

## Detection of Feline Immunodeficiency Virus in Semen from Seropositive Domestic Cats (*Felis catus*)

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**Electroejaculates from experimentally infected domestic cats were evaluated for the presence of feline immunodeficiency virus (FIV). Virus was isolated from cell-free seminal plasma and seminal cells by cocultivation with a feline interleukin-2-dependent CD4<sup>+</sup> T-cell line, in which productive infection was demonstrated by syncytium formation and FIV gag p26 antigen secretion. In addition, an 868-bp segment of the FIV gag provirus gene was identified in cocultured cells by PCR and Southern analysis. A 582-bp fragment of the FIV gag provirus genome was detected by nested PCR and Southern analysis in nonfractionated seminal cells and in sperm purified by a swim-up procedure. This is the first report describing the detection of replication-competent FIV in cell-free and cell-associated forms in domestic cat semen.**

Feline immunodeficiency virus (FIV) is a lentivirus that infects domestic (11, 42, 50) and wild felids (4, 6, 33). Infection in domestic cats shares many of the clinical and immunologic characteristics of human immunodeficiency virus type 1 (HIV-1) infection, including an acute flu-like illness followed by clinical latency and progressive immune dysfunction that may lead to terminal AIDS-like diseases (5, 11, 43). FIV has been isolated from blood (50) and body fluids, such as saliva (27), cerebrospinal fluid (50), and milk (40). Biting is thought to be the major route of exposure in natural infections (35, 50). Experimentally, FIV can be transmitted horizontally via parenteral (10), oral (30, 40), or rectal exposure (30). Transmission in utero (34) and through nursing (40) have also been reported. Although seroepidemiologic studies suggest that FIV is not commonly transmitted through sexual activity (14, 21, 49), experimental infection of queens via intravaginal inoculation indicates that venereal transmission may potentially occur (30).

The clinical progression of FIV infection in nondomestic felids has not been clearly defined, though immunologic changes, including hypergammaglobulinemia, reduced CD4<sup>+</sup>/CD8<sup>+</sup> T-cell ratios (23), and diminished lymphocyte mitogenesis (45) have been demonstrated. Recently, Poli et al. (37) reported lymphoma and pneumonia in a seropositive lion. The route of virus transmission within nondomestic cat populations is unclear. Phylogenetic and epidemiologic analyses of seropositive free-roaming lions suggest horizontal transmission is more likely than vertical transmission (7). Because of the endangered status of most nondomestic felids, assisted reproduction techniques, e.g., artificial insemination and in vitro fertilization, have become invaluable tools for species preservation (3, 18, 19, 47). The spread of pathogens through contact with infected semen could potentially have deleterious consequences for genetically restricted cat populations, such as cheetahs, Florida panthers, and clouded leopards. A domestic cat (*Felis catus*) model of seminal transmission of FIV would provide invaluable information for planning appropriate re-

productive strategies for FIV-infected nondomestic cats. The primary objective of this study was to determine whether FIV could be detected in seminal components from chronically infected domestic cats.

**Isolation of FIV from cell-free seminal plasma.** FIV has been successfully transmitted from body secretions, such as saliva (27, 50) and milk (40), to activated feline lymphocyte cultures. We first attempted to isolate FIV from semen by coculturing cell-free seminal plasma with FCD4E cells, an interleukin-2-dependent feline CD4<sup>+</sup> T-lymphocyte cell line developed in our laboratory that is highly permissive for FIV infection (10). Male cats were obtained from a colony of specific-pathogen-free cats bred at the Laboratory Animal Resources Facility at North Carolina State University (NCSU), College of Veterinary Medicine. Seven perinatally infected males were used for this experiment. An uninfected male served as a control subject. Male cats were group housed on the basis of FIV status in accordance with the American Association for Accreditation of Laboratory Animal Care standards. All cats were negative for feline leukemia virus antigen by enzyme-linked immunosorbent assay (ELISA) (Idexx, Portland, Maine).

