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Effect of xylazine on the retrograde flow of spermatozoa into the bladder and postvasectomy clearance of spermatozoa from electroejaculates of rams

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ABSTRACT

The effect of xylazine on the retrograde flow of spermatozoa into the urinary bladder of rams (Part I, Experiments 1, 2, and 3), and the effect of flushing the vasa deferentia at the time of vasectomy on the rate of clearance of spermatozoa from the electroejaculates of vasectomized rams (Part II, Experiment 4) were studied. In Experiment 1, the spermatozoal concentration, but not the volume or the total number of spermatozoa of the electroejaculate, was decreased (P=0.015) by treatment with xylazine before electroejaculation (EE). Administration of xylazine to rams before EE increased (P=0.017) the retrograde flow of spermatozoa into the bladder. Administration of xylazine to sexually rested rams induced urinary losses of spermatozoa that were not different (P>0.1) from those induced by EE or by giving xylazine before EE. In Experiment 2, treatment of sexually rested rams with xylazine decreased (P=0.0001) the volume of urine voided during micturition and the frequency of micturitions within 60 minutes of treatment. Spermatozoal concentration and total number of spermatozoa in the urine obtained during micturition were not different between control and xylazine-treated rams. In Experiment 3, urine was withdrawn from a catheter implanted in the urinary bladder. Xylazine increased (P=0.006) the total volume of urine obtained from the bladder within 60 minutes of treatment. Xylazine significantly increased the spermatozoal concentration (P=0.048) and total number of spermatozoa (P=0.009) in the urine obtained from the bladder of sexually rested rams. Results of Part I indicate that xylazine induced retrograde flow of spermatozoa into the urinary bladder when administered to sexually rested rams, and that administration of

xylazine to rams before EE increased the retrograde flow of spermatozoa into the bladder. Because of the magnitude of spermatozoal losses in urine induced by xylazine when given to sexually rested rams or when given to rams before EE, the use of xylazine as a sedative to restrain rams during EE is not recommended.

In Experiment 4, flushing the vasa deferentia at the time of vasectomy reduced to zero the number of viable, motile spermatozoa in the electroejaculates by post-vasectomy day 7, whereas the number of viable and motile spermatozoa was reduced to zero by post-vasectomy day 14 for the control rams, which were vasectomized but not flushed. The total number of spermatozoa and number of intact spermatozoa in the electroejaculates were significantly less for the treated than for the control rams on post-vasectomy day 3 ($P<0.0001$) and again on post-vasectomy day 7 ($P<0.002$). However, none of the rams from either treatment group produced two consecutive azoospermic electroejaculates during the experimental period (up to post-vasectomy day 63). Flushing the vasa deferentia at the time of vasectomy may have clinical applications since this technique resulted in the obtainment of electroejaculates devoid of viable and motile spermatozoa one week earlier than vasectomy alone.

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INTRODUCTION

For the purpose of presentation of the results of studies performed for the Master of Science degree, this Thesis is divided into two parts.

In Part I, the results of three experiments (Experiments 1, 2, and 3) conducted to determine effects of xylazine on spermatozoal displacement and retrograde flow of spermatozoa into the urinary bladder of rams are described. Experiment 1 was performed to determine whether administration of xylazine to rams before seminal collection affected spermatozoal displacement during electroejaculation. In subsequent experiments, xylazine was administered to sexually rested rams and the magnitude of urinary spermatozoal losses was determined for urine obtained during micturition (Experiment 2) and for urine obtained from a catheter implanted in the urinary bladder (Experiment 3).

In Part II, the rate of clearance of spermatozoa from the ejaculates of vasectomized rams was determined. Experiment 4 was designed to determine the effects of flushing the vasa deferentia at the time of vasectomy on the rate of spermatozoal clearance in rams and on the viability of spermatozoa in post-vasectomy ejaculates.

PART I. EFFECT OF XYLAZINE ON THE RETROGRADE FLOW OF SPERMATOZOA INTO THE URINARY BLADDER OF RAMS

It is now evident that retrograde flow of spermatozoa into the urinary bladder occurs in several animal species when semen is collected with an artificial vagina (AV), during electroejaculation (EE), or even during mating. Urinary losses caused by the retrograde flow of spermatozoa into the bladder decrease the number of spermatozoa in the ejaculate. Thus, retrograde flow of spermatozoa into the bladder during seminal collection should be evaluated as a component of the breeding soundness examination of rams and other domestic males. Sires releasing oligospermic ejaculates are often culled. However, retrograde flow of spermatozoa into the bladder could be a temporary condition and, therefore, its magnitude should be determined at each breeding soundness examination, particularly for genetically valuable males.

Xylazine is often used as a sedative in clinical practice in Veterinary Medicine to restrain animals. Tranquilizers have been used to restrain unmanageable bulls during EE, and xylazine may, under certain conditions, be used for the same purpose in rams. However, xylazine may affect the electroejaculatory response in rams, because it has been shown that xylazine induces retrograde flow of spermatozoa into the bladder of dogs (1).

Part I of this study was designed to determine whether xylazine: a) affects the electroejaculatory response of rams and b) induces retrograde displacement of spermatozoa from their sites of storage in the epididymides and vasa deferentia to the urethra and from the urethra into the urinary bladder of sexually rested rams.

Literature Review

For all the domestic species studied to date, the daily sperm output (DSO) is lower (2-5) than the daily sperm production (DSP). This discrepancy has been attributed to losses of spermatozoa in the equipment used for seminal collection (2), overestimation of the DSP (2,3), or phagocytosis and reabsorption of spermatozoa in the excurrent ducts (6-8). Although phagocytosis of spermatozoa by leukocytes has been observed (9) in the excurrent ducts after vasectomy, studies on laboratory rodents (9) did not support the reabsorption hypothesis. For the ram, the DSP per gram of testis was estimated (5) as $25 \pm 2 \times 10^6$ spermatozoa, whereas the DSO per gram of testis was only $7 \pm 1 \times 10^6$ spermatozoa. The mean daily sperm output estimated (10) from the number of spermatozoa eliminated daily in the urine of sexually rested rams was 96.6% of the estimated daily sperm production.

Urinary losses of spermatozoa have been long known to occur in rams (10-16), bulls (6), rabbits (17), rats (18), and men (19,20), and the number of spermatozoa voided in the urine decreased during periods of frequent ejaculations in rams (11,16), bulls (6) and rabbits (17). Spermatozoa were observed (16) in the urine obtained from a catheter implanted in the bladder of two rams when semen was collected with an AV. Studies performed to determine spermatozoal losses in the urine have either failed to recognize the retrograde flow of spermatozoa into the urinary bladder and attributed (2,3,6,10-13,17-21) the losses to the washing out by the urine of spermatozoa left in the urethra during ejaculation or between ejaculations, or did not determine for each animal the magnitude of the

ejaculatory retrograde flow of spermatozoa (14-16). Spermatozoa have been recovered from the urinary bladder of rams during sexual rest (14-16), before EE (22,23), and before seminal collection with an AV (23). Spermatozoal losses due to retrograde flow of spermatozoa into the urinary bladder occur during EE in anesthetized cats (24-26), lion-tailed macaques (27) and gorillas (28), during EE in non-anesthetized bulls (29) and rams (22,23), during collection of semen with an AV in rams (23) and cats (26), during collection of semen by digital manipulation of the penis in dogs (1,25), and during natural mating in cats (26). Retrograde ejaculation, with no antegrade displacement of spermatozoa, has been reported in a dog (30) and in men (31-34).

In the absence of ejaculation, daily produced spermatozoa are displaced from the caudae epididymides and vasa deferentia into the pelvic urethra (3,8). In rabbits (35,36) and rams (7,14-16), there is a constant transport of spermatozoa through the vasa deferentia during and between ejaculations. Contractility of the caudae epididymides and vasa deferentia was shown during seminal emission in rats (37). In rabbits (35,36) the vasa deferentia participates actively in establishing a decreasing gradient of spermatozoal concentration toward the distal (urethral) end of the vas deferens during sexual rest. Radiopaque dye injected into the epididymis of rabbits (36) moved slowly during sexual rest until reaching the urethra, but it was transported faster from the epididymis into the vas during sexual arousal. During sexual rest that followed sexual stimulation, with or without ejaculation, the radiopaque dye in the vas was displaced back into the epididymis (36). Contractility of the vasa deferentia and

epididymides of rams (38) can be initiated by stimuli elicited during penile intromission, by increased intraluminal pressure in the vasa, or by administration of oxytocin, vasopressin, or epinephrine. The contractile effect on the vasa deferentia and epididymides (38) was more intense for oxytocin and epinephrine than for vasopressin, and more prolonged for oxytocin and vasopressin than for epinephrine. Oxytocin, but not epinephrine, increased (39) the spermatozoal output of rams when semen was collected with an AV.

The estimated resistance opposed by the pars spongiosa of the urethra of rams to fluid displacement (16) was relatively high (550 to 1740 mm Hg). In anesthetized rats (40), micturition occurred when the intravesicular pressure was increased by saline infusion to 20 mm Hg to overcome the urethral resistance to outflow. Fluid flushed through the vasa deferentia of anesthetized dogs and cats at the time of vasectomy (25) flowed into the urinary bladder. Thus, flow of spermatozoa, and possibly other seminal components, into the urinary bladder during and between ejaculations may follow a pathway of least resistance (22,25).

Retrograde flow of fluid into the bladder was observed when radiopaque fluid was injected into the scrotal vas deferens of marsupials (41) or men (42). Radiopaque fluid flowed into the bladder of anesthetized rams (43,44) when the fluid was deposited directly into the urethra or when the volume of the radiopaque fluid infused into the scrotal vas deferens was sufficient to fill the vas and spill into the pelvic urethra. Radiopaque fluid infused into the scrotal vas deferens of anesthetized rams was also displaced into the urethra during electrical stimulation with an

electroejaculator, and a portion of the radiopaque fluid flowed into the bladder (43,44). These results, again, suggest that the pathway of least resistance is for the retrograde flow of spermatozoa and possibly seminal fluid into the bladder. Furthermore, retrograde flow of spermatozoa into the bladder in sexually rested animals or between ejaculations may be a physiological mechanism to discard aged spermatozoa (22,25).

The percentage of retrograde flow, defined as the percentage of the total number of spermatozoa displaced from the sites of storage in the epididymides and vasa deferentia during ejaculation or EE that flowed into the urinary bladder, varies among animals and method of seminal collection (1,22-27,29). The overall mean percentages of retrograde flow during EE in rams (22) were 28.3 % and 20.1 % for the nonbreeding and breeding seasons, respectively. The percentage of retrograde flow of spermatozoa into the bladder of rams (23) during the nonbreeding season tended to be smaller when semen was collected with an AV (overall mean ± SD = 2.7 ± 4.8 %) than when semen was collected by EE (15.3 ± 29.9 %) from the same rams.

The spermatozoal concentration in sequential samples of urine collected during the first micturition after electroejaculation of bulls (29) and rams (22,23), or in sequential samples of urine obtained by cystocentesis from dogs after ejaculation (1) was not different between consecutive samples, indicating that the spermatozoa had mixed with the urine in the bladder before micturition or cystocentesis. This offers a non-invasive approach to determine the percentage of retrograde flow of spermatozoa into the bladder during the preceding ejaculation (22,23,29). Administration of furosemide to rams, prior to seminal collection with an

AV, did not affect the ejaculatory response, volume of ejaculate and total number of spermatozoa in the ejaculate, total number of spermatozoa in the post-ejaculation urine, or the percentage of retrograde flow of spermatozoa into the bladder (23). Thus, furosemide can be used to induce micturition to facilitate the collection of sequential samples of urine as a non-invasive approach to determine the percentage of retrograde flow in rams and possibly other species. Furosemide exerts its diuretic effect through inhibition of reabsorption of sodium chloride in the ascending limb of Henle's loop of the nephron (45). An additional experimental or clinical advantage for the use of furosemide is that the motility of spermatozoa in the urine may be prolonged (23) because spermatozoa are exposed to a less concentrated urine when furosemide is used. The motility of spermatozoa in samples of human ejaculates ceased when samples were mixed with fresh human urine (46). Neutralization of urinary pH did not protect the spermatozoa from this effect unless the osmolarity of the urine was also isotonically adjusted (46).

Marked differences in the spermatozoal output among animals of the same species may be conditioned by a variable retrograde flow of spermatozoa into the bladder during seminal collection (22). Because the spermatozoal concentration in the urine collected during sexual rest or before ejaculation in bulls (29), cats (26), dogs (1), rams (22,23), and possibly other species is negligible or the urine is azoospermic, the determination of the total number of spermatozoa in the ejaculate and in the urine recovered immediately after ejaculation should provide an accurate (1,22) estimation of the total number of spermatozoa displaced

during the preceding ejaculation. This in turn would provide a non-invasive means to monitor DSP over extended periods of time.

Administration of xylazine to sexually rested dogs induced retrograde flow of spermatozoa into the urinary bladder (1). This finding indicates that spermatozoal displacement and retrograde flow of spermatozoa into the urinary bladder can be induced pharmacologically in the absence of sexual stimulation or ejaculation. In addition, xylazine induced ejaculation ex copula in horses with or without sexual pre-stimulation in 39% or 14% of the trials performed, respectively (47). Xylazine is an α_2-adrenoreceptor agonist that depresses the CNS in dogs (48) and sheep (49,50). Alpha$_2$-adrenoreceptor agonists induce diuresis by reversing vasopressin-induced water retention (51). It has not been determined whether the diuretic effect of α_2-adrenoreceptor agonists is exerted centrally through inhibition of release of vasopressin or peripherally through a direct antagonism of the renal actions of vasopressin (51). Xylazine induced a diuresis that lasted for 5 hours in cattle, and the effect was greater during the first 2 hours after xylazine was given (52). Peripherally, xylazine decreases urethral pressure and resistance to flow of urine in male and female dogs, without altering intravesicular pressure (53).

Alpha-adrenoreceptors mediate the response to adrenergic agonists for the contraction of the vas deferens (54-56), whereas β-adrenoreceptors are thought to mediate relaxation (56). Mediated by α_2-adrenoreceptors, xylazine induced a pre-synaptic inhibition of the contractile response elicited by neural stimulation of the isolated vas deferens of rats, whereas α_1-adrenoreceptor agonists potentiated the pre-existing, neurally

stimulated contraction (54). Alpha-adrenoreceptors are thought (31) to participate in the contraction of the trigone and sphincter of the urinary bladder during ejaculation. Beta-adrenoreceptors predominate (57) in the body of the bladder of 3 to 4 month old male lambs, whereas α_1-adrenoreceptors predominate in the caudal portion of the bladder (bladder base). During sympathetic stimulation, the bladder base of sheep constricts by activation of the α_1-receptors and the detrusor muscle relaxes by activation of β_1 and β_2-receptors, contributing to the filling of the bladder during the storage phase of micturition (57). In horses (58), α_1-adrenoreceptors mediate the contraction of the urethra, α_1- and α_2-adrenoreceptors mediate prostatic contractions, and β_2-adrenoreceptors mediate the relaxation of both organs. In men (59), α-adrenergic agonists have been used to prevent retrograde ejaculation. The combined treatment with a β-adrenoreceptor antagonist and an α-adrenoreceptor agonist increased spermatozoal output in dogs (60) and horses (61). Tolazoline, an α_2-adrenoreceptor antagonist, reverses the xylazine-induced bradycardia, tachypnea and CNS depression in sheep (49,50).

