobulin content by a solid-phase radioimmunoassay (RIA).

A solid-phase RIA for mink IgG was developed for this study. Flat-bottomed multiwell microtiter plates (1-220-29; Cooke Engineering Co., Alexandria, Va.) were incubated for 12 h at 4°C with rabbit anti-mink IgG antiserum IgG (5.0 μg in 50 μl) and washed with PBS, followed by a 2-h incubation at room temperature with PBS containing 1% rabbit serum, 1% BSA, and 0.2% Na-azide (RIA buffer). 50 μl of serial dilutions of mink IgG (standard curve) and of the unknown sample were added to the wells and incubated for 60 min at 20°C. After the wells were washed with PBS, ~0.1 μg (50 μl) of ^{125}I-labeled IgG of rabbit anti-mink IgG antiserum was added, incubated for 60 min, and washed with PBS. The radioactivity bound

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; FITC, fluorescein isothiocyanate; IF, immunofluorescence; PBS, phosphate-buffered saline; RIA, radioimmunoassay.

to the plate was determined in a gamma-spectrometer. The assay generated a linear dose-response curve for mink IgG from 0.04 to 1.25 mg/ml. The IgG content of the unknown sample was derived from the standard curve.

Detection of Sperm Antigens in Testicular Immune Complexes. From the sera of two mink with high levels of anti-sperm antibody whose testes had extensive immune complexes, IgG was isolated by binding to and eluting from a Sepharose protein-A column using 0.1 M citrate buffer, pH 3.2, dialyzed in PBS, concentrated to 10 mg/ml and conjugated with FITC (26). By direct IF, the FITC-conjugated anti-mink sperm antiserum IgG reacted with mink sperm smears and with the acrosomal apparatus of testicular cells in frozen sections of mink testis. To attempt detection of sperm antigen in the IgG deposits in the testis, frozen sections of 10 mink testes with positive IgG deposition were incubated in: (*a*) PBS, pH 7, at 37°C for 30 min; (*b*) 0.1 M citrate buffer, pH 3.2, at 37°C for 30 min; and (*c*) PBS, pH 7, with 3 M NaCl at 37°C for 30 min; rinsed in PBS, fixed in ethanol-ether, stained with FITC-conjugated antiserum IgG to mink sperm, and studied with the Leitz fluorescence microscope (E. Leitz Inc.).

Microbial Cultures of Mink Testis with Orchitis and/or Aspermatogenesis. The testes from four infertile mink, two with orchitis and aspermatogenesis, and two with aspermatogenesis alone, were obtained from the mink at autopsy under sterile conditions, frozen in sterile containers, and subsequently cultured for aerobic, anaerobic, and acid-fast bacteria, fungi, and Coxsackie B and mumps virus according to standard procedures (27–29).

Statistical Analysis. The Student's *t* test was used.

Results

Clinical Classification of the Infertile Black Mink. Mink born in early May were sexually mature 10 mo later. After mating in March, their testes began to regress and were impalpable 2–3 mo later. Some fertile mink served as breeders in subsequent years. The mink used in this study were killed or bled in early April; thus, their reproductive performances were recently determined. Of the 77 infertile black mink, 45 were infertile in their first mating, at the age of 10 mo. These animals were designated as having primary infertility (Table I). The remaining 32 were initially fertile: 21 for 1 yr, 6 for 2 yr, and 2 for 4 yr, but were subsequently infertile. They were designated as animals with secondary infertility (Table II).

Serum Anti-Sperm Antibodies. Male mink had more anti-sperm antibodies as detected by indirect IF than female mink. 72% of male fertile mink, regardless of fur color, had antibodies, whereas 29% of the females had antibodies.

In the females, antibodies of low titer were confined to mink with black and pastel

TABLE I

Summary of Immunopathologic Findings in Mink with Primary Infertility

Number of mink (Percent of total)	Number with abnormal testicular position	Orchitis			Aspermatogenesis*	Testicular immune complexes‡
		Diffuse	Focal	Interstitial		
3 (7%)	0	3	0	0	3	0/2§
8 (18%)	1	0	8	0	5	0/8
6 (13%)	3	0	0	6	6	0/5
22 (49%)	6	0	0	0	22	0/20
6 (13%)	2	0	0	0	0	0/2

* Over 50% of seminiferous tubules are aspermatogenic or hypospermatogenic.

‡ Granular deposits of mink IgG and/or C3 in peritubular basement membrane by IF.

§ Number positive/number studied.

TABLE II

Summary of Immunopathologic Findings in Mink with Secondary Infertility

Number of mink (Percent of total)	Number with abnormal testicular position	Orchitis			Aspermatogenesis*	Testicular immune complexes‡
		Diffuse	Focal	Interstitial		
15 (47%)	1	15	0	0	12	10/11§
8 (25%)	3	0	8	0	7	7/8
5 (15.5%)	0	0	0	1	5	3/5
4 (12.5%)	0	0	0	0	0	0/4

* Over 50% of seminiferous tubules are aspermatogenic or hypospermatogenic.
‡ Granular deposits of mink IgG and/or C3 in peritubular basement membrane by IF.
§ Number positive/number studied.

