Retrograde ejaculation associated spontaneous sperm cystolithiasis in four Rhesus Macaques (*Macaca mulatta*)

Sanjeev Gumber[a,*], **Cynthia L Courtney**[a], **Karen R Strait**[b], **Prachi Sharma**[a], **Julie E Freebersyser**[c], and **Maria M Crane**[d]

[a] Division of Pathology, Yerkes National Primate Research Center, Emory University, 954 Gatewood Road NE, Atlanta, Georgia 30329, USA

[b] Division of Animal Resources, School of Medicine, Emory University, Whitehead Biomedical Research Building, Atlanta, Georgia 30322, USA

[c] Amgen, One Amgen Center Drive, Thousand Oaks, California 91320, USA

[d] Yerkes National Primate Research Center, Emory University, 2409 Taylor Ln, Lawrenceville, Georgia 30043, USA

Abstract

Retrograde ejaculation (RE) has been reported in humans and animals but RE with subsequent sperm calculi has rarely been reported. This report documents clinical and pathological findings of spontaneous sperm cystolithiasis in four rhesus macaques. While this condition has been associated with repeated electroejaculation, spontaneous sperm cystolithiasis is highly unusual. The animals presented with either stranguria, dysuria, hematuria, distended abdomen or lethargy. Ultrasound examination revealed several hyperechoic masses within the lumen of the urinary bladder. The animals were euthanized due to poor prognosis or study end points. Postmortem examination revealed multiple angular, amorphous, soft to firm, pale yellow to greenish-brown and variably sized calculi in the lumen of the urinary bladder or prostatic/penile urethra. Histologically, the calculi were composed of numerous sperm embedded in abundant brightly eosinophilic matrix. Based on gross and histologic findings, RE associated sperm cystolithiasis was diagnosed, with ulcerative urethritis as the major primary apparent etiology. To the authors' knowledge, this is the first report of four spontaneous cases of sperm cystolithiasis in rhesus macaques.

Keywords

Calculi; Ejaculation; Retrograde; Rhesus; Sperm

*Corresponding author: Sanjeev Gumber, Division of Pathology, Yerkes National Primate Research Center, Emory University, 954 Gatewood Road NE, Atlanta, Georgia 30329, USA. Tel.: +1 404 727 7022 Fax: +1 404 727 4531 sgumber@emory.edu.

Introduction

In non-human primates, the routine collection of sperm is often required for scientific investigations. Sperm are produced in the testis and stored in the caudal part of the epididymis. Ejaculation of sperm occurs either during natural mating, masturbation or loss through normal urination (Newman et al. , 1982). Electroejaculation was first developed for primates in the early sixties. It provides an expedient, high volume, high quality sample of sperm compared to other methods (Mastroianni and Manson, 1963). Electroejaculation can be performed by direct penile or rectal probe stimulation. Rectal probe stimulation is routinely used in non-human primates; however, this procedure has been reported to induce retrograde flow of sperm into urinary bladder (Schaffer et al. , 1989b). In rats sperm urolithiasis can be induced by inseminating sperm into the neck of the urinary bladder or ventral prostate. The retention of sperm in the urinary bladder can cause coagulation and subsequently urinary obstruction (Stein-Werblowsky and Ablin, 1994). Mineral urolithiasis is uncommon in non-human primates and has rarely been reported in cynomolgus macaques (Faltas, 2000, Lees et al. , 1995, O'Rourke et al. , 1995, Renlund et al. , 1986, Stephens et al. , 1979). While rare reports exist in the literature of retrograde ejaculation (RE) with subsequent sperm mass or calculi in monkeys that have been electroejaculated (Chandolia et al. , 2007, Frisk et al. , 1974), this is the first report to the authors' knowledge of four spontaneous cases in rhesus macaques. This report describes the clinical and pathological features of spontaneous sperm cystolithiasis caused by RE in four rhesus macaques, and the underlying etiopathogenesis is discussed in comparison to humans and other species.