Neonatal infection of these cats with the NCSU<sub>1</sub> isolate of FIV has been reported in detail by Sellon et al. (40). Two male cats (no. 324 and 325) were orally administered  $2 \times 10^6$  50% tissue culture infective doses of FIV-NCSU<sub>1</sub>-infected, cell-free culture supernatant fluid between 1 and 3 days of age. Five neonatal male cats (no. 309, 310, 311, 315, and 316) were infected after their nursing mothers had been intravenously inoculated with  $5 \times 10^5$  50% tissue culture infective doses of FIV-NCSU<sub>1</sub> culture supernatant fluid within 24 h of delivery of their last kittens. The kittens were periodically monitored for infection by PCR amplification and Southern analysis of an 868-bp segment of the FIV gag provirus genome in peripheral blood mononuclear cells (PBMC) and by detection of FIV-specific serum antibodies by commercial ELISA (Idexx) (40). The kittens were provirus positive by 8 weeks of age and seroconverted by 12 weeks of age. They remained positive for PBMC FIV gag provirus and antibody when tested at 12 and 24 months of age. Compared with uninfected age-matched controls, infected males had lower body weights and persistently

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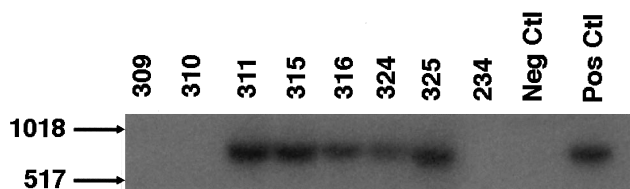


FIG. 1. FIV *gag* provirus in cell-free seminal plasma cocultures from 12-month-old cats infected with FIV-NCSU<sub>1</sub> as neonates. One hundred microliters of seminal plasma was added to  $2 \times 10^6$  FCD4E cells in 24-well plates. When syncytia were observed, or at the end of 4 weeks, cocultured cells were analyzed by PCR and Southern analysis for an 868-bp segment of the FIV *gag* provirus genome. Cats 309, 310, 311, 315, 316, 324, and 325 are FIV infected. Cat 234 is as an uninfected control. PCR controls consisted of PBMC from an uninfected control cat (Neg Ctl) and an FIV-NCSU<sub>1</sub>-infected control cat (Pos Ctl).

low peripheral CD4<sup>+</sup>/CD8<sup>+</sup> T-lymphocyte ratios (data not shown). With the exception of mild lymph node enlargement, seropositive cats were otherwise clinically healthy during this study period.

After the induction of anesthesia by intramuscular injection of 30 to 35 mg of tiletamine-zolazepam (Telazol; Fort Dodge Laboratories, Fort Dodge, Iowa), semen samples were collected by a standardized electroejaculation protocol for domestic cats (15, 16). Ejaculates were immediately diluted in a 1:1- to 1:2-volume ratio with Ham's F-10 medium supplemented with 5% fetal bovine serum, and aliquots were retained for determining sperm concentration. Diluted semen was centrifuged ( $13,000 \times g$  for 5 min), and the supernatant fluid (seminal plasma) was removed for virus isolation. One hundred microliters of seminal plasma was added to  $2 \times 10^6$  FCD4E cells in 2 ml of RPMI 1640 medium (GIBCO, Grand Island, N.Y.) supplemented with 10% fetal bovine serum, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), 2 mM L-glutamine, 0.075% NaHCO<sub>3</sub>,  $2.5 \times 10^{-5}$  M 2-mercaptoethanol, 100 U of penicillin per ml, 100 µg of streptomycin per ml, and 100 U of interleukin-2 per ml (kindly provided by the Biological Resources Branch of the National Institutes of Health) in 24-well plates incubated at 37°C in 5% CO<sub>2</sub>. Cocultures were monitored every 2 to 3 days for syncytium formation, a morphologic change characteristic of productive FIV infection in this cell line (10). When syncytia were evident, or at the end of 4 weeks, cocultured cells were washed in 10 mM Tris-HCl-1 mM EDTA wash buffer (pH 8.0) and then incubated in 100 µl of digestion buffer (1× PCR buffer with 100 µg of proteinase K per ml; Promega, Madison, Wis.) at 56°C overnight (10). An 868-bp fragment of the FIV *gag* provirus genome was amplified from crude coculture digests by PCR and Southern analysis and resolved on 1% agarose gels, as described for PBMC (10, 40). The presence of FIV *gag* p26 antigen in coculture supernatants was determined by a commercial ELISA (Idexx).