PART II. CLEARANCE OF SPERMATOZOA FROM ELECTROEJACULATES OF VASECTOMIZED RAMS AFTER FLUSHING THE VASA DEFERENTIA AT THE TIME OF VASECTOMY

Vasectomized rams are commonly used at the beginning of the breeding season to hasten and synchronize the onset of estrous cyclicity of ewes. Vasectomized rams are also used as teasers to detect estrous ewes during the breeding season for artificial insemination, to carry out programs of controlled mating, and to evaluate the pregnancy rate by detecting estrous ewes after artificial insemination or after the breeding ram has been removed from the flock.

To my knowledge, there are no reported studies that have critically evaluated the time required from vasectomy to the obtainment of azoospermic ejaculates in rams. Spermatozoa have been found in ram ejaculates for as long as 430 days after vasectomy (62). The long and uncertain time lag required after vasectomy for the safe use of vasectomized rams as teasers is conducive to economic losses for the sheep producer and has legal implications for the veterinary practitioner.

This study was designed to determine: a) the time after vasectomy required for the clearance of spermatozoa from the ejaculate, b) whether flushing the vasa deferentia at the time of vasectomy shortens the time from vasectomy to the obtainment of azoospermic ejaculates, and c) the viability of spermatozoa in the ejaculates of vasectomized rams during the period of spermatozoal clearance.

Literature Review

The time elapsed from vasectomy to azoospermia has not been critically determined for the ram. Scattered reports recommend to avoid

the use of rams for matings for 14 days after vasectomy (63) and 30 days after removal of the caudae epididymides (64). However, nonmotile, morphologically normal spermatozoa have been found in ram ejaculates up to 430 days after vasectomy (62). In addition, a claim has been made (65) that rams should be ejaculated at least 3 times after vasectomy to reduce the number of spermatozoa in the ejaculate to "the low levels normally associated with sterility".

The time elapsed from vasectomy to azoospermia (25,66,67) is influenced by the species, variation among animals, and likely by the frequency of seminal collection after vasectomy. Ejaculates from vasectomized dogs (67,68), cats (66,68), rams (62,65), llamas (69), and men (70-73), or ejaculates from epididymally ligated rams (7) and bulls (74,75) contained spermatozoa for variable, but prolonged periods of time after bilateral vasectomy or epididymal ligation. Spermatozoa have been observed in ejaculates of vasectomized men for up to 1 year after vasectomy (70), and in ejaculates of vasectomized rams 430 days after vasectomy (62).

The site of vasectomy (66-68) seems to influence the rate of clearance of spermatozoa from ejaculates of dogs and cats, because the interval from vasectomy to azoospermia appears to shorten (68) as the site of severance or occlusion approaches the urethral end of the vasa deferentia. It has been reported (62) that the well developed ampulla of the vas deferens of the ram is a major site of storage for spermatozoa after vasectomy. However, studies on the time required after vasectomy to clear the ejaculates from spermatozoa in dogs (67,68) and cats (66,68) indicate that the vasa deferentia are minor sites of storage of

spermatozoa, whereas the caudae epididymides are the major source of ejaculated spermatozoa.

The number of spermatozoa released from the vas deferens after vasectomy is likely influenced by the spermatozoal load in the vas deferens at the time of vasectomy. In sexually rested rabbits, there was a decreasing gradient in the spermatozoal content of the vas deferens toward the distal, urethral end of the vas (35). Sexual stimulation without ejaculation increased the number of spermatozoa in the vas deferens, particularly in the abdominal portion of the vas (35). However, immediately after ejaculation, the vas deferens of the rabbit contained fewer spermatozoa than during sexual rest or sexual stimulation, and the number of spermatozoa of the abdominal portion of the vas was similar to that of the scrotal portion (35).

Motile spermatozoa were found in human ejaculates up to the 15th day post-vasectomy in a study (71) in which the frequency of ejaculation was at least 1 ejaculation every 8 days. Spermatozoa capable of fertilization, as assessed by the zona-free hamster egg test, were recovered in human ejaculates as long as 12 days after vasectomy (76). Motility of ram spermatozoa, estimated by the swirling of spermatozoa in whole samples of ejaculates, declined to zero by the third post-vasectomy ejaculate, independently of whether it was collected from 7 hours to 6 days after vasectomy (65). Spermatozoa obtained from the cauda epididymis and vas deferens of rams 60 days after ligation of the corpus-cauda epididymal junction were found to be motile (7).

Flushing the vasa deferentia at the time of vasectomy reduced to zero

the number of intact spermatozoa in the ejaculate by postvasectomy day 6 in the dog (25) and by postvasectomy day 7 in the cat (25), whereas the vasectomized, but non-flushed controls still had intact spermatozoa in their ejaculates by day 14 and detached spermatozoal heads by day 35 in the dog, and intact spermatozoa by day 28 and detached spermatozoal heads by day 63 in the cat. In view of these results (25), it is possible that the time elapsed from vasectomy to azoospermia could be shortened in the ram by flushing the vasa deferentia at the time of vasectomy. Spermatozoa were found in smears of centrifuged samples of human ejaculates 15 weeks after flushing the vasa deferentia at the time of vasectomy (77). These results (25,77) suggest that anatomical differences among species might influence the time of clearance of spermatozoa from the ejaculates because, unlike the vasa deferentia of dogs and cats, the vasa of men and rams have well developed ampullae.

MATERIALS AND METHODS

PART I

Experiment 1. Effect of Xylazine on the Retrograde Flow of Spermatozoa into the Urinary Bladder of Rams during Electroejaculation

Twelve adult rams, including nine western range and three polypay (average body weight ± SD = 90 ± 17 kg) were used to determine whether xylazine: a) affects the electroejaculatory response, and b) induces retrograde flow of spermatozoa into the bladder of sexually rested rams. Rams were quarantined for four weeks, sheared, and treated for external and internal parasites. Rams were fed alfalfa hay supplemented with a mixture of crushed grains. Rams were randomly assigned in a 3 x 4 factorial design, with rams nested within weeks, to three groups of four rams each, as shown in Table 1: Group 1, electroejaculation; Group 2, treatment with xylazine followed by electroejaculation; and Group 3, treatment with xylazine alone. Treatments were replicated four times, once each week.

Table 1. Experimental design (Experiment 1)

	Treatment groups		
Week	Electroejaculation	Xylazine plus electroejaculation	Xylazine
1	4 Rams	4 Rams	4 Rams
2	↓	↓	↓
3	↓	↓	↓
4	↓	↓	↓

The following end points were determined for the electroejaculates obtained from rams of Group 1 and 2: volume (ml), spermatozoal concentration (10^6/ml), and total number of spermatozoa (10^6). The end points determined for the urine obtained from rams of the three treatment groups during the first post-treatment micturition were: volume (ml), spermatozoal concentration (10^6/ml), total number of spermatozoa (10^6), percentage of viable spermatozoa, and number of urine samples containing motile spermatozoa.

Semen was collected from each ram of Groups 1 and 2 using an electrojaculator developed in our laboratory (78). The electroejaculator has an adjustable signal generator to control and monitor the slope, voltage, and duration of each electrical stimulus, and the interval between stimuli. The protocol for electroejaculation (22) was as follows: a commercially available rectal probe (Standard Precision Electronics Inc, Denver, Colo.) was used to apply electrical stimuli to the rams. Rams were confined in a crate and remained standing during seminal collection. Ejaculation was induced by giving each ram a set of five series of 10 electrical stimuli each, with a sinusoidal wave form at 30 Hz. The first series of stimuli was given at 2 V (46 to 90 mA), the second series at 3 V (50 to 130 mA), the third series at 4 V (50 to 170 mA), the fourth series at 5 V (60 to 190 mA), and the fifth series at 6 V (60 to 200 mA). For the 50 electrical stimuli, the interval between the onset of consecutive electrical stimuli was 10 seconds, the slope of stimulus application was 1.5 seconds, and the duration of each stimulus, from 0 V to the selected peak voltage and back to 0 V was 5 seconds. The seminal fluid and

spermatozoa obtained with the 50 electrical stimuli was considered an electroejaculate. The volume of electroejaculate was measured in graduated tubes and the concentration of spermatozoa was determined by hemacytometric counts. The total number of spermatozoa for each electroejaculate was estimated by multiplying the volume by the concentration.

Urine was collected once a week from each ram during micturition. To facilitate urine collection, micturition was induced in each ram of Groups 1, 2, and 3 by an intramuscular (IM) injection of a diuretic (250 mg of furosemide). Rams of Group 1 were given furosemide immediately after EE. Rams of Group 2 received an IM injection of a solution of 20 mg/ml of xylazine at a dose of 0.4 mg/kg of body weight and EE was initiated 15 minutes after xylazine injection. Furosemide was given to rams of Group 2 immediately after EE, approximately 23 minutes after xylazine injection. Rams of Group 3 were not electroejaculated and received an IM injection of xylazine at the same dosage used for the rams of Group 2. To equate time of treatment between groups, furosemide was given 23 minutes after injection of xylazine to rams of Group 3.

On the basis of previous observations, it was anticipated that some rams would become recumbent after injection of xylazine. Tolazoline, an α_2-adrenergic antagonist, was given intravenously at the dose of 1 mg/kg of body weight to prevent or reverse xylazine-induced recumbency in order to facilitate the collection of semen and/or urine from rams of Groups 2 and 3. However, to equate treatments among groups, each ram of Group 1 also received an intravenous injection of tolazoline. The injection of tolazoline was given approximately 1 minute after the injection of

furosemide for rams of Groups 1, 2, and 3, except for 3 of the 4 rams of Group 2 that became recumbent before or during EE in 12 of the 16 seminal collections. In those cases, tolazoline was given at the time of recumbency, so that EE could be performed while rams were standing in the crate, and furosemide was then given immediately after EE. Time of injection of drugs and reaction of rams were recorded.

The post-treatment urine, which consisted of the urine voided during the first micturition after furosemide injection, was collected in plastic, 600 ml cups placed in a wooden support held under the prepuce, while the ram was urinating. The time elapsed from furosemide injection to micturition was recorded. Immediately after micturition, the post-treatment urine was mixed and a sample was taken and immediately examined for motile spermatozoa. The sample was placed on a microscope slide, previously warmed to 37 °C on a slide warmer, and the preparation was observed at a magnification of 400X. Spermatozoa displaying independent movement were considered motile. The time elapsed from urine collection to evaluation of spermatozoal motility was recorded.

Immediately after the examination for spermatozoal motility, an eosin B dye-exclusion assay for spermatozoal viability (79) was done for a sample of the post-treatment urine obtained from each ram. The sample was placed in a chamber made as follows: a 25 x 75 mm microscope slide was warmed to 37 °C on a slide warmer and an area of approximately 18 x 18 mm was ringed on the slide with liquid petrolatum. The petrolatum was applied through a 23-gauge needle attached to a syringe loaded with petrolatum. To facilitate the discharge of petrolatum, the needle was warmed briefly in a

flame. The protocol for the spermatozoal viability assay was as follows: 10 μl of urine were placed inside the ringed area of a warm slide (37 °C). The urine sample was then gently mixed with 10 μl of a warm (37 °C) buffered saline solution containing 0.15 % of eosin B, HEPES (0.015 M), NaCl (0.134 M), and KCl (0.005 M). The pH of this saline solution was previously adjusted to 7.4 with 1N NaOH before adding the eosin B. After mixing the eosin B buffered solution with the urine, the ringed area on the microscope slip was sealed with a 22 x 22 mm coverslip to prevent evaporation of the sample. The preparation was then incubated in the dark at room temperature (18 to 24 °C) for 15 minutes. To determine spermatozoal viability, the preparation was then examined at 400X with a light microscope, using oblique illumination and a didymium filter. The number of stained and unstained spermatozoa were counted for each preparation. When ≤ 200 cells were present, all the spermatozoa in the preparation were evaluated. The counting period lasted for up to 5 minutes after the incubation period. The percentage of unstained spermatozoa in the preparation was recorded and considered to represent the percentage of spermatozoal viability for the sample. To evaluate spermatozoal viability and spermatozoal motility within the viability preparation, motile and nonmotile spermatozoa which remained unstained during exposure to eosin B were considered viable. The number of samples containing spermatozoa displaying independent, either progressive or stationary, movement was determined.

The volume of urine collected during the first micturition after furosemide injection was measured in graduated cylinders. The

concentration of spermatozoa in urine was determined by placing a sample of undiluted urine in each of the two chambers of a hemacytometer and examining the preparation at 400X. If spermatozoa were not found in any of the 25 large squares of each chamber, the urine was considered to be azoospermic. The total number of spermatozoa in urine was estimated by multiplying the volume of urine collected by the concentration of spermatozoa in the urine. To determine the percentage of retrograde flow for rams of Groups 1 and 2, the number of spermatozoa displaced from storage sites in the epididymides and vasa deferentia was estimated by adding the total number of spermatozoa in the electroejaculate to the total number of spermatozoa in the urine. The percentage of retrograde flow was then calculated by dividing the total number of spermatozoa in the urine by the number of spermatozoa displaced.

Statistical analysis

Analysis of variance (ANOVA; 80,81) was used to determine the effect of treatment, week, and the interaction between treatment and week on the volume, spermatozoal concentration, and total number of spermatozoa in the electroejaculates; on the percentage of retrograde flow; and on the urine volume, spermatozoal concentration, total number of spermatozoa, and percentage of viable spermatozoa in the urine. For the ANOVA on percentage of retrograde flow and percentage of spermatozoal viability, percentages were transformed to the arcsin of the square root of 1/100 of the percentages. The sources of variation were: treatment, week, and interaction treatment x week. Ram within treatment was used as the error

term for treatment effect, and the general error was used as the error term for week effect and interaction treatment x week. Significance was established at $P \leq 0.05$. The conservative F value (82) was used to confirm significance for the effect of week and for the interaction treatment x week. χ^2 analysis (80) was used to determine the effect of treatment on the ratio: number of urine samples that had motile spermatozoa in samples of urine that had spermatozoa.

Experiment 2. Effect of Xylazine on the Retrograde Flow of Spermatozoa into the Urinary Bladder of Sexually Rested Rams

The results of Experiment 1 documented retrograde flow of spermatozoa into the bladder of rams after treatment with xylazine in the absence of ejaculation. Because of the experimental design and adjustments made in Experiment 1, a potentiating or interactive effect of tolazoline could not be ruled out. Thus, it became important to determine whether or not xylazine induces retrograde flow of spermatozoa into the bladder of sexually rested rams and gain information regarding ram variation in the retrograde flow of spermatozoa into the bladder in response to xylazine.

The twelve rams used in Experiment 1 were used again in Experiment 2, after a period of at least 14 days of rest between experiments. Rams were maintained as described in Experiment 1. Body weight was not determined for these rams at the beginning of the experiment. Rams were randomly assigned in a 2 x 3 split plot factorial design, with rams nested within weeks, to two treatment groups of 6 rams each: Group 1, control saline solution; Group 2, treatment with xylazine. The two treatments were

replicated three times, once each week. The major factors of the experimental design were two treatments and three weeks, with two subplots (pre- and post-treatment urine), as shown in Table 2.

The following end points were determined in the pre- and post-treatment urine: volume (ml), spermatozoal concentration (10^6/ml), total number of spermatozoa (10^6), percentage of viable spermatozoa, and number of urine samples containing motile spermatozoa. The time elapsed from treatment to the first post-treatment micturition was recorded. The number of post-treatment micturitions was determined as a complementary end point.

