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Semen Evaluation for Verification of Azoospermia After Vasectomy in Chimpanzees (*Pan troglodytes*)

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Standards for the reproductive management of captive chimpanzees stipulate that chimpanzees admitted into the National Chimpanzee Sanctuary System must undergo vasectomy followed by laboratory confirmation of azoospermia. In light of the observations of ourselves and others, we questioned whether azoospermia is a necessary indicator of successful vasectomy. Therefore, the objectives of the present study were to assess how much time is required between vasectomy and semen evaluation for azoospermia to be reached and to determine the percentage of vasectomized chimpanzees that actually are azoospermic. The study population comprised 39 adult male chimpanzees that underwent vasectomy and subsequent semen examination at 0.5 to 24 mo afterward. Overall, spermatozoa were found in the semen of at least 1 chimpanzee in almost every month in which animals were evaluated. Of the animals evaluated repeatedly after vasectomy, 20% had no sperm at any examination, 60% were azoospermic then positive during at least 1 subsequent examination, 13.3% were positive at least once and then azoospermic, and 6.7% were positive at every examination. After 0.5 mo postvasectomy, all sperm observed were nonmotile. The results suggest that azoospermia is not a necessary indicator of successful vasectomy.

Abbreviations: CHIMP, Chimpanzee Health Improvement, Maintenance, and Protection; NCSS, National Chimpanzee Sanctuary System

In 1997, the surplus of chimpanzees residing at research centers prompted a moratorium on the further production of offspring by chimpanzees supported under the National Chimpanzee Biomedical Research Program. In 2000, a sanctuary system to provide for the lifetime care of retired research chimpanzees was mandated by The Chimpanzee Health Improvement, Maintenance, and Protection (CHIMP) Act.[15] In 2002, the National Chimpanzee Sanctuary System (NCSS) was established. A facility was constructed for the care of retired chimpanzees. Facility personnel provide care for chimpanzees no longer used in biomedical research or entertainment or wanted as pets. Provisions of the CHIMP Act state that reproduction of chimpanzees is prohibited in the NCSS. Therefore, all males must be vasectomized or housed apart from females until they are sterilized. Documentation of azoospermia must accompany each vasectomized male accepted into the NCSS.

Challenges associated with this requirement arose when chimpanzees residing at the University of Louisiana at Lafayette, New Iberia Research Center were evaluated. A group of 8 chimpanzees was vasectomized, and histologic examination verified that a section of vas deferens had been removed. However, laboratory evaluation of the semen from these males 9 mo after surgery revealed that a semen sample from each of 3 of the chimps contained 1 nonmotile sperm per 10 high-power fields. These findings are similar to those for vasectomized men.[5] Griffin and coworkers[9] have suggested that for humans, loss of sperm motility and the presence of only nonmotile sperm are indicative of sterility. Currently, however, there is no such allowance in the NCSS requirements for the presence of nonmotile sperm. Therefore, the presence of nonmotile sperm currently precludes admission into the NCSS.

More recently, an adult female chimpanzee residing in the NCSS was found to be pregnant and later gave birth. This adult female chimp appears to have been impregnated by a vasectomized male chimpanzee. Although rare, additional unplanned pregnancies among chimpanzees have occurred after breeding vasectomized males.[2]

Collectively, chimpanzees evaluated postvasectomy at the New Iberia Research Center, reports in the literature pertaining to humans, and the recent impregnation of the female chimpanzee in the NCSS have prompted questions regarding the usefulness of azoospermia as a requirement for entry into the NCSS. For example, how much time is required between vasectomy in the chimpanzee and semen evaluation for a resultant semen evaluation to contain no motile sperm? To what extent should azoospermia be required to verify successful vasectomy of chimpanzees, given that azoospermia is not a criterion for sterility in men? The objectives of this investigation were to evaluate semen from chimpanzees at various time points postvasectomy to determine the approximate period of time required to reach azoospermia and the percentage of vasectomized chimpanzees actually azoospermic. Our hypothesis was that semen evaluation for the absence of spermatozoa is not a necessary indicator of sterility.