As shown in Fig. 1, seminal plasma samples from five of seven FIV-infected cats contained virus as determined by PCR of cocultured FCD4E cells. Indicators of productive infection are summarized in Table 1. Virus was not detected in the seminal plasma of control cat 234. Consistent with active replication of FIV, four of the five PCR-positive cocultures demonstrated syncytium formation within 14 to 27 days of culture. The harvest of cocultures at the first evidence of syncytium formation apparently precluded the accumulation of detectable levels of p26 antigen, as only one of the PCR-positive cocultures (no. 324) contained measurable concentrations of the viral protein. The seminal plasma coculture from cat 316 expressed FIV provirus without syncytia or p26 antigen secretion, indicating that actively replicating virus was absent or at

levels too low to be detected. These results show that FIV is present in cell-free seminal fluid and that semen obtained during the asymptomatic period of infection can establish productive infection of a feline lymphocyte cell line.

**Detection of FIV in seminal cells by nested PCR.** The source of FIV in seminal plasma is undetermined, though virus could originate in productively infected seminal cells. Because most artificial insemination protocols for domestic and nondomestic cats currently utilize washed seminal cells rather than whole ejaculates (3, 15, 18, 19), we addressed the presence of FIV within the cellular fraction of domestic cat semen. Aliquots of pelleted seminal cells from the diluted ejaculates described above (containing approximately  $10^6$  spermatozoa) were washed three times in Tris-EDTA buffer and then digested in 50 µl of proteinase K digestion buffer as described above. Cells other than mature spermatozoa (spermatogenic cells, squamous epithelial cells, and leukocytes) made up fewer than 0.1% of cells in these feline electroejaculates. Lymphocytes, macrophages, and neutrophils were evident, though  $\leq 0.01\%$  of seminal cells were leukocytes (data not shown). PCR to detect FIV *gag* with our single primer set (with a sensitivity of one provirus copy per 20,000 PBMC or cocultured cells [22]) failed to generate identifiable products on a 1% agarose gel; however, an internal 582-bp segment could be successfully amplified from crude nonfractionated seminal cell digests with nested primer pairs described previously (10, 22). The specificity of the gene products was confirmed by Southern analysis (10, 40). As shown in Fig. 2, four of seven seminal cell samples were positive for FIV *gag* provirus by nested PCR. No provirus was evident in control cat 234. These results indicate that the cellular fraction of semen may harbor provirus. The inability to detect FIV *gag* DNA without nested primers suggests that the provirus copy number is lower in semen than in blood ( $10^6$  PBMC), which required a single primer pair to detect provirus in these same cats (data not shown).

Interestingly, the presence of virus in seminal fluid and seminal cells was not necessarily concordant (Table 1). For instance, cat 309 had detectable provirus in seminal cells (Fig. 2),

TABLE 1. Summary of FIV analysis of semen from infected cats at 12 months of age

Cat <sup>a</sup>	Analysis of			
	Seminal plasma cocultures for: <sup>b</sup>			Nonfractionated seminal cells for FIV <i>gag</i> provirus by nested PCR <sup>c</sup>
	Syncytia (day first observed)	FIV <i>gag</i> provirus by PCR	FIV <i>gag</i> p26 antigen secretion by ELISA	
309	Negative	Negative	Negative	Positive
310	Negative	Negative	Negative	Negative
311	Positive (22)	Positive	Negative	Positive
315	Positive (27)	Positive	Negative	Positive
316	Negative	Positive	Negative	Negative
324	Positive (14)	Positive	Positive	Negative
325	Positive (22)	Positive	Negative	Positive

<sup>a</sup> Specific-pathogen-free male cats with neonatally acquired FIV-NCSU<sub>1</sub> infection.

