Effect of flushing the vasa deferentia at the time of vasectomy on the rate of clearance of spermatozoa from the ejaculates of dogs and cats

by

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DEDICATION

This thesis is dedicated to my wife, Deb; without her support and encouragement, I could not have completed it.

A special thanks to my major professor, Dr. M. H. Pineda, for his guidance, advice, and constant encouragement and also to Mr. M. P. Dooley for his continuous help and input into my research.

ABSTRACT

Two experiments were done to determine the effect of flushing of the vasa deferentia at the time of vasectomy on the rate of clearance of spermatozoa from the ejaculates of dogs and toms. In the dog, flushing of the vasa deferentia at the time of vasectomy shortened the time from vasectomy to azoospermia to postvasectomy day 6. In the tom, the number of intact spermatozoa was reduced to zero by postvasectomy day 7, but the time from vasectomy to azoospermia was not shortened.

The fluid used to flush the vasa deferentia was not eliminated through the penile urethra but flowed into the urinary bladder, suggesting that the path of least resistance for the exit of vasal contents is toward the urinary bladder in the anesthetized dog and tom. Both control and treated dogs and toms had spermatozoa in the urine obtained by cystocentesis immediately after ejaculation or ejaculation and flushing of the vasa deferentia. The concentration of spermatozoa found in the urine of control dogs and toms was not significantly different from the concentration of spermatozoa in the urine of treated dogs and toms. The presence of spermatozoa in the urine from both, treated and control dogs and toms indicates that a retrograde flow of spermatozoa into the urinary bladder had occurred during or shortly after ejaculation. The finding that the spermatozoal

concentration in the urine was not different between control and treated dogs and toms indicates that a retrograde flow of spermatozoa into the urinary bladder had occurred prior to the flushing of the vasa deferentia and not as a result of the flushing procedure.

Flushing the vasa deferentia at the time of vasectomy is easy to perform, safe, and can be used in clinical practice to decrease the time from vasectomy to the safe utilization of dogs and toms as teasers. The procedure has potential application to males of other species.

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INTRODUCTION

Vasectomy is a technique of potential application for control of population growth of dogs and cats, and vasectomized animals are used as teasers to detect estrus in both domestic and laboratory animal species. Semen can be collected from vasectomized dogs as soon as 24 hours after vasectomy[24]. Vasectomized males may be capable of breeding shortly after recovery from anesthesia and utilization of these animals as teasers may result in pregnancies from spermatozoa present in the ejaculates of recently vasectomized animals.

Ejaculates from bilaterally vasectomized animals[7,23,24,29,31] and humans[2,9,10,13] or ejaculates from bilateral, epididymally ligated[14,27] or vasal-ligated[19] animals contain spermatozoa for variable but prolonged periods after vasectomy or ligation. Spermatozoa have been observed in the ejaculates of bilaterally vasectomized men 6 months to 1 year following vasectomy[10]. Ejaculates from vasectomized dogs[24] and toms[23] contain spermatozoa for as long as 21 and 49 days after vasectomy, respectively.

The site of vasectomy[23,24,31] appears to have a major influence on the rate that spermatozoa disappear from the ejaculates of vasectomized dogs and toms. The interval from vasectomy to azoospermia appears to decrease as the site of

severance or occlusion approaches the urethral ends of the vasa deferentia[31]. Laparoscopic techniques, utilizing fiberoptic endoscopy, for occlusion of the abdominal vasa deferentia have been advocated[31] for sterilization of dogs and toms. Laparoscopy[31] requires the use of expensive fiberoptic equipment that is not commonly found outside of veterinary teaching hospitals or very large private practices.

The presence of spermatozoa in the ejaculates of bilaterally vasectomized dogs and toms indicates that in these species ejaculated spermatozoa originate from both the epididymides and vasa deferentia, not from the epididymides alone[23,24]. The postejaculatory load of spermatozoa in the vasa deferentia of dogs was reported to be 47×10^6 spermatozoa[17]. A technique that decreases or eliminates the load of spermatozoa in the vasa deferentia at the time of vasectomy would result in an accelerated clearance of spermatozoa from the ejaculates of vasectomized animals, shortening the time from vasectomy to azoospermia. Such a technique would be useful to researchers and clinicians because it would allow the immediate utilization of a vasectomized male as a teaser and would have practical application in programs dealing with the control of pet population growth.

This study was designed to determine 1) whether bilateral flushing of the vasa deferentia at the time of vasectomy would shorten the interval from vasectomy to azoospermia in dogs and toms, and 2) the postvasectomy spermatozoal load of the vasa deferentia and urethra in these two species.

In this thesis, a male dog is called a dog, a male cat is called a tom, a female dog is called a bitch, and a female cat is called a queen.

LITERATURE REVIEW

Pet Population Problem

In 1975 there were an estimated 41.3 million dogs and 23.1 million cats in the United States[30]. The canine population has not decreased significantly in size since 1975 and is predicted to increase to 48.1 million by 1990[15]. The feline population has continued to grow even in areas where greater than 50% of cats were spayed or neutered[30]. In fact, the cat population is projected to increase to 55.1 million by 1990[15]. Unwanted and uncontrolled pets pose a social, economic, and health threat to communities. Free roaming dogs and cats spread zoonotic diseases, inflict bites, pollute public areas, and cause damage to livestock, wildlife, and property[22]. Confinement and disposal of unwanted pets requires money to be channeled from other programs of importance to the community.

A study conducted in Suffolk county, New York in 1981[18] indicated that only one half of the bitches and queens were spayed and that the percentage of owners that did not want their dog neutered was even greater. In Yolo County, California in 1970, 33% of the bitches were spayed yet only 4% of dogs were neutered[8]. These percentages support the contention[30] that the female of a species is looked upon as the major target for population control. Owners of dogs and toms apparently are not concerned with

unwanted pregnancies and litters, and are not likely to spend money to have their pets neutered. Since dogs and toms are sexually active throughout the year, they are capable of mating with a number of females and siring many litters[22].

Methods to Control the Growth of the Pet Population

Orchiectomy and vasectomy are the two surgical procedures available for permanent sterilization of the male dog and cat. Pet owners voice a number of objections to orchiectomy, some real and some imagined, but all contribute to maintain a high percentage of intact males in the pet population[22]. Desirable effects of orchiectomy in the adult male include reduction in roaming tendencies, urine marking, and fighting.

Vasectomy produced azoospermic ejaculates as early as postvasectomy day 7 and as late as postvasectomy day 28 in the dog[24], and as early as postvasectomy day 21 and as late as postvasectomy day 56 in the tom[23]. Confinement for a period of at least 30 days after vasectomy has been recommended[22] to prevent the occurrence of fertile matings and subsequent pregnancies in dogs. An even longer period of confinement would appear to be needed after vasectomy in the tom.

