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Oral imipramine and intravenous xylazine for pharmacologically-induced ex copula ejaculation in stallions

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Abstract

This study is part of ongoing work toward developing pharmacological methods for enhancing and inducing ejaculation in stallions with ejaculatory dysfunction or disabilities that interfere with normal breeding behavior. The objective was to evaluate a treatment regimen involving oral imipramine followed by intravenous xylazine that, in uncontrolled field clinical trials, had shown promise for a higher rate of ejaculation and fewer side effects using a more easily obtained and administered form of imipramine. Eight stallions each underwent eight trials in which treatment consisted of imipramine hydrochloride (3 mg/kg, orally in a small portion sweet feed) followed 2 h later by xylazine hydrochloride (0.66 mg/kg, intravenously). Trials were conducted with the stallion in a stall. Semen was collected using a collection bag secured over the prepuce with a girth band. Overall, 44 of the 64 attempts (68%) resulted in ejaculation. Within-stallion ejaculation rate ranged from 3 of 8 to 7 of 8 attempts. Interval from xylazine treatment to ejaculation ranged from 1.2 to 14 min. As is typical for induced ejaculations in which imipramine is included in the treatment regimen, ejaculates were of low volume, high sperm concentration, and with a higher total number of sperm than for in copula ejaculates of these stallions. These results represent a modest improvement in rate of ejaculation over previous treatment regimens.

Keywords: Chemical ejaculation; Reaction; Stallion

1. Introduction

Over the last decade, our laboratory and others have been exploring methods for pharmacologically enhancing stallion ejaculatory function in copula (natural service or semen

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collection using an artificial vagina or manual stimulation) as well as inducing ejaculation ex copula (without sexual arousal or copulatory effort) as an alternative method for semen collection in stallions. The principal compounds studied to date include α-adrenergics and other smooth muscle-active agents commonly used in equine veterinary practice. All had been known to affect contractions of genital smooth muscle in the horse and had been associated with the occasional side effect of induced-ejaculation. Compounds studied to date include xylazine (McDonnell, 1991; McDonnell and Love, 1991; McDonnell and Odian, 1994; McDonnell and Oristaglio Turner, 1994; Card et al., 1997; Johnston and DeLuca, 1998), imipramine (McDonnell, 1991; McDonnell and Odian, 1994; McDonnell and Oristaglio Turner, 1994; Card et al., 1997; Johnston and DeLuca, 1998), prostaglandin F_{2a} (PGF_{2a}; McDonnell, 1991) and detomidine (Rowley et al., 1999). Regimens for induced ex copula ejaculation of stallions reported include a variety of doses, schedules, routes of administration, combinations of agents, and pre-treatment procedures. Overall, reported rates of ejaculation range approximately 30–75% of attempts. Regimens and success (rate of ejaculation) are summarized by the study in Table 1. For the xylazine regimen, experience suggests that rate of ejaculation is affected by the level of arousal (calm or excited) of the stallion at the time of treatment, with higher success in quiet and undisturbed stallions (McDonnell and Love, 1991). It has also been found that, although these procedures are done while the stallion is not sexually aroused, ejaculation rate can be enhanced by prolonged teasing within a few hours before treatment (McDonnell and Love, 1991).

Ejaculate characteristics vary significantly among treatment regimens, apparently associated with variably enhanced or inhibited smooth muscle contraction of the ampullae and accessory sex glands. Semen obtained using xylazine alone typically has volume, sperm concentration, pH and total sperm number similar to those of ejaculates obtained in copula (McDonnell and Love, 1991). Treatment with imipramine or detomidine appears to enhance contraction of the ampullae, and inhibit contraction of the accessory sex glands, with the resulting ejaculates of lower volume, higher sperm concentration, higher total number of sperm, and lower pH than for in copula ejaculates (McDonnell and Odian, 1994; McDonnell and Oristaglio Turner, 1994; Rowley et al., 1999). PGF_{2a} treatment appears to selectively enhance contraction of the accessory sex glands, resulting in ejaculates of higher volume, lower sperm concentration, similar total number of sperm, copious amounts of gel, and higher pH compared to in copula samples (McDonnell, 1991).

Interval and variability of interval from treatment to ejaculation also vary among treatment regimens. With xylazine alone or with xylazine and imipramine (p.o. or i.v.) combination treatment, most ejaculations occur either within 1–3 min after xylazine treatment as the animal is just becoming sedate or between 15 and 25 min after treatment as the animal is arousing from sedation (McDonnell, 1991; McDonnell and Love, 1991; McDonnell and Odian, 1994; McDonnell and Oristaglio Turner, 1994; Card et al., 1997; Johnston and DeLuca, 1998). Ejaculates following intravenous imipramine treatment alone have generally occurred within 10–45 min (McDonnell and Odian, 1994; McDonnell and Oristaglio Turner, 1994). Ejaculation following PGF_{2a} treatment typically occurs between approximately 10 and 50 min following injection (McDonnell, 1991).

