Reproduction in Domestic Animals

Reprod Dom Anim doi: 10.1111/j.1439-0531.2012.02123.x
ISSN 0936-6768

Efficient Association between PGF2α and Methyl 4-hydroxybenzoate Sex Pheromone Prior to Electroejaculation in Dogs

IP Santos, CLFG Ramos, JLG Ramos, RF Oliveira and ICN Cunha

Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, Rio de Janeiro State, Brazil

Contents

Electroejaculation is a technique that can be used to collect semen from canines, but its use with this group of animals is restricted by low success rate and low semen quality. Here, we evaluated whether pharmacological and sexual sensory stimuli, which may affect ejaculation, can increase electroejaculation efficiency and improve ejaculate quality. We worked with 20 dogs of mixed breed weighing between 5.3 and 22.2 kg, divided into two groups. Both groups were exposed to a spayed female for 10 min, but in the second group, the same spayed female had her vagina impregnated with methyl 4-hydroxybenzoate synthetic pheromone for 10 min and after receiving dinoprost tromethamine IM, 0.1 mg/kg. After stimulation, all dogs were chemically restrained with ketamine, 8 mg/kg, IM; and xylazine, 1 mg/kg, IM, and subjected to electroejaculation protocol. We obtained 100% of antegrade ejaculate in treatments when the spayed female had her vagina impregnated with pheromone and 80% when she did not. Sperm motility was significantly different ($p < 0.05$) between controls and the test group (10.1 ± 4.5 and 43.0 ± 8.3, respectively). We concluded that the adopted electroejaculation protocol was efficient and that the PGF2α associated with sexual sensory stimulation can improve semen quality in dogs undergoing the procedure.

Introduction

Semen collection in dogs is usually performed by digital stimulation of the penis because the process is easy and does not necessarily require prior conditioning of the animal or the presence of an oestrous bitch (Kutzler 2005). Other methods used to collect canine semen require artificial vagina or electroejaculation (Ohl et al. 1994; Goodrowe et al. 1998; Kutzler 2005; Minter and DeLiberto 2005; Newell-Fugate 2009). Although electroejaculation is not the standard method for collecting the semen of domestic dogs, reproductive biotechniques that have been developed for the dog can now be used to collect semen from endangered canines (Minter and DeLiberto 2005). During electroejaculation, the animal needs to be chemically restrained. Anaesthetic combinations such as tiletamine and zolazepam (Goodrowe et al. 1998; Cunha et al. 2008), or ketamine and xylazine (Minter and DeLiberto 2005), or ketamine, medetomidine and atropine (Johnston et al. 2007) or halothane (Ohl et al. 1994) alone, have been used.

The drawbacks of canine semen collection via electroejaculation usually contain a low amount of sperm and/or low motility, which results in semen of low quality. Additionally, the ejaculate is frequently contaminated with urine (Cunha et al. 2008). In an attempt to obtain semen samples of better quality, some authors have used hormones and neurotransmitters that increase male libido (Estienne and Harper 2004; Masoumi et al. 2008), affect the time of ejaculation and amount of semen ejaculated (Yonezawa et al. 2001), or even increase sperm motility (Shimizu et al. 1998; Karahan et al. 2006; Yeste et al. 2008).

Prostaglandin F2-alpha (PGF2α) increases ejaculate volume by intensifying contraction of the smooth muscles responsible for semen discharge (Hess 2006; Kustritz and Hess 2007). Sensory stimuli by sex pheromones of synthetic origin, or secreted by an oestrous bitch, such as methyl 4-hydroxybenzoate (Goodwin et al. 1979), may also increase the amount of semen collected through manual stimulation of the penis (Kutzler 2005; Kustritz and Hess 2007). Herein, we described the combined use of sensory and hormonal stimuli, performed prior to chemical restraint, to improve the efficiency of electroejaculation in dogs.