MATERIALS AND METHODS

Experiment 1

This experiment investigated how flushing the vasa deferentia at the time of vasectomy affected spermatozoal clearance in dogs. Twelve random-source dogs weighing from 8.62 to 31.75 kg of body weight (mean $\pm$ SD = 17.15 $\pm$ 7.59) were used. Dogs were quarantined for at least 4 weeks, and during this period they were vaccinated against rabies, distemper, and parvovirus and treated for external and internal parasites. Dogs were housed in individual cages, fed commercial dog food, and maintained in light- and temperature-controlled rooms. Dogs were randomly assigned to a treated or to a control group of 6 dogs each. Control dogs were bilaterally vasectomized. Treated dogs were bilaterally vasectomized and their vasa deferentia were flushed at the time of vasectomy.

Vasectomy for the treated and control groups was performed as follows: an intramuscular injection of 2.2 mg/kg body weight of xylazine was given as a preanesthetic sedative. Approximately 10 minutes later, anesthesia was induced and maintained with intravenous injection of thiamylal sodium. The dosage was adjusted to achieve the desired level of anesthesia in individual dogs (approximately 10 mg/kg of body weight). The prescrotal area was clipped free of hair, scrubbed, and rinsed three times

using surgical soap (povidone iodine) and an alcohol rinse. The surgical site was draped. A ventral midline incision, 1 to 2 centimeters long, was made through the skin immediately cranial to the scrotum. Blunt dissection was used to isolate the left spermatic cord and the cord was exteriorized through the skin incision. The spermatic cord was fixed by positioning a mosquito forcep between the exteriorized loop of the cord and the skin incision. The vas deferens was visualized through the tunica vaginalis and a small (1-3 mm) incision was made through the tunic over the vas deferens. The vas deferens and associated artery of the vas were pulled through the opening in the tunic and held in place with a gauze sponge moistened with 0.9% physiologic saline solution, while the artery was dissected free of the vas deferens. Two silk ligatures were placed around the vas deferens, approximately one centimeter apart, and the isolated segment of the vas was severed and removed. The ligated ends of the vas deferens and the intact artery of the vas were allowed to retract through the incision in the tunica vaginalis before the tunic was closed using a single silk suture. The spermatic cord was then allowed to retract through the skin incision. This procedure was repeated on the right side, followed by closure of the skin incision using simple, interrupted silk sutures. For treated dogs, the vas deferens was isolated and ligated, as described for control dogs,

except that the ligature on the distal portion of the vas deferens was not tied, but placed loosely around the vas. The vas deferens was then severed between the two ligatures. The distal portion of the vas was held using the ligature and positioned, with the aid of forceps, to visualize the lumen. A 3X magnifying loupe was used to aid in the visualization of the luminal opening of the vas deferens. The distal end of the vas deferens was then cannulated to a depth of approximately 1 centimeter using the blunted end of a 1.0 to 1.5-inch long, 22 to 26 gauge needle. The gauge of the needle was selected according to the apparent diameter of the luminal opening of the vas deferens. The needle was secured in the vas deferens by placing a mosquito forcep around the cannulated vas deferens. Each vas deferens was then flushed with 10 to 12 ml of a 0.007% solution of Trypan Blue dye in 0.9% saline. The infusion of the flushing solution was performed in a pulsatile fashion. The force required to flush each vas deferens was not measured. Fluids were not released from the penile urethra during or immediately after flushing. After flushing, the distal end of the vas deferens was ligated near the tip of the needle and then severed at a point between the tip of the needle and the distal ligature.

To determine if the flushing fluid had flowed in a retrograde direction into the urinary bladder, urine samples were obtained from control and treated dogs by midventral

cystocentesis prior to recovery from anesthesia. The volume and color of each of the urine samples were recorded.

Semen was obtained[20,24] from each dog by digital manipulation using a rubber directing cone attached to a graduated glass centrifuge tube. The rim of the rubber cone was lubricated with a small amount of a sterile lubricating jelly. The directing cone was then placed over the penis as the prepuce was retracted caudally to expose the bulbus glandis. The penis was then encircled with the thumb and index finger caudal to the bulbus glandis, and the other fingers and palm of the hand were used to exert gentle pressure over the bulbus glandis and penile body to simulate a vaginal lock. Digital manipulation was continued for 10 minutes or until 13 ml of ejaculate had been obtained. Semen was obtained from each dog on day 0, immediately prior to vasectomy, and on postvasectomy days 3, 4, 5, 6, 7, and 14. For control dogs, semen was also obtained on postvasectomy days 21, 28, and 35. Obtainment of semen on days 21 through 35 were scheduled, as additional observations, to determine when azoospermic ejaculates would be produced by control dogs. The volume and total number of spermatozoa were determined for each ejaculate. Volume was determined at the time of collection using graduated, collecting tubes. Numbers of intact spermatozoa and detached spermatozoal heads were determined using a hemacytometer. To

determine spermatozoal concentration, samples of prevasectomy semen were diluted 1:100 or 1:200 with distilled water prior to determination of spermatozoal concentration. Spermatozoal concentration in postvasectomy samples was determined using undiluted samples. Both chambers of the hemacytometer were filled with a sample of semen and the hemacytometer was allowed to stand undisturbed for five minutes at room temperature. The number of intact spermatozoa and spermatozoal heads present in the 25 large ruled squares of each chamber of the hemacytometer were counted and the total divided by two. The volume of each of the chambers of the hemacytometer is 0.1 µl. Thus, the finding of one spermatozoa in two chambers represented a spermatozoal concentration of 5.0×10^3 spermatozoa/ml. When no spermatozoa or detached spermatozoal heads were found in the 50 large ruled squares of the two chambers, the ejaculate was considered to be azoospermic. The concentration of spermatozoa in the urine samples obtained by cystocentesis immediately after vasectomy for control dogs and after vasectomy and flushing for treated dogs was determined using a hemacytometer, as described for the determination of the number of spermatozoa in postvasectomy ejaculates.

Spermatozoal motility was evaluated in undiluted semen samples as soon after collection as possible. One drop of undiluted semen was placed on a glass slide warmed to 37 C,

and a coverslip was placed over the sample. The slide was then placed on a temperature-controlled stage plate at 37 to 39 C and examined at 400X for independent motility, using a phase-contrast microscope.

Spermatozoal morphology was not evaluated, except that the number of intact spermatozoa and loose spermatozoal heads present in the semen sample were recorded at the time that spermatozoal counts were made.

The body weight of each dog was determined at the time of seminal collection.

Experiment 2

This experiment investigated how flushing the vasa deferentia at the time of vasectomy affected spermatozoal clearance in cats. Eight random-source toms weighing from 3.40 to 4.17 kg of body weight (mean $\pm$ SD = 3.85 $\pm$ 0.25) were used. Toms were quarantined for at least 4 weeks, and during this period they were vaccinated against rabies and panleukopenia and treated for external and internal parasites. Toms were housed in individual cages, fed commercial cat food, and maintained in light- and temperature-controlled rooms.

Toms were randomly assigned to a treated or to a control group of 4 cats each.

Vasectomy for the treated and control groups was performed as described in Experiment 1 with the following

modifications: 1) a preanesthetic was not used, 2) anesthesia was induced and maintained using an intramuscular injection of 30 mg/kg of body weight of ketamine hydrochloride, and 3) 1-inch long, 24 to 30 gauge needles were used to cannulate the vasa deferentia. The flushing solution, volume, and procedure used were as described for Experiment 1.