<sup>b</sup> Cell-free seminal plasma (100 µl) collected by electroejaculation was added to  $2 \times 10^6$  FCD4E cells, an interleukin-2-dependent CD4<sup>+</sup> feline T-lymphocyte cell line, in 24-well plates. Cocultures were monitored for syncytium formation, an indication of productive FIV infection, every 2 to 3 days. Cocultured cells were harvested when syncytia were observed, or at the end of 4 weeks, and analyzed for an 868-bp fragment of the FIV *gag* provirus genome by PCR and Southern analysis. Culture supernatants were analyzed by commercial ELISA for FIV *gag* p26 antigen.

<sup>c</sup> Nonfractionated seminal cells ( $10^6$ ) were analyzed for a 582-bp fragment of FIV *gag* provirus genome by nested PCR and Southern analysis.

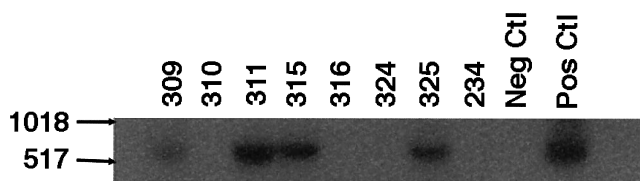


FIG. 2. FIV *gag* provirus in nonfractionated seminal cells from 12-month-old cats infected with FIV-NCSU<sub>1</sub> as neonates. A 582-bp segment of the FIV *gag* provirus gene was amplified from seminal cells by nested PCR, and specificity was confirmed by Southern analysis. Cats 309, 310, 311, 315, 316, 324, and 325 are FIV infected. Cat 234 is as an uninfected control. PCR controls consisted of PBMC from an uninfected control cat (Neg Ctl) and an FIV-NCSU<sub>1</sub>-infected control cat (Pos Ctl).

but seminal plasma coculture results were negative (Fig. 1 and Table 1). Seminal cell samples were provirus negative in cats 316 and 324 (Fig. 2), though FIV was evident in seminal plasma from both cats (Fig. 1 and Table 1). Such results may be due to differences in the methodologies and sensitivities of cocultivation and PCR; e.g., PCR positivity may represent quiescent, defective, or incomplete virus, in addition to replication-competent virions. Alternatively, cell-free virus may be shed by sources other than infected seminal cells, such as the epididymis, ductus deferens, prostate, bulbourethral gland, or other tissues lining the reproductive tract.

**The presence of FIV in seminal plasma and seminal cells obtained 1 year later.** Because the cats in this study were just reaching sexual maturity (11 to 12 months in domestic cats) at the time of the first semen collection, it was possible that the variation in virus expression in seminal components was influenced by the stage of sexual development. In order to confirm the initial isolation and PCR results and to examine virus expression over time, we repeated the FIV analysis of seminal plasma and seminal cells collected from the infected cats at 24 months of age. Three uninfected males served as control subjects. Seminal plasma (200  $\mu$ l) was cocultured with FCD4E cells, and nonfractionated seminal cells ( $10^6$  cells) were analyzed by nested PCR and Southern analysis as described above. The remaining cells were saved for swim-up sperm preparations (see below). None of the samples from the three control cats had evidence of FIV (data not shown). As indicated in Table 2, seminal plasma cocultures from the same five seropositive males were PCR positive 1 year later. Increasing the

volume of seminal plasma in cocultures from 100 to 200  $\mu$ l and incubating all cultures for a total of 4 weeks enhanced detection of replication-competent virus as demonstrated by earlier syncytium formation (11 to 16 days) and p26 antigen secretion by all five PCR-positive cocultures (Table 2). The results of nested PCR and Southern analysis of nonfractionated seminal cells collected at 1 (Fig. 2) and 2 years (Table 2) of age were unchanged in four cats (315, 316, 324, and 325). Although provirus positive at 12 months of age, nonfractionated seminal cells from cats 309 and 311 did not express detectable levels of FIV *gag* DNA at 24 months of age. Conversely, cat 310 was negative for seminal cell provirus at 12 months postinfection and positive 1 year later. These findings confirm that semen from chronically infected, sexually mature males contains infectious FIV and viral DNA. Although only two time points were examined, the data suggest that virus expression in seminal plasma may be more consistent over time than provirus expression in seminal cells.

