

Documented and anecdotal effects of certain pharmaceutical agents used to enhance semen quality in the dog

Milan Hess *

Colorado Veterinary Specialists, 223 W. County Line Rd., Littleton, CO 80129, USA

Abstract

Prostaglandin $F_{2\alpha}$, gonadotropin releasing hormone, cabergoline and various nutraceuticals have all been recommended by reproductive practitioners to improve sperm motility and morphology and to increase sperm numbers in the ejaculate of the dog. Increasing sperm quantity and quality in the canine ejaculate would benefit all assisted reproductive techniques used in this species. The purpose of this manuscript is to review the documented and anecdotal effects of certain pharmaceuticals used to enhance semen quality in the dog.

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1. Introduction

The demand for canine reproductive services including semen cryopreservation, chilled semen shipment and artificial insemination appears to be increasing, based on the number of litters reported by the American Kennel Club to result from such techniques. Coincidental with this increased demand is the increase in number of dogs with poor semen quality that are evaluated by practitioners. When presented with a patient with oligozoospermia, asthenozoospermia or teratozoospermia, practitioners are frequently asked by their clients what they can do to improve the dog's semen quality. Improvement in the number and quality of sperm obtained during semen collection would benefit most areas of assisted reproduction. Multiple therapeutic regimes are routinely recommended but are not substantiated in the literature. This manuscript describes the documented and anecdotal effects of a few

of the more commonly recommended pharmaceuticals used to enhance semen characteristics in the dog.

2. Prostaglandin $F_{2\alpha}$

2.1. Sexual preparation

The use of sexual preparation in conjunction with semen collection has been shown to optimize the number of sperm in the ejaculate of the rabbit and bull [1–3]. In the dog, sexual preparation includes the use of a bitch in estrus or the use of pheromones in the form of vaginal discharge from a bitch in estrus (preserved on bedding or other absorbent materials). Unfortunately, the availability of teaser bitches and pheromones is frequently limited and clinicians are therefore forced to collect semen from dogs without sexual preparation. Collections obtained in this manner often have a decreased volume and total sperm number compared to collections obtained with sexual preparation. This decrease in sperm cell quantity without sexual preparation is seen in multiple species including the rabbit and bull [2,3].

* Tel.: +1 303 794 1188; fax: +1 303 794 4881.

E-mail address: showdogvet@aol.com.

Table 1

Summary of effects of prostaglandin $F_{2\alpha}$ administration on ejaculate characteristics in multiple species

Species	Dose (mg)	Route	Treatment to collection interval	Sperm/ ejaculate	Sperm concentration	Semen volume	Author(s)
Buffalo	0.5–2.0	IM	10 min	↑	Unchanged	↑	Ibrahim [4]
Bull	40 and 2×40	SC	1 h; 1 and 2 h	↑	↑	Unchanged	Marshall and Hafs [6]
Rabbit	5	SC	2 and 4 h	↑	↑	Unchanged	Reichard et al. [8]
Stallion	10	IM	1 h	↑	↑ (not significant)	↑ (not significant)	Cornwell et al. [9]
Ram	1 and 2	IM	30 min	↑	Unchanged	↑	Mekonnen et al. [7]
Rabbit	2.5	SC	2 h	↑	↑	Unchanged	Hafs et al. [5]
Bull	40 or 80	IM	30 min	↑	↑	Unchanged	Hafs et al. [5]

To attempt to counter the decrease in sperm numbers seen when collecting semen without sexual preparation, multiple researchers have evaluated the effect of $PGF_{2\alpha}$ administration prior to semen collection. Administration of $PGF_{2\alpha}$ increased sperm numbers in the ejaculate of multiple species as shown in Table 1 [4–9]. Total sperm numbers are increased as a function of greater sperm concentration, ejaculate volume, or both.