Pre- and post-treatment urine was collected from each ram, once a week for three weeks. To collect urine, all rams were placed in a home-made harness before injection of xylazine or saline to support the xylazine-treated rams as they became recumbent. A hole in the harness allowed the collection of urine from the preputial opening while the rams

Table 2. Experimental design (Experiment 2)

		Treatment groups	
Week	Urine sample	Saline	Xylazine
1	Pre	6 Rams ↓	6 Rams ↓
	Post		
2	Pre		
	Post		
3	Pre		
	Post		

were urinating. To facilitate the collection of urine, micturition was induced by treatment with furosemide. Each ram was given an IM injection of 250 mg of furosemide and the time of injection of furosemide was recorded. The pre-treatment urine consisted of the urine obtained from the first micturition after furosemide injection. The pre-treatment urine was collected in a 1200 ml plastic cup held in close apposition to the prepuce by elastic straps. These straps allowed for the placement or removal of the cup without disturbing the ram. The time of the pre-treatment micturition was recorded. Immediately after the pre-treatment micturition, control rams (Group 1) received an IM injection of 2 ml of 0.9 % physiologic saline solution, and treated rams (Group 2) were given an IM injection of a solution of 20 mg/ml xylazine, at a dose of 0.4 mg/kg of body weight. The time of injection of xylazine or the saline solution was recorded.

The first post-treatment micturition was defined as the earliest micturition occurring beyond 6 minutes after xylazine or saline injection, because the estimated half life of distribution of xylazine after IM injection in sheep was reported (83) to be 5.45 minutes. Time elapsed from treatment with saline or xylazine injection to the first post-treatment micturition was recorded. The post-treatment urine was collected in a 4 liter plastic container connected to a plastic funnel by a 1 m long, flexible plastic tube. The funnel was held in close apposition to the prepuce by elastic straps. In addition, and to satisfy the purposes of this experiment, the post-treatment urine consisted of all the urine voided during the micturition or micturitions that occurred within 60 minutes

after xylazine or saline injection, regardless the number of micturitions that occurred during that post-treatment period.

Immediately after the pre- and post-treatment urine were collected, viability of spermatozoa was determined in the pre- and post-treatment urine, respectively, using the eosin B dye-exclusion assay (79) as described for Experiment 1. However, because in Experiment 1 it was found that dead spermatozoa stained within the first 5 minutes of the 15 minute incubation time that was allowed, the incubation period was shortened to 5 minutes for Experiment 2, to minimize the potential toxic effects of exposure of ram spermatozoa to urine and eosin B. Thus, a complementary evaluation of motility for spermatozoa in urine without using any staining, as it was done for Experiment 1, was considered to be unnecessary. A filter system consisting of 1 or 2 cyan filters, instead of the didymium filter used for Experiment 1, was placed over the light source of the microscope to increase the color contrast of the preparation. The degree of color saturation of the cyan blue filter system was adjusted for each preparation by placing either 1 or 2 filters, such that the red background produced by eosin B was neutralized to clearly differentiate stained or unstained spermatozoa. The number of urine samples containing spermatozoa displaying independent, either progressive or stationary, movement was determined as described for Experiment 1. The volume, spermatozoal concentration, and total number of spermatozoa in the pre- and post-treatment urine were determined as for Experiment 1.

Statistical analysis

ANOVA (80,81) was used to determine the effect of treatment, week, and the interaction between treatment and week on the volume of pre- and post-treatment urine, and on the spermatozoal concentration, total number of spermatozoa, and percentage of viable spermatozoa in the pre- and post-treatment urine. ANOVA was also used to determine the effect of treatment, week, and interaction between treatment and week on the time elapsed from treatment to the first post-treatment micturition, and on the number of post-treatment micturitions. For the ANOVA on percentage of spermatozoal viability, percentages were transformed to the arcsin of the square root of 1/100 of the percentages. The sources of variation were: treatment, week, and interaction treatment x week. Ram within treatment was used as the error term for treatment effect, and the general error was used as the error term for the effect of week and interaction treatment x week. Significance was established at $P \leq 0.05$. The conservative F value (82) was used to confirm significance for the effect of week and for the interaction treatment x week. χ^2 analysis (80) was used to determine the effect of treatment on the ratio: number of urine samples that had motile spermatozoa in samples of urine that had spermatozoa.

Experiment 3. Effect of Xylazine on the Retrograde Flow of Spermatozoa into the Urinary Bladder of Sexually Rested, Catheterized Rams

The possibility of interactions among drugs (xylazine, tolazoline, and furosemide) affecting the response of rams to treatment in Experiments 1 and 2 of this study, and the delayed and possibly incomplete micturitions

of rams which were treated with xylazine in Experiment 2, prompted the design of another experiment to test for an effect of xylazine on retrograde flow of spermatozoa into the bladder without using any other drug that could interact with xylazine. To facilitate urine collection, this experiment was done in rams which had a permanent catheter surgically implanted in the urinary bladder.

Eight adult rams, including five western range and three polypay, were used in this study, which was initiated 10 months after Experiment 2 was completed. Seven of these rams were from Experiment 2, and another ram was purchased for the present experiment. Rams were housed in individual pens and fed as described in Experiment 1. The average body weight (± SD) at the start of Experiment 3 was 96 ± 17 kg.

A catheter was surgically implanted (22) into the urinary bladder of each ram. Before surgery, rams were given an IM injection of xylazine (0.4 mg/kg of body weight) and then were anesthetized with sodium pentobarbital (7.5 mg/kg of body weight, IV), 10 minutes after administration of xylazine. An indwelling catheter was placed in the jugular vein and used to administer maintenance doses of sodium pentobarbital, as needed during surgery. Xylazine (0.2 mg/kg of body weight, IM) was given hourly during surgery. The jugular catheter was also used to infuse a 0.9 % saline solution containing 24 mEq of sodium bicarbonate/l (5 ml/kg of body weight/h) during surgery (22). The midventral and surrounding skin areas were clipped free of wool, washed, disinfected, and draped. A longitudinal incision (10 to 15 cm long) extending cranially from the base of the scrotum was made through the abdominal wall. The incision was parallel to

the penile sheath and approximately 3 to 4 cm lateral from it. A Tygon (S-50 HL tubing, Norton Co, Plastics and Synthetics Division, Akron, OH) tube (2.38 mm ID, 3.97 mm OD, and 120 cm long) was used to catheterize the urinary bladder. The proximal (bladder) end of the catheter had a Dacron pad (Troy Mills Inc, Troy, NH 03465) glued (Silastic Medical Adhesive, Dow Corning Corp, Midland, MI) to the outside of the catheter to suture and secure the catheter to the bladder. The urinary bladder was exteriorized, the catheter was then inserted through a hole made with a sharp probe in the centrum verticis of the bladder, and the catheter was then anchored to the bladder with sutures. The proximal end of the catheter extended approximately 4 cm into the bladder and had approximately 10 additional oval openings to facilitate urine withdrawal. The distal end of the catheter was passed through the abdominal wall, directed under the skin with the aid of a metal tube, and then exteriorized in the right lumbar, paravertebral area as previously described (22). The exteriorized catheter was plugged and maintained in a cloth pouch sutured to the skin. After the abdominal incision was closed, rams were returned to their pens and given 1 mg of tolazoline/kg of body weight, IV to hasten recovery from anesthesia (22). Each ram was allowed at least 7 days to recover from surgery. During the first day of this period of recovery, the catheter was allowed to drain continuously. The distal end of the catheter was plugged on the second day of recovery, and the catheter was then monitored periodically for patency for the duration of the study. After each urine withdrawal, the catheter was filled with approximately 4.0 ml of a 0.02 % solution of nitrofurazone, plugged, and replaced in the pouch.

After recovery from surgery, rams were randomly assigned, in a 2 x 5 split plot factorial design with rams nested within periods of collection, to two treatment groups of 4 rams each: Group 1, control saline solution; Group 2, treatment with xylazine. The two treatments were replicated five times over a period of fifteen days, once every three or four days, alternatively, as shown in Table 3. The major factors were two treatments and five periods of collection, with two subplots: pre- and post-treatment urine. The pre-treatment urine consisted of a single sample, and the post-treatment urine consisted of three sequential samples, which were collected

Table 3. Experimental design (Experiment 3)

Period of collection	Urine sample	Minutes	Treatment groups: Saline	Treatment groups: Xylazine
1st	Pre		4 rams	4 rams
(day 1)	Post	20	↓	↓
		40		
		60		
2nd	Pre			
(day 4)	Post	20		
		40		
		60		
3rd	Pre			
(day 8)	Post	20		
		40		
		60		
4th	Pre			
(day 11)	Post	20		
		40		
		60		
5th	Pre			
(day 15)	Post	20		
		40		
		60		

at 20, 40, and 60 minutes after treatment with saline or xylazine, as shown in Table 3.

For each ram and day of collection, the following end points were determined in the pre-treatment sample and in each of the three post-treatment samples of urine obtained from the bladder: volume (ml), spermatozoal concentration (10^6/ml), total number of spermatozoa (10^6), and number of urine samples containing motile spermatozoa.

To enhance the withdrawal of measurable volumes of fluid from the bladder at each urine collection, 60 ml of physiologic saline solution were infused into the bladder through the catheter immediately before withdrawal of each pre- or post-treatment sample of urine. At each collection day, the pre-treatment urine was collected from the catheter by evacuating the bladder. Immediately after collection of the pre-treatment urine, control rams (Group 1) received an IM injection of 2 ml of 0.9 % physiologic saline solution, and treated rams (Group 2) were given an IM injection of a solution of 20 mg/ml xylazine, at a dose of 0.4 mg/kg of body weight. The time of injection was recorded. To allow for maximal effects of xylazine (83), post-treatment urine was obtained from control and treated rams by evacuating the bladder at 20, 40, and 60 minutes after saline or xylazine injection.

Urine was examined at 400X for motile spermatozoa immediately after the pre- and each of the post-treatment samples of urine was collected. The number of urine samples containing motile spermatozoa was determined as described for Experiment 1. The eosin B assay for viability of spermatozoa was not done in this experiment. For each ram and day of collection, the

volume of urine, spermatozoal concentration, and total number of spermatozoa in the pre-treatment urine and in each of the post-treatment samples of urine, obtained at 20, 40, and 60 minutes after treatment, were determined as described for Experiment 1. For each ram and day of collection, the overall volume of post-treatment urine was estimated by adding the volume of urine obtained for each of the post-treatment samples, and the overall total number of spermatozoa in the post-treatment urine was estimated by adding the total number of spermatozoa in the urine obtained for each of the post-treatment samples. For each ram and day of collection, the overall concentration of spermatozoa in the post-treatment urine was then estimated by dividing the overall total number of spermatozoa in the post-treatment urine by the overall volume of post-treatment urine.

Rams were observed for masturbation on each day of collection. Only on one occasion, a ram (Group 2) was observed masturbating shortly before the planned beginning of urine collection. For this ram, the urinary bladder was flushed with saline through the catheter to induce micturition. The treatment and collection of urine samples were delayed for 40 minutes. The pre-treatment urine for this ram was azoospermic. All data from this ram were included in the statistical analyses and in Tables.

Statistical analysis

ANOVA (80,81) was used to determine the effect of treatment, day of collection, and the interaction between treatment and day of collection on the volume of urine, spermatozoal concentration, and total number of

spermatozoa in the pre- and post-treatment urine. The sources of variation were: treatment, day of collection, and interaction treatment x day of collection. Ram within treatment was used as the error term for treatment effect, and the general error was used as the error term for day of collection and the interaction treatment x day of collection. Significance was established at $P \leq 0.05$. The conservative F value (82) was used to confirm significance for the effect of day of collection and for the interaction treatment x day of collection.

ANOVA was also used to determine the effect of time of sampling (20, 40, or 60 minutes after treatment) and the interaction treatment x time of sampling on the volume of urine, spermatozoal concentration, and total number of spermatozoa in the sequential samples of post-treatment urine. The general error was used as the error term for time of sampling and treatment x time of sampling. The conservative F value was also used to confirm significance for the effect of time of sampling and for the interaction treatment x time of sampling. Tukey's ω test (80,81) was used to test for differences between means of treatment groups or differences in means within treatment groups for volume, spermatozoal concentration, and total number of spermatozoa of the sequential samples of post-treatment urine when ANOVA indicated a significant F ratio for treatment or for time of sampling. Regression lines (80,81) were calculated to characterize the rate of change of the end points determined in the sequential samples of post-treatment urine for which ANOVA indicated a significant F ratio for time of sampling.

χ^2 analysis (80) was used to determine the effect of treatment on the

ratio: number of urine samples that had motile spermatozoa in samples of urine that had spermatozoa.

PART II

Experiment 4. Clearance of Spermatozoa from Electroejaculates of Vasectomized Rams after Flushing the Vasa Deferentia at the Time of Vasectomy

Ten adult rams, including seven western range and three polypay, were used in this study to determine the time after vasectomy required for clearance of spermatozoa from the ejaculate and to determine whether flushing the vasa deferentia at the time of vasectomy shortens the time elapsed from vasectomy to azoospermia. Nine of these rams were previously used in Experiment 1 and also in Experiment 2, and 7 of these rams were used again in Experiment 3, including the ram purchased for Experiment 3. Experiment 4 was initiated 14 days after Experiment 3 was completed. Rams were maintained as described in Experiment 1. The average (± SD) body weight at the start of Experiment 4 was 101 ± 17 kg. Rams were randomly assigned in a 2 x 11 days of collection factorial design, with rams nested within periods of collection, to two groups of five rams each, as shown in Table 4: control group, vasectomy; treated group, vasectomy plus flushing of the vasa deferentia. Pre-vasectomy electroejaculates were obtained from each ram immediately before surgery on day 0. Post-vasectomy electroejaculates were obtained on post-vasectomy days 3 and 7, and then at 7 day intervals (Table 4) until day 63.

The following end points were determined in the pre- and post-vasectomy electroejaculates: volume (ml), spermatozoal concentration (10^6/ml), number of intact spermatozoa (10^6), total number of spermatozoa (10^6), percentage of viable spermatozoa, and number of electroejaculates containing motile spermatozoa.

Table 4. Experimental design (Experiment 4)

Day of collection	Control group	Treated group
0	5 Rams	5 Rams
3	↓	↓
7		
14		
21		
28		
35		
42		
49		
56		
63		

Vasectomy for treated and control rams was performed as follows: rams were anesthetized as described for Experiment 3. The scrotum and surrounding areas were then clipped, washed and disinfected. Rams were positioned in dorsal recumbency and the scrotum was pushed caudally. A 4 cm incision was made on the midline of the scrotal neck, beginning approximately 3 cm distal to the abdominal wall. Each vas deferens was approached from this single midline incision, exposed by blunt dissection and then fixed with two forceps. For the control rams, each vas deferens was isolated, double-ligated with silk suture, and then severed between the ligatures. The scrotal incision was then closed. For the treated rams, each vas deferens was isolated, and then ligated with silk suture, as described for the control rams, except that the ligature on the distal, abdominal end of the vas deferens was not tied, but placed loosely around the vas. The vas deferens was then severed between the ligatures, as

described for the control rams. The loosely ligated, distal end of the vas deferens was held by the ligature and positioned with forceps, so that the opening of the lumen could be visualized. The vas deferens was cannulated with the blunted end of a 1.0 inch (2.54 cm) long, 22-gauge needle and flushed with 25 ml of a 0.01 % solution of trypan blue in 0.9 % isotonic saline solution (25). To determine post-vasectomy viability of spermatozoa in rams of the treated group, a spermicidal agent was not added to the flushing solution. The infusion of the flushing solution was done in a pulsating fashion, using 15 ml syringes to help overcoming the resistance opposed to flushing. No attempts were made to measure this resistance. As it was anticipated (25), most of the flushing solution retrograded into the bladder, and only trace amounts of blue flushing solution were seen at the preputial opening during or immediately after flushing in two of the five treated rams. Immediately after flushing, the distal end of each vas deferens was ligated. The scrotal incision was sutured once this process was completed for both vasa deferentia.