No fluid was recovered from the penile urethra of the tom during or immediately after flushing. To determine if the flow of flushing fluid was in a retrograde direction, the urinary bladder of all control and treated toms was emptied, prior to recovery from anesthesia, using midventral cystocentesis. The volume and color of each of the urine samples were recorded.

Semen was collected by electroejaculation. The electroejaculator, rectal probes, and anesthetic protocol have been described[4,5]. A brief description follows: At each seminal collection, each tom was given 3 series of 60 electrical stimuli of 6.0 volts each, for a total of 180 stimuli, utilizing a sinusoidal wave form at a frequency of 30 Hz. There was an interval of three seconds between stimuli, and the duration of each stimulus, from 0 volts to the corresponding voltage for the series and back to 0 volts, was 1.5 seconds. A rest period of 5 minutes was allowed between each series of 60 stimuli. An electroejaculate was

defined as the pooled seminal fluid and spermatozoa obtained from the set of 180 electrical stimulations. An electroejaculate was obtained from each tom immediately prior to vasectomy (PVD 0). The first postvasectomy electroejaculate (PVD 1) was obtained from control toms, immediately after vasectomy and cystocentesis and from treated toms, immediately after vasectomy, flushing of the vasa deferentia, and cystocentesis. Electroejaculates were then obtained at weekly intervals until postvasectomy day 63.

The volume and total number of spermatozoa were determined for each electroejaculate. Volume was measured using a 500 µl syringe attached to a disposable Pasteur pipette. Total number of spermatozoa and number of loose heads were determined as described for Experiment 1.

The total number of spermatozoa in the urine, spermatozoal motility in undiluted samples of postvasectomy ejaculates, and body weight were determined, as described in Experiment 1.

Statistical Analyses

Analyses of variance[28] were used to determine the effect of treatment, day of collection, and the interaction treatment X day of collection on the volume of ejaculate and body weight for Experiments 1 and 2 of the present study. The animal within treatment mean square was used as the error term for the effect of treatment. For the purposes of this

study, significance of F ratios was established at $P \leq 0.05$. The conservative F value[11] was used to establish significance for the effect of day of collection.

Statistical analyses to determine effect of treatment, day of collection, and the interaction treatment X day of collection were not performed on data for total number of spermatozoa, number of intact spermatozoa, and detached spermatozoal heads per ejaculate due to the large number of early azoospermic ejaculates in the treatment groups of Experiment 1 and 2. Regression lines were calculated[28], however, to compare and characterize the relationship between the rate of clearance (day of collection = independent variable) and the number of intact spermatozoa (dependent variable) in the postvasectomy ejaculates of control and treated dogs or toms of Experiments 1 and 2. For computational purposes, data were transformed to natural logarithm using the expression: $\ln [(\text{number of intact spermatozoa } (10^6) + 0.0001) \times 1 \times 10^6]$. Due to the large number of azoospermic ejaculates, the regression lines were computed as follows: Experiment 1 -- regression lines for the control and treated groups were determined from values obtained on those days in which the mean number of intact spermatozoa in the postvasectomy ejaculates was a nonzero value; Experiment 2 -- regression lines for the control and treated groups were determined using all values up to and

including the first postvasectomy seminal collection in which no intact spermatozoa were found in any of the seminal samples for the control or the treated groups, because none of the treated toms had intact spermatozoa in their ejaculates on postvasectomy day 7.

After the fact, and because we found that there was retrograde flow of flushing fluid into the urinary bladder, analyses of variance were used to determine the effect flushing the vasa deferentia had on the concentration of spermatozoa in the urine from the control or treated dogs or toms of Experiments 1 and 2. For presentation purposes, the number of spermatozoa in the urine samples from the dogs was estimated for a volume normalized to 13.0 ml, due to variation among dogs in the volume of urine samples collected. For the toms, the total number of spermatozoa was estimated from the total volume of urine obtained by emptying the urinary bladder.

RESULTS

Experiment 1

The volume of the ejaculate was not affected by treatment or day of collection ($P>0.05$), and the interaction treatment X day of collection was also not significant ($P>0.05$, Table 1).

The number of spermatozoa in the ejaculate decreased rapidly after vasectomy or vasectomy and flushing (Table 1). No consistent relationship was observed between the number of spermatozoa in prevasectomy ejaculates and the time of the first azoospermic ejaculate (Table 2). For instance, control dog number 2 had greater than four times the number of spermatozoa in his prevasectomy ejaculate than dog number 1 (Table 4), but he became azoospermic on postvasectomy day 14; dog number 1 had intact spermatozoa in his ejaculate until postvasectomy day 21 and did not produce an azoospermic ejaculate by postvasectomy day 35 when the seminal collections were discontinued (Table 3).

All treated dogs had azoospermic ejaculates by postvasectomy day 6 and continued to be azoospermic through post vasectomy day 14 when seminal collections were discontinued for these dogs (Table 2). Dog number 8 had an azoospermic ejaculate on postvasectomy day 3, the first postvasectomy ejaculate collected, and continued to have azoospermic ejaculates through postvasectomy day 14

(Table 2). Three dogs (numbers 9, 11, and 12) had intact motile or nonmotile spermatozoa in their ejaculates on postvasectomy day 3 and had azoospermic ejaculates on postvasectomy day 4 through day 14 (Table 2). The ejaculates from dog number 10 had intact, nonmotile spermatozoa on postvasectomy day 3 and intact, motile spermatozoa on postvasectomy day 4. The ejaculates obtained from postvasectomy day 5 through day 14 were azoospermic (Table 2). Intact, motile spermatozoa were present in the ejaculates from dog number 7 on postvasectomy days 3, 4, and 5 and the ejaculates from postvasectomy day 6 through day 14 were azoospermic (Table 2).

None of the control dogs, whose vasa deferentia were not flushed at the time of vasectomy, had azoospermic ejaculates by postvasectomy day 6 (Table 2). Motile spermatozoa were detected in the ejaculates from control dogs (numbers 1, 2, 3, and 4) on postvasectomy day 7. The ejaculates from dog number 6 were azoospermic on postvasectomy days 7 and 14 (Table 2). Three dogs (numbers 2, 4, and 5) had azoospermic ejaculates on postvasectomy day 14 (Table 2). The ejaculate from dog number 3 contained loose heads and the ejaculate from dog number 1 contained intact, nonmotile spermatozoa and loose heads on postvasectomy day 14 (Table 2). In the additional observations on the control dogs (Table 3), three dogs (numbers 2, 3, and 5) had azoospermic ejaculates from

postvasectomy day 21 through postvasectomy day 35 when seminal collections ended. The ejaculate from dog number 6 was azoospermic on postvasectomy day 21; contained loose heads on postvasectomy day 28, and was azoospermic on postvasectomy day 35. Intact, nonmotile spermatozoa were present in the ejaculate of dog number 4 on postvasectomy day 21, loose heads were present on postvasectomy day 28, and the ejaculate was azoospermic on postvasectomy day 35. The ejaculate from dog number 1 contained intact, nonmotile spermatozoa and loose heads on postvasectomy day 21, and loose heads on postvasectomy day 28 and day 35.