Materials and methods

Case no. 1

A 7-year old, male rhesus macaque presented to the Yerkes National Primate Research Center (YNPRC) Veterinary Service with acute hematuria and stranguria. The monkey was enrolled in a research protocol that studied modulation of HIV immunity with dendritic cells in Simian immunodeficiency virus (SIV) infected animals. The subject was SIV positive, infected in with SIVmac251 intra-rectally. The viral load after 12 weeks of infection was 238421.2 copies/ml of blood.

Case no. 2

An 11.5-year old, male rhesus macaque presented to YNPRC Veterinary Service with the history of chronic weight loss, diarrhea, dysuria and a distended abdomen. The monkey was SIV negative and assigned to a study examining the role of hormones in sex differences in adaptability.

Case no. 3

A 7.5-year old, male rhesus macaque developed severe lethargy. The animal was SIV negative, an alpha male and was not assigned to any research protocol.

Case no. 4

A 10.5-year old, male rhesus macaque presented with an enlarged prepuce, stranguria, distended urinary bladder, swollen and dark purple distal penis. The animal was SIV negative and was not assigned to any research protocol.

Animals 1 and 2 were born at YNPRC and enrolled in research protocols that were approved by the Institutional Animal Care and Use Committee of Emory University. Animals 3 and 4 were acquired from Oregon National Primate Research Center and Mannheimer Foundation/ Haman Ranch (Florida, USA), respectively for breeding and genetic diversification in the Yerkes colony. All animals were fed a standard diet of commercial primate chow (5037 Old World Primate Diet, PMI International, Brentwood, MO) supplemented daily with various fresh fruits and vegetables. Water was provided ad libitum by means of lixits. Room conditions included a 12:12 hour light: dark cycle, temperature of 24.6°C to 29°C and 10-15 air changes per hour. A complete physical examination, complete blood cell count (CBC), serum biochemical panel, urethral catheterization, survey radiographs or double contrast cystography and abdominal ultrasonography were performed on these animals. Due to the poor prognosis or because of study end points, the animals were euthanized. A complete necropsy was performed on these animals. For histopathologic examination, various tissue samples were fixed in 10% neutral buffered formalin, routinely processed, paraffin-embedded, sectioned at 5 μm, and stained with hematoxylin and eosin. Immunohistochemical staining of formalin-fixed, paraffin-embedded tissue sections of urinary bladder and prostatic urethra of Case 1 was performed for SIV (SIVgag mAb (clone FA2; AIDS Reagent Repository Program) using the streptavidin-biotin complex peroxidase method as described by the manufacturer (Dako North America Inc., Carpinteria, CA).

Results

Case no. 1

Physical examination revealed an enlarged, firm urinary bladder in the caudal abdomen. A sterile 5 French urinary catheter was successfully passed through the urethra to rule out an obstruction. Urinalysis revealed severe hematuria and proteinuria, and 3-5 white blood cells (WBC) /high power field (hpf). No bacteria were isolated on bacteriological culture. No significant findings were noted on the CBC and serum biochemical panel. Survey radiographs of abdomen were within normal limits and no urinary calculi were seen. A double contrast cystogram (Omnipaque, GE Healthcare) revealed numerous, irregularly shaped, radiolucent filling defects in the lumen of the urinary bladder. An abdominal ultrasound examination (8C transducer; GE Medical Systems Co, LTD., Jiangsu, China) showed a distended urinary bladder containing hyperechoic masses in the bladder lumen not associated with the urinary bladder mucosa.