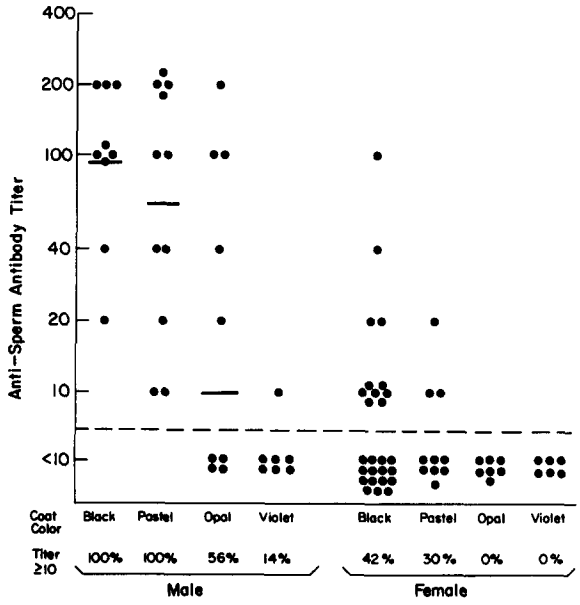

FIG. 1. Comparison between the prevalence and mean anti-sperm antibody titer (horizontal bars) and between fertile male and female minks of four different fur colors. The difference between the mean antibody titer of male black and male pastel minks is not statistically significant.

fur (Fig. 1). Anti-sperm antibody presence correlated with fur color in the male (Fig. 1). Black mink and pastel mink (which are genetically most related to the black mink) had the highest prevalences of anti-sperm antibodies, and their mean antibody titers were comparable.

Anti-sperm antibodies were found in both fertile and infertile black mink (Fig. 2). Although the prevalence of anti-sperm antibodies were comparable between different groups of black mink, their mean antibody titers differed. Although fertile animals, animals with secondary infertility, and animals with orchitis had comparable mean anti-sperm antibody titers, the levels of antibody were low in animals with primary infertility ($P < 0.001$). Finally, when both primary and secondary infertile mink are

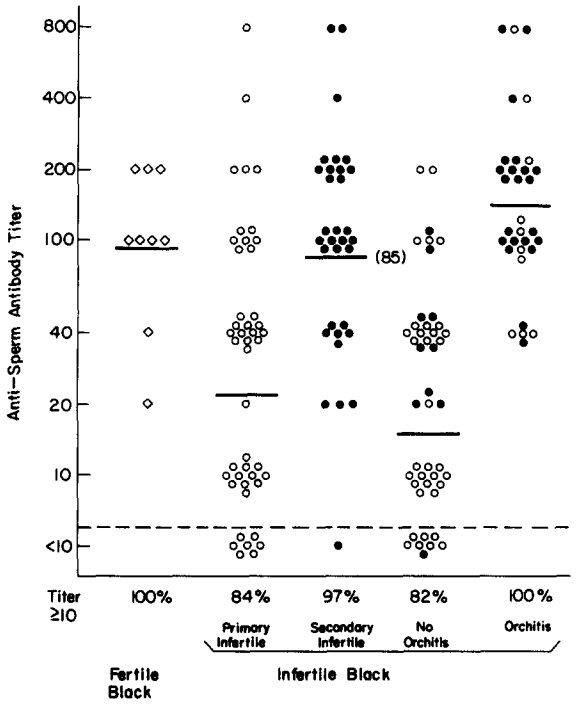

FIG. 2. Comparison between the prevalence and mean titer (horizontal bars) of anti-sperm antibodies detected by indirect IF in fertile and infertile black minks. ○, minks with primary infertility; ●, minks with secondary infertility. Significant difference ($P < 0.001$) exists between primary infertile and secondary infertile minks, and between minks with orchitis and those without orchitis. The mean antibody titers between fertile minks and those with secondary infertility are similar.

taken into consideration, the mean antibody levels of animals without orchitis was significantly below those with orchitis ($P < 0.001$).

Pathology of the Infertile Black Mink. The testes from 8 of 77 (10%) infertile mink were in the scrotal position and had normal histology. Nevertheless, no spermatozoa or a few immobile spermatozoa were found in the semen. Four of these had primary infertility and the other four had secondary infertility. Six of these animals had significant serum anti-sperm antibodies. The following pathology was found in the remaining infertile mink.

Cryptorchism. 16 (21%) of the 77 infertile mink had unilaterally undescended testes: 13 were inguinal and 3 were abdominal. Anti-sperm antibodies were detected in six of these animals. Cryptorchism was found slightly more frequently in mink with primary infertility (27%) than in those with secondary infertility (13%).

Orchitis. Orchitis was detected in 41 of 77 (53%) infertile mink. In seven, sparse lymphocytic infiltration was confined to the interstitium. Leydig cells adjacent to the inflammatory cells contained pynotic nuclei and eosinophilic cytoplasma, changes that suggest cellular degeneration (interstitial orchitis).