Materials and Methods

Vasectomy procedure. All procedures were performed after approval by the institutional animal care and use committees of the University of Louisiana at Lafayette and Louisiana State University. Both institutions are fully accredited by the Association for the Assessment and Accreditation of Laboratory

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Table 1. Ejaculates collected from 39 chimpanzees at various times after vasectomy

Time after vasectomy (mo)	No. of semen collections	% of collections positive for sperm	Semen volume (ml)[a]	Average sperm count (per high-power field)
0.5	11	9.1	0.21 (0.1–0.5)	12.0
1	15	60	0.33 (0.05–2.0)	4.2 (1.0–13.0)
2	13	46.2	0.29 (0.05–1.0)	1.8 (1.0–5.0)
3	6	33.3	0.57 (0.1–2.0)	0.65 (0.3–1.0)
4	1	100	2.0	2.0
5	1	0	1.5	NA
6	1	100	1.0	1.0
12	6	33.3	ND	ND
18	2	0	0.1	NA
24	15	26.7	0.36 (0.1–2.0)	1.25 (1.0–2.0)

NA, not applicable; ND, not determined.
After 0.5 mo, all sperm observed were nonmotile.
[a]Values are presented as mean (range).

Animal Care International. Vasectomies were performed as minor surgical procedures on animals deeply sedated (Telazol, 5 mg/kg intramuscularly, Fort Dodge, Fort Dodge, Iowa). The vasectomy procedure was performed as described by others.[10] Briefly, the vas deferens was isolated, and approximately 3 to 5 cm was removed. The remaining ends of the vas were ligated and the edges cauterized. The section of vas deferens removed was placed in 10% formalin and prepared for histologic evaluation. All animals received an anti-inflammatory drug (ibuprofen, 200 mg orally 3 times daily; Motrin, McNeil-PPC, Fort Washington, PA) and an analgesic (nalbuphine hydrochloride, 10 mg intramuscularly every 4 to 6 h, Hospira, Lake Forest, IL) as needed during the first 48 h after recovery from sedation.

Semen collection. The study included 39 male chimpanzees housed at the New Iberia Research Center. Animals were grouped according to length of time postvasectomy until semen evaluation. An electroejaculator (model 303, with 0–10 and 0–50 voltmeters, a 0–250 mAmp current meter, 0–1.5 ammeter, and a #5 sized rectal probe; P-T Electronics, Sandy, OR) was used to obtain semen samples during rectal probe electrostimulation.[8] The stimulator used has been well tolerated by adult male chimpanzees and was capable of providing a controlled sine wave stimulus of 1–50 V amplitude at an alternating current frequency of 20–60 Hz. Each chimpanzee was sedated (Telazol, 2 to 5 mg/kg intramuscularly) and placed in lateral recumbency. The preputial area was cleaned with alcohol-soaked gauze. The probe was lubricated using a sterile, water-based lubricant (Surgilube, E Fougera and Company, Melville, NY) and introduced into the rectum with the electrodes positioned towards the animal's ventrum. The penis was exposed by manual reflection of the prepuce. Stimulation was provided by rhythmic pulsation from the stimulator starting at 5 V and increasing to a maximum of 30 V. The effect of the stimulus was determined by the penile response. After collection of the semen sample, the urethra was cleared manually to ensure extrusion of any coagulum remaining in the urethra. If after approximately 3 min there was no evidence of penile stimulation or fluid emission, the current was turned to 0 and the probe removed from the rectum. After the procedure was completed, the animal recovered in a single cage and was observed for signs of pain or discomfort for 24 to 48 h. Any animal that appeared to be in discomfort was given an analgesic (nalbuphine, 10 mg intramuscularly every 4 h).