2.2. Mechanism of action

The mechanism behind the increase in ejaculate volume and/or concentration in response to $PGF_{2\alpha}$ is not fully understood. It is thought that $PGF_{2\alpha}$ acts directly on the contractile tissues of the testicular capsule and epididymis, causing an increased rate of sperm passage from the epididymis to the deferent ducts. Prostaglandin receptors in the epididymis are most plentiful in the distal segments, making these areas more sensitive to changes in $PGF_{2\alpha}$ concentration [10]. Therefore, it is reasonable to speculate that endogenous prostaglandins exert more of an effect in the cauda epididymis, the portion of the epididymis that acts as a site of storage for mature sperm. When the cauda epididymis contracts in response to $PGF_{2\alpha}$, mature sperm are moved into the deferent duct where they are available for ejaculation.

There is convincing *in vivo* support for the effect of $PGF_{2\alpha}$ on epididymal contractility. In a series of experiments, Hafs et al. used anesthetized rabbits to demonstrate that exogenous $PGF_{2\alpha}$ significantly increased the movement of sperm from the epididymis to the deferent duct [11]. In the first experiment, one testicle and associated epididymis and deferent duct were removed. The second testicle, epididymis and deferent duct were removed 10, 30 or 60 min after injection of 5 mg $PGF_{2\alpha}$ into the tunica vaginalis surrounding the remaining testicle. The second experiment was similar to the first, except that $PGF_{2\alpha}$ was administered subcutaneously (SC) 10 or 30 min prior to

removal of the testicle, epididymis and deferent duct. The number of sperm in the deferent duct, cauda epididymis and corpus-caput epididymis were determined. Following peritesticular $PGF_{2\alpha}$ injection, the number of sperm in the deferent duct was more than twice that of the controls. Thirty minutes after SC injection of $PGF_{2\alpha}$, the number of sperm in the deferent duct was 2.5-fold greater than that of controls. In a non-anesthetized study, rabbits were given 10 mg $PGF_{2\alpha}$ SC three times at 20-min intervals. The rabbits were then killed and the distribution of sperm in the epididymis and deferent was determined. As with the anesthetized rabbits, the number of sperm in the deferent duct of treated rabbits was almost three-fold greater than saline-treated controls.

In addition to the effects that $PGF_{2\alpha}$ has on smooth muscle of the epididymis, the testicular capsule also contracts in response to $PGF_{2\alpha}$ [12–14]. The dog is known to have a prominent supply of contractile cells in the testicular capsule [15,16]. It is likely that contraction of the testicular capsule in response to $PGF_{2\alpha}$ plays a role in increasing the number of sperm available for ejaculation.

The use of $PGF_{2\alpha}$ prior to collection may optimize the number of sperm in a collection by enhancing sperm movement from the epididymis to the deferent duct where they are available for ejaculation. The dosages and intervals from $PGF_{2\alpha}$ administration to collection have varied widely among reports. Response to treatment has also varied among reports and with collection method.

2.3. Effects on libido

In addition to increased sperm numbers in the ejaculate following $PGF_{2\alpha}$ administration, some researchers noted that treated animals had more libido at the time of semen collection [7,11,17]. Libido was assessed using quantifiable observations, such as time to initial false mount and time to ejaculation in buffalo, and time for collection in rams.

2.4. Effects on cooling and cryopreservation

Use of PGF_{2α} to increase sperm numbers in the ejaculate is only beneficial if the resulting sperm have normal fertilizing and cryopreservation ability. In the rabbit, it has been reported that cauda epididymal sperm incubated with PGF_{2α} have normal fertilizing ability [18]. Treatment of intact male hamsters with 50 µg of PGF_{2α} once daily for 10 days had no effect on fertilizing ability in vivo [19]. Post-thaw motility of cryopreserved buffalo and bull semen was unaffected by administration of PGF_{2α} prior to semen collection and freezing [4,6,17].

2.5. Prostaglandin F_{2α} administration in the dog

In the dog, the administration of PGF_{2α} (Lutalyse[®] dinoprost tromethamine; Upjohn, Kalamazoo, MI, USA) has been shown to increase the total number of sperm in the ejaculate when sexual preparation was not used [20]. When 0.1 mg/kg of PGF_{2α} was given SC 15 min prior to semen collection, total sperm numbers increased 271% compared to saline-treated controls. In addition, the ease at which the PGF_{2α}-treated dogs were collected was improved compared to the saline-treated controls. The characteristics of chilled and frozen semen from treated dogs did not differ from the controls.