Postmortem examination revealed a thickened urinary bladder wall and the lumen contained multiple angular, amorphous, soft, and greenish masses measuring approximately 2-5 cm in diameter (Fig 1A). Similar calculi were present near the ejaculatory duct opening in the proximal urethra (Fig 1B). Pinpoint ulcers were present on the trigone area of the urinary bladder mucosa (Fig 1B). Seminal vesicles, epididymis, and testes were unremarkable. No

other significant macroscopic findings were observed. Histologically, the urinary bladder calculi were composed of numerous sperm embedded in abundant brightly eosinophilic matrix (Fig 1 D). The submucosa of the urinary bladder was moderately edematous and multifocally infiltrated by moderate numbers of neutrophils. The prostatic urethra proximal to the seminal vesicles was multifocally ulcerated and the underlying submucosa was expanded with moderate edema, numerous neutrophils, macrophages, lymphocytes, plasma cells, and semen matrix in the lumen (Fig 1E). The prostatic urethral section directly over the ejaculatory duct and distal to the site of mucosal ulceration had marked mucosal hyperplasia with submucosal edema and acute inflammation. Other histological findings included moderate multifocal neutrophilic prostatitis and renal interstitial lymphoplasmacytic inflammation. Immunohistochemical staining for SIV was negative on the urinary bladder and prostatic urethra.

Case no. 2

On physical examination, the animal had enlarged/swollen tip of the penis extruding from the foreskin. The urinary bladder was enlarged and firm. Attempts at urethral catheterization were unsuccessful but produced two large (2-4cm in length) pale yellow to white casts upon flushing with sterile saline. Gritty material was blocking approximately 6 cm of the urethra. Urinalysis revealed severe hematuria, moderate proteinuria, and 2-4 WBC/hpf but bacterial cultures remained negative. The CBC results revealed moderate leukocytosis (WBC count, $17.4 \times 10^3/\mu l$, reference (ref.) interval: $5.8\text{-}10.4 \times 10^3/\mu l$) characterized by moderate neutrophilia ($15.7 \times 10^3/\mu l$, ref. interval: $2.2\text{-}6.3 \times 10^3/\mu l$). The other hematological and serum biochemical panel findings were within normal limits. The hematological and serum biochemical panel reference intervals were used as described by Association of Primate Veterinarians (Lee and Doane, 2012). Abdominal ultrasound examination revealed a large amount of hyperechoic material in the dorsal (dependent) aspect of the bladder.

On necropsy, the abdominal wall adhered to the dorsal wall of the markedly distended urinary bladder. The urinary bladder contained 40-50 ml of slightly brown-tinged urine and two 1-2 cm, angular or dumbbell shape, greenish-brown calculi. The urinary bladder wall was slightly thickened and the tip of penis was markedly swollen. No other remarkable gross findings were noticed. Histologically, the calculi were consistent with sperm cystoliths as described above with multifocal areas of calcification (data not shown). Additional histological findings were moderate prostatic urethral ulceration with overlying semen matrix, marked fibrinosuppurative pyelonephritis, and moderate necrosuppurative inflammation of the penis.

Case no. 3

On physical examination, the rhesus macaque was weak, lethargic and dehydrated. Urethral catheterization revealed hematuria. The CBC results revealed moderate leukocytosis (WBC count, $15.4 \times 10^3/\mu l$, reference (ref.) interval: $5.8\text{-}10.4 \times 10^3/\mu l$) characterized by moderate neutrophilia ($13.4 \times 10^3/\mu l$, ref. interval: $2.2\text{-}6.3 \times 10^3/\mu l$). Results from the serum biochemical panel revealed markedly elevated creatinine (11 mg/dL, ref. interval: 1.0-1.4 mg/dL) and moderately increased BUN (130 mg/dL, ref. interval: 22-30 mg/dL). Bacteriological culture from urine samples revealed heavy growth of *Staphylococcus*

aureus. An abdominal ultrasound examination showed a markedly distended urinary bladder and many hyperechoic masses in the bladder lumen, multiple calculi were retrieved by cystotomy.