The relationship between the logarithmically transformed number of intact spermatozoa in the ejaculate and time after vasectomy is shown in Figure 1. The regression equations predicting the rate of disappearance of intact spermatozoa from the ejaculate ($\hat{Y}$) as a function of time after vasectomy (X, postvasectomy day = PVD) were linear and can be represented by the equations: $\hat{Y}$ = antilog [16.4086 + (-0.7897 X PVD)] - 100 for control dogs ($r=0.79$, $n=36$, $P<0.0001$) and $\hat{Y}$ = antilog [17.6477 +(-2.4506 X PVD)] - 100 for treated dogs ($r=0.54$, $n=18$, $P<0.025$).

The total number of spermatozoa in the prevasectomy ejaculate, first postvasectomy ejaculate, and in urine samples obtained by cystocentesis after vasectomy or vasectomy and flushing are shown in Table 4.

The retrograde flow of flushing fluid into the urinary bladder in treated dogs and the high concentration of spermatozoa present in urine samples obtained by cystocentesis from both treated and control dogs were unexpected findings. The mean concentration of spermatozoa per ml of urine (not shown in tables) for control (mean $\pm$ SD = $3.75 \pm 3.91 \times 10^6$) and treated (mean $\pm$ SD = $3.40 \pm 3.01 \times 10^6$) dogs were not significantly ($P>0.05$) different. Urine samples of 12.5 to 13.5 ml were obtained from 11 dogs and a urine sample of 33.0 ml was obtained from 1 dog. Urine volumes were normalized to 13.0 ml for purposes of estimating the total number of spermatozoa present in the urine (Table 4). The total number of spermatozoa that were displaced from the epididymides and vasa deferentia was then calculated by adding the number of spermatozoa present in the ejaculate with the estimated number of spermatozoa present in the urine. The percent of the total number of spermatozoa displaced that were present in the urine was then calculated. The mean percentage of the total number of spermatozoa displaced that were found in the urine of control (mean $\pm$ SD = $21.27 \pm 38.84\%$; range = 0.1% to 99.7%) and treated (mean $\pm$ SD = $23.65 \pm 33.01\%$; range = 0.01% to 85.3%) groups were not significantly different ($P>0.05$). In 5 of 6 treated dogs, the urine obtained by cystocentesis was colored blue. The remaining dog (number 12) urinated blue-tinted urine before

the cystocentesis sample of urine could be obtained.

Body weight of dogs was not affected by day of collection but there were trends ($P<0.1$) for an effect of treatment and for the interaction treatment X day of collection (Table 1).

Because none of the fluid used to flush the vasa deferentia of treated dogs was eliminated through the penile urethra during or immediately after flushing, the postejaculatory load of the vasa deferentia and penile urethra could not be estimated.

Experiment 2

The volume of the ejaculate was not affected by treatment or day of collection ($P>0.05$) and the interaction treatment X day of collection was also not significant ($P>0.05$, Table 5).

The number of spermatozoa in the ejaculate decreased rapidly after vasectomy and vasectomy and flushing (Table 5). No consistent relationship was observed between the number of spermatozoa in prevasectomy ejaculates and the time of the first azoospermic ejaculate (Tables 6 through 8).

None of the treated toms had intact spermatozoa in their postvasectomy ejaculates from postvasectomy day 7 through postvasectomy day 63 when the seminal collections were discontinued (Table 6). Treated tom number 7 had an azoospermic ejaculate on postvasectomy day 1, immediately

following vasectomy and flushing of the vasa deferentia (Table 6). The ejaculates from tom number 7 were devoid of intact spermatozoa through postvasectomy day 63 when data collection ended; however, his ejaculates contained loose heads on postvasectomy days 7, 21, and 28. The ejaculate from tom number 5 contained intact motile spermatozoa on postvasectomy day 1 and the remaining ejaculates on postvasectomy days 7 through 63 were azoospermic (Table 6). Tom number 8 had intact, nonmotile spermatozoa in his ejaculate on postvasectomy day 1 and had loose heads in his ejaculate on postvasectomy day 7 (Table 6). The ejaculates from tom number 6 contained intact, nonmotile spermatozoa and loose heads on postvasectomy day 1, and only loose heads on postvasectomy days 7, 21, and 49 (Table 6).

The ejaculates from control tom number 2 contained intact, motile spermatozoa and loose heads on postvasectomy day 1; were azoospermic on postvasectomy days 7 through 28; contained loose heads on postvasectomy day 35; and were azoospermic from postvasectomy day 42 through 63, when data collection ended (Table 7). Tom number 3 had intact, nonmotile spermatozoa in his ejaculates on postvasectomy day 1; intact, motile spermatozoa and loose heads on postvasectomy day 7; his ejaculates were azoospermic on postvasectomy days 14 through 35; loose heads were present in in his ejaculate on postvasectomy day 42; and the ejaculates

were azoospermic from postvasectomy days 49 through 63 (Table 7). The ejaculates from tom number 1 contained intact, motile spermatozoa on postvasectomy day 1; intact, motile spermatozoa and loose heads on postvasectomy days 7 and 14; intact, nonmotile spermatozoa and loose heads on postvasectomy day 21; only loose heads on postvasectomy day 28; and were azoospermic from postvasectomy day 35 through 63 (Table 7). Tom number 4 had intact, nonmotile spermatozoa and loose heads in his ejaculate on postvasectomy day 1; had intact, nonmotile spermatozoa in his ejaculates on postvasectomy days 14 through 28; had only loose heads present on postvasectomy day 35; the ejaculates were azoospermic from postvasectomy day 42 through day 56; and had only loose heads in his ejaculate on postvasectomy day 63 (Table 7).

The relationship between the logarithmically transformed number of intact spermatozoa in the ejaculate and time after vasectomy is shown in Figure 2. The regression equations predicting the rate of disappearance of intact spermatozoa from the ejaculate ($\hat{Y}$) as a function of time after vasectomy (X = PVD), were linear and can be represented by the equations: $\hat{Y}$ = antilog [10.4416 + (-0.1873 X PVD)] - 100 for the control toms ($r = 0.75$, $n = 24$, $P < 0.0001$) and $\hat{Y}$ = antilog [7.1863 + (-0.3687 X PVD)] - 100 for the treated toms ($r = 0.62$, $n = 8$, $P<0.05$).

The total number of spermatozoa in the prevasectomy ejaculate, first postvasectomy ejaculate, and in the urine obtained by cystocentesis after vasectomy or vasectomy and flushing are shown in Table 8.

The retrograde flow of flushing fluid into the urinary bladder in treated toms and the high concentration of spermatozoa present in the urine obtained by cystocentesis from both treated and control toms were, as in the dog, unexpected findings. The mean concentration of spermatozoa per ml of urine (not shown in tables) for control (mean $\pm$ SD = 2.44 $\pm$ 2.61 X 10^6) and treated (mean $\pm$ SD = 0.89 $\pm$ 0.72 X 10^6) toms were not different ($P>0.05$). The mean total number of spermatozoa in the urine from control and treated toms is shown in Table 8.