In the remaining 34 testes, heavy infiltrations of lymphocytes, monocytes, plasma cells, and polymorphonuclear neutrophils were found around and inside the seminiferous tubules (Fig. 3). Severe destruction was accompanied by replacement of the

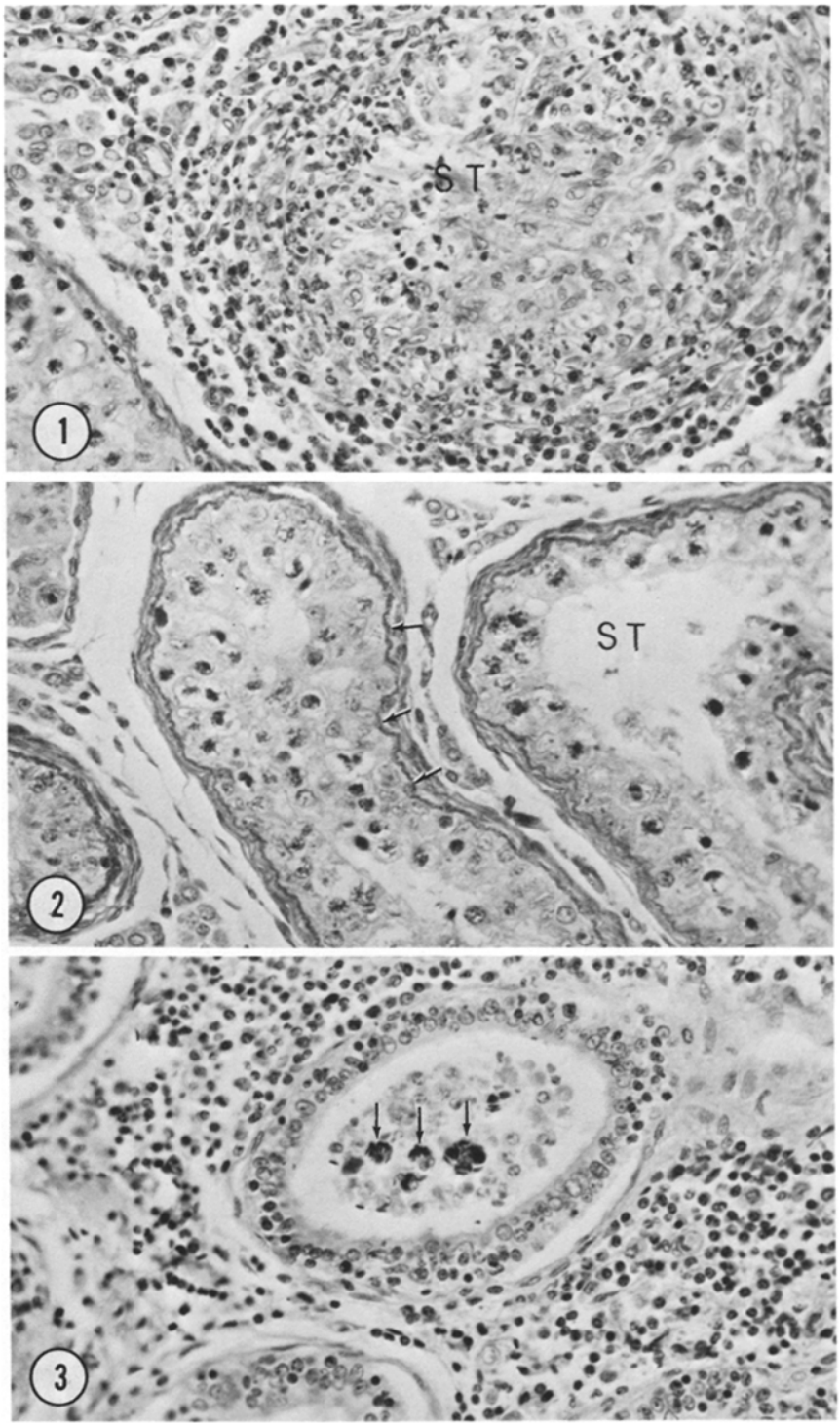

FIG. 3. Histopathology of minks with secondary infertility. In frame 1, severe orchitis is characterized by heavy infiltrations of lymphocytes, macrophages, neutrophils, and plasma cells around and inside the seminiferous tubules (ST), which have no recognizable germ cells. Frame 2 shows aspermatogenic ST without orchitis. Arrows point to wrinkled and thickened tubular basement membrane. This testis has diffuse immune complexes surrounding all ST. Frame 3 illustrates monocytic epididymyitis. Note macrophages with phagocytosed spermatozoa (arrows) in duct lumen. × 250.

germinal epithelium with numerous macrophages. Orchitis in some testes was focal, involving five seminiferous tubules or less, but in the majority, it was diffuse.

The incidence of different types of orchitis differed between mink with primary and secondary infertility (Tables I, II, and III). Patchy and diffuse orchitis with destruction of the seminiferous tubules was found almost exclusively in animals with secondary infertility ($P < 0.001$). On the other hand, interstitial orchitis was found more frequently in mink with primary infertility than in mink with secondary infertility.

Aspermatogenesis. Aspermatogenesis involving >50% of the seminiferous tubules was found in 60 of 77 (78%) infertile black mink (Fig. 3). The overall prevalence of aspermatogenesis between primary and secondary infertility was comparable (Table I). However, aspermatogenesis in the absence of orchitis or testicular immune complexes (see below) was found almost exclusively in mink with primary infertility ($P < 0.001$) (Table III).