Postmortem examination revealed severe fibrous adhesions between the abdominal wall and the serosa of the urinary bladder. The urinary bladder was markedly distended with approximately 50-60 ml of serosanguineous fluid containing multiple clumps of fibrin plaques and pale yellow to dark brown, soft to firm calculi (1-3 × 0.5-2 cm). There were multifocal ulcerations and hemorrhages in the mucosa of the urinary bladder (Fig 1C). The mucosal surface at neck of the urinary bladder and prostatic urethra had multifocal ulcerations and contained small amounts of greenish-brown, amorphous material. Histologically, the calculi were composed of numerous sperm embedded in brightly eosinophilic matrix as described earlier intermixed with moderate amounts of necrotic cellular debris, fibrin, numerous degenerate neutrophils, clusters of bacterial cocci and sloughed transitional epithelial cells. There was moderate suppurative to lymphoplasmacytic pyelonephritis, and the renal lymphatics were plugged with fibrin intermixed with moderate numbers of degenerate and viable neutrophils, small numbers of lymphocytes, plasma cells and fewer macrophages (Fig 1F). No sperm were detected in fibrin emboli using Periodic acid Schiff-hematoxylin staining. The other histological findings included severe multifocal to coalescing ulcerative fibrinosuppurative cystitis, serositis and prostatic urethral ulceration with overlying semen matrix. No other significant microscopic findings were seen in this rhesus macaque.

Case no. 4

On physical examination, the urinary bladder was markedly enlarged and approximately 400 ml urine was collected via cystocentesis. Digital rectal examination revealed the presence of a palpable mass at pelvic inlet. Several attempts to pass the catheter through urethra were unsuccessful. Urinalysis revealed severe hematuria and proteinuria, and 5-7 WBC/hpf but no bacteria were cultured. The CBC results revealed moderate leukocytosis (WBC count, 16.7 × 10^3/μl, ref. interval: 5.8-10.4 × 10^3/μl) characterized by moderate neutrophilia (14.7 × 10^3/μl, ref. interval: 2.2-6.3 × 10^3/μl). Results from the serum biochemical panel were within normal limits. Abdominal ultrasound examination revealed a discrete hyperechoic mass in the ventral urinary bladder.

On necropsy, the urethral mucosa was diffusely hemorrhagic and the foreskin contained approximately 2 ml of unclotted blood. The lumen of the urinary bladder had a pale yellow viscous gelatinous calculus (3 ×1.5 cm) with a broad base and a tapering tail towards the urethra. On microscopic evaluation, the semen calculus was composed of eosinophilic coagulum containing numerous sperm as described above. The penile urethral epithelium was diffusely ulcerated and infiltrated by moderate numbers of neutrophils and the lumen contained semen matrix. The other histological findings included moderate multifocal neutrophilic aggregates intermixed with hemorrhage in the preputial dermis. No other significant microscopic findings were seen in this rhesus macaque.

The clinical, gross and histopathological findings were consistent with RE associated sperm cystolithiasis in all the four rhesus macaques.

Discussion

In human males, the prostatic urethra begins at the neck of the urinary bladder and includes all of the secretion that passes through the prostate gland. It is the widest and most dilatable part of the male urethral canal. Once sperm are produced, they travel through the epididymis, and then through the vas deferens, which joins the seminal vesicles to form the ejaculatory duct. The seminal vesicles produce a fluid that provides nutrients for the sperm, lubricates the urethra, and constitutes the bulk of the seminal fluid (Newman et al. , 1982).

