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# Transmission of Feline Immunodeficiency Virus in Domestic Cats via Artificial Insemination

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**The objective of this study was to determine whether semen from male domestic cats infected with feline immunodeficiency virus (FIV) can transmit virus to females. Twelve inseminations were performed by an intrauterine laparoscopic technique with fresh or cryopreserved electroejaculates from asymptomatic males chronically infected with the $NCSU_1$ strain of FIV. Of six inseminations performed with fresh semen, three resulted in infection of queens, as indicated by seroconversion, expression of FIV *gag* provirus in peripheral blood leukocytes, and reduced peripheral $CD4^+/CD8^+$ T-lymphocyte ratios. None of the six inseminates with thawed cryopreserved semen resulted in infection. Two infected queens and one uninfected queen became pregnant. Virus was not evident in the seven offspring. We conclude that FIV can be transmitted horizontally by artificial insemination with fresh semen.**

Domestic cats (3, 39) and many wild feline species, such as lions, leopards, jaguars, cheetahs, bobcats, and cougars (2, 4, 23), are susceptible to infection with the lentivirus feline immunodeficiency virus (FIV). In domestic cats, the virus is most likely transmitted through saliva in bite wounds (3, 38) and like human immunodeficiency virus type 1 (HIV-1) is associated with progressive immune suppression leading to clinical AIDS-like disorders (9). Immunologic perturbations have been reported also in nondomestic cats (20), although the clinical ramifications of infection and primary routes of transmission remain to be defined for these populations.

In addition to animal-to-animal transmission through biting, FIV has been experimentally transmitted from queens to their kittens in utero (24) and through milk (24, 28). It is unknown whether FIV, like HIV-1, can be spread by sexual contact. There are no reports documenting transmission through mating, and current epidemiologic evidence does not indicate that naturally acquired infections are more prevalent in sexually intact domestic cats than in neutered cats (12, 17). However, several experimental findings suggest that venereal transmission is possible. First, our laboratory has recently isolated replication-competent FIV in semen from chronically infected domestic cats by cocultivation with a feline $CD4^+$ lymphocyte cell line (19). Second, virus has been identified in vaginal washes obtained from infected females (24). Third, females can be infected by intravaginal inoculation with infected lymphocytes (21). Thus, male and female cats shed virus in genital tract fluids, and the female reproductive tract is susceptible to mucosal transmission.

Although the impact of venereal transmission of FIV in domestic cat populations is unclear, dissemination of a potentially immunosuppressive virus through natural or artificial breeding in cheetahs, Florida panthers, clouded leopards, and other genetically restricted felid populations could have serious consequences for their long-term survival. The maintenance of genetic vigor in threatened and endangered mammalian populations may eventually rely, in part, on assisted reproduction, including artificial insemination (AI), embryo transfer, and in vitro fertilization (34, 36). For these species it is critical to determine whether seminal transmission of potential pathogens such as FIV can occur and, if so, to develop strategies for eliminating or reducing the risk of disease transmission.

The domestic cat has been used extensively as a model for the investigation of reproductive technologies for rare feline species (35). A laparoscopic intrauterine insemination technique developed in domestic cats was found to enhance pregnancy rates obtained with intravaginal AI in anesthetized felids from approximately 10 to 50% (14, 25). Subsequently, this intrauterine AI method has been performed using freshly collected ejaculates to produce offspring in many endangered cats, such as the cheetah (16), snow leopard (27), and clouded leopard (15). AI with cryopreserved semen in the leopard cat (34) and ocelot (31) has also been reported. This study was designed to determine whether domestic cats undergoing this AI procedure using fresh or thawed cryopreserved semen from FIV-positive males are at risk for infection.

Ten adult female cats were artificially inseminated. Five random-source queens (animals 524, 525, 526, 527, and 581) were purchased from a licensed dealer. Additionally, five specific-pathogen-free, adult, female cats (BXX, MOF, BWF, MNS, and BUO) were obtained from Liberty Laboratories (Liberty, N.J.). Random-source and specific-pathogen-free females were housed in separate rooms in individual cages. Prior to insemination, all females were negative for antibodies to FIV by enzyme-linked immunosorbent assay (ELISA) (Idexx, Portland, Maine) and negative for feline leukemia virus antigen by ELISA (TechAmerica, Omaha, Nebr.).