Pathology in the Epididymis. 67 of 77 (87%) infertile black mink exhibited pathology in the caput and/or cauda epididymides. The cauda epididymides in 66% of the testes contained no spermatozoa. Epididymitis in the caput epididymides consisted of heavy periductal and intraepithelial lymphocytic infiltration and intraductal macrophages that had phagocytosed spermatozoa (Fig. 3). It was found in 43 animals, slightly more frequently in primary than in secondary infertile animals (Table I).

In summary, the immunopathologic findings in the testes of infertile mink are complex. However, severe orchitis and testicular immune complexes are more frequently found in mink with secondary infertility, whereas aspermatogenesis unaccompanied by orchitis is more prevalent in mink with primary infertility (Table III). In addition, the data summarized in Tables I and II indicate that mink with primary or secondary infertility are not a homogenous group, but that each kind is composed of several subgroups with respect to their pathologic findings.

TABLE III

*Comparison of Frequency of the Immunologic and Pathologic Findings between Minks with Primary and Secondary Infertility**

Findings	Primary infertile minks		Secondary infertile minks		*P* value‡
	Number	Percent positive	Number	Percent positive	
Fluorescent anti-sperm antibodies	45	84 (22)§	32	97 (85)§	0.001
Testicular immune complexes	37	0	28	71	0.001
Orchitis, diffuse or patchy	45	7	32	47	0.001
Orchitis, focal	45	18	32	25	0.05
Orchitis, interstitial	45	13	32	3	0.05
Epididymitis	45	27	32	16	0.05
Cryptorchism, unilateral	45	27	32	13	0.05
Aspermatogenesis, without diffuse or focal orchitis or testicular immune complexes	45	62	32	15.5	0.001

* Of nine fertile dark minks, only one had focal orchitis and epididymitis.
‡ Between minks with primary and secondary infertility.
§ Geometric means of anti-sperm antibody titers.

Findings Outside the Testis. No pathology was found in the thyroid, adrenal, brain, spinal cord, kidney, liver, spleen, or lung.

Pathology of the Fertile Black Mink. Of nine fertile mink, the testes and the epididymides in eight mink were within normal limits. Focal orchitis and lymphocytic epididymides were present in one animal.

Immunohistochemical Findings. The testes of 3 fertile black mink, 37 mink with primary infertility, and 28 with secondary infertility were studied by direct IF for evidence of in vivo binding of mink IgG and C3. Heavy, granular deposits of IgG and/or C3, typical of immune complexes, were found in the basal lamina surrounding the seminiferous tubules in 20 mink with secondary infertility (71%) (Fig. 4), and in none of the 37 mink with primary infertility. The extent of immune complex deposition varied: in nine, essentially 100% of the seminiferous tubules were surrounded by IgG and/or C3 deposits; in four, about 50% of the seminiferous tubules were positive; and in the remaining seven, which had extensive peritubular fibrosis and markedly thickened basement membrane, 5–20% of seminiferous tubules contained granular IgG or C3. In all instances, immune complexes were absent in the region of the rete testis (Fig. 4 a), and were free of detectable IgA or IgM.

Some correlations existed between the nature of the immune deposit and histopathologic findings. Thus, IgG was found mainly in the aspermatogenic testes with little or focal orchitis (9 of 11 mink), whereas C3 alone was found mainly in testes with diffuse orchitis (8 of 10 mink). Inflammatory cells, stained positively for IgG and occasionally for IgA and IgM, surrounded and infiltrated the seminiferous tubules of all testes with orchitis (Fig. 5). By IF, neither IgG nor C3 was detected in the kidneys.

Nature of Testicular Immune Deposits. The immune complex nature of the immunoglobulin deposits in secondary infertility was suggested by the finding that the immunoglobulin in the frozen section of the testis was completely eluted by either buffer at acid pH or buffer with high concentration of NaCl, but not by PBS at pH 7. The result of quantitative elution of antibody from testes with immune complexes indicated that the immune complexes contained anti-sperm antibody. The IgG content in the PBS extracts of these testes declined to an extremely low level after nine washings. At this point, further extraction of tissue with citrate buffer (pH 3) resulted in a definite elution of mink IgG, which was not observed in the parallel PBS extract (Fig. 6). More significantly, the IgG of the testicular acid eluate was found to contain 10 times more anti-sperm antibody activity than the serum IgG of the same animals (Table IV). The acid eluted antibody reacted mainly with the sperm acrosome (Fig. 7). Sperm antigen was not detected in the testicular immune complexes by indirect IF either before or after removal of stainable IgG by buffer of pH 3 or buffer of high ionic strength.

Microbial Culture of the Mink Testes. Four testes, two with orchitis, and two with aspermatogenesis, were negative for bacteria, fungi, and Coxsackie B or mumps viruses.