Semen evaluation. Semen was collected into a sterile conical tube, and the sample was weighed. Semen samples were taken immediately to the clinical laboratory of the New Iberia Research Center for evaluation. A drop of semen was placed on a glass slide and 10 fields examined by light microscopy (magnification, ×40) for the presence of sperm. Morphology and motility were not uniformly recorded, because the requirements of the NCSS concern simply the presence or absence of sperm. If spermatozoa were noted, a cell count was performed and recorded as number of sperm per high-power field.

Statistical methods. Kendall's tau-c test was used to determine whether knowing the month postvasectomy resulted in a proportional reduction in error in predicting the presence or absence of sperm. Next, a binomial test was conducted to determine whether the probability of an ejaculate containing nonmotile sperm in any given month postvasectomy (1, 2, 3, 4, 5, 6, 12, 18, or 24) was different than the expected probability of 0.33 reported for human males 3 mo after vasectomy.[6] Lastly, regression analysis was conducted to evaluate the relationship between month postvasectomy (0.5, 1, 2, 3, 4, 5, 6, 12, 18, or 24) and volume of semen collected. Likewise, regression analysis was conducted to evaluate the relationship between month postvasectomy and number of sperm present in those samples where sperm were found. For all statistical tests, significance was assigned P values less than 0.05 (Statistical Package for the Social Sciences software, Student Version 14.0; SPSS, Chicago, IL).

Results

The present study was conducted to determine how much time is required between vasectomy and semen evaluation for azoospermia to be reached and the percentage of postvasectomized chimpanzees actually azoospermic. A total of 39 male chimpanzees were vasectomized and underwent a total of 71 semen evaluations (Table 1). Samples were not collected at scheduled time points if there was a clinical problem, chimps

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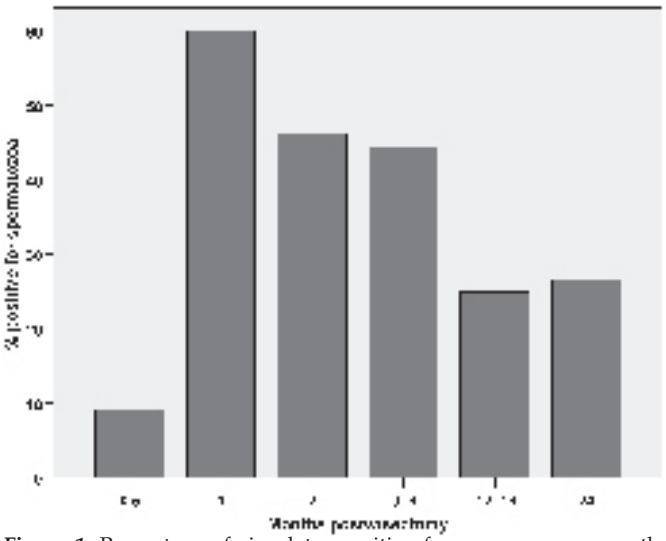

Figure 1. Percentage of ejaculates positive for sperm versus month postvasectomy. Because of the small number of samples for months 4, 5, 6, and 18, data from months 3, 4, 5, and 6 were combined into a single group (months 3–6). Likewise, data from months 12 and 18 were combined into a single group (months 12–18). After 0.5 mo, all sperm present were nonmotile.

were sent to the retirement sanctuary, or in 1 case, death had occurred unrelated to study activity. Fifteen chimpanzees were evaluated 2 or more times at 0.5, 1, 2, and 3 mo postvasectomy, with 1 chimpanzee further evaluated at 4, 5, and 6 mo. Twenty-four chimpanzees were evaluated once each at 0.5 (n = 1), 12, (n = 6), 18 (n = 2), or 24 (n = 15) mo postvasectomy. Overall, spermatozoa were found in the semen of at least 1 chimpanzee every month, with the exception of months 5 and 18 (Figure 1). Of the 15 animals evaluated multiple times between 0.5 and 3 mo postvasectomy, 3 animals (20%) were negative at every examination, 9 animals (60%) were negative then positive during at least 1 subsequent examination, 2 animals (13.3%) were positive at least once and then negative, and 1 animal (6.7%) was positive at every examination (Table 2). Although sperm morphology and motility were not uniformly recorded, the observers noted that after 0.5 mo postvasectomy, all sperm present were nonmotile.