Three FIV-seropositive, 1- to 2-year-old specific-pathogen-free cats served as semen donors. These males were bred at the Laboratory Animal Resources Facility at the North Carolina State University College of Veterinary Medicine and were orally infected with the $NCSU_1$ FIV isolate soon after birth as described elsewhere (19, 28). FIV was previously isolated in electroejaculates from these cats (19). Infected males were

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TABLE 1. AI with semen from FIV-infected male domestic cats

| Insemination[a] | Queen[b] | Donor | Semen[c] | No. of inseminated sperm ($10^6$) | Queen infected[d] | Pregnancy (no. of kittens)[e] |
|---|---|---|---|---|---|---|
| 1 | 526 (1st) | 324 | Frozen (11 mo) | 9.0 | No | No |
| 2 | 526 (2nd) | 325 | Fresh | 76.8 | Yes | Yes (2) |
| 3 | 527 (1st) | 325 | Frozen (11 mo) | 9.5 | No | No |
| 4 | 527 (2nd) | 315 | Frozen (1 h) | 4.6 | No | No |
| 5 | 581 | 324 | Fresh | 13.3 | No | No |
| 6 | 525 | 325 | Fresh | 90.0 | Yes | Yes (1) |
| 7 | 524 | 315 | Fresh | 9.5 | No | Yes (4) |
| 8 | BXX | 315 | Fresh | 6.5 | Yes | No |
| 9 | MOF | 324 | Frozen (4 mo) | 2.0 | No | No |
| 10 | BWF | 315 | Frozen (4 mo) | 3.8 | No | No |
| 11 | MNS | 325 | Frozen (4 mo) | 30.7 | No | No |
| 12 | BUO | 324 | Fresh | 27.9 | No | No |

[a] Insemination was performed by an intrauterine laparoscopic technique (see the text).
[b] Two inseminations were performed with queens 526 and 527 at 6-month intervals.
[c] The length of cryopreservation prior to AI is indicated in parentheses. Inseminates administered to queens 527 (2nd) and BXX originated from the same ejaculate from donor 315.
[d] Infection was determined by persistent seroconversion and expression of FIV *gag* provirus DNA in peripheral blood leukocytes (see the text).
[e] FIV infection was not evident in the offspring.

asymptomatic during this study, although they had decreased peripheral $CD4^+/CD8^+$ T-lymphocyte ratios and lower body weights compared with those of uninfected male siblings (data not shown). Male cats were group housed in a room separate from females. All animals were maintained in accordance with the American Association for Accreditation of Laboratory Animal Care standards.

Semen was collected by an electrostimulation protocol standardized for domestic cats as previously described (13, 19). Electroejaculates were immediately diluted in a 1:1- or 1:2-volume ratio with Ham's F-10 medium supplemented with 5% fetal bovine serum. After determination of sperm concentrations, samples were centrifuged (300 × *g* for 10 min) and seminal plasma was removed. For fresh inseminations, seminal cell pellets (containing >99.9% spermatozoa) were resuspended in 300 μl of fresh Ham's F-10 medium at room temperature and shielded from light until AI. Cryopreserved inseminates were prepared by resuspending cell pellets in 300 μl of cryodiluent (20% [vol/vol] egg yolk, 11% [vol/vol] lactose, and 4% [vol/vol] glycerol in distilled water), followed by cooling to 5°C for 30 min and then pellet freezing on dry ice (35). Frozen samples were stored in liquid nitrogen for periods of 1 h to 11 months (Table 1). Immediately prior to AI, frozen specimens were thawed in Ham's F-10 medium at 37°C and centrifuged (300 × *g* for 10 min). Cell pellets were resuspended in 300 μl of fresh Ham's F-10 medium, and sperm counts were repeated.

From each male, three to four ejaculates were collected over an 11-month period. For males 324 and 325, two specimens from each donor were used immediately for insemination as fresh inseminates and two additional ejaculates were cryopreserved prior to insemination (Table 1). Three ejaculates were collected from male 315; one was used as a fresh inseminate, and the second ejaculate was cryopreserved. The third ejaculate from this male was divided, with one half used immediately as a fresh inseminate and the other half cryopreserved prior to insemination.