Materials and Methods

Animals

We used 20 mongrel dogs presenting no signs of clinical abnormalities and considered suitable for semen collection via electroejaculation. Reproductive history of the study animals could not be obtained because all dogs came from local shelters. The dogs were divided in groups, without any randomizing procedure, of four, and once a week, they were taken to the Veterinary Hospital and each animal underwent clinical evaluation and ultrasound of testis and prostate, before undergoing semen collection.

Animals were held for 3 days in individual cages to get adapted. During the first 2 days, the animals received water and rations twice a day (morning and afternoon). A pre-anaesthetic fasting regime was implemented on the third day and the dogs were then subjected electroejaculation, as described below. After electroejaculation, all animals were castrated and returned to the local shelters for future adoption.

The experiment was approved by the Ethics Committee on Animal Use/UENF (protocol number 67/2009) and performed in the summer of 2009/2010, from November to March.

Semen collection

Samples were collected at a controlled temperature of ±23°C. The electroejaculator (Santa Lydia Laboratories, Presidente Prudente, SP, Brazil) was manufactured

to the following specifications: voltage 0–10.9 V, current of 100 and 200 mA, sine waves with a frequency of 50 and 60 Hz and probe with two parallel 8-cm longitudinal electrodes. The probe had a total length of 25.4 cm and diameter of 1.6 cm, similar to that previously described devices (Ohl et al. 1994; Newell-Fugate 2009).

Before electroejaculation was started, the dogs were divided into two groups. Each group received a different sensory and pharmacological treatment to prepare them for semen collection. Differently than the test group (n = 10), the control group (n = 10) was not exposed to pheromone. The control group underwent the procedure prior to the test group to avoid interference from synthetic pheromone residues that might have remained in the environment.

PGF2α + pheromone group

A swab moistened with saline was inserted into a bottle of powdered pheromone methyl 4-hydroxybenzoate sex pheromone (cat. H5501; Sigma-Aldrich®, Saint Louis, MO, USA) and then inserted into the vagina of a spayed bitch at a depth of 2 cm. Then, 10 dogs that received a dose of dinoprost tromethamine (Lutalyse®) at 0.1 mg/kg IM were exposed to this bitch for 10 min. Next, anaesthesia and electroejaculation were applied, as described next.

Anaesthesia was performed using a combination of 8 mg/kg ketamine hydrochloride (Cetamin®; Syntec, Hortolândia, SP, Brazil) and 1 mg/kg xylamine hydrochloride (Xilazin®; Syntec, Hortolândia, SP, Brazil). After anaesthesia, catheterization of the radial vein for fluid therapy with 5 ml/kg/h saline was performed.

Electroejaculation The probe, lubricated with KY gel® (Johnson & Johnson, São José dos Campos, SP, Brazil), was placed rectally above the prostate of each animal. The complete protocol consisted of 10 series with 10 electrical stimuli at a current of 100 mA and frequency of 50 Hz that lasted for 3 s followed by 3 s of rest. The electrical voltages applied to the 1–10 series were 1.5 V; 2.4 V; 3.0 V; 4.0 V; 4.2 V; 4.9 V; 5.9 V; 7.3 V; 8.8 V and 10.9 V. Stimulation intensity was gradually increased until each dog started to ejaculate. Then, electrical stimuli were continuously applied at the same voltage until ejaculation ceased. When ejaculation was over, the electrical stimuli ceased, regardless of the status of the 10 series sequence. Before the two last stimuli of each series, the probe was removed and placed back on the prostate. Then, the last two electrical stimuli were applied, and a new series of 10 stimuli at a higher voltage started.

The correct probe positioning was observed by perianal muscle contraction in the first three series and easing of hind legs, especially after the fourth series. Induction, semen collection and returning from anaesthesia altogether lasted up to 20 min. If the animal awoke during electroejaculation, a half dose of the ketamine and xylazine combination was intravenously administered.

Control group

Ten dogs were individually exposed to the same spayed bitch for 10 min and anesthetized as already described.