Because of the small numbers of samples for months 4, 5, 6, and 18, data from months 3, 4, 5, and 6 were combined into 1 group (months 3 to 6). Likewise, data from months 12 and 18 were combined into a single group (months 12 to 18). The Kendall's tau-c test was used to determine whether knowing the month postvasectomy resulted in a proportional reduction in error in predicting the presence or absence of sperm. When all months were included, the tau-c score was –0.038 ($P = 0.761$); scores with absolute values of less than 0.25 indicate a weak association between month postvasectomy and the presence or absence of sperm.[5] However, when only months 1 to 24 were included in the model, the tau-c score was –0.292 ($P = 0.032$). Scores with absolute values between 0.25 to 0.50 indicate a moderate association between month postvasectomy and the presence or absence of sperm.[5]

A binomial test was conducted to determine whether the probability of an ejaculate containing nonmotile sperm in any given month postvasectomy was different than the expected probability of 0.33 reported for men.[6] The observed probability of obtaining a chimp semen sample positive for nonmotile sperm was 0.42, which was not significantly different from the expected probability of 0.33.

Table 2. Presence (+) or absence (–) of spermatozoa in the ejaculates of chimpanzees examined at various time points after vasectomy

	Time after vasectomy (mo)			
Animal	0.5	1	2	3
1	–	–	–	ND
2	–	+	+	ND
3	–	–	–	ND
4	–	+	+	ND
5	–	+	–	ND
6	–	+	ND	ND
7	ND	+	+	+
8	–	+	ND	–
9	–	–	–	–
10	–	+	+	–
11	+	+	–	–
12	ND	–	+	ND
13	ND	–	–	+
14	ND	–	+	ND
15	ND	+	–	ND

ND, not determined.
After 0.5 mo, all sperm observed were nonmotile.
Chimpanzee 10 was evaluated further and was +, –, and + for sperm at 4, 5, and 6 mo, respectively, postvasectomy.

Semen volumes were recorded for 65 of the 71 (91.5%) ejaculates and ranged from 0.05 to 2.0 ml (mean ± 1 standard deviation, 0.38 ± 0.48 ml; Table 1). Regression analysis revealed no relationship between month postvasectomy and semen volume. Sperm counts were recorded for 24 of the 26 (92.3%) semen samples in which sperm were identified. Sperm counts ranged from 0.10 to 12.0 (2.22 ± 3.10) sperm per high-power field (Table 1). Regression analysis revealed no relationship between month postvasectomy and sperm count.

Discussion

The objectives of our investigation were to evaluate semen from chimpanzees at various time points after vasectomy to determine the length of time required to achieve azoospermia and the percentage of vasectomized chimpanzees that actually are azoospermic. The results from this study do not allow us to state the time required for chimpanzees to consistently reach azoospermia, because many chimpanzees either were azoospermic postvasectomy but not at subsequent evaluation, were releasing sperm at initial examination but were azoospermic at subsequent examination, were consistently azoospermic or were never azoospermic over the period of evaluation, or had data points missing. Most chimpanzees were azoospermic at 0.5 mo postvasectomy but positive for nonmotile sperm at 1 mo postvasectomy, and many were positive at subsequent collections. When results from 0.5 mo were excluded, there was a moderate association between month postvasectomy and the presence of sperm, with the percentage of positive samples declining over time. This outcome is supported by Figure 2, which shows a temporal decline in the percentage of chimpanzees with nonmotile sperm.