To synchronize folliculogenesis and ovulation, queens received 100 IU of pregnant mares' serum gonadotropin intramuscularly and 80 h later were administered 75 IU of human chorionic gonadotropin intramuscularly (13, 14). AI was performed under general anesthesia approximately 38 h after the last injection by a laparoscopic intrauterine technique as described previously (13, 14). Inseminates were split into two aliquots (containing approximately 150 μl per aliquot) and deposited into each uterine horn via percutaneous transabdominal catheterization. Six inseminations were performed with freshly collected sperm, and six inseminations were performed with thawed cryopreserved sperm (Table 1). Eight queens were inseminated once. AI was repeated in two queens (526 and 527) that were determined to be uninfected within 6 months after the first insemination (i.e., they failed to express anti-FIV antibody and FIV *gag* provirus in peripheral blood leukocytes [see below]).

After AI, queens were monitored every 2 to 4 weeks for 5 months for evidence of infection as determined by detection of plasma anti-FIV antibodies by ELISA (Synbiotics Corp., San Diego, Calif.) and confirmation by Western blot (immunoblot) analysis (22) and by PCR amplification of an 868-bp segment of the FIV *gag* provirus genome in peripheral blood leukocytes as previously reported (8, 28). Cats were examined daily for evidence of clinical illness and monitored for pregnancy by palpation. Prior to and periodically after insemination, blood was collected for flow cytometric analysis of lymphocyte subpopulations using a panel of monoclonal antibodies developed in our laboratory (32). Mean peripheral $CD4^+/CD8^+$ T-lymphocyte ratios for the uninfected and infected queens were compared by nonparametric testing (Mann-Whitney U test; Statistix 3.1; Analytical Software, St. Paul, Minn.). Statistical significance was established at $P < 0.05$.

Results are summarized in Table 1. By 12 weeks postinsemination, three queens exposed to fresh semen (525, 526, and BXX) demonstrated persistent plasma anti-FIV antibodies by ELISA and Western blot analysis (Fig. 1) and expressed detectable levels of FIV *gag* provirus DNA in peripheral blood leukocytes (Fig. 2). Plasma from these queens demonstrated strong antibody responses against major FIV *gag* proteins p26 and p15 (Fig. 1). Infection was not evident in the remaining queens. Three of six fresh inseminations resulted in infection, while none of the six inseminations performed with frozen-thawed semen resulted in infection. Infection was established with inseminates ranging from $6.5 \times 10^6$ to $90.0 \times 10^6$ spermatozoa (Table 1). Semen from two of the three males (one ejaculate from cat 315 and two ejaculates from 325) transmitted infectious virus. Fresh semen from male 315 infected

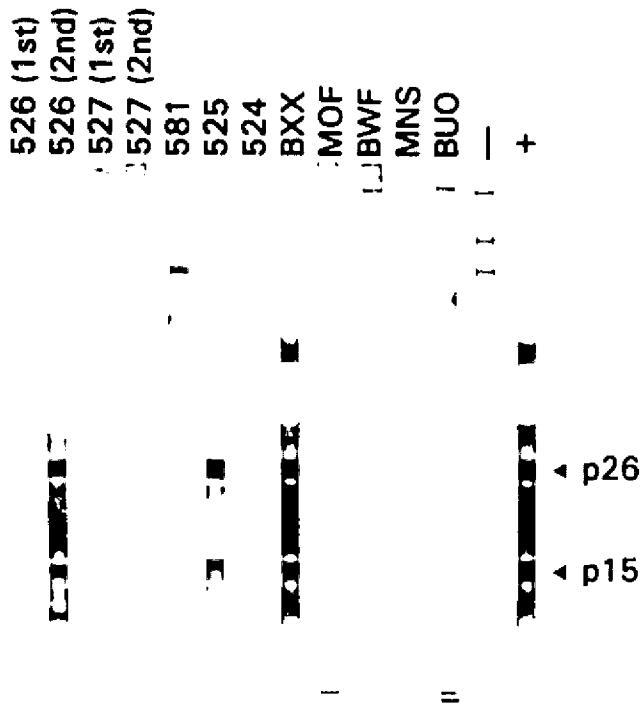


FIG. 1. Western blot analysis of FIV antibodies in plasma from cats artificially inseminated with semen from FIV-seropositive males. Position and size of major FIV *gag* viral proteins are indicated on the right. The negative and positive strips represent an uninfected control cat and an FIV ($NCSU_1$)-infected control cat, respectively.

queen BXX, while cryopreserved cells from the same ejaculate did not infect queen 527 (Table 1).