There are two distinct physiological events of ejaculation controlled by sympathetic efferent fibers: 1) emission and 2) expulsion- through contraction of penile musculature. In the normal course of ejaculation, urethral and penile musculature contract synchronously to succeed in the propulsion of semen in the anterior direction (McDonnell, 1992). During the expulsive phase, it is necessary that the bladder neck (internal urethral sphincter) be closed to prevent reflux of semen into the urinary bladder as the urethral pressure increases. Failure of the urinary bladder neck to close and resulting reflux of semen into the urinary bladder is known as RE (Yavetz et al. , 1994). RE is relatively common in humans and in select other species, including cats (Dooley et al. , 1991), dogs (Dooley et al. , 1990), pigs (Martin et al. , 1994), stallion (Brinsko, 2001), sheep (Pineda et al. , 1987), cattle (Dooley et al. , 1986), and non-human primates (Schaffer et al. , 1989a). In non-human primates though, RE has primarily been associated with routine electroejaculation which induces a lack of synchronous flow potentially due to asynchronous stimulation of nerve tracts, leading to trapping of semen in the posterior urethra (Chandolia et al. , 2007, Schaffer et al. , 1989a). This causes retrograde flow of semen into the urinary bladder. The urinary losses of sperm due to their retrograde flow into the urinary bladder varied considerably among species (Dooley et al. , 1990, 1991, Pineda et al. , 1987, Schaffer et al. , 1989a), and for certain males in these studies, 50-90% of sperm flowed into the urinary bladder during ejaculation or electroejaculation. Only boars do not retrograde significant numbers of sperm (less than 0.2%) into the urinary bladder during electroejaculation (Martin et al. , 1994). In men, the backflow of sperm into the urinary bladder has been reported in individuals with partial urethral dysfunction and urolithiasis has been shown to be a side effect in males after vasectomy surgery (Kronmal et al. , 1997). In humans, RE accounts for less than 2% of cases of subfertility presenting to a fertility clinic (Yavetz et al. , 1994). RE occurs in 75% of the male patients who have undergone transurethral resection of the prostate (Bettocchi et al. , 2008).

Processes that cause interference with competency of the urethral sphincter may result in RE. These processes can be neurogenic, myogenic or neuromuscular. RE has been reported due to congenital malformation of the posterior urethra, spinal trauma, bladder neck surgery, chronic inflammation or can be idiopathic (Yavetz et al. , 1994). In dogs, RE has been associated with hypothyroidism (Root et al. , 1994) and sedation with xylazine (Dooley et al. , 1990). It is theorized that spontaneous sperm cystolithiasis in the current report could be due to formation of a plug in the outflow track causing RE into the urinary bladder and producing coagulation of semen with subsequent urethral obstruction. This finding has been reported previously in cynomolgus macaques in which electroejaculation was used for sperm collection (Chandolia et al. , 2007). The other possible reason is because of the

unique semen characteristics in rhesus macaques. In primates, sperm are entrapped in the coagulum at the time of ejaculation. The coagulum is a fibrinous material produced by mixing of fluids from accessory sex glands and the degree of coagulation varies among species (Schaffer et al. , 1989b). Interestingly, the coagulum of rhesus semen does not undergo complete lysis as seen for human and great apes semen, and coagulation may occur in the urethra, thereby producing strands of plug during collection (Valerio et al. , 1970). Human semen liquefies because the prostate gland secretes the proteolytic enzyme fibrinolysin which is absent from rhesus macaque semen (Clarke, 1982, Price and Ashman, 1961). Therefore, the unique rhesus semen features along with ulcerative urethritis in the current report might have caused blockage of the urethra with a semen plug with subsequent RE and sperm cystolithiasis.