Pre- and post-vasectomy electroejaculates were collected for each ram as described in Experiment 1. Immediately after semen collection, the electroejaculates were examined at 400X for motile spermatozoa. Viability of spermatozoa in the electroejaculates was evaluated using the eosin B dye-exclusion assay (79), as described in Experiment 1 with the modifications introduced in Experiment 2 for the estimation of spermatozoal viability in urine. A 1/10 or 1/100 semen dilution was made, as needed, by diluting a sample of electroejaculate in the buffered saline solution described in Experiment 1. Depending on the spermatozoal concentration of

the electroejaculate, 10 μl of a diluted (1/10 or 1/100) or undiluted seminal sample were placed inside the ringed area of the chamber and were then gently mixed with 10 μl of 0.15 % eosin B solution. The incubation period was 5 minutes before evaluation. Spermatozoal viability and motility within the viability preparation were determined as described in Experiment 2. The volume, concentration of spermatozoa, and total number of spermatozoa were estimated for each electroejaculate in diluted or undiluted samples as described in Experiment 1. Electroejaculates were considered to be azoospermic when spermatozoa were not found in duplicate undiluted samples of post-vasectomy electroejaculates.

Statistical analysis

ANOVA (80,81) was used to determine the effect of treatment, day of collection, and the interaction between treatment and day of collection on the volume of the electroejaculates, and on the spermatozoal concentration, number of intact spermatozoa, total number of spermatozoa, and percentage of viable spermatozoa in the electroejaculates. For the ANOVA on percentage of spermatozoal viability, percentages were transformed to the arcsin of the square root of 1/100 of the percentages. ANOVA was also used to determine the effect of treatment on the cumulative total number of spermatozoa released to the post-vasectomy electroejaculates. The sources of variation were: treatment, day of collection, and interaction treatment x day of collection. Ram within treatment was used as the error term for treatment effect, and the general error was used as the error term for day of collection and interaction treatment x day of collection. Significance

was established at $P \leq 0.05$. The conservative F value (82) was used to confirm significance for the effect of day of collection and for the interaction treatment x day of collection.

ANOVA (80,81) was also used to test for post-vasectomy differences in the rate of change between treatment groups in the number of intact spermatozoa, total number of spermatozoa, and cumulative total number of spermatozoa in the electroejaculates.

Regression lines (80,81) were calculated for each treatment group to characterize the rate of change between PVD and the number of intact spermatozoa, total number of spermatozoa, and cumulative total number of spermatozoa in the post-vasectomy electroejaculates. Data on total number of spermatozoa and number of intact spermatozoa were transformed to natural logarithm, using the expression (25) $\ln [(\text{variable } (10^6) + 0.0001) \times (1 \times 10^6)]$.

χ^2 analysis (80) was used to determine the effect of treatment on the ratio: number of electroejaculates that had motile spermatozoa in electroejaculates that had spermatozoa.

RESULTS

PART I

Experiment 1

The volume of electroejaculates, collected from rams of Groups 1 and 2, was not affected ($P>0.1$, Table 5) by treatment or week, and the interaction treatment x week was also not significant ($P>0.1$). The spermatozoal concentration in the electroejaculates was affected ($P=0.015$, Table 5) by treatment, but was not affected ($P>0.1$) by week, and the interaction treatment x week was also not significant ($P>0.1$). The total

Table 5. Volume of electroejaculates and concentration and total number of spermatozoa in the electroejaculates of rams (Experiment 1)

		Electroejaculate		
Treatment group		Volume (ml)	Spermatozoal concentration (10^6/ml)	Total No. of spermatozoa (10^6)
Electroejaculation (Group 1)		5.0 ± 2.5	1458 ± 535	7387 ± 4695
Xylazine + electroejaculation (Group 2)		4.0 ± 1.6	848 ± 407	3321 ± 1835
Source	**df**		**P**	
Treatment	1	>0.1	=0.015	>0.1
Week	3	>0.1	>0.1	>0.1
Week x trt	3	>0.1	>0.1	>0.1

df = degrees of freedom; P = probability; trt = treatment. Data presented as overall mean ± SD; values over time and across rams, N = 16 observations for each treatment group.

number of spermatozoa in the electroejaculates was not affected (P>0.1, Table 5) by treatment or week, and the interaction treatment x week was also not significant (P>0.1).

The volume of urine collected from rams of Groups 1, 2, and 3 during the first post-treatment micturition was not affected (P>0.1, Table 6) by

Table 6. Volume of urine, concentration and total number of spermatozoa in the urine collected during the first post-treatment micturition, and percentage of retrograde flow of spermatozoa into the urinary bladder of rams (Experiment 1)

	Urine			
Treatment group	Volume (ml)	Spermatozoal concentration (10^6/ml)	Total no. of spermatozoa (10^6)	Retrograde flow (%)
Electroejaculation (Group 1)	387 ± 245	1.90 ± 1.60	897 ± 1152	12 ± 11
Xylazine + Electroejaculation (Group 2)	412 ± 175	3.15 ± 2.05	1131 ± 721	29 ± 18
Xylazine (Group 3)	204 ± 130	5.94 ± 11.47	567 ± 887	NA

Source	df	P	df	P	df	P	df	P
Treatment	2	>0.1	2	>0.1	2	>0.1	1	=0.017
Week	3	>0.1	3	>0.1	3	>0.1	3	>0.1
Week x trt	6	>0.1	6	>0.1	6	>0.1	3	>0.1

NA = not applicable, semen was not collected from rams of Group 3.
df = degrees of freedom; P = probability; trt = treatment. Data presented as overall mean ± SD; values over time and across rams, N = 16 observations for each treatment group. For Groups 1 and 2, all urine samples contained spermatozoa. For Group 3, only 7 of 16 urine samples contained spermatozoa.

treatment or week, and the interaction treatment x week was also not significant ($P>0.1$). The concentration and total number of spermatozoa of spermatozoa in the urine collected during the first post-treatment micturition were not affected ($P>0.1$, Table 6) by treatment or week, and the interaction treatment x week were also not significant ($P>0.1$). The percentage of retrograde flow of spermatozoa into the urinary bladder of rams of Groups 1 and 2 was affected ($P=0.017$, Table 6) by treatment, but was not affected ($P>0.1$) by week, and the interaction treatment x week was also not significant ($P>0.1$).

The urine obtained during the first post-treatment micturition from rams of Group 3 was azoospermic (footnote, Table 7) in 9 of 16 collections. One of the 4 rams of Group 3 (data not shown in tables) did not have spermatozoa in the urine in any of the four weekly collections, 2 of the remaining 3 rams had spermatozoa in the urine on alternate weeks, and 1 ram had spermatozoa in the urine in 3 of the 4 weekly collections.

The percentage of viable spermatozoa in the urine obtained during the first post-treatment micturition was not affected ($P>0.1$, Table 7) by treatment or week, and the interaction treatment x week was also not significant ($P>0.1$). For the urine collected during the first post-treatment micturition, the ratio: number of urine samples that had motile spermatozoa in samples of urine that had spermatozoa was not different ($P>0.1$, Table 7) between treatment groups.

Table 7. Percentage of viable spermatozoa in the urine collected from rams during the first post-treatment micturition and ratio of urine samples that contained motile spermatozoa (Experiment 1)

Treatment group	Spermatozoal viability in urine (%)	Spermatozoal motility in urine
Electroejaculation (Group 1)	46 ± 24	16 / 16
Xylazine + Electroejaculation (Group 2)	45 ± 25	15 / 16
Xylazine (Group 3)	50 ± 18	6 / 7

Source	df	P	df	P[a]
Treatment	2	>0.1	2	>0.1
Week	3	>0.1		
Week x trt	6	>0.1		

df = degrees of freedom; P = probability; trt = treatment. Data for spermatozoal viability is presented as overall mean ± SD; values over time and across rams, N = 16 observations for each treatment group, except for rams of Group 3, N = 7, because of 9 azoospermic samples.

[a]χ^2 analysis for the ratio: number of urine samples that had motile spermatozoa from the number of samples that had spermatozoa.

Experiment 2

The volume of pre-treatment urine was not different (P>0.1, Table 8) between treatment groups, was not affected (P>0.1) by week, and the interaction treatment x week was also not significant (P>0.1).

The post-treatment urine consisted of all the urine voided within 60 minutes after treatment, except for 7 collections from 5 rams of Group 2, which did not urinate during the 60 minute period and were maintained under

observation until the first post-treatment micturition occurred. For these 7 collections, however, the volume of urine was considered to be zero for the statistical analysis or tables for volume of post-treatment urine. The volume of post-treatment urine was affected (P=0.0001, Table 8) by treatment, but was not affected (P>0.1) by week, and the interaction treatment x week was also not significant (P>0.1).

Thirteen micturitions from 9 rams occurred before 6 minutes. These micturitions were not included in the analysis. The time elapsed from treatment to the first post-treatment micturition was affected (P=0.0001,

Table 8. Volume of pre- and post-treatment urine of sexually rested rams (Experiment 2)

Treatment group	Volume of urine (ml)	
	Pre-treatment	Post-treatment
Saline-controls (Group 1)	356 ± 175	1743 ± 366
Xylazine-treated (Group 2)	400 ± 198	152 ± 195

Source	df	P	
Treatment	1	>0.1	=0.0001
Week	2	>0.1	>0.1
Week x trt	2	>0.1	>0.1

df = degrees of freedom; P = probability; trt = treatment. Data presented as overall mean ± SD; values over time and across rams, N = 18 observations for each treatment group, except for post-treatment urine of control rams , N = 17, because a datum was not recorded.

Table 9) by treatment, but was not affected (P>0.1) by week, and the interaction treatment x week was also not significant (P>0.1). The number of post-treatment micturitions within the period of 60 minutes was affected (P=0.0001, Table 9) by treatment, but was not affected (P>0.1) by week, and the interaction treatment x week was also not significant (P>0.1).

The spermatozoal concentration (Table 10) and total number of spermatozoa (Table 11) in the pre- and post-treatment urine were not

Table 9. Time elapsed from treatment to the first post-treatment micturition, and number of micturitions within 60 minutes after treatment of sexually rested rams (Experiment 2)

Treatment group		Post-treatment: Time to first[a] micturition (min.)	Post-treatment: No. of micturitions within 60 min.
Saline-controls (Group 1)		13 ± 3	5.4 ± 2.2
Xylazine-treated (Group 2)		54 ± 21	0.9 ± 0.9
Source	**df**	**P**	
Treatment	1	=0.0001	=0.0001
Week	2	>0.1	>0.1
Week x trt	2	>0.1	>0.1

df = degrees of freedom; P = probability; trt = treatment. Data presented as overall mean ± SD; values over time and across rams, N = 18 observations for each treatment group.

[a]Only micturitions that occurred after 6 minutes from saline or xylazine injection were included in the analysis.

Table 10. Concentration of spermatozoa in pre- and post-treatment urine of sexually rested rams (Experiment 2)

Treatment groups		Spermatozoal concentration (10^6/ml)	
		Pre-treatment urine	Post-treatment urine
Saline-controls (Group 1)		0.37 ± 1.42	0.006 ± 0.022
Xylazine-treated (Group 2)		0.19 ± 0.58	0.096 ± 0.239
Source	**df**	P	
Treatment	1	>0.1	>0.1
Week	2	>0.1	>0.1
Week x trt	2	>0.1	>0.1

df = degrees of freedom; P = probability; trt = treatment. Data presented as overall mean ± SD; values over time and across rams, N = 18 observations for each treatment group. For the post-treatment urine, 16 of 18 urine samples from rams of Group 1 and 11 of 18 urine samples from rams of Group 2 were azoospermic (χ^2, 1 df, $P<0.1$).

different (P>0.1) between treatment groups and were not affected (P>0.1) by week, and the interactions treatment x week were also not significant (P>0.1).

The pre-treatment urine contained spermatozoa in 2 of 18 collections from rams of Group 1, and in 5 of 18 collections from rams of Group 2. The post-treatment urine contained spermatozoa in 2 of 18 collections from rams of Group 1, and in 7 of 18 collections from rams of Group 2.

The percentage of viable spermatozoa in the pre-treatment urine was

Table 11. Total number of spermatozoa in pre- and post-treatment urine of sexually rested rams (Experiment 2)

Treatment group	Total No. of spermatozoa (10^6)	
	Pre-treatment urine	Post-treatment urine
Saline-controls (Group 1)	97 ± 369	6 ± 21
Xylazine-treated (Group 2)	39 ± 119	25 ± 68

Source	df	P	
Treatment	1	>0.1	>0.1
Week	2	>0.1	>0.1
Week x trt	2	>0.1	>0.1

df = degrees of freedom; P = probability; trt = treatment. Data presented as overall mean ± SD; values over time and across rams, N = 18 observations for each treatment group. For the post-treatment urine, 16 of 18 urine samples from rams of Group 1 and 11 of 18 urine samples from rams of Group 2 were azoospermic (χ^2, 1 df, P<0.1).

not different (P>0.1, Table 12) between treatment groups. There was a trend (P=0.087, Table 12) for an effect of treatment on the percentage of viable spermatozoa in the post-treatment urine, but the percentage of viable spermatozoa in the post-treatment urine was not affected (P>0.1) by week. The ratio: number of urine samples that had motile spermatozoa in samples of urine that had spermatozoa was not different (P>0.1, Table 12) between treatment groups for either the pre- or post-treatment urine.

Table 12. Percentage of viable spermatozoa in the pre- and post-treatment urine of sexually rested rams and ratio of urine samples that contained motile spermatozoa (Experiment 2)

	Spermatozoal viability (%)		Spermatozoal motility	
Treatment group	Pre-treatment urine	Post-treatment urine	Pre-treatment urine	Post-treatment urine
Saline-controls (Group 1)	17 ± 13	7 ± 12	2 / 2	1 / 2
Xylazine-treated (Group 2)	17 ± 24	28 ± 32	2 / 5	5 / 7

Source	df	P	df	P	df	P[a]	df	P[a]
Treatment	1	>0.1	1	=0.087	1	>0.1	1	>0.1
Week	2	ND	2	>0.1				

df = degrees of freedom; P = probability; ND = not determined because of the number of azoospermic samples. Data for spermatozoal viability is presented as overall mean ± SD; values over time and across rams; for the pre-treatment urine, n = 2 (Group 1) or n = 5 (Group 2), and for the post-treatment urine, n = 2 (Group 1) or n = 7 (Group 2), because of 16, 13, 16, and 11 azoospermic samples, respectively.

[a]χ^2 analysis for the ratio: number of urine samples that had motile spermatozoa from the number of samples that had spermatozoa.