The total number of spermatozoa displaced during electroejaculation and the percentage of the total number of spermatozoa displaced that were found in the urine were estimated for each tom, as described for the dogs of Experiment 1. The mean percentage of the total number of spermatozoa displaced that were found in the urine of control toms (mean $\pm$ SD = 72.20 $\pm$ 6.70% ; range = 67.7% to 79.9%) was not different ($P>0.05$, Table 8) from the mean percentage of the total number of spermatozoa displaced by electro-ejaculation and flushing that were found in the urine of treated toms (mean $\pm$ SD = 57.05 $\pm$ 48.97% ; range = 10.5% to

99.6%). As for the dogs, the fluid used to flush each vas deferens was not eliminated through the penile urethra, but retrograded into the urinary bladder. The urine collected by cystocentesis from each of the treated toms was colored blue.

There was a significant effect of day of collection on body weight of toms ($P<0.05$, Table 5).

Since none of the fluid used to flush the vasa deferentia of treated toms was eliminated through the penile urethra during or immediately after flushing, the post ejaculatory load of the vasa deferentia and penile urethra could not be estimated.

TABLES

Table 1. Volume and total number of spermatozoa per ejaculate and body weight of control and treated dogs (Experiment 1)

	Volume (ml)		No. of spermatozoa (10^6)[a]		Body weight (kg)	
PVD	Controls	Treated	Controls	Treated	Controls	Treated
0	4.02±2.50	6.88±5.69	926.6667±935.7141	942.3758±1,106.7897	13.46±2.08	20.84±9.47
3	4.87±2.50	7.00±4.60	2.7373± 1.9941	0.2157± 0.2461	13.57±2.13	20.85±9.37
4	4.65±2.82	6.28±3.68	1.1441± 1.6687	0.0775± 0.1380	13.25±2.19	20.41±9.44
5	3.73±1.84	6.50±3.53	0.8795± 1.3555	0.0460± 0.1127	13.12±2.32	20.80±9.27
6	3.35±1.76	6.18±4.18	0.3361± 0.3447	0 ----	12.95±2.35	20.56±9.22
7	3.68±2.87	6.15±3.99	0.3929± 0.5852	0 ----	13.73±2.22	20.35±9.25
14	4.23±2.38	4.53±5.93	0.0105± 0.0169	0 ----	13.85±2.15	20.35±8.71
P						
Treatment effect		NS[b]		ND[c]		<0.1
Day effect		NS		ND		NS
Interaction		NS		ND		<0.1

[a]Total number includes intact spermatozoa and detached spermatozoal heads. Data are expressed as mean±SD, n=6 for each mean.

[b]NS = Not significant (P>0.05).

[c]ND = Not determined.

Table 2. Number of intact spermatozoa and detached spermatozoal heads (10^6) in the postvasectomy ejaculates of control and treated dogs (Experiment 1)

Control dogs

	No. 1		No. 2		No. 3		No. 4		No. 5		No. 6	
PVD	Intact	Heads	Intact	Heads	Intact	Heads	Intact	Heads	Intact	Heads	Intact	Heads
3	1.1790	0	6.5000	0	2.8125	0	2.5350	0	0.9600	0	2.4375	0
4	0.2960	0.0740	4.4900	0	1.0750	0	0.3190	0	0.1705	0	0.4125	0.0275
5	0.4400	0	2.4900	1.0600	0.2520	0	0.9263	0.0570	0	0.0280	0.0235	0
6	0.8710	0.0130	0.6000	0	0.0500	0.0500	0.3360	0	0.0115	0	0.0850	0
7	0.4070	0.0130	1.0850	0.4650	0.1230	0	0.2025	0	0.0620	0	0	0
14	0.0130	0.0260	0	0	0	0.0240	0	0	0	0	0	0

Treated dogs

	No. 7		No. 8		No. 9		No. 10		No. 11		No. 12	
PVD	Intact	Heads	Intact	Heads	Intact	Heads	Intact	Heads	Intact	Heads	Intact	Heads
3	0.5700	0	0	0	0.0320	0	0.0360	0.0090	0.4730	0	0.1740	0
4	0.1250	0	0	0	0	0	0.3400	0	0	0	0	0
5	0.0920	0.1840	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0

Table 3. Number of intact spermatozoa and detached spermatozoal heads (10^6), and total number of spermatozoa (10^6) in the postvasectomy ejaculates of control dogs. Additional observations to Experiment 1

	Dog 1		Dog 2		Dog 3		Dog 4		Dog 5		Dog 6		Total No. spermatozoa per ejaculate[a]
PVD	Intact	Heads	Intact	Heads	Intact	Heads	Intact	Heads	Intact	Heads	Intact	Heads	
21	0.0065	0.0195	0	0	0	0	0.0230	0	0	0	0	0	0.0082±0.0127
28	0	0.0100	0	0	0	0	0	0.0245	0	0	0	0.0450	0.0133±0.0183
35	0	0.0015	0	0	0	0	0	0	0	0	0	0	0.0003±0.0006

[a]Data are mean±SD, n=6 dogs.

Table 4. Total number of spermatozoa in the prevasectomy ejaculate, in the first postvasectomy ejaculate, and in samples of urine collected by cystocentesis after vasectomy or vasectomy followed by flushing the vasa deferentia of the dog (Experiment 1)

Controls					Treated				
	Total No. spermatozoa (10^6)					Total No. spermatozoa (10^6)			
	Ejaculate					Ejaculate			
Dog No.	Pre-vasectomy	First post-vasectomy	Urine[a]	% of total[b]	Dog No.	Pre-vasectomy	First post-vasectomy	Urine[a]	% of total[c]
1	442.00	1.18	19.50	4.2	7	2,360.00	0.57	83.20	3.4
2	1,950.00	6.50	22.10	1.1	8	39.81	0	21.45	35.0
3	346.50	2.81	65.98	16.0	9	580.00	0.03	7.15	1.2
4	2,257.00	2.54	3.04	0.1	10	327.60	0.05	66.95	17.0
5	564.00	0.96	39.33	6.5	11	14.85	0.47	86.45	85.3
6	0.50	2.44	143.00	99.7	12	2,332.00	0.17	0.26	0.01
Mean±SD	926.67±935.71	2.74±1.99	48.83±50.84	21.27±38.84		942.38±1,106.79	0.22±0.25	44.24±39.10	23.65±33.01

[a]Volume of urine normalized to 13.0 ml to estimate the total number of spermatozoa in the sample.

[b]The percentage of the estimated total number of spermatozoa displaced during the prevasectomy ejaculation that were recovered in the urine sample.

[c]The percentage of the estimated total number of spermatozoa displaced during the prevasectomy ejaculation and flushing of the vasa deferentia that were recovered in the urine sample.