**Detection of FIV *gag* provirus in spermatozoa purified by a swim-up procedure.** Potential cellular reservoirs of FIV in ejaculates include spermatozoa and nonspermatozoal cells, such as lymphocytes, macrophages, immature sperm cells, and epithelial lining cells. Previous work has shown that CD4<sup>+</sup> T lymphocytes, CD8<sup>+</sup> T lymphocytes, B cells, and monocytes/macrophages are infected with FIV in vivo (10). Virus also replicates in cells of epithelial origin in vitro, such as Crandell feline kidney cells (8). We, therefore, hypothesized that cell-associated virus is present in seminal nonsperm cell populations, rather than in spermatozoa. Because feline nonsperm cells are uncommon and difficult to purify in sufficient quantity, we approached this problem by utilizing a swim-up technique to assess whether FIV *gag* provirus could be detected in mature sperm. A swim-up procedure is commonly used by reproductive specialists to enrich inseminates for highly motile, morphologically normal spermatozoa prior to artificial insemination, and a protocol has been adapted for cat semen (17). Cells that fail to swim up, or migrate upwards through a fluid interface, include nonsperm cells and spermatozoa with various structural morphologies and motilities (e.g., some sperm may be motile, but because of morphologic abnormalities, they may swim inefficiently).

Seminal plasma was removed from the ejaculates described above, and fresh Ham's F-10 medium was carefully layered over seminal cell pellets. Samples were incubated for 1 h at

TABLE 2. Summary of FIV analysis of semen from infected cats at 24 months of age<sup>a</sup>

Cat	Analysis of							
	Seminal plasma cocultures for: <sup>b</sup>			Nonfractionated seminal cells <sup>c</sup> for FIV <i>gag</i> provirus by nested PCR	Swim-up sperm <sup>d</sup>	Nonswim-up seminal cell cocultures for: <sup>e</sup>		
	Syncytia (day first observed)	FIV <i>gag</i> provirus by PCR	FIV <i>gag</i> p26 antigen secretion by ELISA			Syncytia (day first observed)	FIV <i>gag</i> provirus by PCR	FIV <i>gag</i> p26 antigen secretion by ELISA
309	Negative	Negative	Negative	Negative	Positive	Positive (15)	Positive	Positive
310	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
311	Positive (15)	Positive	Positive	Negative	Negative	Positive (18)	Positive	Positive
315	Positive (11)	Positive	Positive	Positive	Positive	Positive (15)	Positive	Positive
316	Positive (16)	Positive	Positive	Negative	Negative	Negative	Negative	Negative
324	Positive (15)	Positive	Positive	Negative	Positive	Negative	Negative	Negative
325	Positive (12)	Positive	Positive	Positive	Positive	Positive (19)	Positive	Positive

<sup>a</sup> Electroejaculates were obtained from perinatally infected cats at 24 months of age.

<sup>b</sup> Cell-free seminal plasma (200  $\mu$ l) was cocultured with FCD4E cells and monitored as described in the text. At the end of 4 weeks, cocultured cells were evaluated for the presence of an 868-bp segment of the FIV *gag* provirus genome and FIV *gag* p26 antigen.

<sup>c</sup> Nonfractionated seminal cells ( $10^6$ ) were analyzed for a 582-bp segment of the FIV *gag* provirus genome by nested PCR and Southern analysis.

<sup>d</sup> Sperm purified by a swim-up procedure (see the text) were analyzed by nested PCR and Southern analysis for a 582-bp segment of the FIV *gag* provirus genome.