Bladder catheterization and semen collection during electroejaculation

To search for the presence of sperm before and after electroejaculation, the bladder of each animal was catheterized with urethral probe no. 4 or 6 (EMBRAMED, Brazil) (depending on the dog's size); contents were aspirated using a 20-ml syringe and then transferred to 50-ml tubes. The standard aspirated volume was 10 ml because most dogs urinated and defecated as soon as they went out of the cage.

During electroejaculation, one of the team members kept the animal's penis exposed and a 50-ml plastic tube, pre-warmed at 37°C, was placed over the glands to collect the semen samples.

Monitoring of animals

All animals were monitored during electroejaculation. The observed variables were respiratory and heart rates, both measured every 5 min and rectal temperature, measured before and after the procedure.

Ejaculate assessment

To establish the efficiency of the electroejaculation protocol, we checked for the presence of sperm in urine, in addition to antegrade ejaculation. Antegrade ejaculation was considered as the ejection of any liquid contents through the urethra. The material collected was classified as: (i) ejaculates with or without sperm: transparent liquids, (ii) pure semen: white liquid and (iii) semen contaminated with urine: yellow liquids (we noted the presence of sperm in all yellow samples). Total sperm motility (%) and vigour (motility quality, score of 0–5) of all samples (regardless of the classification) were evaluated by three different observers. We also evaluated sperm morphology after dilution of sample in formalin–saline (1 : 10) between slide and coverslip under phase contrast microscopy at 1000× magnification (Nikon, model Eclipse 80*i*), using the classification proposed by Oettlé (1993); sperm concentration in a Neubauer chamber after dilution of sample in formalin–saline (1 : 200); pH, using colorimetric pH stripes at a scale of 0–14 (Universalindikator; Merck; Darmstadt, Germany); and osmolarity, using an osmometer, model 5004 Micro-osmett™ (Precision Systems, Inc., Natick, MA, USA).

Statistical analysis

Data were tabulated in Microsoft Excel® spreadsheets and evaluated in environment R version 2.11.1 (2010). After testing for normality with the Shapiro–Wilk test ($\alpha = 0.05$), data that did not normalize even after transformation (for example: total motility, vigour, total sperm count, osmolarity, pH and normal sperm)

were compared by Wilcoxon rank sum test. Normal data (example: sample volume) were compared by *t*-test. Contingency tables of data from success rate of ejaculation were analysed by chi-square. Data are presented with their respective means ± standard errors.

Results

Behavioural and clinical aspects during the procedure

The average weight and body score (scale of 1–5) of experimental animals in both groups were similar ($p > 0.05$): control, 15.7 ± 1.5 kg/3.9 ± 0.2 and PGF2α + pheromone, 12.6 ± 1.3 kg/3.5 ± 0.3. Immediately after applying dinoprost tromethamine (Lutalyse®), the following side effects were observed: salivation (n = 10) and defecation of loose faeces (n = 4). There were no effects that could be related to an association between synthetic prostaglandin and anaesthetics. There was no sexual behaviour observed between the dogs and the bitch; for example, there were no attempted mounts, even though she was impregnated. Temperature of the animals before and after treatment was 39.0 ± 0.1 and 38.2 ± 0.2 in control and 38.9 ± 0.1 and 38.7 ± 0.1 in PGF2α + pheromone ($p > 0.05$).

Heart rate ranged from 96.0 ± 6.7 to 77.6 ± 4.9 in control, and 89.1 ± 7.5 and 81.6 ± 6.9 in PGF2α + pheromone ($p > 0.05$). Respiratory rate ranged from 23.1 ± 1.9 to 14.8 ± 2.9 in control, and 21.6 ± 2.1 and 15.2 ± 1.4 in PGF2α + pheromone ($p > 0.05$). Vocalization occurred only after returning from anaesthesia for all dogs, beginning at the eighth series of electrical stimulation, and when vocalization occurred, half the initial dose of anaesthetic was applied. Once the animal returned from anaesthesia, electrical stimuli were applied to the voltage that resulted in ejaculation. One of the dogs in the control group did not ejaculate during the sequence of electrical stimuli at 100 mA, and a new sequence at 200 mA was initiated until he ejaculated. The animal ejaculated during the tenth series.