Pyelonephritis was observed in two animals in this case report and one animal had concurrent severe ulcerative bacterial cystitis and fibrin plugs in the renal lymphatics. However, in the majority of cases, infection has not been documented as a cause for semen retrograde flow into the urinary bladder in non-human primates (Schaffer et al. , 1989a). Since one rhesus macaque was SIV positive in this report, this infection might have contributed to this clinical presentation. It has been shown that the testis and epididymis of juvenile macaques infected with SIVmac251 are active in viral uptake, and this affects the spermatogenesis and the maturity of the male genital tract post-infection (Shehu-Xhilaga et al. , 2007). However, the SIV infected animal in the current report was 7-year old. Acute renal failure is common in HIV-infected patients and is associated with advanced immunodeficiency (Roe et al. , 2008). Further, HIV patients receiving highly active antiretroviral therapy (HAART) can develop urolithiasis. Indinavir in particular, causes mineral urolithiasis in 5-25% of HIV-positive patients treated with the drug (Kohan et al. , 1999). However, the SIV positive rhesus macaque in this report did not receive HAART, and among the hundreds of SIV infected rhesus macaques necropsied at Yerkes, this has been the first case. Additionally, the calculi in this case were composed of myriad sperm embedded in semen matrix. Histopathology and composition analysis of sperm calculi did not reveal any evidence of mineral in this case. However, calcification of calculi was noticed in one SIV negative animal (case 2), suggesting chronicity of the lesion. The effect of SIV infection on semen characteristics and sperm coagulation is currently unknown.

Medical management of RE aims at increasing the tone of the bladder neck and therefore preventing retrograde flow of semen into urinary bladder by either stimulating sympathetic tone or blocking parasympathetic stimulation (Jefferys et al. , 2012). It has been suggested that after electroejaculation in monkeys, the urinary bladder may be scanned ultrasonographically to assess the retrograde flow of sperm and a Foley catheter can be used to evacuate the bladder for sperm recovery (Chandolia et al. , 2007). The unique characteristics of sperm calculi in this report such as the pale yellow to greenish-brown color and soft to firm texture can assist in gross diagnosis of sperm cystolithiasis in rhesus macaques.

Acknowledgements

References

Bettocchi C, Verze P, Palumbo F, Arcaniolo D, Mirone V. Ejaculatory disorders: pathophysiology and management. Nat Clin Pract Urol. 2008; 5:93–103. [PubMed: 18259187]

Brinsko SP. Retrograde ejaculation in a stallion. J Am Vet Med Assoc. 2001; 218:551–3. 27. [PubMed: 11229508]

Chandolia RK, Luetjens CM, Wistuba J, Semjonow A, Puhse G, Nieschlag E. Blockage of urine by intravesical ejaculate in cynomolgus monkeys. J Med Primatol. 2007; 36:21–4. [PubMed: 17359462]

Clarke GN. Liquefaction of viscous semen. Fertil Steril. 1982; 37:855–6. [PubMed: 6211375]

Dooley MP, Pineda MH, Hopper JG, Hsu WH. Retrograde flow of spermatozoa into the urinary bladder of dogs during ejaculation or after sedation with xylazine. Am J Vet Res. 1990; 51:1574–9. [PubMed: 2240779]

Dooley MP, Pineda MH, Hopper JG, Hsu WH. Retrograde flow of spermatozoa into the urinary bladder of cats during electroejaculation, collection of semen with an artificial vagina, and mating. Am J Vet Res. 1991; 52:687–91. [PubMed: 1854092]

Dooley MP, Pineda MH, Naurer RR, Lunstra DD. Evidence for retrograde flow of spermatozoa into the urinary bladder of bulls during electroejaculation. Theriogenology. 1986; 26:101–9. [PubMed: 16726174]

Faltas NH. Urolithiasis in cynomolgus monkey (Macaca fascicularis): a case report. Contemp Top Lab Anim Sci. 2000; 39:18–9. [PubMed: 11040869]

Frisk CS, Manning PJ, Wagner JE. Semen matrix calculi in a rhesus monkey (Macaca mulatta). Lab Anim Sci. 1974; 24:679–81. [PubMed: 4369162]

Jefferys A, Siassakos D, Wardle P. The management of retrograde ejaculation: a systematic review and update. Fertil Steril. 2012; 97:306–12. [PubMed: 22177462]

Kohan AD, Armenakas NA, Fracchia JA. Indinavir urolithiasis: an emerging cause of renal colic in patients with human immunodeficiency virus. J Urol. 1999; 161:1765–8. [PubMed: 10332431]