One uninfected queen (BWF) developed pyometra within 39 days after AI. After unsuccessful treatment with prostaglandin $F_{2\alpha}$ (Lutalyse; Upjohn Co., Kalamazoo, Mich.), this queen was ovariohysterectomized at 48 days post-AI with no further complications. One infected, pregnant queen (526) exhibited mild hemorrhagic vaginal discharge at 5.5 weeks after the second AI. This resolved after cesarean section at 8 weeks of gestation. All other queens were clinically normal. As shown in Fig. 3, mean peripheral $CD4^+/CD8^+$ T-lymphocyte ratios in infected queens were significantly lower than ratios in uninfected queens by 16 weeks after insemination. Both decreases in $CD4^+$ T cells and elevations in $CD8^+$ T cells contributed to the lower ratios (data not shown).

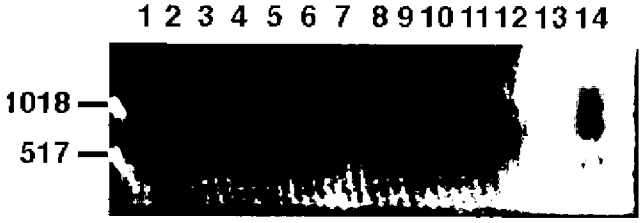


FIG. 2. Circulating FIV *gag* provirus DNA in queens 12 weeks after AI with semen from seropositive donors. An 868-bp segment of the FIV *gag* provirus gene was amplified from peripheral blood leukocytes by PCR and confirmed by Southern blot analysis. Samples 1 through 12 are listed in the order that they appear in Table 1. PCR controls include peripheral blood leukocytes from an uninfected control cat (lane 13) and Crandell feline kidney cells infected with FIV (Petaluma) (lane 14).

Three queens (524, 525, and 526) that received fresh semen from donors 315 and 325 became pregnant. Queen 524 gave birth to four kittens, and cat 525 delivered one kitten. These kittens were monitored for infection beginning at birth and every 2 to 4 weeks until 4 months of age. At 8 to 10 weeks of age, the kittens were weaned and housed separately from their mothers. All five kittens were healthy and remained negative for provirus. Anti-FIV antibodies were detected only in the kitten of queen 525 from 1 day of age through 8 weeks of age, presumably because of ingestion of maternal antibodies in colostrum and milk, as it was seronegative thereafter (data not shown). As part of an ongoing vertical transmission study, queen 526 underwent cesarean section at 8 weeks of gestation for analysis of FIV provirus in fetal tissues by PCR, as described by Sellon et al. (28). Tissues (blood, spleen, liver, and thymus) from the two fetuses from this cat were normal and negative for provirus. No pregnancies occurred after insemination with frozen semen specimens.

Previously, our laboratory identified replication-competent FIV in the cellular and cell-free fractions of semen from domestic cats by cocultivation of seminal cells and seminal plasma with a feline $CD4^+$ T-lymphocyte cell line (19). Productive infection of cocultured lymphocytes was indicated by syncytium formation, *gag* p26 antigen secretion, and expression of a segment of the FIV *gag* provirus genome. This AI study confirms that infectious FIV is present in semen from asymptomatic, seropositive domestic cats and provides the first in vivo demonstration of seminal transmission of FIV.

The 25% rate of transmission (three infections from 12 inseminations) in this study was unexpected in light of the apparent infrequency of transmission during natural breeding. Intrauterine deposition of semen by percutaneous catheterization may circumvent protective factors within the vaginal environment. For instance, virus could gain entry via the uterine or cervical mucosa and conceivably via the peritoneal cavity if seminal components leak through catheter puncture sites in the uterine wall. Additionally, hematogenous dissemination of virus at puncture sites in the abdominal wall or uterus cannot be excluded, although semen leakage and hemorrhage were not observed during the procedures. Thus, this experiment confirms that queens exposed to semen from infected donors during intrauterine AI are at risk of infection, although the location of target tissues within susceptible female hosts remains to be defined.