Electroejaculation protocol efficiency

Antegrade ejaculation occurred in 80% of the animals in the control group and 100% in the PGF2α + pheromone group, and did not differ between groups (Table 1). All performed procedures (n = 20) were efficient, and resulted in samples with sperm, even when it come from retrograde ejaculation resulted in samples with sperm, even if due to retrograde ejaculation. The dogs subjected to electroejaculation responded similarly to electrical stimulation regardless of the treatment (Table 2). Sperm number and motility found in the bladder before electroejaculation were always lower than that observed after the procedure was performed. This indicates that sperm collected in the bladder was expelled during the procedure. Motility differed significantly between treatments ($p = 0.019$), revealing greater effectiveness in PGF2α + pheromone, as compared to control (Table 1). Other variables did not differ between treatments (Tables 1 and 2).

Table 1. Seminal characteristics of antegrade ejaculate from dogs subjected to electroejaculation before being stimulated by PGF2α/-methyl 4-hydroxybenzoate pheromone association and control (mean ± standard error)

Variables	Control	PGF2α + pheromone	p value
Success rate (n)	8 (10)	10 (10)	0.456
Volume (ml)	1.9 ± 0.8	1.5 ± 0.5	0.744
pH	7.6 ± 0.3	7.4 ± 0.2	0.473
Total motility (%)	10.1 ± 4.5*	43.0 ± 8.3*	0.019*
Vigour (0–5)	0.5 ± 0.3	1.4 ± 0.3	0.066
Total sperm count ($\times 10^6$)	68.1 ± 23.8	103.7 ± 36.0	1.000
Osmolarity (mOsmol/l)	442.8 ± 66.7	656.5 ± 79.5	0.170
Normal sperm (%)	59.8 ± 13	42.7 ± 8.8	0.324

*Are statistically different, with values of $p < 0.05$.
Ejaculation occurred beginning in the fifth stimulation series in the control group and in the eighth series in the PGF2α + pheromone group, as shown in Table 2.

Discussion

Known side effects of PGF2α, such as salivation and defecation (Kustritz and Hess 2007), occurred soon after the administration of PGF2α and were brief; thus, during those 10-min which the animals were kept in the presence of the spayed bitch, these side effects were not evident. We did not observe any difference between groups for the vital signs, such as heart and respiratory rate or rectal temperature.

The success rate of the electroejaculation was similar for both treatments (Table 2). This may be due to the fact that electroejaculation was performed with sine

Table 2. Percentage of antegrade electroejaculations and ejaculate characteristics per series in dogs previously stimulated by the PGF2α and methyl 4-hydroxybenzoate compared to controls

Variable	Treatment	Classification	Series									
			1th	2th	3th	4th	5th	6th	7th	8th	9th	10th
Ejaculation (%)	Control	Absent	100	100	100	100	90	80	80	70	30	10
		Semen	0	0	0	0	10	10	0	10	30	30
		Urine	0	0	0	0	0	0	0	0	0	0
		Semen + urine	0	0	0	0	0	0	0	0	20	10
		EE[a]	0	0	0	0	0	10	20	20	20	50
	PGF2α + pheromone	Absent	100	100	100	100	100	100	100	60	40	0
		Semen	0	0	0	0	0	0	0	10	20	10
		Urine	0	0	0	0	0	0	0	10	0	0
		Semen + urine	0	0	0	0	0	0	0	20	10	40
		EE[a]	0	0	0	0	0	0	0	0	30	50

[a]EE, ejaculates already evaluated and recorded in previous series.

waves, which uses lower voltage to induce ejaculation, as compared with other equipment that uses other wave types (Furman et al. 1975). The electrical pulse sequence based on the work by Newell-Fugate (2009), which has no rest interval between series, also made the whole process continuous and progressive, without loss of intensity between series. Different protocols of electroejaculation used in canids, as used by Cunha et al. (2008), have some rest between the series that allows to loose erection during the procedure and can reduce the performance of the electrical stimuli aiming the ejaculation. It should be noted that probe repositioning before the last two stimuli in each series (Newell-Fugate 2009) seems to be essential for keeping a homogeneous and intimate contact between electrodes and the rectal mucosa during the procedure.