Kronmal RA, Krieger JN, Coxon V, Wortley P, Thompson L, Sherrard DJ. Vasectomy is associated with an increased risk for urolithiasis. Am J Kidney Dis. 1997; 29:207–13. [PubMed: 9016891]

Lee DR, Doane CJ. Non Human Primate Formulary. Association of Primate Veterinarians. 2012

Lees CJ, Carlson CS, O'Sullivan MG. Urinary calculus caused by plant material in a cynomolgus monkey. Lab Anim Sci. 1995; 45:441–2. [PubMed: 7474888]

Martin PA, Dooley MP, Hembrough FB, Pineda MH. Retrograde flow of spermatozoa into the urinary bladder of boars during collection of semen by electroejaculation. Theriogenology. 1994; 41:869–77. [PubMed: 16727440]

Mastroianni L Jr. Manson WA Jr. Collection of monkey semen by electroejaculation. Proc Soc Exp Biol Med. 1963; 112:1025–7. [PubMed: 13933495]

McDonnell SM. Ejaculation. Physiology and dysfunction. Vet Clin North Am Equine Pract. 1992; 8:57–70. [PubMed: 1576554]

Newman HF, Reiss H, Northup JD. Physical basis of emission, ejaculation, and orgasm in the male. Urology. 1982; 19:341–50. [PubMed: 6122300]

O'Rourke CM, Brammer DW, St Romain G, Peter GK, Hofing GL. Calcium carbonate urolithiasis in an adult male cynomolgus monkey. Lab Anim Sci. 1995; 45:222–4. [PubMed: 7603030]

Pineda MH, Dooley MP, Hembrough FB, Hsu WH. Retrograde flow of spermatozoa into the urinary bladder of rams. Am J Vet Res. 1987; 48:562–8. [PubMed: 3592354]

Price, D.; Ashman, HG. The accessory reproductive glands of mammals.. In: Young, WC., editor. Sex and internal secretions. 3rd ed.. Williams & Wilkins; Baltimore: 1961.

Renlund RC, McGill GE, Cheng PT. Calcite urolith in a cynomolgus monkey. Lab Anim Sci. 1986; 36:536–7. [PubMed: 3773469]

Roe J, Campbell LJ, Ibrahim F, Hendry BM, Post FA. HIV care and the incidence of acute renal failure. Clin Infect Dis. 2008; 47:242–9. [PubMed: 18540821]

Root MV, Johnston SD, Olson PN. Concurrent retrograde ejaculation and hypothyroidism in a dog: case report. Theriogenology. 1994; 41:593–600. [PubMed: 16727415]

Schaffer NE, Cranfield M, Fazleabas AT, Jeyendran RS. Viable spermatozoa in the bladder after electroejaculation of lion-tailed macaques (Macaca silenus). J Reprod Fertil. 1989a; 86:767–70. [PubMed: 2760902]

Schaffer NE, Cranfield M, Meehan T, Kempske S. Semen Collection and Analysis in the Conservation of Endangered Nonhuman Primates. Zoo Biogy Supplement. 1989b; 1:47–60.

Shehu-Xhilaga M, Kent S, Batten J, Ellis S, Van der Meulen J, O'Bryan M, et al. The testis and epididymis are productively infected by SIV and SHIV in juvenile macaques during the post-acute stage of infection. Retrovirology. 2007; 4:7. [PubMed: 17266752]

Stein-Werblowsky R, Ablin RJ. Sperm-induced urolithiasis in the rat. Eur J Epidemiol. 1994; 10:605–7. [PubMed: 7859862]

Stephens EC, Middleton CC, Thompson LJ. Urinary cystic calculi in a cynomolgus monkey (Macaca fascicularis): a case report. Lab Anim Sci. 1979; 29:797–9. [PubMed: 119114]

Valerio DA, Leverage WE, Munster JH. Semen evaluation in Macaques. Lab Anim Care. 1970; 20:734–40. [PubMed: 4247970]