Discussion

The process of breeding for mink with fine black fur has selected for the undesirable phenotype of male infertility. Some mink are infertile soon after puberty (primary infertility), whereas others become infertile after a period of proven fertility (secondary infertility). Mink with primary infertility have low levels of anti-sperm antibodies,

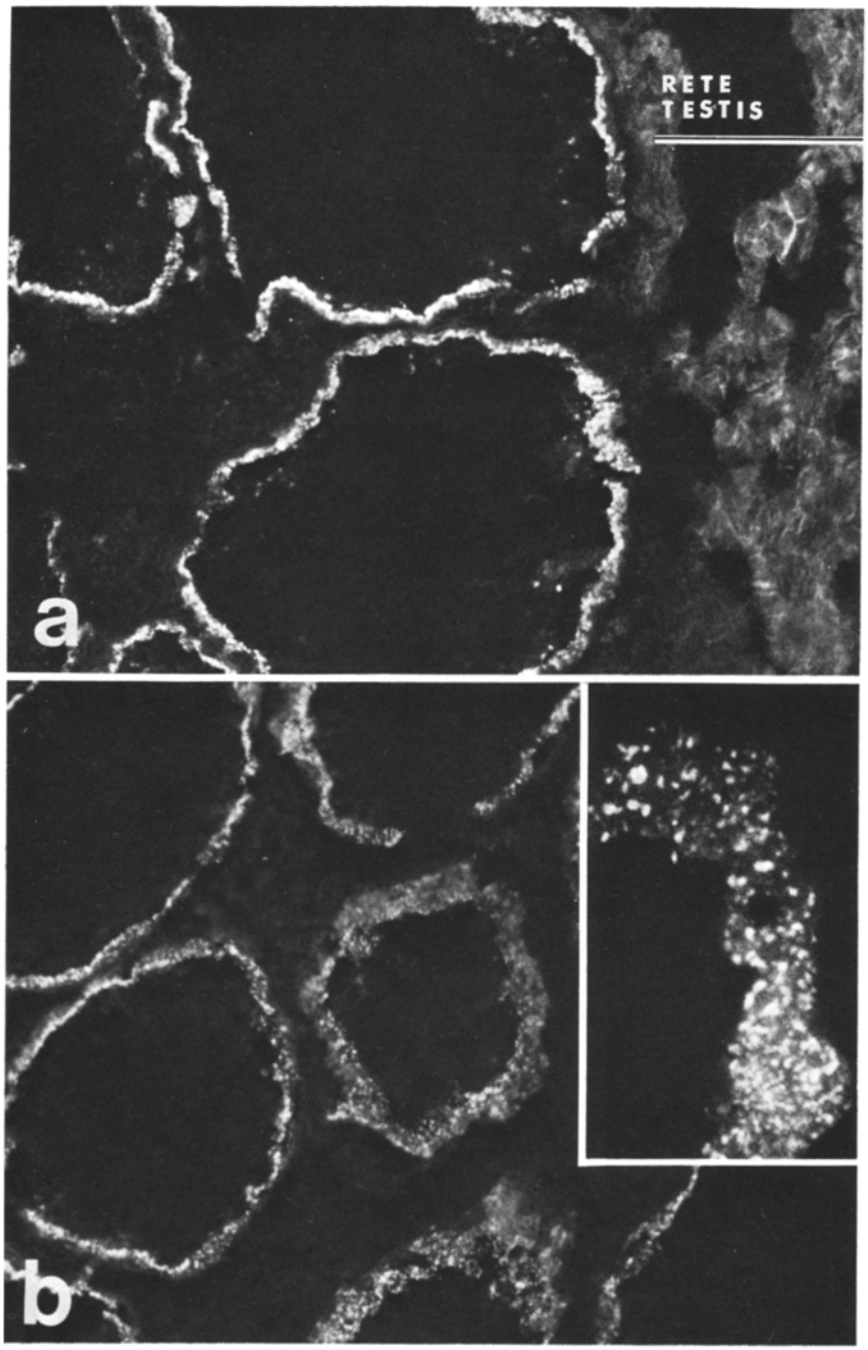

FIG. 4. Immunofluorescence photomicrographs showing massive, diffuse granular (see insert of frame b) deposits of mink IgG in the basement membrane surrounding seminiferous tubules. As shown in (a), the rete testis is free of immune deposits. × 250.