Although our study population is small, these preliminary data suggest that productive FIV infection is more efficiently transmitted with fresh versus thawed cryopreserved inseminates. Indeed, while an aliquot of fresh semen from male 315 infected queen BXX, an equivalent volume of the same specimen subjected to cryopreservation and thawing failed to infect the exposed queen. Whether this is a consequence of reduced concentrations of infectious virus particles in the frozen samples or a result of qualitative alterations in infectivity of virions during the freeze-thaw process will require further investigation.

Inadvertent transmission of HIV-1 by AI with fresh (6, 7) and frozen (30) ejaculates has been described. An association between the duration of cryopreservation and virus transmission has been observed (30). Of eight women inseminated with cryopreserved semen from the same HIV-positive donor, four seroconverted. The infected women had received specimens stored for 4 months or less, while the women who received semen stored for 16 to 17 months did not seroconvert. In order to reduce the risk of HIV-1 transmission by AI, the Centers for Disease Control and Prevention currently recommend that

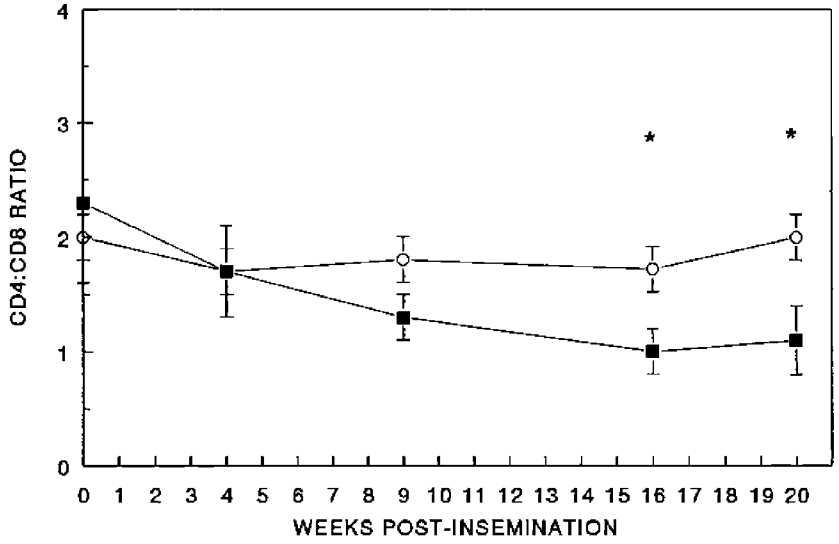


FIG. 3. Mean peripheral $CD4^+/CD8^+$ T-lymphocyte ratios (± standard errors) in queens artificially inseminated with semen from seropositive males. Mean values for uninfected queens are indicated with open circles, and mean values for infected queens are indicated with black squares. Asterisks indicate differences in means that are statistically significant ($P < 0.05$) by the Mann-Whitney U test.

clinics cryopreserve semen for at least 6 months and retest donors for HIV-1 antibody prior to use (5).

As we and others (25) have observed, pregnancy rates in cats may be suboptimal with cryopreserved inseminates; thus, there is a need to explore methods for eliminating virus in fresh ejaculates without severely limiting fertility. In a controversial report, Semprini and colleagues (29) did not observe HIV-1 transmission during 50 artificial inseminations with fresh ejaculates from infected donors after subjecting semen specimens to gradient centrifugation and washing, followed by a swim-up sperm separation procedure. Previous attempts in our laboratory to eliminate FIV from ejaculates after swim-up and washing have been unsuccessful (19). This may be the result of differences in seminal virus expression. Leukocytes are relatively common in human ejaculates (37), and HIV-1 has been cultivated from seminal mononuclear cells obtained by density centrifugation (10, 11, 26). However, attempts to isolate virus from the purified spermatozoa of seropositive men by culture methods have so far been unsuccessful (1). In contrast, seminal leukocytes are uncommon in cats (19), and FIV has been isolated from feline swim-up sperm specimens by coculture (18). Thus, elimination of FIV from cat semen will most likely require techniques other than spermatozoal purification.