Table 5. Volume and total number of spermatozoa per ejaculate and body weight of control and treated cats (Experiment 2)

	Volume (ml)		No. of spermatozoa (10^6)[a]		Body weight (kg)	
PVD	Controls	Treated	Controls	Treated	Controls	Treated
0	0.09±0.05	0.28±0.11	8.0500±8.8800	31.6534±37.9887	3.74±0.27	3.97±0.19
1	0.07±0.04	0.20±0.13	0.9713±1.7745	0.0303± 0.0598	--- ---	--- ---
7	0.10±0.05	0.21±0.10	0.0214±0.0197	0.0007± 0.0006	3.61±0.16	3.79±0.25
14	0.12±0.07	0.23±0.15	0.0059±0.0103	0 ---	3.68±0.19	3.73±0.29
21	0.14±0.08	0.18±0.09	0.0013±0.0015	0.0004± 0.0004	3.73±0.23	3.86±0.25
28	0.15±0.09	0.19±0.07	0.0004±0.0006	0.0002± 0.0004	3.81±0.14	3.89±0.45
35	0.09±0.01[+]	0.21±0.09	0.0003±0.0003	0 ---	4.10±0.06	4.23±0.30
42	0.11±0.02	0.23±0.10	0.0002±0.0003	0 ---	4.03±0.09	4.22±0.27
49	0.14±0.04	0.21±0.10	0 ---	0.0003± 0.0006	3.89±0.06	4.04±0.50
56	0.14±0.10	0.23±0.10	0 ---	0 ---	4.01±0.10	4.05±0.42
63	0.13±0.05	0.25±0.09	0.0003±0.0006	0 ---	4.16±0.17	4.17±0.26
P						
Treatment effect		NS[b]		ND[c]		NS
Day effect		NS		ND		<0.05
Interaction		NS		ND		NS

[a]Total number includes intact spermatozoa and detached spermatozoal heads. Data are expressed as mean±SD, n=4 cats for each mean, except where indicated[+], n=3 due to urine contamination of the ejaculate of 1 cat.

[b]NS = Not significant (P>0.05).

[c]ND = Not determined.

Table 6. Spermatozoal motility and number of intact spermatozoa and detached spermatozoal heads (10^6) in the postvasectomy ejaculates of treated cats (Experiment 2)

	No. 5			No. 6			No. 7			No. 8		
PVD	Intact	Motility	Heads	Intact	Motility	Heads	Intact	Motility	Heads	Intact	Motility	Heads
1[a]	0.0007	Yes	0	0.0600	No	0.0600	0	---	0	0.0006	No	0
7	0	---	0	0	---	0.0015	0	---	0.0005	0	---	0.0008
14	0	---	0	0	---	0	0	---	0	0	---	0
21	0	---	0	0	---	0.0008	0	---	0.0007	0	---	0
28	0	---	0	0	---	0	0	---	0.0008	0	---	0
35	0	---	0	0	---	0	0	---	0	0	---	0
42	0	---	0	0	---	0	0	---	0	0	---	0
49	0	---	0	0	---	0.0011	0	---	0	0	---	0
56	0	---	0	0	---	0	0	---	0	0	---	0
63	0	---	0	0	---	0	0	---	0	0	---	0

[a]The first postvasectomy ejaculate was obtained immediately after vasectomy and flushing of the vasa deferentia.

Table 7. Spermatozoal motility and number of intact spermatozoa and detached spermatozoal heads (10^6) in the postvasectomy ejaculates of control cats (Experiment 2)

	No. 1			No. 2			No. 3			No. 4		
PVD	Intact	Motility	Heads	Intact	Motility	Heads	Intact	Motility	Heads	Intact	Motility	Heads
1[a]	0.2000	Yes	0	0.0035	Yes	0.0014	3.6300	No	0	0.0500	No	0.0001
7	0.0471	Yes	0.0007	0	---	0	0.0171	Yes	0.0018	0.0188	No	0
14	0.0158	Yes	0.0054	0	---	0	0	---	0	0.0025	No	0
21	0.0019	No	0.0010	0	---	0	0	---	0	0.0022	No	0
28	0	---	0.0005	0	---	0	0	---	0	0.0012	No	0
35	0	---	0	0	---	0.0005	0	---	0	0	---	0.0005
42	0	---	0	0	---	0	0	---	0.0006	0	---	0
49	0	---	0	0	---	0	0	---	0	0	---	0
56	0	---	0	0	---	0	0	---	0	0	---	0
63	0	---	0	0	---	0	0	---	0	0	---	0.0011

[a]The first postvasectomy ejaculate was obtained immediately after vasectomy.

Table 8. Total number of spermatozoa in the prevasectomy ejaculate, in the first postvasectomy ejaculate, and in the urine obtained by cystocentesis immediately after vasectomy or vasectomy followed by flushing the vasa deferentia of the domestic cat (Experiment 2)

Controls					Treated				
	Total No. spermatozoa (10^6)					Total No. spermatozoa (10^6)			
	Ejaculate					Ejaculate			
Cat No.	Pre-vasectomy	First post-vasectomy	Urine	% of total[a]	Cat No.	Pre-vasectomy	First post-vasectomy	Urine	% of total[b]
1	18.00	0.20	37.80	67.7	5	77.90	0.0007	18.33	19.0
2	0.09	0.01	0.20	69.0	6	0.41	0.1200	94.70[c]	99.6
3	13.05	3.63	52.00	79.9	7	0.68	0	77.05	99.1
4	1.05	0.05	---[d]	---	8	47.63	0.0006	5.60	10.5
Mean±SD	8.05±8.88	0.97±1.77	30.00±26.77	72.20±6.70		31.66±37.99	0.03±0.06	48.92±43.59	57.05±48.97

[a]The percentage of the total number of spermatozoa displaced during the prevasectomy electroejaculation that were recovered in the urine.

[b]The percentage of the total number of spermatozoa displaced during the prevasectomy ejaculation and flushing of the vasa deferentia that were recovered in the urine.

[c]Includes 11.00×10^6 spermatozoa that were estimated to be in the urine recovered from the penis after flushing of the vasa deferentia; the color of urine was blue.

[d]Urine sample could not be obtained by cystocentesis.