The dogs used in this study began to ejaculate when reaching 4.0 V stimulation, different from results observed by Ohl et al. (1994) in which the dogs began to ejaculate at 8 V and 93 mA. Regarding semen flow during ejaculation, Ohl et al. (1994) observed 34 retrograde ejaculations in 35 electroejaculation procedures. In this experiment, 100% of dogs from the PGF2α + pheromone group and 80% from the control group presented antegrade ejaculation, confirming what has already been seen that probably wave type and series procedure for applying stimuli were responsible for more satisfactory results. The maximum voltage and current used, 10.9 V and 100 mA, were similar to those used by Ohl et al. (1994): 12 V and 149 mA. It is noteworthy that 40% of dogs from the control group ejaculated at the tenth stimuli series when maximum voltage was used.

There is divergence among results concerning libido improvement in studies in which animals received similar PGF2α analogues prior to semen collection. Estienne and Harper (2004) used dinoprost tromethamine repeatedly to increase efficiency of semen collection from boars, but found no effects of this drug on semen characteristics such as concentration, count, motility and velocity. However, in previous research, authors observed that dinoprost tromethamine effectively increased libido of sexually inexperienced boars (Kozink et al. 2002). In studies with dogs, neither PGF2α nor oxytocin analogues were observed to increase sperm number in ejaculate (Traas and Kustritz 2004), but when in the presence of an oestrous bitch, sperm number was higher when just dinoprost tromethamine was used. In this study, we observed that dogs showed little interest in females impregnated with synthetic pheromone, while receiving dinoprost tromethamine. Furthermore, the dogs tended to adopt typical rest behaviour, as to keep sitting or lying. To contrast this observation with a natural condition of male exposure to an oestrous female, we conducted a parallel experiment (unpublished data) in which we observed four dogs in the presence of an oestrous bitch. We found that reaction time was the same for all animals, including the jumping and attempted mounting, and this behaviour lasted no more than 5 min (Ohl et al. 1994).

Semen motility in dogs from the PGF2α + pheromone group was greater than from those in the control group. Yeste et al. (2008) observed increased progressive sperm motility in boars after the addition of PGF2α to semen. Authors of experiments that used PGF2α to improve dog semen collection found no association between PGF2α and significant improvement in motility before semen collection (Traas and Kustritz 2004). The effect eicosanoids have on sperm motility differs between target species and prostaglandin structure. While E_1 and E_2 prostaglandins were responsible for increased human sperm motility by enabling a calcium influx and consequent sperm capacitation (Shimizu et al. 1998), PGF did not alter semen motility in pigs (Maes et al. 2003) and demonstrated equivocal effects on bovine semen motility, depending on the dose, according to *in vitro* tests (Karahan et al. 2006).

Total sperm number did not differ between treatments. These results are consistent with previous studies that found no positive effects of PGF2α (singly) on sperm concentration in dogs (Traas and Kustritz 2004). However, in another work, it was observed that the association of PGF2α application and the presence of an oestrous bitch increased total sperm number in ejaculate (Kustritz and Hess 2007) and that sperm production is linked to the animal body weight (Olar et al. 1983). Kustritz and Hess (2007) argue that the difference in sperm number could have been caused by the large difference in weight between dogs from different groups. The decision to evaluate ejaculate osmolarity was made considering this variable as an indicator of contamination by urine, which could reduce sperm motility. However, no significant difference between osmolarity values was found between groups. Because ejaculate osmolarity in PGF2α + pheromone was slightly larger, as compared to control, motility was higher in PGF2α + pheromone.