Vertical transmission was not documented in this study, although this could be related to the small sample size (only three offspring were obtained from seropositive queens), stage of maternal infection, or virus strain. During acute maternal infection, transmission of FIV from mothers to kittens can exceed rates of 60% (24, 28), while chronically naturally infected queens may infect their kittens infrequently (33, 39). Experimental FIV strains $NCSU_1$ (28) and CSU-2771 (24) can be transmitted through milk. However, in utero transmission has been demonstrated only with CSU-2771 (24). As observed in our study and in previous experiments (28), virus could not be detected in fetuses from queens infected with the $NCSU_1$ isolate. Interestingly, reproductive complications similar to those observed in this report have been described with the CSU-2771 isolate, which has been also associated with abortion, stillbirth, and fetal death (24).

In summary, this is the first report demonstrating that FIV may be transmitted in domestic cats upon exposure to semen by an intrauterine AI technique. Infected queens seroconverted and expressed FIV *gag* provirus sequences in peripheral blood leukocytes within 12 weeks after AI and demonstrated reduced peripheral $CD4^+/CD8^+$ T-lymphocyte ratios. Future investigations will examine further the potential for transmission with cryopreserved ejaculates and determine factors that influence susceptibility of female hosts. These results indicate that insemination of female domestic or nondomestic felids with fresh or cryopreserved semen from FIV-infected donors should be avoided.

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## REFERENCES

1. **Anderson, D. J., H. Wolff, J. Pudney, Z. Wenhao, A. Martinez, and K. Mayer.** 1990. Presence of HIV in semen, p. 167–180. *In* N. Alexander, J. Galemick, and J. Spieler (ed.), Heterosexual transmission of AIDS. Alan R. Liss, New York.
2. **Barr, M., P. P. Calle, M. E. Roelke, and F. W. Scott.** 1989. Feline immunodeficiency virus infection in nondomestic felids. J. Zoo Wildl. Med. **20**:265–272.
3. **Bendenelli, M., M. Pistello, S. Lombardi, A. Poli, C. Garzelli, D. Matteucci, L. Ceccherini-Nelli, G. Malvaldi, and F. Tozzini.** 1995. Feline immunodeficiency virus: an interesting model for AIDS studies and an important cat pathogen. Clin. Microbiol. Rev. **8**:87–112.
4. **Brown, E. W., M. Sriyanie, and S. J. O'Brien.** 1993. Prevalence of exposure to feline immunodeficiency virus in exotic felid species. J. Zoo Wildl. Med. **24**:357–364.
5. **Centers for Disease Control.** 1988. Semen banking, organ and tissue transplantation and HIV antibody testing. Morbid. Mortal. Weekly Rep. **37**:57–63.
6. **Centers for Disease Control.** 1990. HIV-1 infection and artificial insemination with processed semen. Morbid. Mortal. Weekly Rep. **39**:249–256.
7. **Chiasson, M. A., R. L. Stoneburner, and S. C. Joseph.** 1990. Human immunodeficiency virus transmission through artificial insemination. J. Acquired Immune Defic. Syndr. **3**:69–72.
8. **English, R. V., C. M. Johnson, D. H. Gebhard, and M. B. Tompkins.** 1993. In vivo lymphocyte tropism of feline immunodeficiency virus. J. Virol. **67**: 5175–5186.