FIGURES

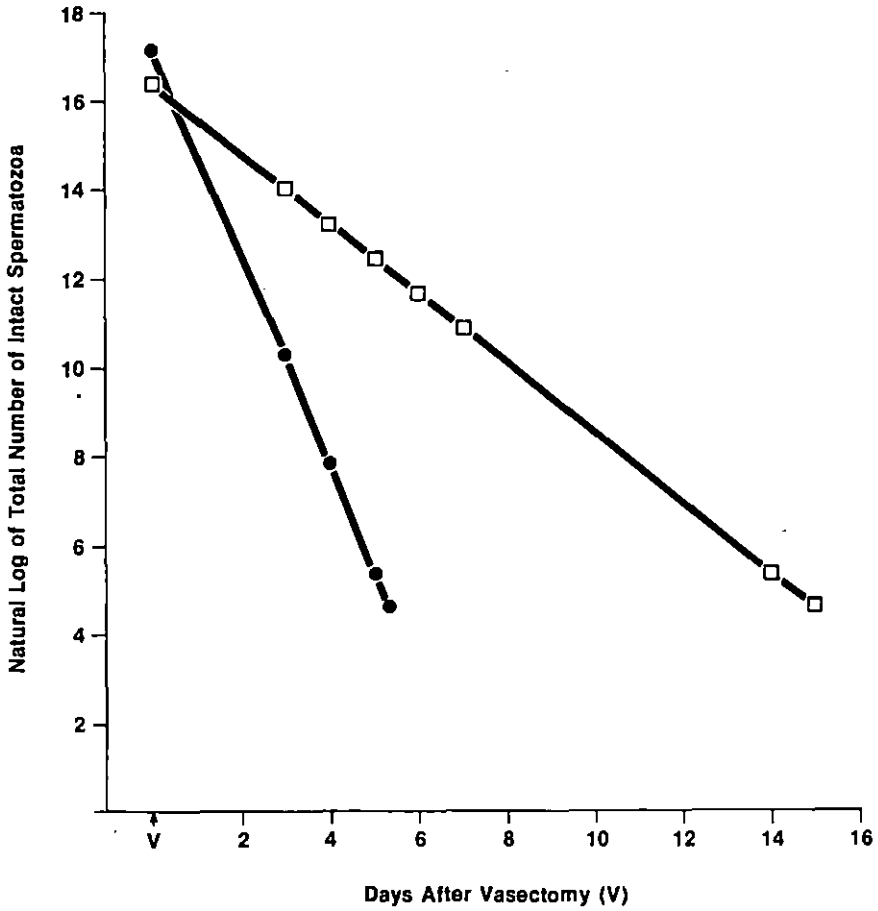

Figure 1. Regression lines for the rate of clearance of intact spermatozoa from the ejaculates after vasectomy of control □——□ and treated ●——● dogs, experiment 1

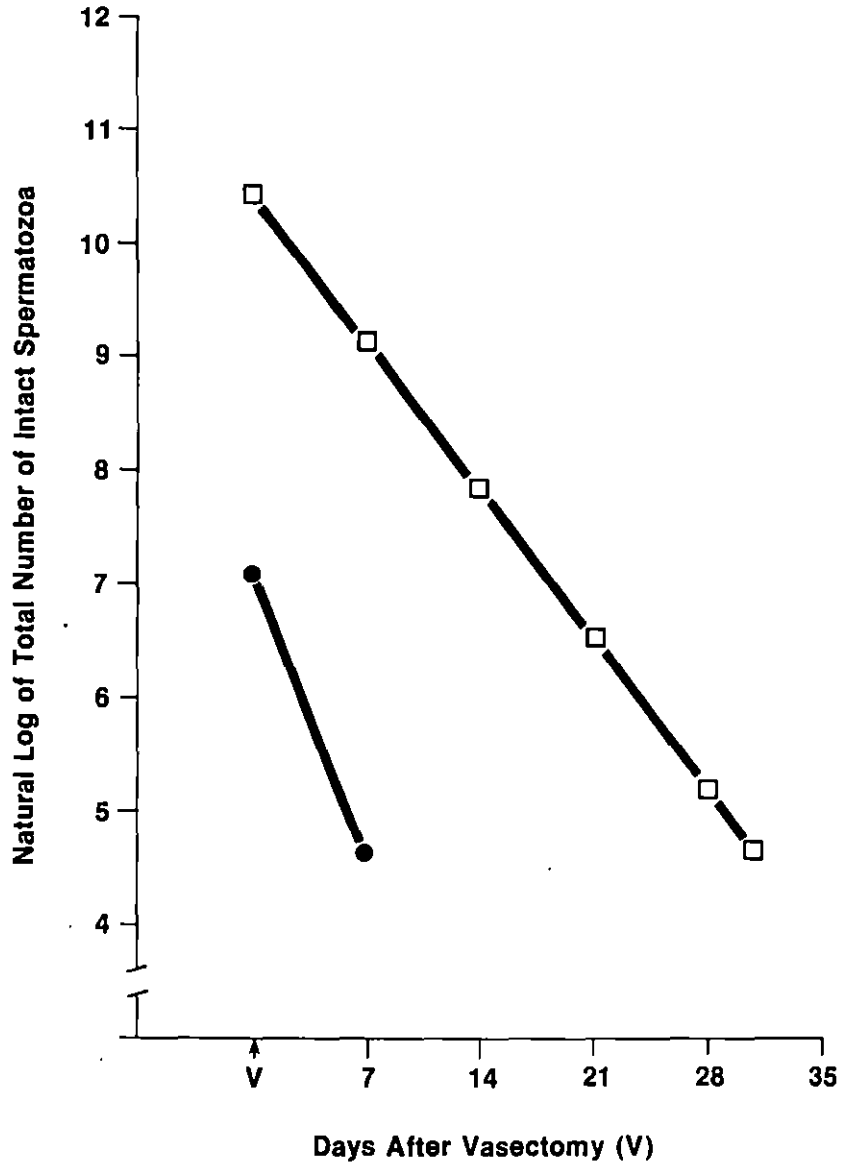

Figure 2. Regression lines for the rate of clearance of intact spermatozoa from the ejaculates after vasectomy of control □——□ and treated ●——● cats, experiment 2. Only 2 points are shown for the regression line of the treated cats because these cats had no intact spermatozoa in their 2nd postvasectomy ejaculates

DISCUSSION

The volume of ejaculate from dogs and toms was not affected by treatment or day of collection. There was no significant interaction treatment X day of collection. It was anticipated that the volume of the ejaculate would not change following vasectomy or vasectomy and flushing because the majority of the volume of the ejaculate in the dog[24] and the electroejaculate in the tom[23] is contributed by the accessory sex glands.

Flushing the vasa deferentia at the time of vasectomy rapidly reduced to zero the number of intact, motile or nonmotile spermatozoa in the postvasectomy ejaculates of dogs and toms and considerably shortened the time from vasectomy to azoospermia for the dog but not for the tom. The persistence of spermatozoa or spermatozoal heads in the ejaculate confirms prior findings[23,24] in that a prolonged postvasectomy period is required to clear spermatozoa from the ejaculate in these two species. It is likely that dogs and toms producing ejaculates containing only spermatozoal remnants are sterile and that utilization of dogs on postvasectomy day 6 and toms on postvasectomy day 7 would not result in pregnancies.

No consistent relationship was observed between the number of spermatozoa in the prevasectomy ejaculate and the time of the first azoospermic ejaculate. Dogs and toms with

relatively low numbers of spermatozoa in their prevasectomy ejaculate took as long, or in some cases longer, to produce an azoospermic ejaculate than dogs and toms with high numbers of spermatozoa in their prevasectomy ejaculate. Treated toms, whose vasa deferentia were flushed at the time of vasectomy, continued to have loose heads in their postvasectomy ejaculates for as long as 49 days after vasectomy. Production of earlier azoospermic ejaculates by the dog than by the tom may have been due to several factors: 1) spermatozoa and loose heads may be more tightly bound in the vasa deferentia of the tom; 2) spermatozoa and loose heads may be present in other areas of the excurrent ducts in the tom, such as the bulbourethral glands, which may not have been reached by the flushing fluid, although reported data on the presence of spermatozoa in the bulbourethral glands of toms could not be found; or 3) it may be the result of the method of seminal collection used, which may induce varying degrees of stimulation and contraction of the excurrent ducts. Possibly electroejaculation did not induce in toms as much stimulation and contraction of the musculature of the vasa deferentia as was produced in dogs when ejaculation was induced using digital manipulation.