In clinical settings, PGF_{2α} is effectively used to obtain ejaculates from reluctant or inexperienced dogs that otherwise could not be collected (M. Hess, N. Roskin, B. Purswell, personal communication). The resulting ejaculate is highly concentrated with low volume. Side effects observed following subcutaneous PGF_{2α} administration at 0.1 mg/kg included panting and salivation; they persisted for 10–20 min and were considered mild. Owners should be made aware of the typical and potentially serious side effects before PGF_{2α} is given. The more severe side effects of PGF_{2α} administration include vomiting, urination, diarrhea, tachycardia, dyspnea, anxiety, restlessness and abdominal pain.

3. Gonadotropin releasing hormone

3.1. Mechanism of action

Gonadotropin releasing hormone (GnRH) is produced by the hypothalamus and signals the release of LH and follicle stimulating hormone (FSH) from the anterior pituitary. Luteinizing hormone acts as a trigger for testosterone release by the Leydig cells in the testicle. The surge in LH occurs 30 min following administration of GnRH in the dog, whereas testosterone peaks 1 h after the administration of GnRH [21–23].

3.2. Gonadotropin releasing hormone administration in the dog

Gonadotropin releasing hormone has been used clinically prior to collection to improve the performance of dogs with poor libido [23]. The testosterone surge following GnRH administration is the proposed cause for the improvement in libido. Gonadotropin releasing hormone has also been used to improve sperm quantity in dogs with oligozoospermia. A Bearded Collie with 60×10^6 total sperm in the ejaculate was given 3.3 µg/kg of gonadorelin diacetate tetrahydrate (Cystorelin; Abbott Laboratories, North Chicago, IL, USA) IM once weekly for 4 months; after treatment, the dog's sperm count was 180×10^6 (Beverly Purswell, personal communication). Six months after treatment, the dog became oligozoospermic again, but the protocol was repeated with success. Kawakami and colleagues evaluated the effects of a GnRH agonist (GnRH-A) on semen parameters. Treatment of a collie with idiopathic oligozoospermia with one IM injection of 1 µg/kg GnRH-A was reported to markedly increase semen volume, sperm number, sperm motility and viability, as well as decreasing the percentage of morphologically abnormal sperm [24]. These changes were apparent 4 weeks after GnRH-A treatment. Other clinicians (C. Lopate, R. Van Hutchison) report variable success treating subfertility using intramuscular or subcutaneous Cystorelin[®] or and injectable deslorelin preparation (BET Laboratories).

4. Prolactin inhibitors

In men, hyperprolactinemia induces hypogonadism by inhibiting GnRH secretion, which, in turn, inhibits FSH, LH, and testosterone secretion. Consequently, hyperprolactinemic patients have oligoasthenospermia, teratozoospermia, decreased libido and decreased ejaculate volume [25,26]. Treatment with cabergoline, a synthetic ergoline-derived dopamine agonist that inhibits prolactin secretion, normalizes serum prolactin levels and restores gonadal function and fertility. In people, hyperprolactinemia results most frequently from macroadenomas [25,26].

Prolactin inhibitors such as cabergoline have also been used by some clinicians with anecdotal success (R. Fayrer-Hosken, R. Van Hutchison, personal communications). Dr. Van Hutchison's protocol is to use 5 µg/kg orally every other day for 3 weeks and reevaluate. If improvement is seen, the drug is continued. If there has been no increase in sperm numbers seen after 6 weeks of treatment, the cabergoline is discontinued. The

number of dogs that respond to cabergoline treatment is not known and the compounded drug is frequently cost-prohibitive, making investigation in a clinical setting difficult.

5. Nutraceuticals

5.1. Supplementation with Glyco-Flex[®] and other glycosaminoglycans

Glyco-Flex[®] is a supplement containing *Perna canaliculus* (New Zealand Green-Lipped Mussel), glucosamine, and minerals. According to the manufacturer Vetri-Science (Essex Junction, VT, USA) the product is a good source of glycosaminoglycans (GAGs) and is used to support proper joint function. Glyco-Flex[®] is prescribed by multiple reproductive practitioners to improve semen quality in the dog (D. Bleifer, N. Roskin, K. Kampschmidt, personal communication). Other GAG supplements, without methylsulfonylmethane (MSM) are recommended for treatment of subfertility (C. Lopate, personal communication). Although commonly advocated by a variety of veterinarians, there is little hard evidence supporting the use of these products. To further confuse the issue, there is no standard protocol for the type of case in which Glyco-Flex[®] or other GAG supplements are prescribed.