9. **English, R. V., P. Nelson, C. M. Johnson, M. Nasisse, W. A. Tompkins, and M. B. Tompkins.** 1994. Development of clinical disease in cats experimentally infected with feline immunodeficiency virus. J. Infect. Dis. **170:**543–552.
10. **Hamed, K. A., M. A. Winters, M. Holodniy, D. A. Katzenstein, and T. D. Merigan.** 1993. Detection of human immunodeficiency virus type 1 in semen: effects of disease stage and nucleoside therapy. J. Infect. Dis. **167:**798–802.
11. **Ho, D. D., R. T. Schooley, T. R. Rota, J. C. Kaplan, and T. Flynn.** 1984. HTLV-III in the semen and blood of a healthy homosexual man. Science **226:**451–453.
12. **Hosie, M. J., C. J. Robertson, and O. Jarrett.** 1989. Prevalence of feline leukaemia virus and antibodies to feline immunodeficiency virus in cats in the United Kingdom. Vet. Rec. **128:**293–297.
13. **Howard, J.** 1992. Feline semen analysis and artificial insemination, p. 929–938. *In* R. W. Kirk and J. W. Bonagura (ed.), Current veterinary therapy XI. The W. B. Saunders Co., Philadelphia.
14. **Howard, J. G., A. M. Barone, A. M. Donoghue, and D. E. Wildt.** 1992. The effect of pre-ovulatory anaesthesia on ovulation in laparoscopically inseminated domestic cats. J. Reprod. Fertil. **96:**175–186.
15. **Howard, J. G., A. P. Byers, J. L. Brown, S. J. Barrett, M. Z. Evans, R. J. Schwartz, and D. E. Wildt.** 1996. Successful ovulation induction and laparoscopic intrauterine artificial insemination in the clouded leopard (*Neofelis nebulosa*). Zoo Biol. **15:**55–69.
16. **Howard, J. G., A. M. Donoghue, M. A. Barone, K. L. Goodrowe, E. S. Blumer, K. Snodgrass, D. Starnes, M. Tucker, M. Bush, and D. E. Wildt.** 1992. Successful induction of ovarian activity and laparoscopic intrauterine artificial insemination in the cheetah (*Acinonyx jubatus*). J. Zoo Wildl. Med. **23:**288–300.
17. **Ishida, T., T. Washizu, K. Toriyabe, S. Motoyoshi, I. Tomoda, and N. C. Pedersen.** 1989. Feline immunodeficiency virus infection in cats of Japan. J. Am. Vet. Med. Assoc. **194:**221–225.
18. **Jordan, H., J. Howard, M. Barr, S. Kennedy-Stoskopf, and W. A. F. Tompkins.** 1996. Detection of feline immunodeficiency virus in semen from experimentally and naturally infected domestic cats, p. 44. *In* Proceedings of the Third International Feline Retrovirus Research Symposium, Ft. Collins, Colo.
19. **Jordan, H. L., J. Howard, W. A. Tompkins, and S. Kennedy-Stoskopf.** 1995. Detection of feline immunodeficiency virus in semen from seropositive domestic cats (*Felis catus*). J. Virol. **69:**7328–7333.
20. **Kennedy-Stoskopf, S.** 1994. Feline immunodeficiency virus infection in the African lion (*Panthera leo*), p. 227–230. *In* C. L. Davis Symposium on Veterinary and Comparative Pathology, Blacksburg, Va.
21. **Moench, T. R., K. J. Whaley, T. D. Mandrell, B. D. Bisbop, C. J. Witt, and R. A. Cone.** 1993. The cat/feline immunodeficiency virus model for transmucosal transmission of AIDS: nonoxynol-9 contraceptive jelly blocks transmission by an infected cell inoculum. AIDS **7:**797–802.
22. **Novotney, C., R. English, J. Housman, M. G. Davidson, M. P. Nasisse, C.-R. Jeng, W. C. Davis, and M. B. Tompkins.** 1990. Lymphocyte population changes in cats naturally infected with feline immunodeficiency virus. AIDS **4:**1213–1218.
23. **Olmsted, R. A., R. Langley, M. E. Roelke, R. M. Goeken, D. Adger-Johnson, J. P. Goff, J. P. Albert, C. Packer, M. K. Laurenson, T. M. Caro, L. Scheepers, D. E. Wildt, M. Bush, J. S. Martenson, and S. J. O'Brien.** 1992. Worldwide prevalence of lentivirus infection in wild feline species: epidemiologic and phylogenetic aspects. J. Virol. **66:**6008–6018.
24. **O'Neil, L. L., M. J. Burkhard, L. J. Diehl, and E. A. Hoover.** 1995. Vertical transmission of feline immunodeficiency virus. AIDS Res. Hum. Retroviruses **1:**171–182.