Experiment 3

The volume of pre-treatment urine was not different (P>0.1, Table 13) between treatment groups, was not affected (P>0.1) by day of collection, and the interaction treatment x day of collection was also not significant (P>0.1). The total volume of post-treatment urine collected during the 60 minute period was affected by treatment (P=0.006, Table 13), but was not

Table 13. Volume of pre-treatment urine and total volume of post-treatment urine obtained from the bladder of sexually rested rams within 60 minutes after treatment (Experiment 3)

Treatment group	Volume of urine (ml)	
	Pre-treatment	Post-treatment
Saline-controls (Group 1)	128 ± 95	263 ± 72
Xylazine-treated (Group 2)	70 ± 34	864 ± 347

Source	df	P	
Treatment	1	>0.1	=0.006
Day	4	>0.1	>0.1
Day x trt	4	>0.1	>0.1

df = degrees of freedom; P = probability; trt = treatment. Data presented as overall mean ± SD; values over time and across rams, N = 20 observations for each treatment group.

affected ($P>0.1$) by day of collection, and the interaction day of collection x treatment was also not significant ($P>0.1$). The volume of urine obtained 20, 40, and 60 minutes after treatment was affected by treatment ($P=0.006$, Table 14), time of sampling ($P=0.001$), and the interaction time x treatment was also significant ($P=0.0001$, Table 14). For the saline control group, the mean volume of post-treatment urine collected at 20 minutes was larger ($P<0.05$, Table 14) than the mean volume of post-treatment urine collected at 60 minutes, but was not different ($P>0.05$) from the volume of urine collected at 40 minutes. However, the

Table 14. Volume of urine obtained from the bladder of sexually rested rams at 20, 40, and 60 minutes post-treatment (Experiment 3)

Treatment group	Volume of post-treatment urine (ml) At 20 min.	At 40 min.	At 60 min.
Saline-controls (Group 1)	98 ± 41[a]	86 ± 24[ab]	80 ± 18[b]
Xylazine-treated (Group 2)	217 ± 121[c]	337 ± 153[d]	310 ± 101[d]

Source	df	P
Treatment	1	=0.006
Time	2	=0.001
Time x trt	2	=0.0001

df = degrees of freedom; P = probability; Time = 20, 40, or 60 minutes; trt = treatment. Data presented as mean ± SD; values over time and across rams, N = 20 observations for each treatment group. Means within columns or rows which do not have a common superscript letter are significantly different, $P<0.05$.

mean volume of post-treatment urine collected from the xylazine-treated group was smaller ($P<0.05$, Table 14) at 20 minutes than the mean volume of urine collected at 40 or 60 minutes. For each time period, the mean volume of post-treatment urine collected from the xylazine-treated group was 55% to 74% larger ($P<0.05$, Table 14) than the volume of post-treatment urine collected from the saline control group.

The linear regression predicting the rate of change in volume of urine ($\hat{Y}$) as a function of the time elapsed after treatment (X = 20, 40, or

60 minutes), and the fitting of the data to the regression lines were as follows: $\hat{Y}$ (ml) = 106 - 0.5X for the rams of Group 1 (r=0.24, N=20, P=0.054); and $\hat{Y}$ (ml) = 195 + 2.3 X for the rams of Group 2 (r=0.28, N=20, P=0.03).

The concentration of spermatozoa in the pre-treatment and in the post-treatment urine and the total number of spermatozoa in the pre-treatment urine were not different (P>0.1, Table 15) between treatment groups, were not affected (P>0.1) by day of collection, and the interactions day of collection x treatment were also not significant (P>0.1). However, there was a trend approaching significance (P=0.083, Table 15) for an effect of treatment on the total number of spermatozoa in the urine obtained within 60 minutes after treatment. In addition, there were more azoospermic urine collections from the control rams (12 of 20) than from the treated rams (5 of 20, P<0.05, footnote, Table 15). The effect of day and the interaction day x treatment were not significant (P>0.1, Table 15).

According to the level of significance established in Materials and Methods, the F ratio for effect of treatment on the total number of spermatozoa in the urine obtained within 60 minutes after treatment indicated only a trend for an effect of treatment. However, I feel justified to claim that this treatment effect is significant on the basis that the mean of the total number of spermatozoa in the post-treatment urine obtained within 60 minutes after treatment was 460 times higher (Table 15) for rams of the treated group than for rams of the control group. I attribute the lack of a statistical significance to the variation

Table 15. Concentration and total number of spermatozoa in the pre-treatment urine and in the post-treatment urine obtained from the bladder of sexually rested rams within 60 minutes after treatment (Experiment 3)

Treatment group	Spermatozoal concentration (10^6/ml)		Total No. of spermatozoa(10^6)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Saline-controls (Group 1)	0.01 ± 0.02	0.01 ± 0.03	1.6 ± 3.8	3.2 ± 7.9
Xylazine-treated (Group 2)	0.05 ± 0.10	1.9 ± 4.0	4.0 ± 7.0	1471 ± 2210

Source	df	P		P	
Treatment	1	>0.1	>0.1	>0.1	=0.083
Day	4	>0.1	>0.1	>0.1	>0.1
Day x trt	4	>0.1	>0.1	>0.1	>0.1

df = degrees of freedom; P = probability; trt = treatment. Data presented as overall mean ± SD; values over time and across rams, N = 20 observations for each treatment group. For the post-treatment urine obtained within 60 minutes after treatment, 12 of 20 urine collections from rams of Group 1 and 5 of 20 urine collections from rams of Group 2 were azoospermic (χ^2, 1 df, $P<0.05$).

among rams within treatment groups, as evidenced by the magnitude of the SD (Table 15). Because the total volume of post-treatment urine obtained during the 60 minutes of collection was significantly affected by treatment (P=0.006, Table 13) and the total number of spermatozoa in the urine is a function of the volume and spermatozoal concentration of the urine, ANOVA F tests for contrasts of data (80,81) for spermatozoal concentration and

total number of spermatozoa in pre-treatment versus post-treatment urine were done after the fact (Table 16) to further evaluate whether or not there was an effect of treatment. The spermatozoal concentration and total number of spermatozoa in the urine obtained from the saline control rams were not different (P>0.1, Table 16) between the pre-treatment and the post-treatment urine collections. Treatment with xylazine, however, significantly affected the spermatozoal concentration (P=0.048, Table 16) and total number of spermatozoa (P=0.009) in the post-treatment urine. The spermatozoal concentration in the post-treatment urine collected at 20, 40, and 60 minutes was not affected by treatment or time of sampling (P>0.1, Table 17), and the interaction time of sampling x treatment was also not significant (P>0.1). The total number of spermatozoa in the post-treatment urine collected at 20, 40, and 60 minutes was not affected (P>0.1, Table 17) by time of sampling, and the interaction time x treatment was also not significant (P>0.1). However there was a trend approaching significance (P=0.083, Table 17) for an effect of treatment. This trend was independent of time of sampling, as indicated by a nonsignificant (P>0.1, Table 17) time x treatment interaction. The variation among rams in response to treatment may have precluded the statistical detection of a treatment effect because, for all means displayed in Table 17, the magnitude of the SD exceeded that of the mean.

The pre-treatment urine from rams of Group 1 contained spermatozoa in 10 of 20 collections, and in 16 of 20 collections for the pre-treatment urine collected from rams of Group 2 (data not shown in tables, χ^2, 1 df, P<0.05). The post-treatment urine from rams of Group 1 obtained during the

Table 16. Statistical analysis (F contrasts) of data for concentration and total number of spermatozoa in the pre-treatment urine and in the post-treatment urine obtained from the bladder of sexually rested rams within 60 minutes after treatment (Experiment 3)

		Spermatozoal concentration (10^6/ml)		Total No. of spermatozoa(10^6)	
Urine		Control rams	Treated rams	Control rams	Treated rams
Pre-treatment		0.01 ± 0.02	0.05 ± 0.10	1.6 ± 3.8	4.0 ± 7.0
Post-treatment		0.01 ± 0.03	1.9 ± 4.0	3.2 ± 7.9	1471 ± 2210
Contrast	df	P		P	
Pre vs. post	1	>0.1	=0.048	>0.1	=0.009

df = degrees of freedom; P = probability. Data are from Table 15 and are presented as overall mean ± SD; values over time and across rams, N = 20 observations for each treatment group.

60 minute period contained spermatozoa in 8 of 20 collections, and in 15 of 20 collections for the post-treatment urine collected from rams of Group 2 (data not shown in tables, χ^2, 1 df, P<0.05).

χ^2 analysis for the ratio: number of samples that had motile spermatozoa in samples of pre-treatment urine that had spermatozoa was not different (P>0.1, Table 18) between treatment groups. However, the ratio: number of samples that had motile spermatozoa in each of the time periods of collection of post-treatment urine that had spermatozoa was greater at

Table 17. Concentration and total number of spermatozoa in the urine obtained from the bladder of sexually rested rams at 20, 40, and 60 minutes post-treatment (Experiment 3)

	Post-treatment urine					
	Spermatozoal concentration (10^6/ml)			Total No. of spermatozoa (10^6)		
Treatment groups	At 20 min.	At 40 min.	At 60 min.	At 20 min.	At 40 min.	At 60 min.
Saline-controls (Group 1)	0.001 ± 0.003	0.01 ± 0.04	0.02 ± 0.06	0.1 ± 0.3	1.0 ± 3.9	2.2 ± 5.9
Xylazine-treated (Group 2)	2.2 ± 5.0	2.5 ± 5.8	1.4 ± 2.9	380 ± 634	673 ± 1143	418 ± 820

Source	df	P	P
Treatment	1	>0.1	=0.083
Time	2	>0.1	>0.1
Time x trt	2	>0.1	>0.1

df = degrees of freedom; P = probability; Time = 20, 40, or 60 minutes; trt = treatment. Data presented as mean ± SD; values over time and across rams, N = 20 observations for each treatment group.

Table 18. Number of urine samples that contained motile spermatozoa in the pre- and post-treatment urine obtained from the bladder of sexually rested rams (Experiment 3)

Treatment group		Motility of spermatozoa in urine				
		Pre-treatment	Post-treatment			
			At 20 min.	At 40 min.	At 60 min.	Overall
Saline-controls (Group 1)		0 / 10	NA	0 / 4	0 / 7	0 / 11
Xylazine-treated (Group 2)		2 / 16	8 / 14	10 / 13	7 / 14	25 / 41
Treatment effect	df	P[a]				
	1	>0.1	NA	<0.01	<0.025	<0.0005

NA = not applicable because of azoospermic samples; df = degrees of freedom; P = probability. Data presented as number of urine samples that had motile spermatozoa from the number of samples that had spermatozoa; 20 samples were collected for each treatment group per each time period (pre-treatment and 20, 40, and 60 minutes post-treatment). For the overall total of post-treatment urine, N = 60.

[a]χ^2 analysis for the ratio: number of urine samples that had motile spermatozoa from the number of urine samples that had spermatozoa.

40 minutes (P<0.01, Table 18) and again at 60 minutes (P<0.025) for the treated than for the control group. The overall ratio: number of post-treatment samples of urine that had motile spermatozoa from the total number of post-treatment urine samples that had spermatozoa was greater (P<0.0005, Table 18) for the treated group than for the control group. In addition, for all samples of post-treatment urine collected from each ram

for all time periods, more (data not shown in tables, χ^2, 1 df, $P<0.0005$) samples of urine containing spermatozoa were obtained from treated rams (41 of 60) than from control rams (11 of 60).

PART II

Experiment 4

The pre-vasectomy volume of electroejaculates and the spermatozoal concentration, total number of spermatozoa, and total number of intact spermatozoa on day 0 were not different (P>0.1, Tables 19 through 21) between treatment groups. Because one ram, which was not used in Experiment 1, consistently voided small amounts of urine during seminal collection, data on volume and spermatozoal concentration of the electroejaculates of this ram were not included in the statistical analyses for these end points. The volumes (Table 19), spermatozoal concentrations (Table 20), total numbers of spermatozoa (Table 21), and total numbers of intact spermatozoa (Table 21) in the post-vasectomy ejaculates collected up to day 63 were not affected (P>0.1) by treatment or day of collection, and the interactions day x treatment were also not significant (P>0.1), when data were statistically analyzed by the factorial ANOVA. However, the concentration, total number of spermatozoa, and total number of intact spermatozoa decreased rapidly from the pre-vasectomy seminal collection to PVD 63 for both, control and treated rams (Tables 20 and 21). The overall contrasts pre- versus post-vasectomy (analysis not shown in tables) were significant for volume of electroejaculates (P=0.0017), spermatozoal concentration (P= 0.0001), total number of spermatozoa (P=0.0001), and number of intact spermatozoa (P=0.0001) in the electroejaculates. Furthermore, from day 0, the major decline in spermatozoal concentration, total number of spermatozoa, and total number of intact spermatozoa appeared to have occurred earlier for the treated rams, which had their

Table 19. Volume of the pre- and post-vasectomy electroejaculates of rams (Experiment 4)

	Volume of electroejaculate (ml)	
Collection days	Control rams	Treated rams
Pre-vasectomy		
0	7.6 ± 3.3	4.6 ± 2.0
Post-vasectomy		
3	6.3 ± 2.4	3.8 ± 1.3
7	3.8 ± 1.8	5.1 ± 2.9
14	5.0 ± 2.4	3.7 ± 1.3
21	5.0 ± 3.4	3.8 ± 1.8
28	4.6 ± 2.1	4.2 ± 1.0
35	5.0 ± 2.4	4.6 ± 1.5
42	3.2 ± 0.9	3.2 ± 0.6
49	6.2 ± 4.3	3.4 ± 0.5
56	4.9 ± 2.6	4.2 ± 0.6
63	3.8 ± 1.2	3.3 ± 0.6

Pre-vasectomy	df	P
Treatment	1	>0.1
Post-vasectomy		
Treatment	1	>0.1
Day	9	>0.1
Day x treatment	9	>0.1

df = degrees of freedom; P = probability. Data presented as mean ± SD; values across rams, N = 5 observations per day of collection for each treatment group, except for treated rams, N = 4, because of urine contamination of electroejaculates.

Table 20. Spermatozoal concentration in the pre- and post-vasectomy electroejaculates of rams (Experiment 4)

	Spermatozoal concentration (10^6/ml)	
Collection days	Control rams	Treated rams
Pre-vasectomy		
0	833.10 ± 410.34	1086.55 ± 1135.55
Post-vasectomy		
3	91.07 ± 138.19	1.11 ± 1.52
7	2.39 ± 3.05	0.17 ± 0.22
14	0.63 ± 0.36	0.28 ± 0.24
21	0.46 ± 0.31	0.09 ± 0.09
28	0.43 ± 0.51	0.24 ± 0.13
35	0.49 ± 0.51	0.09 ± 0.07
42	0.36 ± 0.26	0.07 ± 0.06
49	0.20 ± 0.15	0.05 ± 0.04
56	0.17 ± 0.16	0.11 ± 0.11
63	0.09 ± 0.10	0.07 ± 0.05

Pre-vasectomy	df	P
Treatment	1	>0.1
Post-vasectomy		
Treatment	1	>0.1
Day	9	>0.1
Day x treatment	9	>0.1

df = degrees of freedom; P = probability. Data presented as mean ± SD; values across rams, N = 5 observations per day of collection for each treatment group, except for treated rams, N = 4, because of urine contamination of electroejaculates.

Table 21. Total number of spermatozoa and number of intact spermatozoa in the pre- and post-vasectomy electroejaculates of rams (Experiment 4)

Collection days	Total No. of spermatozoa (10^6)		No. of intact spermatozoa (10^6)	
	Controls	Treated	Controls	Treated
Pre-vasectomy				
0	6763.8 ± 4803.0	4678.8 ± 3298.2	6678.7 ± 4709.5	4628.9 ± 3293.2
Post-vasectomy				
3	642.8 ± 877.4	5.8 ± 6.9	597.8 ± 834.6	4.9 ± 6.5
7	10.5 ± 13.2	1.7 ± 2.0	8.7 ± 11.3	1.4 ± 1.8
14	2.9 ± 1.9	1.1 ± 0.8	1.8 ± 1.2	0.9 ± 0.8
21	2.4 ± 1.9	1.8 ± 3.5	1.2 ± 0.8	1.2 ± 2.1
28	1.8 ± 1.8	0.8 ± 0.5	1.0 ± 1.0	0.6 ± 0.5
35	2.3 ± 2.4	0.4 ± 0.4	1.1 ± 1.0	0.3 ± 0.3
42	1.2 ± 0.8	0.2 ± 0.2	0.8 ± 0.6	0.1 ± 0.1
49	1.1 ± 0.7	0.3 ± 0.2	0.5 ± 0.3	0.2 ± 0.1
56	0.8 ± 0.7	1.0 ± 1.2	0.4 ± 0.4	0.5 ± 0.5
63	0.4 ± 0.4	0.2 ± 0.1	0.2 ± 0.3	0.1 ± 0.1

	df	P	df	P
Pre-vasectomy				
Treatment	1	>0.1	1	>0.1
Post-vasectomy				
Treatment	1	>0.1	1	>0.1
Day	9	>0.1	9	>0.1
Day x trt	9	>0.1	9	>0.1

df = degrees of freedom; P = probability; trt = treatment. Data presented as mean ± SD; values across rams, N = 5 observations per day of collection for each treatment group.

vasa deferentia flushed at the time of vasectomy, than for the control rams.