Significant side effects also vary among treatment regimens. With PGF_{2a} regimens tested so far, sweating, abdominal cramping, and urine dripping are important complicating side

Table 1
Summary of pharmacologically-induced-ejaculation regimens for stallion and rates of success

	Regimen	Rate of ejaculation (number of stallions)
Xylazine McDonnell and Love, 1991 (laboratory study)	0.66 mg/kg i.v.	27% ($n = 28$)
Detomidine Rowley et al., 1999 (laboratory study)	0.02 mg/kg i.m. followed by 0.01 mg/kg 15 min later	50% ($n = 1$)
Imipramine McDonnell and Turner, 1994 (laboratory study)	2 mg/kg i.v.	42% ($n = 5$)
Imipramine followed by Xylazine McDonnell and Odian, 1994 (laboratory study)	2 mg/kg imipramine i.v. followed 10 min later with 0.3 mg/kg i.v. xylazine	33% ($n = 8$)
McDonnell and Turner, 1994 (laboratory study)	2 mg/kg imipramine i.v. followed 60 min later with 0.3 mg/kg i.v. xylazine	53% ($n = 5$)
Johnston and DeLuca, 1998 (farm practice)	0.75–2.0 mg/kg p.o. followed 1–3 h later with 0.3 mg/kg i.v. xylazine	57% ($n = 6$)
Prostaglandin F_{2a} McDonnell 1992 (laboratory study)	0.01–0.15 mg/kg i.m. (individual stallion titration trials)	75% ($n = 8$)

effects (McDonnell, 1991). Beyond the discomfort to the stallion, these side effects pose some practical challenges to obtain a sample free of contamination of urine or perspiration dripping from the prepuce. For xylazine treatment, the major adverse side effect is deep standing sedation, lasting about 15 min. For intravenous imipramine treatments, the most significant adverse side effect noted has been mild hemolysis following injection (McDonnell and Odian, 1994). The minor side effect of prolonged yawning is typical with imipramine and with PGF_{2a} treatment.

Recently, Johnston and DeLuca (1998) reported good results inducing ejaculation in stallions in clinical farm practice using imipramine orally followed by xylazine intravenously. Oral administration of imipramine has several advantages over injection of imipramine. Imipramine in the oral form is readily available, it is relatively inexpensive, more easily administered by unskilled personnel, and without the adverse side effect of hemolysis associated with intravenous administration of imipramine (McDonnell and Odian, 1994). The objective of the current study was to evaluate a treatment regimen similar to that of Johnston and DeLuca in more controlled laboratory trials.

2. Methods

2.1. General design

Each of eight mature stallions was given a series of eight induced-ejaculation trials at 2- to 3-day intervals during October and November of 1999. Rate of ejaculation was described and resulting ex copula ejaculate characteristics were compared within subjects to baseline in copula ejaculates obtained in the week immediately preceding these trials.

2.2. Subjects

Six pony (180–275 kg) and 2 horse stallions (480 and 500 kg), housed in individual stalls or kept in groups at pasture at the Hofmann Center, University of Pennsylvania, were used as subjects. Stallions ranged in age from 3 to 19 years and were all sexually experienced. During the week before the start of the study, three semen samples were collected in copula from each stallion using a dummy mount and manual stimulation to obtain baseline measures of volume, sperm concentration, pH, total sperm number, and visually estimated total and progressive motilities, against which to compare ex copula ejaculates. Manual stimulation was done using hot compresses as described previously to ejaculates similar to those obtained with an artificial vagina (McDonnell and Love, 1990).

2.3. Trials

Induced-ejaculation trials were conducted with the stallion in a box stall with no animals in adjacent stalls. Treatment consisted of imipramine hydrochloride (3 mg/kg, orally in a small portion of sweet feed) followed 2 h later by xylazine hydrochloride (0.66 mg/kg intravenously). Semen was collected into a non-spermicidal plastic bag (Nasco Tail Mitt,

Nasco Farm and Ranch, Atkinson, WI) that was mounted on a 5-inch plastic embroidery hoop secured over the prepuce with a girth band (VetRap, 3M, St. Paul, MN). Stallions were observed continuously either directly or remotely on video surveillance monitors until ejaculation occurred or for up to 2 h after administration of xylazine. Ejaculates were removed from the collection device within 1 min after ejaculation. Semen volume, sperm concentration, pH, total sperm number, and visually estimated total and progressive motilities were estimated as described for baseline in copula ejaculates.