Dr. Roger Kendall, a biochemist with Vetri-Science, cites numerous anecdotal reports of improved semen quality in dogs, horses and bulls treated with Glyco-Flex[®]. Dr. Kendall theorizes that the product enhances cellular reactions and amino acid uptake, which may improve semen quality and motility. Although currently not documented, users frequently report improvement in sperm motility and in the quality of cryopreserved or chilled semen after supplementation with Glyco-Flex[®]. A controlled trial is needed to definitively determine the effects of Glyco-Flex[®] on semen characteristics in the dog.

5.2. Omega 3 fatty acids and antioxidants

Several reproductive practitioners suggest a form of Omega 3 fatty acid supplementation to enhanced semen quality. Dr. Kit Kampschmidt advocates a product called OM3 Gold[®] (Cepav Laboratórios, São Paulo, SP, Brazil; <http://www.cepav.com.br>); this product contains linoleic acid, linolenic acid, oleic acid and Vitamin E. In Dr. Kampschmidt's experience, 50% of dogs with a high percentage of proximal droplets will show significant improvement after 6 weeks of treatment with OM3 Gold[®] (personal communication). Other clinicians

simply advocate administration of Vitamins C and E for their potential beneficial effects on semen quality.

Research has been conducted in the stallion evaluating the effect of feeding a supplement rich in docosahexaenoic acid (DHA) on semen quality, freezability and cooling ability [27]. In this study, stallions fed a DHA supplement had improvements in motility following cooling and freezing. PROSPERM[®] (Minitube America, Inc., Minneapolis, MN, USA) has been shown to increase semen concentration, total sperm number in the ejaculate and sperm motility in the boar (Spermnotes, volume V, issue 1, Spring 2001 4–5). PROSPERM[®] is a supplement that contains DHA, Vitamin E and selenium and is advocated as a treatment for subfertility in the dog by one clinician (C. Lopate, personal communication). The mechanism of action is not fully understood and to date similar studies have not been conducted to determine the effects of Omega 3 fatty acid supplementation on canine semen parameters.

Clinicians also advocate the administration of Cell Advance[®] (Vetri-Science) an antioxidant supplement that the manufacturer claims protects cells from being altered or destroyed by free radicals (N. Roskin, D. Bleifer, personal communication). Motility Plus[®] (Platinum Performance, Buellton, CA, USA) a carnitine supplement is also prescribed by some practitioners to enhance sperm motility (D. Bleifer, personal communication). In both cases, the mechanism of action has not been documented and the effects of the supplement on canine semen quality are currently unsubstantiated.

6. Research challenges

Multiple obstacles exist that impede a controlled clinical trial evaluating the effects of drug administration or nutraceutical supplementation on semen parameters in the dog. For clinicians, client compliance and need for untreated controls make conducting research difficult. Researchers face difficulty funding lengthy studies and obtaining dogs with suitable semen quality for evaluation in a comparative trial. Both clinicians and researchers face the confounding effects of heat, health, age, and genetics on semen quality. These challenges will undoubtedly persist until large-scale controlled trials document the effects and determine the mechanism of action of various pharmaceuticals used to enhance canine semen quality.

7. Conclusions

Despite similarities in recommendations among the clinicians referenced in this manuscript, there are no

standard protocols for the use of nutraceuticals, cabergoline or GnRH in the dog. The limited availability of information related to the effects pharmaceuticals have on canine semen quality forces clinicians to draw inferences from research in humans and other animal species. Most clinicians prescribe nutraceuticals with the attitude that “it may not help but it won’t hurt.” We must remember that advocating excessive quantities of any pharmaceutical could be deleterious and clients should be made aware that many of these products have unsubstantiated effects on semen parameters in the dog.

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