In our study, motile sperm were not observed beyond 0.5 mo postvasectomy. Similar results have been reported for men. In 1 study, functionally competent human sperm were not recovered from ejaculates beyond 12 d postvasectomy.[14] In another study, 56.3% of 309 men had motile sperm in initial postvasectomy semen samples but not in subsequent samples.[11] Comparable

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results have been reported.[14] In contrast, others have reported the persistence of motile sperm after vasectomy, prompting repeat of the surgical procedure.[4,7] More rarely, motile sperm may transiently appear after vasectomy. In 1 study, 6 of 1000 (0.6%) of men had sperm in their semen 1 y after initial tests showed azoospermia; the authors attributed this finding to late recanalization. Tests were repeated for 5 of these men, and all results were negative.[12]

In general, the probability of finding sperm in the ejaculates of chimpanzees in our study ($P = 0.42$) was not different from the results for men whose ejaculates were evaluated 12 wk postvasectomy ($P = 0.33$). The finding of nonmotile sperm after initial azoospermia has also been reported after vasectomy of men. In 1 study, the mean time to azoospermia was 6.4 mo, and nonmotile sperm were found in the ejaculates of 5 of 65 (7.7%) of men after initial azoospermia;[6] the authors thus concluded that azoospermia was not an appropriate criterion for sterility. In 1 study in which azoospermia was defined as the absence of sperm in 2 consecutive tests, 3% of those tested had not produced 2 consecutive negative tests by 4 mo postvasectomy. Despite this result, no paternities were reported.[3] In another study using similar criteria, roughly 1% of 5233 men still had sperm in their ejaculates 1 y after vasectomy.[1]

The temporary reappearance of nonmotile sperm has also been reported to occur in men. In 1 study, small numbers of sperm appeared intermittently in the semen of men tested repeatedly over prolonged periods.[1] Sperm appearing in the semen of previously azoospermic men were nonmotile; but in that study, 4 pregnancies occurred, at an average of 5.5 y postvasectomy.

Regression analysis failed to reveal a relationship between month postvasectomy and semen volume. This finding is similar to that in men, in whom ejaculate volume is not significantly different before and after vasectomy.[14] Likewise, regression analysis revealed no relationship between month postvasectomy and sperm count, supporting the observation that nonmotile sperm continue to be released into the semen. A study involving both human and nonhuman primates revealed that the seminal vesicles serve as storage sites for nonmotile sperm.[13] In that study, 11 of 24 (45.8%) nonhuman primate seminal vesicles, including 1 of 6 (16.7%) chimpanzee seminal vesicles, contained sperm at necropsy. Seminal vesicles from some nonhuman primate species were more likely to contain nonmotile sperm than vesicles from other species, suggesting a species effect, but the numbers of specimens included were too small to allow statistical comparisons. Strikingly similar to the results from chimpanzees, 1 of 7 (14.3%) men who had died suddenly had sperm in the distal seminal vesicle. Therefore, storage in the seminal vesicles may account for the continued finding of nonmotile sperm in the ejaculates of chimpanzees in our study. Further research will be required to determine whether this notion is in fact the case. In addition, comparative studies using samples obtained from chimpanzees by voluntary means versus rectal probe electrostimulation may provide useful information pertaining to spermatozoa found in normal postvasectomy evaluations.

Our hypothesis was that semen evaluation for the absence of spermatozoa is not a necessary indicator of sterility postvasectomy. The results of our study support that hypothesis and provide a useful context for evaluating vasectomized chimpanzees. Histologic confirmation of vasectomy combined with a finding of azoospermia or only nonmotile sperm after electroejaculation may be more reasonable criteria on which to base entry of chimpanzees into the NCSS. Nonmotile sperm may remain functional, and in primates, are transported by contractions of the uterus and oviduct to the ampulla as effectively as motile sperm. However, because nonmotile sperm cannot traverse the cervical mucus, they are not likely to fertilize the ovum. In addition, recanalization of the vas deferens likely occurs, albeit rarely, in chimpanzees as it does in men,[6] and may result in unexpected pregnancies. However, this outcome may occur regardless of whether a chimpanzee was azoospermic postvasectomy. Indeed, the male chimpanzee that caused the recent pregnancy in an NCSS facility had been vasectomized and had demonstrated azoospermic prior to admission.

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