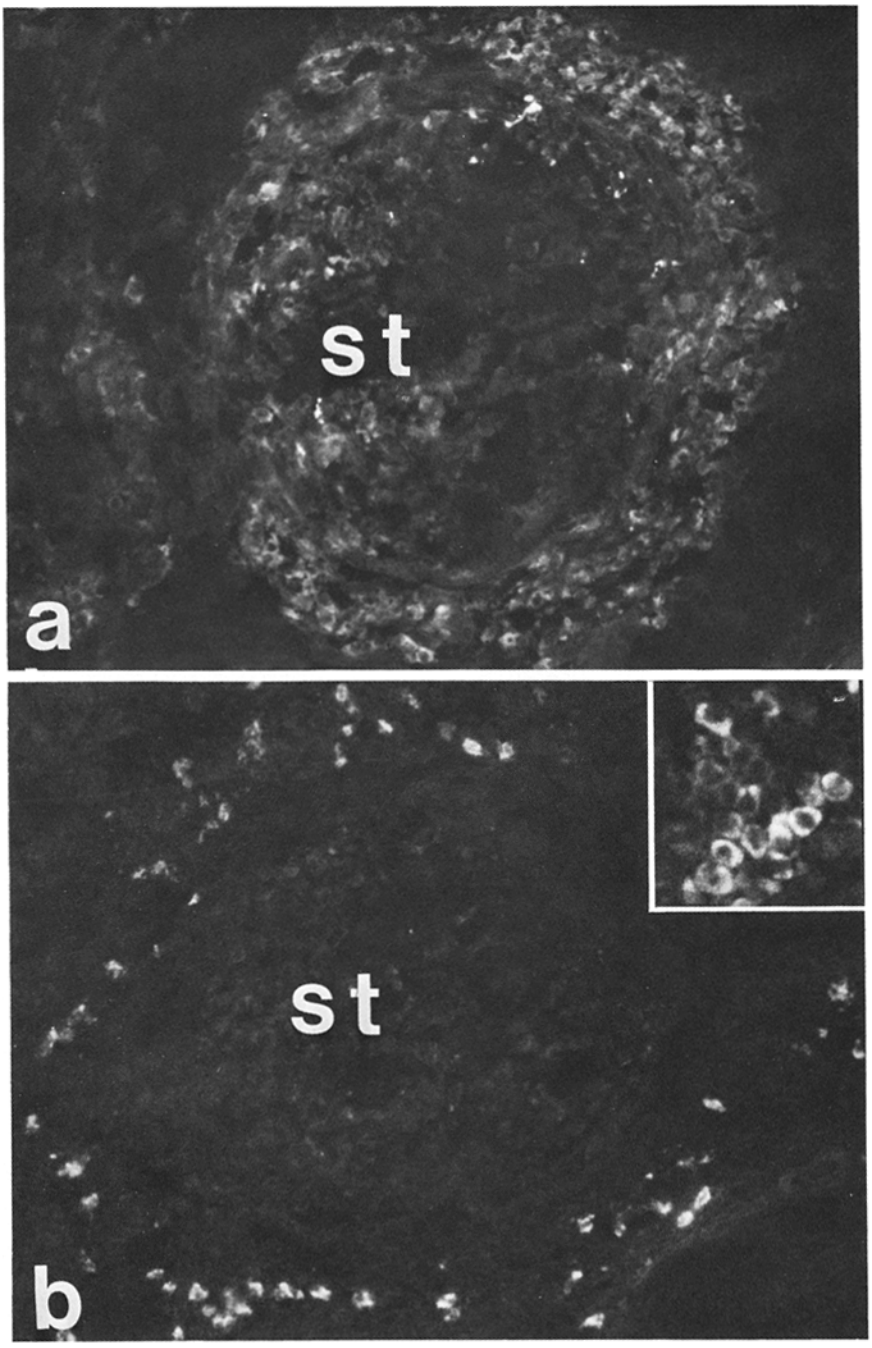

FIG. 5. IF photomicrographs of mink testis with severe orchitis. Seminiferous tubules (ST) are surrounded (b) or infiltrated (a) by IgG-associated inflammatory cells. Some of these cells (insert of b) have intracellular IgG, resembling plasma cells. × 250.

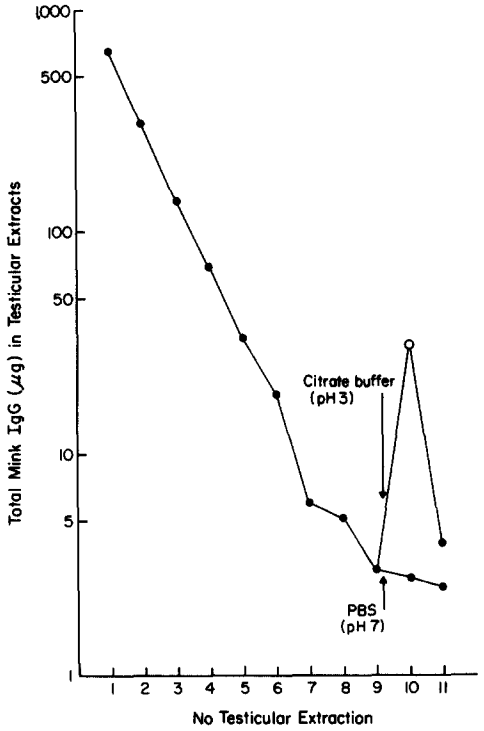

FIG. 6. Quantitation of mink IgG in extracts of mink testis after PBS (●) and citrate buffer, pH 3 (○).

TABLE IV

*Quantitation of Anti-Sperm Antibody in Acid Eluate of Testes and Serum from Mink with Secondary Infertility**

Source	IF anti-sperm anti-body titer‡	IgG content§	Minimum amount of IgG re-quired for positive IF‖
		μg/ml	
Serum	1:200	9140.0	45.7
Testicular acid eluate¶	1:8	37.7	4.7

* Study was based on testes and sera of minks 70, 71, 73, 75, 79, and 80 (Table II).

‡ Indirect IF detected anti-sperm antibody of IgG class.

§ As determined by a solid-phase RIA.

‖ Derived from b × c.