The percentage of viable spermatozoa was not different ($P>0.1$, Table 22) between control and treated rams on pre-vasectomy day 0, but the percentage of viable spermatozoa collected on PVD 3 tended, approaching significance ($P=0.06$, Table 22) to be lower than the percentage of viable spermatozoa present in the electroejaculates of the vasectomized, but not flushed, control rams. The χ^2 analysis of the ratios formed by the number of electroejaculates that had motile spermatozoa in the post-vasectomy electroejaculates collected on PVD 3 and PVD 7 that had spermatozoa were not different ($P>0.1$, Table 22).

Control rams produced 2 azoospermic electroejaculates and treated rams produced 4 azoospermic electroejaculates during the post-vasectomy period (data for individual rams not shown in tables). However, none of the rams produced 2 consecutive azoospermic electroejaculates or even 2 consecutive electroejaculates having only detached spermatozoal heads.

Inspection of data presented in Tables 20 through 22 suggested that the number of spermatozoa, as well as the percentage of viability of spermatozoa and the number of electroejaculates that had motile spermatozoa decreased faster for the treated than for the control rams. In addition, the magnitude of standard deviations for the spermatozoal end points (Tables 20 through 22) approached or exceeded that of the means, suggesting that the lack of significance was due to the variation among rams in response to vasectomy or to vasectomy plus flushing of the vasa deferentia. Thus, because of biological implications, a test of homogeneity of variance

Table 22. Percentage of viable spermatozoa and presence of motile spermatozoa in the pre- and post-vasectomy electroejaculates of rams (Experiment 4)

Collection days	Spermatozoal viability (%)		Spermatozoal motility	
	Controls	Treated	Controls	Treated
Pre-vasectomy				
Day 0	37.1 ± 17.2	40.4 ± 9.4	5 / 5	5 / 5
Post-vasectomy				
Day 3	1.4 ± 1.5	0.3 ± 0.6	3 / 5	1 / 5
Day 7	1.1 ± 2.4	0 ± ...	1 / 5	0 / 4
Day 14 to 63	0 ± ...	0 ± ...	0 / 38	0 / 37

Treatment effect for the collection days:	df	P[a]	df	P[b]
0	4	>0.1	-	NA
3	4	=0.06	1	>0.1
7	-	ND	1	>0.1

df = degrees of freedom; ND = probability for the variance ratio not determined because on day 7 all of the spermatozoa in the electroejaculates from the treated group were dead; NA = not applicable because ratios are identical.

Data for spermatozoal viability is presented as mean ± SD; values across rams (day 0 to 7), and values over time and across rams (day 14 to 63), N = 5 observations per day of collection for each treatment group, except for control rams on days 28 and 56, and treated rams on days 7, 35, 42, and 63, for which N = 4 observations because of azoospermic electroejaculates. The df of variance ratios were established as N - 1.

[a]Probabilities for the variance ratios for the percentage of viable spermatozoa from the number of spermatozoa present in the electroejaculate.

[b]Probabilities for χ^2 analysis for the ratio: number of electroejaculates that had motile spermatozoa from the number of electroejaculates that had spermatozoa.

(80) was used after the fact (Tables 22 through 24), to further define and determine the effect of treatment on spermatozoal concentration, number of intact spermatozoa, and on the total number of spermatozoa in the electroejaculates obtained on pre-vasectomy day 0 and PVD 3, which were the only collection days in which viable spermatozoa were present in the electroejaculates of both control and treated rams, and on PVD 7, because one electroejaculate from a control ram had viable spermatozoa (Table 22).

The test of homogeneity of variance indicated a trend, approaching significance ($P=0.06$, Table 23) for a higher spermatozoal concentration in the electroejaculates on pre-vasectomy day 0 for rams assigned to the treated group than for control rams, and there was a significant ($P<0.0001$, Table 23) decline on PVD 3 and again on PVD 7 ($P=0.0007$) due to treatment in the concentration of spermatozoa for the rams that had their vasa deferentia flushed at the time of vasectomy.

When analyzed by the test of homogeneity of variance, the total number of spermatozoa and number of intact spermatozoa in the electroejaculates collected on pre-vasectomy day 0 were not different ($P>0.1$, Table 24) between treatment groups. However, the total number of spermatozoa and number of intact spermatozoa were significantly less for the treated than for the control rams on PVD 3 ($P<0.0001$, Table 24) and again on PVD 7 ($P<0.002$).

The relationship between the logarithmically transformed total number of spermatozoa in the electroejaculate and the time after vasectomy is shown in Figure 1. The linear equations of regression predicting the rate of disappearance of spermatozoa ($\hat{Y}$) from the ejaculate as a function of the

Table 23. Spermatozoal concentration in the pre- and post-vasectomy electroejaculates of rams until post-vasectomy day 7 (Experiment 4)

	Spermatozoal concentration (10^6/ml)	
Collection days	Control rams	Treated rams
Pre-vasectomy		
0	833.10 ± 410.34	1086.55 ± 1135.55
Post-vasectomy		
3	91.07 ± 138.19	1.11 ± 1.52
7	2.39 ± 3.05	0.17 ± 0.22

Treatment effect for the collection days:	df	P[a]
0	3	=0.06
3	3	<0.0001
7	3	=0.0007

df = degrees of freedom. Data presented as mean ± SD; values across rams, N = 5 observations per day of collection for each treatment group, except for treated rams, N = 4, because of urine contamination of electroejaculates. For the variance ratios, df were established as N - 1 for the lowest component of the ratio.

[a]Probabilities for the variance ratios.

time elapsed after vasectomy (X = PVD), and the fitting of the logarithmically transformed data to the straight lines of regression were as follows: $\hat{Y}$ = antilog [15.805 + (-0.063 x PVD)] - 100 (r=0.44, N=50, P=0.0017) for the control rams, and $\hat{Y}$ = antilog [13.607 + (-0.042 x PVD)] - 100 (r=0.28, N=50, P=0.047) for the treated rams. The analysis of slopes using F test showed no significant difference (P>0.1) between the slopes of these two regression lines.

The relationship between the logarithmically transformed number of

Table 24. Total number of spermatozoa and number of intact spermatozoa in the pre- and post-vasectomy electroejaculates of rams until post-vasectomy day 7 (Experiment 4)

Collection days	Total No. of spermatozoa (10^6)		No. of intact spermatozoa (10^6)	
	Controls	Treated	Controls	Treated
Pre-vasectomy				
0	6763.8 ± 4803.0	4678.8 ± 3298.2	6678.7 ± 4709.5	4628.9 ± 3293.2
Post-vasectomy				
3	642.8 ± 877.4	5.8 ± 6.9	597.8 ± 834.6	4.9 ± 6.5
7	10.5 ± 13.2	1.7 ± 2.0	8.7 ± 11.3	1.4 ± 1.8

Treatment effect for the collection days:	df	P[a]	df	P[a]
0	4	>0.1	4	>0.1
3	4	<0.0001	4	<0.0001
7	4	=0.002	4	=0.002

df, degrees of freedom. Data presented as mean ± SD; values across rams, N = 5 observations per day of collection for each treatment group. The df of variance ratios were established as N - 1.

[a]Probabilities for the variance ratios.

intact spermatozoa in the electroejaculate and the time after vasectomy is shown in Figure 2. The linear equations of regression predicting the rate of disappearance of intact spermatozoa ($\hat{Y}$) from the ejaculate as a function of the time elapsed after vasectomy (X = PVD), and the fitting of the logarithmically transformed data to the straight lines of regression were as follows: $\hat{Y}$ = antilog [15.217 + (-0.068 x PVD)] - 100 (r=0.41, N=50,

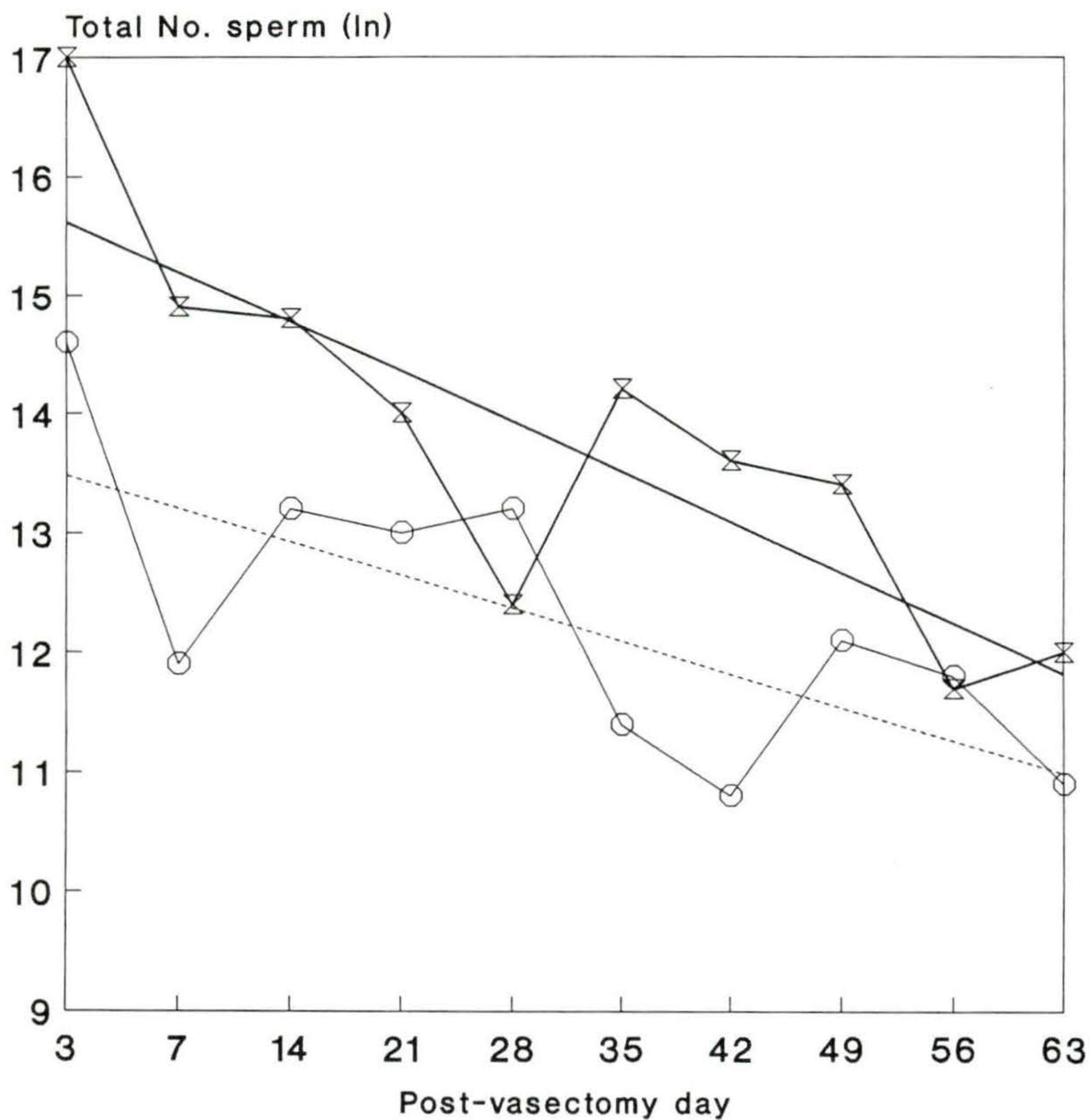


Figure 1. Mean values and regression lines for the rate of clearance of spermatozoa from the electroejaculates of control and treated rams (Experiment 4)

$P=0.003$) for the control rams, and $\hat{Y}$ = antilog [12.960 + (-0.037 x PVD)] - 100 ($r=0.24$, $N=50$, $P<0.1$) for the treated rams. The analysis of slopes using F test showed no significant difference ($P>0.1$) between the slopes of these two regression lines.

The cumulative total number of spermatozoa released into the post-vasectomy electroejaculates up to PVD 63 was (mean ± SD, data not shown in tables) $666.1 \pm 888.8 \times 10^6$ spermatozoa for the control rams, and $13.3 \pm 12.0 \times 10^6$ spermatozoa for the treated rams. The relationship between the cumulative total number of spermatozoa released into the electroejaculates and the time after vasectomy is shown in Figure 3a for control and 3b for treated rams. The linear equations of regression predicting the increase in the cumulative total number of spermatozoa ($\hat{Y}$) that had been released into the electroejaculates from PVD 3 to PVD 63 as a function of the time elapsed after vasectomy (X = PVD), and the fitting of the data to the straight lines of regression were as follows: $\hat{Y}$ (10^6 spermatozoa) = 649.482 + 0.314 x PVD ($r=0.01$, $N=50$, $P>0.1$) for the control rams, and $\hat{Y}$ (10^6 spermatozoa) = 6.947 + 0.114 x PVD ($r=0.22$, $N=50$, $P>0.1$) for the treated rams. The fitting of the data corresponding to both treatment groups to quadratic regressions was also not significant (not shown in figures or tables). However, the analysis of slopes using F test showed a significant difference ($P=0.02$) between the slopes of these two regression lines. This indicates that flushing the vasa deferentia at the time of vasectomy accelerated the clearance of spermatozoa from the post-vasectomy electroejaculates.