Studies performed in the rabbit[26] indicate that radiopaque contrast media directed distally toward the urethra during injection at the vasal-epididymal junction,

initially moved in a proximal direction toward the testes until all the contrast media was contained within the cauda epididymis. This contrast media was then slowly removed from the vas, toward the urethra, during sexual rest, or rapidly removed from the vas during ejaculation. Earlier studies in the rabbit[25] indicate that during sexual rest, spermatozoa are transported into and through the vasa deferentia and that the vasa deferentia maintained a decreasing spermatozoal gradient toward the urethra. Nonejaculatory displacement of vasal spermatozoa toward the urethra has also been reported for the ram[3]. These studies[3,25,26] would suggest that spermatozoa remaining in the vasa deferentia immediately after ejaculation would be slowly eliminated via the urethra during sexual rest. If postvasectomy transport of spermatozoa through the vasa deferentia occurs in this manner in the dog and tom after vasectomy then a slow release of spermatozoa resulting in a prolonged period of time from vasectomy to azoospermia would be expected. This appears to be the case for control dogs and toms in this study, and is consistent with previous reports[23,24].

In dogs, the trend for an effect of treatment and for the interaction of treatment X day of collection was probably due to the assignment of heavier dogs to the treatment group by random chance. The significant effect of day of collection on body weight in toms appeared to be the result

of weight loss in toms after surgery, followed by recovery from surgery and normal growth. This assumption is supported by the lack of effect of treatment and interaction of treatment X day of collection.

A spermatozoal load of 47 $\pm$ 9 X 10^6 (mean $\pm$ SEM) has been reported[17] in the vasa deferentia of the dog. The spermatozoal load of the vasa deferentia of the tom has not been reported. The mean cumulative total number of spermatozoa in the postvasectomy ejaculate of dogs and toms calculated from data in previous studies was 2.63 $\pm$ 1.2 X 10^6 for the dog[24] (mean $\pm$ SD) and 0.25 $\pm$ 0.37 X 10^6 (mean $\pm$ SD) for the tom[23]. The mean cumulative total number of spermatozoa in the postvasectomy ejaculates for control dogs and toms in the present study was 5.50 $\pm$ 5.60 X 10^6 and 1.01 $\pm$ 1.78 X 10^6 (mean $\pm$ SD), respectively. The discrepancy between the mean cumulative total number of spermatozoa present in the postvasectomy ejaculate of dogs, calculated from this and the previous study[24] and the reported[17] spermatozoal load of the vasa deferentia in the dog could be due to loss of spermatozoa from the vasa deferentia into the urethra during the period between seminal collections, phagocytosis of spermatozoa by macrophages within the vasa deferentia[16], the site of ligation, ejaculation technique or other unforeseen factors. For these reasons, estimates of the spermatozoal load of the vasa deferentia based on the

calculated mean cumulative total number of spermatozoa in the postvasectomy ejaculates would be inaccurate.

Flushing of the vasa deferentia at the time of vasectomy was easy to perform and appears to be safe. No complications were observed in any of the treated dogs or toms following vasectomy and flushing. This procedure may be useful in clinical practice to decrease the time from vasectomy until dogs and toms may be used safely as teasers or may be returned to the animal colony without fear of pregnancy resulting from fertile matings. This technique may have application to farm animals where teasers are routinely used for estrous detection and artificial insemination or to man where repeated seminal analysis in postvasectomized individuals are required for medico-legal reasons.

Spermatozoa remain in the excurrent ducts of the reproductive tract after vasectomy[2,7,9,10,13,23,24,29,31], vasal[19], or epididymal ligation[14,27]. As a result, some currently ill-defined interval must be maintained from the time the sterilization procedure is performed until the onset of azoospermia. Both the site of severance or occlusion and the spermatozoal load of the vasa deferentia may influence the rate of spermatozoal clearance in the dog and the tom. Flushing of the vasa deferentia at the time of vasectomy reduces the number of spermatozoa in the postvasectomy ejaculates and increases the rate of clearance of intact

spermatozoa from the ejaculate in dogs and cats.

Additional studies are needed to evaluate the fertilizing capability of spermatozoa present in post-vasectomy ejaculates in dogs and toms as well as in other species. The inclusion of spermicidal agents in the flushing fluid to augment the effect of flushing also needs investigation.

Due to the retrograde flow of the flushing fluid into the urinary bladder, instead of through the penile urethra, an accurate estimation of the total number of spermatozoa remaining in the excurrent tract after ejaculation could not be made in the present study.

The lack of fluid recovery from the distal end of the penile urethra and the recovery of blue tinted fluid via cystocentesis from the urinary bladder provides strong evidence that the path of least resistance for the exit of fluid flushed through the vasa deferentia in the anesthetized dog and tom is toward the urinary bladder.

The presence of spermatozoa in the urine of both treated and control dogs and toms was an unexpected finding. Assuming that these spermatozoa were not present in the urinary bladder of control dogs and toms before ejaculation, these results indicate that the retrograde flow of spermatozoa and seminal fluid had occurred during or shortly after ejaculation or electroejaculation. A study[6] was done

to confirm whether there was a retrograde flow of semen during electroejaculation in the ketamine-anesthetized tom. In that study[6], it was estimated that 39% to 94% of the spermatozoa displaced during electroejaculation retrograded into the urinary bladder.

In the dog, vasectomy does not have the advantages of orchiectomy in relation to desirable behavioral changes[21]. However, vasectomy may provide many dog owners with a method of sterilization more acceptable to them than orchiectomy.

In the cat, vasectomy may be a more effective method of population control than orchiectomy[23]. The queen is an induced ovulator[12]; stimulation from the male's penile spines and the frequency of mating regulates the time of ovulation and the number of oocytes released[12]. A queen which is not stimulated to ovulate will continue to have recurring periods of estrus[32]. The longer a queen is in estrus, the greater the chances of pregnancy through matings with fertile toms. Orchiectomy reduces libido and causes atrophy of the penile spines[1], which appear to be important components of the vaginal stimulation for ovulation. Vasectomized toms do not lose libido or have atrophy of penile spines and they mate readily with estrous queens[31]. Infertile matings with a vasectomized tom would induce ovulation and pseudopregnancy, which lasts, on the average, for 45 days[32]. Thus, the queen would be safe from becoming

pregnant during the period of pseudopregnancy. Mating with a vasectomized tom would effectively remove queens from the breeding population for an average of 45 days following each such mating and thus reduce the overall rate of pregnancy. Since vasectomized males are capable of repeated matings with a number of estrous queens, it is likely that the introduction of a large number of vasectomized males would have an impact on population control[22] in this species.

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