¶ After the testicular tissue was extensively washed with PBS (see Fig. 6), it was extracted with citrate buffer (0.02 M, pH 3) for 45 min at 20°C. The eluate was concentrated by precipitation in ammonium sulfate.

and their main testicular abnormality is aspermatogenesis. Because orchitis occurs rarely and immune deposits are not detected, anti-sperm autoimmunity is unlikely to be the cause of infertility. In contrast, mink with secondary infertility have signifi-

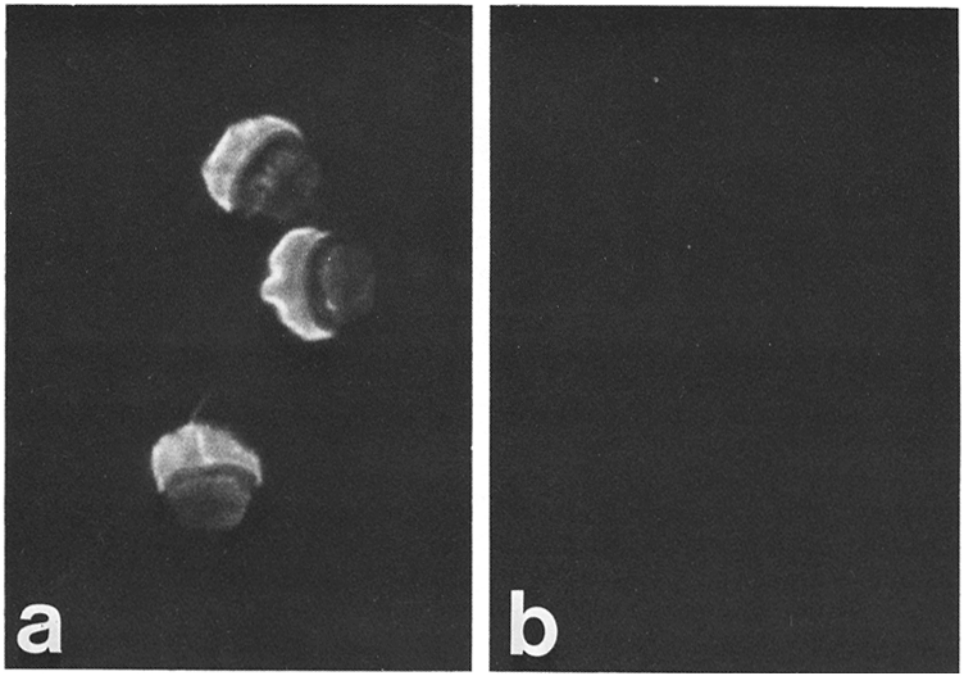

FIG. 7. IF photomicrograph showing anti-sperm antibody in acid eluate of the mink testis (a) and not in PBS eluate of the same tissue (b). Both eluates were studied at 40 μg/ml. × 400.

cantly higher levels of anti-sperm antibodies. Many testes with severe orchitis and/or aspermatogenesis have peritubular granular deposition of mink IgG and C3, presumptive evidence of immune complexes (30). Although sperm antigen was not detected in the immune complexes, acid elution of testes with immune complexes clearly recovered IgG that was enriched in anti-sperm antibody activity. 10 times more anti-sperm antibody activity was found in the acid eluted IgG than the serum IgG of the same animals. Furthermore, PBS failed to elute anti-sperm antibody from the same pool of testicular tissues. These findings are thus consistent with *in situ* deposition of sperm antigen-antibody complexes in the peritubular basement membrane of the testis.

Autoimmune testicular disease in the mink is clearly an important feature of the infertile state, but it is unclear how the autoimmune disease evolves. There are at least two possibilities, and they are not mutually exclusive. First, the testicular autoimmunity may be the primary event that provides the etiologic basis for secondary infertility. If this proved to be true, it would be logical to extrapolate that genes that code for fur characteristics are closely linked to immune response genes for testicular autoantigens relevant to orchitis induction, possibly within the major histocompatibility complex. In support of this hypothesis is the finding that the severity of the Aleutian mink disease, an immunologic disease associated with aberrant immune responses to the Aleutian disease parvovirus, is also related to fur color (31). Furthermore, a recent study in vasectomized guinea pigs indicates that the antoantibody response to sperm surface antigens is determined by a single autosomal, or X-linked, gene (32). As a second possibility, the primary defect may lie with some nonimmu-

nologic mechanisms that determine the development and the maintenance of normal testicular functions. Primary mink infertility may be another experimental model of abnormal development of the gonad that is genetically determined. In this context, it is comparable to backcross mice with the t^{w18} haplotype of the T/t locus gene (33), rats with the growth productive system gene (34), and the W/W^v mouse (35). The pathogenesis of primary mink infertility may be related to malfunction of the hypophysial-pituitary-testicular axis. In consequence, the testis fails to develop, to descend normally, or to respond appropriately to gonadotropins. In addition, failure to maintain the normal blood-testis barrier might lead to excessive release of testis-specific antigens during the seasonal regenerative phase of the testis. Autostimulation by testis-specific antigens could then lead to autoimmune testicular disease, characteristically found in mink with secondary infertility. Dissection of any relationship between immunologic (secondary) and nonimmunologic (primary) bases of the infertile state may be facilitated by determination of whether the progeny of mink with secondary infertility are also infertile, and if they are, whether they develop only secondary infertility or both primary and secondary infertility. It is pertinent to point out that autoimmune thyroiditis in the obese strain of chicken is another disease wherein nonimmunologic abnormality of the target organ leads to autoimmune disease (36).The foregoing discussion would suggest that infertility of the black mink is genetically determined. However, any prediction of the nature of genetic control is premature because of the complexity of the etiology of the infertile state. Furthermore, this question is not readily explorable because mink breeding is a yearly event, and because the major histocompatibility complex of mink is yet poorly defined.