Yavetz H, Yogev L, Hauser R, Lessing JB, Paz G, Homonnai ZT. Retrograde ejaculation. Hum Reprod. 1994; 9:381–6. [PubMed: 8006123]

Ethical Statement

All animal studies were approved by the Institutional Animal Care and Use Committee of Emory University, Atlanta, GA, USA. Emory University and Yerkes national Primate Research Center are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.
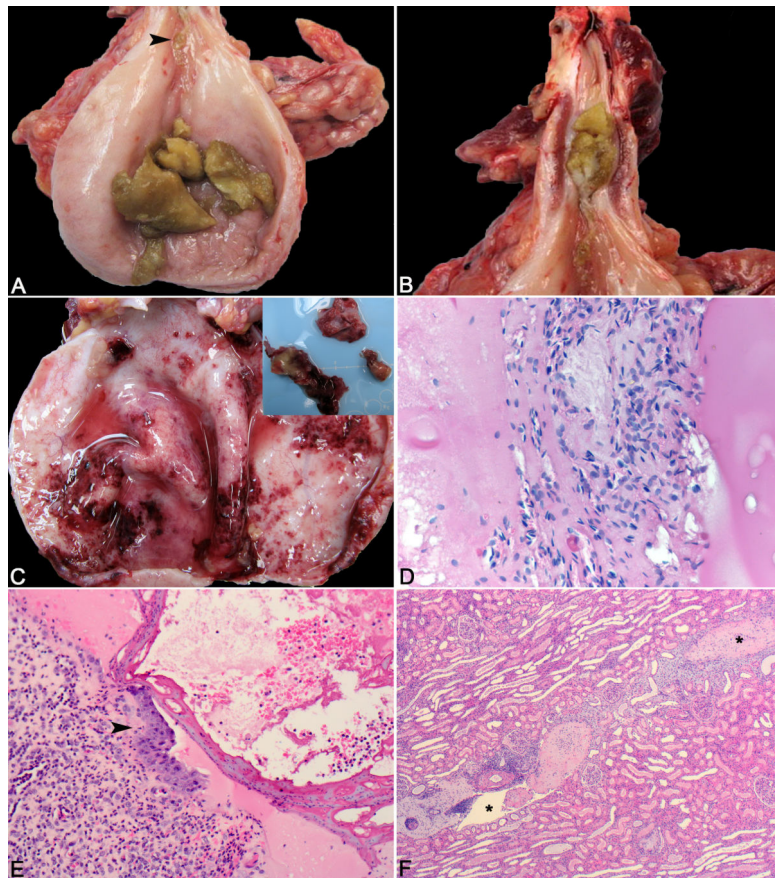
Figure 1.
(A) Urinary bladder lumen from case 1 contains multiple approximately 2-5 cm in diameter soft angular greenish sperm calculi. The neck of the urinary bladder has focal ulceration (arrowhead). **(B)** Prostatic urethra is plugged with soft amorphous semen calculi. **(C)**. Urinary bladder from case 3 has multifocal mucosal erosions and hemorrhages. Inset: Sperm calculi and fibrin plaques collected from the distended urinary bladder. **(D)** Sperm calculi are composed of myriad sperm embedded in brightly eosinophilic laminated matrix and within the lacunae (HE, 400×). **(E)** Prostatic urethral mucosa is ulcerated and contains semen matrix in the lumen. There is a raft of transitional epithelial cells present adjacent to the ulcerated mucosa (arrowhead) and submucosa is expanded by numerous neutrophils, lymphocytes, plasma cells and macrophages (HE, 200×). **(F)** Renal lymphatics (*) in the cortex and medulla are plugged with fibrin intermixed with moderate numbers of degenerate neutrophils. The renal interstitium multifocally contains small to moderate infiltrates of lymphocytes, plasma cells and few neutrophils (HE, 40×).