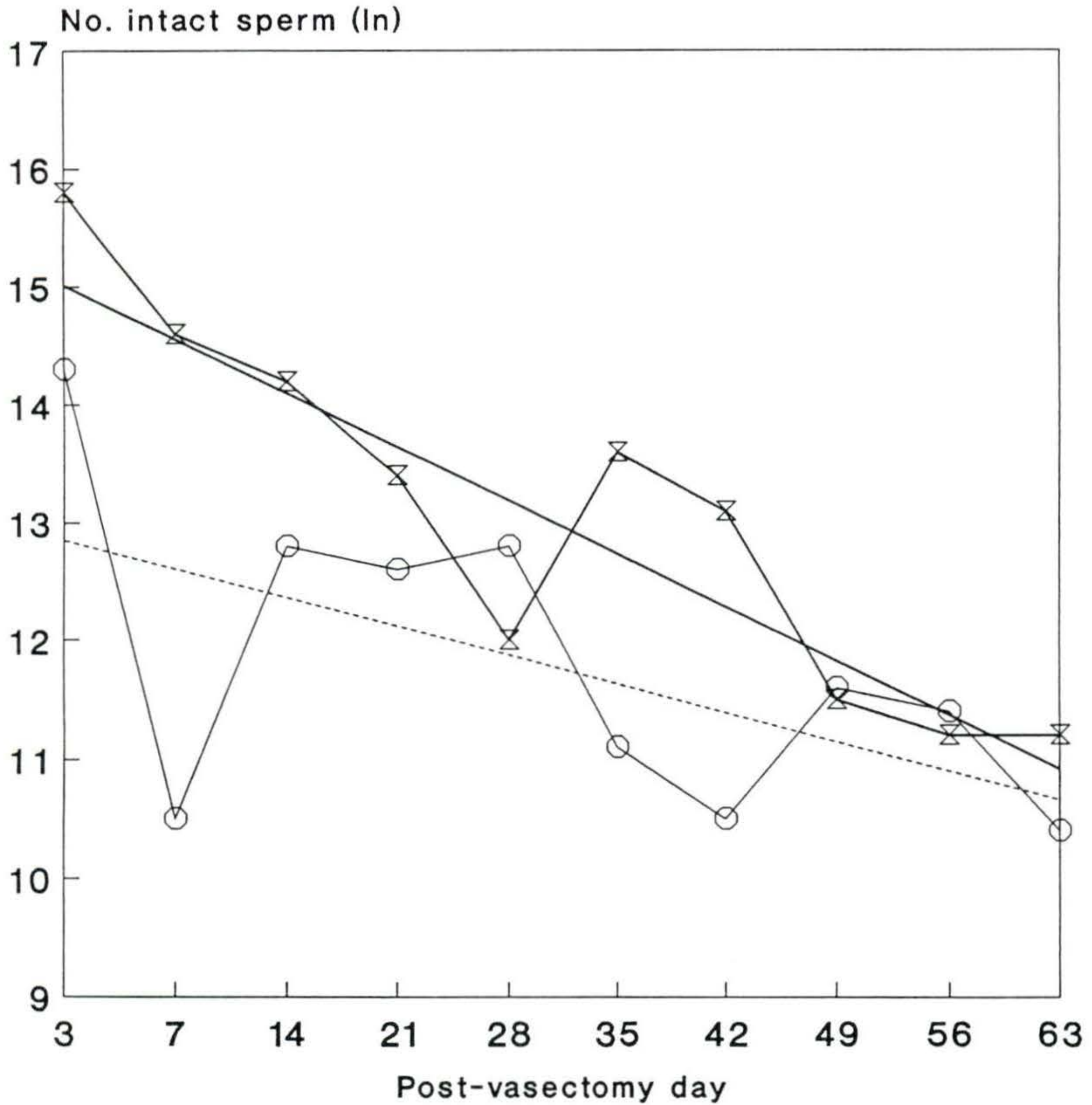
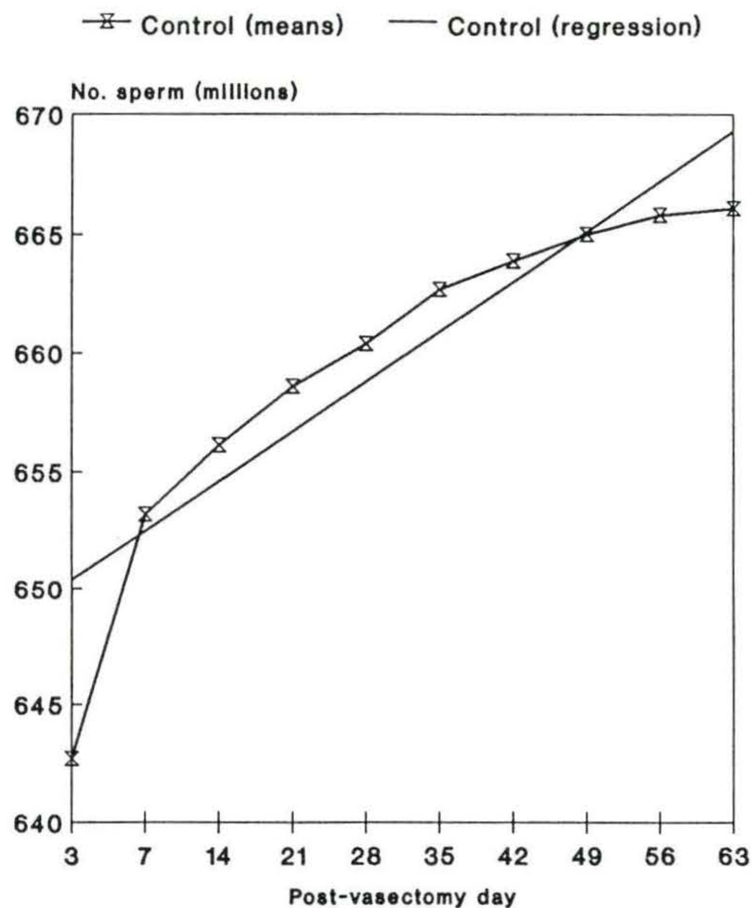
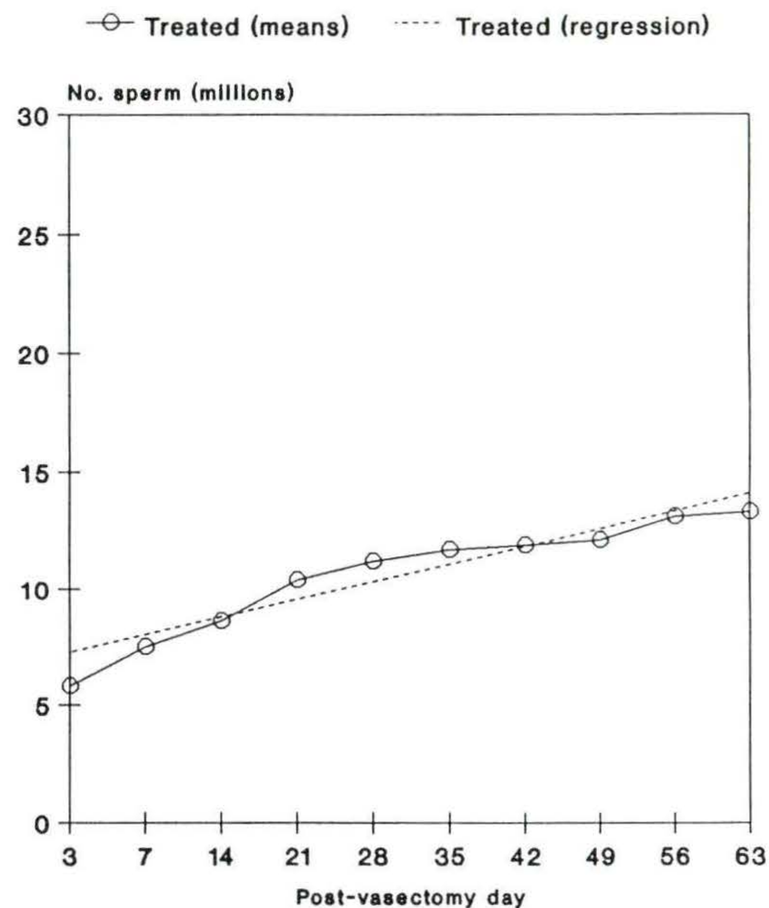


Figure 2. Mean values and regression lines for the rate of clearance of intact spermatozoa from the electroejaculates of control and treated rams (Experiment 4)



a



b

Figure 3. Mean values and regression lines for the rate of increase of the cumulative number of spermatozoa released into the electroejaculates of control (a) and treated (b) rams after vasectomy (Experiment 4)

DISCUSSION

Overall, the results of the studies corresponding to Part I of this Thesis show that xylazine induces retrograde flow of spermatozoa into the urinary bladder when administered to sexually rested rams and affects the electroejaculatory response when given to rams before electroejaculation. The results of Part II indicate that flushing the vasa deferentia at the time of vasectomy affects the rate of clearance of spermatozoa from the ejaculates of vasectomized rams. For the purpose of discussing the results of the research performed for this Thesis, the experiments are grouped as follows:

Part I

Experiment 1. Effect of Xylazine on the Retrograde Flow of Spermatozoa into the Urinary Bladder of Rams during Electroejaculation.

Experiment 2. Effect of Xylazine on the Retrograde Flow of Spermatozoa into the Urinary Bladder of Sexually Rested Rams.

Experiment 3. Effect of Xylazine on the Retrograde Flow of Spermatozoa into the Urinary Bladder of Sexually Rested, Catheterized Rams.

Part II

Experiment 4. Clearance of Spermatozoa from Ejaculates of Vasectomized Rams after Flushing the Vasa Deferentia at the Time of Vasectomy.

PART I

Experiment 1

The lack of an effect of treatment on volume of electroejaculate suggests that the administration of xylazine before EE does not influence the antegrade displacement and volumetric contribution of accessory sex gland secretions to the electroejaculate. The spermatozoal concentration in the electroejaculate was significantly decreased by treatment with xylazine, but the total number of spermatozoa in the electroejaculate was not affected by treatment. These results are difficult to interpret in view of the lack of an effect of xylazine on the volume of electroejaculate. An effect of xylazine on the total number of spermatozoa in the electroejaculate may not have been detectable because of the considerable variation among rams within and between treatment groups. The lack of an effect ($P>0.1$, Table 5) of week and treatment x week interaction support this contention. However, the percentage of retrograde flow of spermatozoa into the bladder was greater ($P=0.017$, Table 6) for the rams that were given xylazine before EE than for the rams of Group 1, which were not given xylazine, indicating that xylazine contributed to decrease the number of spermatozoa in the electroejaculate. In addition, rams of Group 1, which were not given xylazine, received an injection of tolazoline after EE was completed, whereas 3 of 4 rams of Group 2 were given tolazoline before EE in 12 of the 16 seminal collections to counteract the recumbency induced by xylazine. Thus, a potential counteractive effect of tolazoline on the spermatozoal displacement induced by xylazine during electroejaculation cannot be ruled out for rams of Group 2.

Rams of Group 1 had viable and motile spermatozoa in the first post-EE micturition. It has been previously reported (22,23) that spermatozoa are present in the urine collected from the bladder of catheterized rams after EE or in consecutive samples of urine collected during micturition from rams immediately after EE or seminal collection with an AV. In the present experiment, the percentage of retrograde flow of spermatozoa into the bladder for rams of Group 1 (mean ± SD = 12 ± 11%) is comparable to the percentage of retrograde flow during EE previously reported (22,23). Thus, administration of tolazoline after EE to rams of Group 1 apparently did not contribute to the retrograde flow of spermatozoa into the bladder.

The volume of urine and the concentration and total number of spermatozoa in the urine collected during the first post-xylazine + EE micturition (Group 2) or first post-xylazine micturition (Group 3) were not different from those of rams of Group 1. Nevertheless, administration of xylazine, followed by tolazoline 15 to 23 minutes after xylazine, before EE to rams of Group 2 resulted in an increased percentage (29 ± 18 %, P=0.0017, Table 6) of retrograde flow of spermatozoa into the bladder. This suggests that tolazoline, when given before EE, did not counteract the peripheral effects of xylazine on retrograde flow and may have even potentiated them. Administration of xylazine to sexually rested rams (Group 3) induced, per se and in the absence of EE, losses of spermatozoa in urine that were not different from the spermatozoal losses that occurred during EE in rams in this experiment and in previous studies (22,23). However, the magnitude of urinary losses of spermatozoa for rams of Group 3 in response to xylazine and/or tolazoline treatment was erratic.

A potential interactive role of tolazoline and xylazine on spermatozoal displacement must be also taken in consideration, although it is difficult to interpret in view of the mixed α_1- and α_2-blocking properties of tolazoline, currently considered (84) as a non-selective α-adrenoreceptor antagonist. On purely speculative basis, tolazoline may have counteracted the central, but not the peripheral effects of xylazine. Further research designed to study dose-response effects is needed to determine whether tolazoline exerts a counteractive effect on xylazine-induced retrograde flow of spermatozoa into the bladder. Even though a potential facilitatory effect of tolazoline on the retrograde flow of spermatozoa into the bladder cannot be discarded at this time, an interactive effect of furosemide and tolazoline seems unlikely in view of a reported (23) lack of interference of furosemide with the ejaculatory or electroejaculatory responses of rams.

The percentage of viable spermatozoa and the presence of motile spermatozoa in the post-treatment urine was not different between treatment groups, which provides additional support for an effect of both xylazine and EE on retrograde flow of spermatozoa into the bladder.

Results of Experiment 1 document significant losses of spermatozoa into the urinary bladder after administration of xylazine to sexually rested rams or when xylazine is given to rams before EE as an aid in the collection of semen for the breeding-soundness examination. Because of the magnitude of losses of spermatozoa caused by xylazine, its use as a sedative to restrain rams during EE is undesirable.

Experiment 2

The reduced volume of urine and decreased frequency of micturitions for the rams given xylazine is in disagreement with the reported (52) diuretic properties of xylazine in cattle. These effects could be ascribed to species differences in the diuretic response to xylazine, to an impairment in the evacuation of the bladder because of the recumbency induced by xylazine, or to inhibition of the micturition reflex in rams. $Alpha_2$-mediated inhibition of bladder parasympathetic ganglia causes relaxation of the detrusor muscle of the bladder (85). Thus, xylazine may have delayed micturition by preventing constriction of the bladder. However, I observed in this experiment that the first post-xylazine micturition was initiated shortly after the rams stood up after the xylazine-induced recumbency. This micturition was often followed, almost immediately, by one or more short, interrupted micturitions. This observation seems compatible with the hypothesis that the decreased frequency of micturitions may have been caused by the recumbency which prevented bladder evacuation. Furosemide exerts its diuretic effect through inhibition of reabsorption of sodium chloride in the ascendent limb of Henle's loop of the nephron (45). The diuretic effect of furosemide is thought to be mediated by prostaglandins (86), whereas α_2-adrenoreceptor agonists, like xylazine, induce diuresis by reversing vasopressin-induced water retention (51). Thus, an antidiuretic, antagonistic interaction between furosemide and xylazine cannot be ruled out because of the design of this experiment. Further studies are needed to clarify a potential antagonistic effect of xylazine on diuresis induced by furosemide.

The concentration and total number of spermatozoa in the pre-treatment urine were not different between treatment groups. The spermatozoal concentration in pre-treatment urine corresponded to data reported (22,23) in the literature for concentration of spermatozoa in the urine collected from rams immediately before ejaculation or EE. However, the concentration and total number of spermatozoa in the post-treatment urine were also not different between treatment groups. These results must be interpreted with caution, because the post-treatment micturitions may have been incomplete for the xylazine-treated rams. In addition, interactive effects between furosemide and xylazine may have affected the response to xylazine on the displacement of spermatozoa from the sites of spermatozoal storage. This possibility also cannot be ruled out because of the design of the experiment.

The number of azoospermic collections of post-treatment urine for the xylazine-treated rams of Group 2 suggests an erratic response to dose of xylazine which could be an individual trait. For instance, one of the two xylazine-treated rams, that had azoospermic urine for all the collections in Experiment 2, was also given xylazine in Experiment 1 and its urine was also azoospermic in all of the 4 collections performed at that time. In addition, the effect of xylazine on the total number of spermatozoa in the urine of xylazine-treated rams in Experiment 2 appeared to be less intense than for the rams of Group 3 in Experiment 1, which were given tolazoline 23 minutes after xylazine injection. Indeed, the post-xylazine urine obtained from the xylazine-treated rams of Group 3 in Experiment 1 contained spermatozoa in 7 of 16 collections (footnote in Table 6), with a

mean of 567 x 10^6 spermatozoa (N=16, Table 6), whereas the post-treatment urine obtained from the xylazine-treated rams in Experiment 2 contained spermatozoa in 7 of 18 collections (footnote in Table 11), with a mean of only 25 x 10^6 spermatozoa (N=18, Table 11).

The trend approaching significance (P=0.087, Table 12) for an effect of xylazine on the percentage of viable spermatozoa in the post-treatment urine, provides additional support for an effect of xylazine on retrograde flow of spermatozoa into the bladder. Even though not significant (P>0.1, Table 12), there appeared to be more samples of post-treatment urine that had motile spermatozoa for the xylazine-treated (71%) than for the saline control rams (50%). Here, again, the longer exposure of spermatozoa to urine in the bladder due to delayed micturitions may have precluded the finding of more samples with motile spermatozoa in the post-treatment urine for the xylazine-treated rams.