Although tissue-adjuvant-induced autoimmune disease of the testis, experimental allergic orchitis, has been extensively studied in the past three decades (7), mink with secondary infertility is the first example of a spontaneous autoimmune testicular disease known to be associated with infertility. Although orchitis of unknown cause has been described in other situations, such as in man after mumps infection (37), in a colony of beagle dogs (38), and in the backcross t-locus mice with the w^{18} haplotype (33), there is not yet firm evidence to indicate that these diseases are mediated by immunologic mechanisms (reviewed in 39).

Testicular immune complexes have been found in rabbits after vasectomy (19) or immunization with heterologous testicular antigens in complete Freund's adjuvant (40). As in these diseases, the pathogenetic significance of immune complexes in the infertile mink remains unclear. The coexistence of C3 in immune complexes and orchitis suggests complement activation, presumptive release of biologically active complement fragments, and chemotaxis of inflammatory cells that constitute orchitis. However, this interpretation does not take into consideration the finding of IgG and C3 in other testes that lack orchitis. In testes of mink with secondary infertility, one is impressed with the extensive distribution of testicular immune complexes. In nine testes, 100% of seminiferous tubules were surrounded by grandular IgG or C3. Thus, immune complex formation might have been preceded by some event(s) that led to a generalized defect of the blood-testis barrier. Sperm-specific antigens, normally sequestered in the seminiferous tubules, may leak outside the barrier and precipitate with circulating anti-sperm antibody to form immune complexes *in situ*. On the other hand, orchitis, which is often focal or absent in testes with extensive immune complex deposition, is unlikely to be the mechanism leading to the loss of the blood-testis

barrier. It is also of interest that testicular immune complexes are detected only in the seminiferous tubules, where active production of sperm antigens occurs. Immune complexes are absent in the rete testis, even though the epithelial barrier at the rete or the adjacent ductus efferentes is generally more permeable than the barrier in the seminiferous tubules (41, 42). Sertoli cells and their intercellular tight junctions are the major blood-testis barriers in most species (43). It will be important to assess the status of the blood-testis barrier and the physiologic mechanisms that normally preserve this tissue barrier in fertile and infertile black mink.

Our data also indicate that humoral antibody to spermatozoa, detectable by indirect IF, is insufficient to mediate disease of the testis and infertility. Thus, many black and pastel mink with high levels of serum anti-sperm antibodies are fertile. It is possible that additional immunologic factors, such as antibody to sperm surface antigens or cell-mediated immunity to sperm antigens are required for the development of orchitis, aspermatogenesis, and immune complexes. Alternatively, testicular disease may develop only when the blood-testis barrier is defective.

The model of infertile male mink shares characteristics with infertility in men. The testicular findings in both species are mixed and suggest that different and multiple pathogenetic mechanisms may be involved. The end-point of these abnormalities, and also the most frequent testicular finding in both species, is aspermatogenesis. As in infertile man, undescended testes are found in the mink. Finally, a small group of mink and men have normal testicular and epididymal morphology, but are infertile. We believe, therefore, that further elucidation in the pathogenesis of the infertile mink will provide insight into the complex problem of male infertility in man.

Summary

Breeding for fine black fur has generated a colony of mink wherein 20–30% of the males are infertile. Two clinical groups are distinguishable: one being infertile from the start (primary infertility), and the other infertile after one or more years of fertility (secondary infertility). Although the etiology of primary infertility is unknown, the available data indicate that secondary infertility is associated with an autoimmune disease of the testis. Thus, male mink with secondary infertility have (*a*) higher prevalence and levels of anti-sperm antibody when compared with animals with primary infertility, and the antibody prevalence varies with fur color; (*b*) severe monocytic orchitis (47%) and/or aspermatogenesis (75%) with negative cultures for bacterial, fungal, mumps, or Coxsackie B viral organisms; (*c*) massive and extensive granular deposits of mink IgG and/or C3 (71%), typical of immune complexes, along the basal lamina of seminiferous tubules; (*d*) testes that when eluted with buffer or low pH yielded IgG that was 10-fold enriched in anti-sperm antibody activity as compared with serum IgG; and (*e*) no immunopathologic evidence of Aleutian mink disease. Although the sperm antigen-antibody complexes in the testis may be important as a pathogenetic mechanism of the testicular disease, there is no correlation between fluorescent anti-sperm antibody detection in the serum and the infertile state. The infertile black mink is a new model of infertility associated with naturally occurring autoimmune disease of the testis.

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