<sup>e</sup> Aliquots ( $0.5 \times 10^6$  to  $1.0 \times 10^6$  cells) of nonswim-up seminal cells (see the text) were cocultured with  $2 \times 10^6$  FCD4E cells and monitored as described in the text.

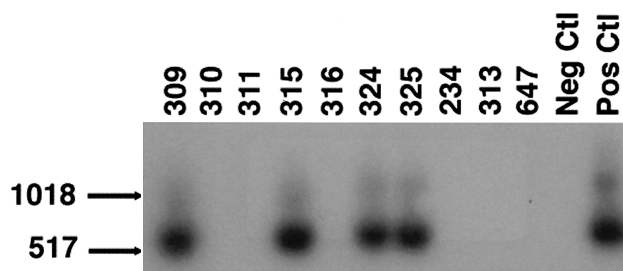


FIG. 3. Swim-up sperm specimens from perinatally infected cats containing FIV *gag* provirus. A 582-bp segment of the FIV *gag* provirus gene was amplified by nested PCR, and specificity was confirmed by Southern analysis. Cats 309, 310, 311, 315, 316, 324, and 325 are FIV infected. Cats 234, 313, and 647 are uninfected controls. PCR controls consisted of PBMC from an uninfected control cat (Neg Ctl) and an infected control cat (Pos Ctl). Total swim-up sperm counts ( $10^6$ ) were as follows: cat 309, 0.9; cat 310, 6.3; cat 311, 0.4; cat 315, 4.9; cat 316, 0.8; cat 324, 38.4; cat 325, 4.4; control cat 234, 0.2; control cat 313, 2.0; and control cat 647, 9.4.

25°C. Spermatozoa which migrated into the medium above the pellet were collected and counted. Because of low sperm recovery in many specimens (recoveries ranged from  $0.2 \times 10^6$  to  $38.4 \times 10^6$  sperm; see the legend to Fig. 3), the entire swim-up sperm sample from each cat was washed and digested in 50  $\mu$ l of proteinase K buffer and analyzed by nested PCR (10- $\mu$ l digest per 100- $\mu$ l PCR mixture) and Southern analysis. Cells other than spermatozoa were not detected during microscopic examination of several swim-up specimens ( $5 \times 10^5$  spermatozoa per slide; data not shown). As shown in Fig. 3, four of seven seropositive males had FIV provirus in swim-up sperm specimens. Provirus was not evident in control cat samples. These data indicate that swim-up sperm preparations from infected cats contain FIV DNA.

The origin of virus DNA in swim-up sperm samples is uncertain. Not all specimens were sufficient to permit a cytologic evaluation of purity, and it is possible that infected nonsperm cells were present at concentrations of  $<1/500,000$  sperm. Alternatively, studies using *in situ* PCR indicate HIV-1 may be present within spermatozoal precursors (32); thus, provirus may be generated during spermatogenesis. A third possibility is that viral DNA may be produced within free virus particles that have adhered to the sperm cell membrane. Structures resembling HIV-1 virions have been observed on the surface of spermatozoal cells by electron microscopy (2), and reverse transcription by extracellular HIV-1 isolated from seminal plasma and other physiologic fluids has been documented (51).

**Seminal cells transmit FIV *in vitro*.** Our PCR data demonstrated the presence of the proviral form of FIV in nonfractionated seminal cells and in motile sperm preparations. To determine whether the seminal cell fraction, like seminal plasma, also contains replication-competent virus, we cocultured nonswim-up cells from the swim-up preparation pellets described above. Washed nonswim-up cells ( $0.5 \times 10^6$  to  $1.0 \times 10^6$  cells) from the seven infected cats and three uninfected control cats (containing  $\leq 0.1\%$  nonsperm cells; data not shown) were added to  $2 \times 10^6$  FCD4E cells in 24-well plates and monitored as described above for seminal plasma. As shown in Table 2, nonswim-up seminal cell cocultures from four of seven infected males expressed provirus and exhibited evidence of productive FIV infection. Virus was not evident in uninfected cat semen. Thus, in addition to provirus, seminal cells harbor replication-competent FIV. Whether infectious virus is associated with sperm, nonsperm cells, or both cell groups is unclear.