Individual or species differences in the number and distribution of α-adrenoreceptors between the epididymides (87) and proximal and distal vasa deferentia (54) maybe influence the magnitude of spermatozoal displacement into the bladder in response to xylazine. Xylazine induced retrograde flow of spermatozoa into the bladder in the absence of any sexual stimulation in 9 of 10 sexually rested dogs (1). However, xylazine induced ex-copula ejaculation in sexually rested stallions (47).

Experiment 3

The overall total volume of urine obtained during the first 60 minutes of collection after treatment and the volumes of each sequential

sample obtained at 20 minutes, 40 minutes, or 60 minutes after treatment were significantly larger for the xylazine-treated rams of Group 2 than for the control rams of Group 1. In addition, the volume of urine increased with time from the first to the second sequential samples taken after xylazine injection. These findings are consistent with the reported (52) diuretic effect of xylazine, but are in contrast to those of Experiment 1, in which no significant differences were detected, and with those of Experiment 2, in which the volume of urine was significantly decreased by xylazine. However, it must be noticed that for Experiment 3 neither furosemide nor tolazoline were given to the rams, and urine was obtained directly from the bladder. Thus, interference caused by recumbency or by interactive actions between furosemide, xylazine, and tolazoline did not play a role.

The mean (± SD) spermatozoal concentration in the pre-treatment urine was $0.01 \pm 0.02 \times 10^6$/ml (N = 20 observations) for rams of Group 1, and $0.06 \pm 0.10 \times 10^6$/ml (N = 19 observations) for rams of Group 2. These spermatozoal concentrations are in agreement with data reported in the literature (22,23). For this particular comparison, but not for Tables 15 and 16 or for the statistical analyses, one datum on spermatozoal concentration was discarded because it was artificially obtained by flushing the urinary bladder of a ram of Group 2 with saline, as explained in Materials and Methods. For the present experiment, the low concentration of spermatozoa and the occasional presence of motile spermatozoa in the pre-treatment urine are also consistent with the hypothesis (25) that the retrograde flow of spermatozoa into the urinary

bladder in sexually rested animals may be a physiological mechanism to discard aged spermatozoa.

The concentration of spermatozoa in the total volume of urine obtained from the bladder within 60 minutes after treatment was not significantly different between treatment groups when analyzed by ANOVA. The considerable variation among rams in spermatozoal concentration, as evidenced by the magnitude of the standard deviations, possibly precluded the detection of a treatment effect on the overall post-treatment concentration of spermatozoa in the urine.

There was a trend approaching significance (P=0.083, Table 15) for a larger total number of spermatozoa in the post-treatment urine obtained from xylazine-treated rams during the 60 minute period of collection than for the control rams when data was analyzed by ANOVA. Here again, the lack of significance was possibly due to ram variation, as evidenced by the magnitude of the standard deviations. Contrast ANOVA, performed after the fact, detected a significant effect of treatment on spermatozoal concentration and total number of spermatozoa (P=0.048 and P=0.009, respectively, Table 16) in the post-treatment urine for the xylazine-treated rams.

The post-treatment urine obtained during the 60 minute period of collection from the xylazine-treated rams in Experiment 3 contained spermatozoa in 15 of 20 collections (footnote in Table 15), with a mean of 1471 x 10^6 spermatozoa (N=20, Table 15). This response to xylazine treatment, regarding retrograde flow of spermatozoa into the bladder, resembles more closely data from xylazine-treated rams of Group 3 in

Experiment 1 (Table 6) than that from the xylazine-treated rams of Experiment 2 (Table 11). For Experiment 3, the response to xylazine was erratic for 2 treated rams, whose post-treatment urine was azoospermic in 1 of 5 and 4 of 5 collections, respectively.

The increased spermatozoal concentration and the increased total number of spermatozoa in post-treatment urine confirm that xylazine, when given alone, without interference from other drugs, induced retrograde flow of spermatozoa into the bladder of rams. The retrograde displacement of spermatozoa into the bladder caused by xylazine may last for a period longer than 60 minutes, because the samples of urine collected at 20, 40, and 60 minutes had spermatozoa, suggesting that the retrograde flow-inducing effect of xylazine may continue beyond the 60 minutes of collection.

No attempts were made to discard the fluid remaining in the bladder catheter, which may have contained part of the 4 ml of the 0.02% nitrofurazone solution used to fill the catheter after the last urine withdrawal for each collection day. Thus, a potential for a detrimental effect of any residual nitrofurazone on spermatozoal motility in the urine cannot be ignored. However, if such a detrimental effect existed, it was minimized because saline was infused into the bladder and then, the urine and saline in the bladder were evacuated. Furthermore, motile spermatozoa were found in the urine. The presence of motile spermatozoa in the post-treatment urine of xylazine-treated rams provides additional support for an effect of xylazine on retrograde flow of spermatozoa into the urinary bladder, because it indicates that these motile spermatozoa were recently

exposed to the urine in the bladder. The conclusions inferred from data on the end points analyzed in the urine obtained from the bladder of rams in this experiment are reinforced by the fact that each pre- and post-treatment collection of urine was done by first infusing saline and then evacuating the bladder. Thus, the spermatozoa found in each of the sequential samples of post-treatment urine retrograded into the bladder as a consequence of treatment with xylazine.

PART II

Experiment 4

The volume of electroejaculates decreased (P=0.0017) after vasectomy or vasectomy plus flushing of the vasa deferentia when compared to pre-vasectomy values. This is consistent with a previous report (65) in which a significant reduction of 40% to 50% in volume was reported for ejaculates of rams collected with an artificial vagina between 3 hours and 6 days after vasectomy. The volume of ejaculate was unaffected for the control, sham-operated rams (65). The post-vasectomy reduction in volume of ejaculate is in contrast with previous reports for dogs (25,66,67) and cats (25,66), and probably reflects species differences in the volumetric contribution of seminal components from the accessory sex glands.

The volume of the post-vasectomy electroejaculates from PVD 3 to PVD 63 was not affected by flushing of the vasa deferentia or by day of collection. The lack of an interaction treatment x day of collection provides additional support for the lack of an effect of treatment.

The total number of spermatozoa and number of intact spermatozoa in the electroejaculates obviously decreased rapidly from the pre- to the first post-vasectomy collections and then decreased gradually to PVD 63 for both control and treated rams. However, the variation in responses among rams and between treatment groups on concentration of spermatozoa, total number of spermatozoa, and on number of intact spermatozoa in the post-vasectomy electroejaculates was considerable, as evidenced by the magnitude of the standard deviations. This precluded, when factorial ANOVA was used, the detection of an effect of treatment or of day on these end points for

the post-vasectomy electroejaculates obtained from PVD 3 to PVD 63. Nevertheless, the overall contrast pre- versus post-vasectomy analysis detected significant (P=0.0001) differences for spermatozoal concentration, total number of spermatozoa, and number of intact spermatozoa in the electroejaculates. In view of these pre- versus post-vasectomy differences, tests of homogeneity of variances were done after the fact to detect treatment effects for pre-vasectomy day 0, PVD 3, and PVD 7. As expected, the variances for these end points were homogeneous between control and treated rams on day 0 (Tables 23 and 24). However, the variances were no longer homogeneous on PVD 3 nor on PVD 7. Thus, the control and treated rams no longer belonged to the same population after the vasa deferentia of the rams from the treated group were flushed at the time of vasectomy. This also explains the low power of ANOVA to detect differences between treatment groups during the post-vasectomy period, because the use of ANOVA is based on the assumption of the homogeneity of variance (80) among treatment groups.

The slopes of regression of data for the total number of spermatozoa and number of intact spermatozoa in the post-vasectomy electroejaculates for post-vasectomy days (from PVD 3 to PVD 63) were not different between control and treated rams. However, the mean of the cumulative total number of spermatozoa present in the electroejaculates by the end of the post-vasectomy period was approximately 50 times higher for control than for treated rams. Indeed, the slopes of the regression of cumulative total number of spermatozoa versus post-vasectomy days (PVD) were different between treatment groups, providing an additional evidence for an effect of

flushing the vasa deferentia at the time of vasectomy on the rate of clearance of spermatozoa from the electroejaculates of vasectomized rams.

The prolonged presence of spermatozoa in the post-vasectomy electroejaculates of control rams, whose vasa deferentia were not flushed, and that of treated rams, whose vasa were flushed during vasectomy, resembles the rate of clearance of spermatozoa from ejaculates of vasectomized cats (66). However, the rate of clearance of spermatozoa from the ejaculates of vasectomized rams approaches more closely the rate of clearance of spermatozoa from ejaculates of men after vasectomy and vasal flushing (77). Spermatozoa were found in smears of centrifuged samples of semen of men 105 days after vasectomy or vasectomy and flushing the vasa deferentia (77). In the present experiment, intact spermatozoa were found in the ejaculates of control and treated rams for a longer post-vasectomy period than that reported for dogs (25,67) and cats (25,66), suggesting that a factor or factors contributed by vasa deferentia or ampulla protects ram spermatozoa against head detaching.

Flushing the vasa deferentia at the time of vasectomy reduced to zero the number of intact spermatozoa and detached spermatozoal heads in the ejaculate by PVD 6 in the dog (25). In cats (25), flushing the vasa deferentia reduced the number of intact spermatozoa to zero by PVD 7, but detached spermatozoal heads were found up to PVD 49, whereas the non-flushed controls had detached spermatozoal heads by PVD 63. This approaches the prolonged rate of clearance of spermatozoa from the ejaculates of vasectomized rams, whose vasa where flushed. Some possible factors involved in the delayed spermatozoal clearance for the ejaculates

of vasectomized rams could be the following. First, spermatozoa may be more tightly lodged to the walls of the vasa deferentia, possibly because of the convoluted inner surface of their well developed ampullae (14,16). Thus, the flushing of the vasa may have not been sufficient in volume or efficient in the strength of the jet of passing fluid to dislodge the spermatozoa. The vasa deferentia of men have also well developed ampullae, and the vasal flushing in men (77) was not as efficient as it was reported (25) for dogs and cats. Second, storage of spermatozoa in the vesicular and bulbourethral glands after vasectomy seems less likely, because accumulation of spermatozoa was not observed in the excurrent ducts (43,44) during radiographic studies in live rams, although accumulation of spermatozoa was found in the vesicular and bulbourethral glands of rams after slaughter (62).

The rapidly decreasing mean values for total number of spermatozoa in the electroejaculates of treated rams from PVD 3 to PVD 7 contrast dramatically (Table 21) with the means for the same period for the control rams. After PVD 7, the means for the remaining post-vasectomy period for both treatment groups appeared to decline at a similar rate. Thus, flushing the vasa deferentia may have washed off only those spermatozoa that were relatively free in the lumen of the vasa deferentia, leaving a population of spermatozoa that were more tightly bound to the walls of the vasa, which were then slowly released after vasectomy.

Mean spermatozoal loads were estimated at post-mortem examination for several portions of the genito-urinary tract of intact rams (16,62), and these ranged from 30 to 315 x 10^6 spermatozoa for the ampullae of the vasa

deferentia, and from 99 to 164 x 10^6 spermatozoa for the remainder of the vasa deferentia (16,62). In another study (12), 3245 x 10^6 spermatozoa were found in the whole vasa deferentia, including the ampullae, and 5014 to 5122 x 10^6 spermatozoa were recovered from the urinary bladder and urethra (12,16). In a post-mortem examination of a single vasectomized ram (62), 0.35 x 10^6 spermatozoa were found in the ampullae, and 0.09 x 10^6 spermatozoa in the remainder of the vasa deferentia. If one estimate, from data reported in two of these studies (16,62), the mean spermatozoal load of the vasa deferentia, including the ampullae, to be approximately 250 x 10^6, this mean would be lower than the mean cumulative total number of 666 x 10^6 spermatozoa released into the electroejaculates of the vasectomized control rams during the post-vasectomy collections up to PVD 63. This disparity, if real, could be explained on the basis of determination of spermatozoal load in necropsy specimens, retrograde flow of spermatozoa into the urinary bladder induced by death (12,16), or even due to increased spermatozoal load of the vasa deferentia before vasectomy caused by the immediately preceding electroejaculation. Studies on the post-ejaculation distribution of spermatozoa in the vasa deferentia of rabbits (35), and on spermatic flow through cannulated vas deferens in rams (16) suggest that the spermatozoal displacement into the vas caused by the pre-vasectomy EE may have delayed the spermatozoal clearance.

The electroejaculates of control rams had viable and motile spermatozoa until PVD 7, whereas for treated rams, viable and motile spermatozoa were found in the electroejaculates only until PVD 3. Furthermore, a significantly higher number of electroejaculates containing

motile spermatozoa were obtained on PVD 3 from control rams than from treated rams.

Most of the blue-marked fluid used to flush the vasa deferentia of rams at the time of vasectomy retrograded into the urinary bladder. This indicates that for anesthetized rams, as also reported (25) for dogs and cats, the pathway of least resistance for the fluid flushed through the vasa deferentia is toward the urinary bladder.

In conclusion, flushing the vasa deferentia at the time of vasectomy accelerated the rate of clearance of spermatozoa from the electroejaculates of vasectomized rams, but it did not induce azoospermia by PVD 63, time at which the post-vasectomy collections were discontinued. However, flushing the vasa deferentia at the time of vasectomy reduced to zero by PVD 7 the presence of viable, motile spermatozoa in the electroejaculates in the treated rams, whereas the number of viable and motile spermatozoa was reduced to zero by PVD 14 in the control rams.

Flushing the vasa deferentia was easy to perform in rams and did not appear to induce any complication after surgery. This technique may have clinical application for the sheep producer because the electroejaculates became devoid of viable and motile spermatozoa one week earlier than vasectomy alone. The following modifications may improve the efficiency of the flushing of the vasa deferentia: 1) the use of a larger gauge needle to cannulate the vasa, 2) an increase in the volume and speed of the flow of flushing fluid, and 3) the use of a flushing fluid containing a non-irritant spermicidal agent.

SUMMARY AND CONCLUSIONS

PART I

The experiments of Part I of this study showed 1) that xylazine induces retrograde flow of spermatozoa into the urinary bladder of sexually rested rams, causing urinary losses of spermatozoa that are similar to those induced by EE, and 2) that administration of xylazine to rams before EE contributes to decrease the number of spermatozoa in the electroejaculates. Urinary losses caused by retrograde flow of spermatozoa into the bladder during EE decrease the number of spermatozoa in the ejaculate, hence the use of xylazine as an aid in the collection of semen by EE in breeding-soundness examinations is not recommended.

PART II

Vasectomy is commonly used to render rams sterile for use as teaser males for detecting estrous ewes and for hastening and synchronizing the onset of estrous cyclicity of ewes. To safely use a vasectomized ram without the risk of inducing unwanted pregnancies, however, it is essential to ascertain the time required for the ejaculates to become azoospermic or devoid of viable spermatozoa after vasectomy. In addition, shortening the time of clearance of spermatozoa from the ejaculates of vasectomized rams increases the advantages of vasectomy for the reproductive management of sheep. This study showed that the time and number of seminal collections after vasectomy required for the induction of azoospermia in rams is more than 9 weeks and 10 collections, respectively. Flushing the vasa deferentia at the time of vasectomy accelerated the rate of clearance of

viable spermatozoa from the ejaculates of vasectomized rams. Flushing the vasa deferentia at the time of vasectomy may have clinical applications since this technique resulted in the obtainment of electroejaculates devoid of viable and motile spermatozoa one week earlier than vasectomy alone. Flushing the vasa deferentia is easy to perform in rams and do not appear to cause complications after surgery.

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