Yellow colour in ejaculate indicates the presence of contamination by urine, which was observed in 50% of dogs in the control group and in 80% of dogs in the PGF2α + pheromone group. We found that even though a great percentage of ejaculates in the PGF2α + pheromone were contaminated by urine, this did not interfere with sperm motility. Our results indicate that the association of xylazine, α-2 adrenergic receptor agonist and ketamine was not effective in preventing urination during ejaculation. These findings differ from those reported by Romagnoli (2002), in which the use of α-2 adrenergic agonists might be able to close the bladder neck and prevent urination during ejaculation. As shown in Table 2, there was no interference by dinoprost tromethamine during the latency period, different from what was observed when using α_2-adrenergic antagonists to investigate the effects that these drugs have on ejaculation in dogs (Yonezawa et al. 2001).

Future studies are needed to confirm our findings regarding vigour, percentage of normal sperm and osmolarity given that our sample size (n = 20) was relatively small and the selection of study animals was non-randomized. Finally, we conclude that improvement in sperm motility and total number obtained in antegrade ejaculations prior to PGF2α stimulation before electroejaculation indicates that the association of these eicosanoids with anaesthetics is safe for use in

dogs. The association between PGF2α analogue and methyl 4-hydroxybenzoate sex pheromone was shown to be effective in improving sperm motility in dogs used in this study, and other evaluated parameters indicate that it is possible to achieve better electroejaculation results when dogs receive ejaculatory stimulation prior to electroejaculation; the adopted electroejaculation procedure, regardless of animals' exposure to pre-ejaculatory stimuli, resulted in an average of 90% antegrade ejaculations and 100% total efficiency when we consider retrograde ejaculation.

Acknowledgements

We are grateful to the veterinary doctor Monica Luz and student Priscilla Carla Santos Costa for their assistance in surgical castration and to CNPq and FAPERJ for financial support.

Conflict of interest

All authors disclose does not have financial and personal relationships with other people or organizations that could inappropriately influence this work.

Author contributions

This article is based on the doctoral thesis conducted at UENF's Animal Science Program of Israel Pereira dos Santos; Israel Pereira dos Santos substantially contributed to conception and design of the study; acquisition, analysis and interpretation of data; drafting the article; and final approval of the version to be published. Cristina Leite Francisco Gualberto Ramos, José Leonardo Gualberto Ramos and Rodrigo Fortunato de Oliveira substantially contributed to the acquisition of data. Isabel Candia Nunes da Cunha, doctoral thesis supervisor, substantially contributed to conception and design of the study; analysis and interpretation of data; revising manuscript critically for important intellectual content; and final approval of the version to be published.

References

Cunha ICN, Morato RG, Santos IP, 2008: Biometry of the reproductive system and the ejaculation response of maned wolf (*Chrysocyon brachyurus*) to electroejaculation procedure. In: 6th International Symposium on Canine and Feline Reproduction, Viena, pp. 68–69.

Estienne MJ, Harper AF, 2004: Semen characteristics and libido in boars treated repeatedly with PGF2α. J Anim Sci **82,** 1494–1498.

Furman JW, Ball L, Seidel GE, 1975: Electroejaculation of bulls using pulse waves of variables frequency. J Anim Sci **40,** 665–670.

Goodrowe KL, Hay MA, Platz CC, Behrns SK, Jones MH, Waddell WT, 1998: Characteristics of fresh and frozen-thawed red wolf (Canis rufus) spermatozoa. Anim Reprod Sci **53,** 299–308.

Goodwin M, Gooding KM, Regnier F, 1979: Sex pheromone in the dog. Science **203,** 559–561.

Hess M, 2006: Documental and anecdotal effects of certain pharmaceutical agents used to enhance semen quality in the dog. Theriogenology **66,** 613–617.

Johnston SD, Ward D, Lemon J, Gunn I, MacCallumb CA, Keeley T, Blyde D, 2007: Studies of male reproduction in captive African wild dogs (*Lycaon pictus*). Anim Reprod Sci **100,** 338–355.