25. **Platz, C. C., D. E. Wildt, and S. W. J. Seager.** 1978. Pregnancy in the domestic cat after artificial insemination with previously frozen spermatozoa. J. Reprod. Fertil. **52:**270–282.
26. **Rasheed, S., L. Zbiliang, and D. Xu.** 1995. Human immunodeficiency virus load: quantitative assessment in semen from seropositive individuals and in spiked seminal plasma. J. Reprod. Med. **40:**747–757.
27. **Roth, T. L., D. L. Armstrong, M. T. Barrie, D. M. Garell, and D. E. Wildt.** 1996. Seasonal effects on ovarian responsiveness to exogenous gonadotropins and successful artificial insemination in the snow leopard (*Panthera uncia*). Theriogenology **45:**246.
28. **Sellon, R. K., H. L. Jordan, S. Kennedy-Stoskopf, M. B. Tompkins, and W. A. F. Tompkins.** 1994. Feline immunodeficiency virus can be transmitted via milk during acute maternal infection. J. Virol. **68:**3380–3385.
29. **Semprini, A. E., P. Levi-Setti, M. Bozzo, M. Ravizza, A. Taglioretti, P. Sulpizio, E. Albani, M. Oneta, and G. Pardi.** 1992. Insemination of HIV-negative women with processed semen of HIV-positive partners. Lancet **340:**1317–1319.
30. **Stewart, G. J., A. L. Cunningham, J. P. P. Tyler, A. L. Cunningham, J. A. Barr, G. L. Driscoll, J. Gold, and B. J. Lamont.** 1985. Transmission of human T-cell lymphotropic virus type III (HTLV-III) by artificial insemination by donor. Lancet **ii:**581–584.
31. **Swanson, W. F., J. G. Howard, T. L. Roth, K. Kaemmerer, T. Alvarago, J. Burton, D. Starnes, L. Sims, D. Oc, and D. E. Wildt.** 1996. Ovarian responsiveness to exogenous gonadotropins and laparoscopic artificial insemination with frozen-thawed spermatozoa in the ocelot (*Felis pardalis*). J. Reprod. Fertil. **106:**87–94.
32. **Tompkins, M. B., D. H. Gebhard, H. R. Bingham, M. J. Hamilton, W. C. Davis, and W. A. F. Tompkins.** 1990. Characterization of monoclonal antibodies to feline T lymphocytes and their use in the analysis of lymphocyte tissue distribution in the cats. Vet. Immunol. Immunopathol. **26:**305–317.
33. **Ueland, K., and L. L. Nesse.** 1992. No evidence of vertical transmission of naturally acquired feline immunodeficiency virus infection. Vet. Immunol. Immunopathol. **33:**301–308.
34. **Wildt, D. E., S. L. Monfort, A. M. Donoghue, L. A. Johnston, and J. G. Howard.** 1992. Embryogenesis in conservation biology—or, how to make an endangered species embryo. Theriogenology **37:**161–184.
35. **Wildt, D. E., M. C. Schiewe, P. M. Schmidt, K. L. Goodrowe, J. G. Howard, L. G. Phillips, S. J. O'Brien, and M. Bush.** 1986. Developing animal model systems for embryo technologies in rare and endangered wildlife. Theriogenology **25:**33–51.
36. **Wildt, D. E., U. S. Seal, and W. F. Rall.** 1993. Genetic resource banks and reproductive technology for wildlife conservation, p. 159–173. *In* J. G. Cloud and G. H. Thorgaard (ed.), Genetic conservation of salmonid fishes. Plenum Press, New York.
37. **Wolff, H., and D. Anderson.** 1988. Immunohistologic characterization and quantitation of leucocyte subpopulations in human semen. Fertil. Steril. **49:**497–504.
38. **Yamamoto, J. K., H. Hansen, E. W. Ho, T. Y. Morishita, T. Okuda, T. R. Sawa, R. M. Nakamura, and N. C. Pedersen.** 1989. Epidemiologic and clinical aspects of feline immunodeficiency virus infection in cats from the continental United States and Canada and possible mode of transmission. J. Am. Vet. Med. Assoc. **194:**213–220.
39. **Yamamoto, J. K., E. Sparger, E. W. Ho, P. R. Anderson, P. O'Connor, C. P. Mandell, L. Lowenstine, R. Munn, and N. C. Pedersen.** 1988. The pathogenesis of experimentally induced feline immunodeficiency virus (FIV) infection in cats. Am. J. Vet. Res. **49:**1246–1258.