This report demonstrates that infectious FIV can be readily

isolated from the semen of experimentally infected cats during the clinically asymptomatic period. However, a role for seminal transmission of FIV in natural settings has yet to be demonstrated. Ishida et al. found no differences in rates of infection between sexually intact and neutered cats in Japan (21). In a survey of cats from the United Kingdom, neutering did not alter the likelihood of a male cat being FIV positive, though, interestingly, neutered females had an increased likelihood of being positive, relative to intact females (14). Susceptibility of queens to intravaginal transmission was experimentally demonstrated by Moench et al. (30). Six of seven females became infected after FIV-positive PBMC were instilled in the vaginal vault. *In vivo* studies will, therefore, be required to assess whether infected semen transmits virus to female cats through sexual intercourse or artificial insemination and to characterize which factors, if any, are important in conferring protection against venereal infection in queens.

Seminal FIV was evident in 100% of the cats (7 of 7) 24 months after infection. Similarly, HIV-1 may be present in semen from over 80% of seropositive men (13, 28). Both viruses can be found in cell-free and/or cell-associated forms in male genital fluid (24, 44), though the specific mechanisms of virus expression remain to be defined. Likely reservoirs of HIV-1 include CD4<sup>+</sup> lymphocytes and macrophages commonly found in human semen (1, 26, 48). Virus-positive mononuclear cells have been identified in ejaculates (26) and within epithelial and connective tissues of reproductive tissues from patients with AIDS (39). The role of sperm cells in the dissemination of HIV-1 is controversial. HIV-related protein (9) and nucleic acids (32) have been associated with germ cells in testes from individuals with AIDS. However, despite the appearance of virus-like particles on and within sperm by electron microscopy (2), HIV-1 has not been demonstrated in purified mature spermatozoa by cocultivation (1) or PCR (28). In contrast, known FIV target cells, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, B cells, and macrophages (10) are apparently rare in feline ejaculates. Indeed, the presence of FIV DNA in swim-up sperm samples suggests that sperm may harbor virus in the cat. Whether these apparent species differences are due to variations in virus tropism or host characteristics is unclear. Feline and human ejaculates differ cytologically; however, anatomically, the feline and human male reproductive tracts are quite similar (46). Differences may, therefore, be related to additional factors, such as mucosal immunity, type of sexual activity, or exposure to sexually transmitted pathogens.

In addition to FIV and HIV-1, simian immunodeficiency virus, murine retroviruses, bovine immunodeficiency virus, and equine infectious anemia have been detected in semen, though for most of these lentiviruses sexual transmission appears to be an inefficient mode of virus dissemination (12). Simian immunodeficiency virus has been isolated from semen and vaginal secretions from experimentally infected macaques (29), and transmission via mating has been reported (25, 36). Several murine type C leukemia retroviruses are spread through sexual contact in susceptible mouse strains (12, 38). Nash et al. (31) recently detected bovine immunodeficiency virus provirus in leukocytes from cryopreserved stud bull semen. Equine infectious anemia has been transmitted to ponies subcutaneously inoculated with semen from an equine infectious anemia-positive donor and to mares bred with a seropositive stallion (41).

In summary, this is the first report describing detection of FIV in semen from chronically infected, asymptomatic cats at 12 and 24 months postinfection. Infectious virus was demonstrated *in vitro* in both cell-free seminal plasma and seminal cells by cocultivation with a feline CD4<sup>+</sup> T-cell line. FIV *gag* provirus was identified in seminal cells by nested PCR. The

detection of provirus in swim-up specimens suggests that virus may be associated with motile spermatozoa. Virus expression varied among seropositive individuals and was not necessarily consistent within different fractions of semen from the same individual over time. These data indicate that assisted reproduction in felids with semen from seropositive donors should be avoided pending additional study.

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