Karahan I, Türk G, Gür S, 2006: *In vitro* effects of prostaglandin F2α and metamizol on the motility of diluted bull semen. Turk J Vet Anim Sci **30,** 271–278.

Kozink DM, Estienne MJ, Harper AF, Knight JW, 2002: The effect of lutalyse on the training of sexually inexperienced boars for semen collection. Theriogenology **58,** 1039–1045.

Kustritz MVR, Hess M, 2007: Effect of administration of prostaglandin F2alpha or presence of an estrous teaser bitch on characteristics of the canine ejaculate. Theriogenology **67,** 255–258.

Kutzler MA, 2005: Semen collection in the dog. Theriogenology **64,** 747–754.

Maes DGD, Mateusen B, Rijsselaere T, De Vliegher S, Van Soom A, de Kruif A, 2003: Motility characteristics of boar spermatozoa after addition of prostaglandin F2α. Theriogenology **60,** 1435–1443.

Masoumi R, Towhidi A, Javaremi AN, Nabizadeh H, Zhandi M, 2008: Cloprostenol injection improves reproductive characteristics in low libido Iranian Holstein bulls. Pak J Biol Sci **11,** 1027–1031.

Minter LJ, DeLiberto TJ, 2005: Influence of extender, freezing rate, and thawing rate on post-thaw motility, viability and morphology of coyote (Canis latrans) spermatozoa. Theriogenology **64,** 1898–1912.

Newell-Fugate AE, 2009: The effects of two formulations of deslorelin on the reproduction of male African wild dogs (*Lycaon pictus*). Master's Dissertation. University of Pretoria, South Africa.

Oettlé EE, 1993: Sperm morphology and fertility in the dog. J Reprod Fertil Suppl **47,** 257–260.

Ohl DA, Denil J, Cummins C, Menge AC, Seager SWJ, 1994: Electroejaculation does not impair sperm motility in the beagle dog: a comparative study of electroejaculation and collection by artificial vaginal. J Urol **152,** 1034–1037.

Olar TT, Amann RP, Pickett BW, 1983: Relationships Among Testicular Size, Daily Production and Output of Spermatozoa, and Extragonadal Spermatozoal Reserves of the Dog. Biol Reprod **29,** 1114–1120.

Romagnoli S, 2002: Infertility in the male *dog* – A diagnostic approach. Proceedings of the Veterinary Sciences Congress, SPCV, Oeiras, Portugal. pp. 171–176.

Shimizu Y, Yorimitsu A, Maruyama Y, Kubota T, Aso T, Bronson RA, 1998: Prostaglandins induce calcium influx in human spermatozoa. Mol Hum Reprod **4,** 555–561.

Traas AM, Kustritz MVR, 2004: Effect of administrating oxytocin or prostaglandin $F_{2\alpha}$ on characteristics of the canine ejaculate. Can Vet J **45,** 999–1002.

Yeste M, Briz M, Pinart E, Sancho S, Garcia-Gil N, Badia E, Bassols J, Pruneda A, Bussalleu E, Casas I, Bonet S, 2008: Boar spermatozoa and prostaglandin F2α Quality of boar sperm after the addition of prostaglandin F2α to the short-term extender over cooling time. Anim Reprod Sci **108,** 180–195.

Yonezawa A, Ando R, Watanabe C, Furuta S, Kutsuwa M, Sakurada S, Kimura Y, 2001: α_2-Adrenoceptor antagonistis: effects on ejaculation, penile erection and pelvic thrusting behavior in dogs. Pharmacol Biochem Behav **70,** 141–147.

Submitted: 21 November 2011; Accepted: 8 May 2012

Author's address (for correspondence): Isabel Candia Nunes da Cunha, Universidade Estadual do Norte Fluminense – Avenida Alberto Lamego, 2000, Campos dos Goytacazes – RJ, CEP 28013-600, Brazil. E-mail: cunhaicn@uenf.br