3. Results

3.1. Ejaculation rate

Overall, 44 of the 64 attempts (68%) resulted in ejaculation. Within-stallion ejaculation rate ranged from 3 of 8 to 7 of 8 attempts. Interval from xylazine treatment to ejaculation ranged from 1.2 to 14 min.

3.2. Comparison of semen with baseline values

Results are summarized in Table 2. Semen volume of induced ejaculates ranged from 6 ml (pony) to 27 ml (horse). Mean volume of induced ejaculates was significantly lower than that of in copula ejaculates (dependent t-tests, 7 d.f., $P < 0.05$). Sperm concentration of induced ejaculates ranged from 380 to 1450 million per milliliter, in all cases higher than either the mean or any of the baseline samples (within-stallion). Mean concentration of sperm in ex copula ejaculates ranged among stallions from 132 to 400% greater than in their in copula ejaculates. Total number of sperm was also higher in ex copula ejaculates than in in copula ejaculates, with the total number of sperm in ex copula ejaculates 12–57% greater than that for in copula ejaculates. The difference was significant (dependent t-tests, 7 d.f., $P < 0.05$). For each stallion, the pH of each ex copula ejaculate was lower than that of all in copula ejaculates, with the mean pH for ex copula ejaculates ranging among stallions from 6.73 to 6.96 compared to in copula ejaculates that ranged from 7.11 to 7.35. Mean total and progressive motilities (visually estimated) were similar among induced and in copula ejaculates (dependent t-tests, 7 d.f., $P > 0.10$).

Table 2
Within-stallion comparison of in copula baseline and ex copula induced ejaculates ($n = 8$)

	In copula comparison ejaculates		Ex copula induced ejaculates		Difference
	Mean	Range	Mean	Range	P
Gel-free semen volume (ml)	32	12–168	19	6–27	<0.05
Gel volume (ml)	17	0–25	0	0	<0.001
Sperm concentration (10^6/ml)	194	89–276	689	380–1450	<0.001
Total sperm (10^9)	7.4	2–8.7	9.3	2.6–9.0	<0.05
pH	7.23	7.11–7.35	6.87	6.73–6.96	<0.001

4. Discussion

The current findings with this imipramine and xylazine regimen represent another minor improvement in rate of ejaculation for pharmacologically induced ex copula ejaculation in stallions. In work with a larger number of horse stallions on breeding farms, this same regimen achieved a similar overall rate (71%) of ejaculation (Dutra et al., personal communication, 2000). In earlier work, we found that titration of dose to individual stallions significantly improves the rate of ejaculation for any given treatment (McDonnell, 1991). In subsequent work (unpublished trials) in which individual titration studies with this protocol were done with 4 stallions, rate of ejaculation ranged from 8 of 10 (80%) to 10 of 10 (100%) attempts at 2–day intervals. For each stallion, the titration to establish "best response" consisted of a series of 8 trials at 2- to 3-day intervals. Four trials were with 3 mg/kg imipramine p.o. 2 h before xylazine at 0.50, 0.60, 0.65, and 0.70 mg/kg i.v. (in random order). The remaining four trials were with similar xylazine treatments, but with 5 mg/kg imipramine, again given at 2 h before the xylazine. While titration studies represent a considerable amount of work, they may be worthwhile in situations where pharmacologically induced ejaculation will be used for extended periods of time with pressure for high rate of success.

Oral administration of imipramine has the advantage of no adverse side effect of hemolysis (unpublished clinical and laboratory observations). Oral administration of imipramine is more easily administered than intravenous administration by non-veterinary personnel in the field. This represents an important practical consideration, since it would reduce the amount of time the veterinarian was required on-site for the induced ejaculation procedure.

The semen characteristics of lower volume, higher sperm concentration, higher total number of sperm, and lower pH for ejaculates associated with imipramine treatment compared to baseline in copula ejaculates are similar to those of earlier reports of ejaculates involving imipramine regimens (McDonnell, 1991; McDonnell and Odian, 1994; McDonnell and Oristaglio Turner, 1994; Card et al., 1997; Johnston and DeLuca, 1998). Ejaculates obtained following a similar protocol using intravenous imipramine and intravenous xylazine have been found to compare favorably for cryopreservation to in copula ejaculates (McDonnell and Oristaglio Turner, 1994). For cryopreservation, the high concentration and high total number of sperm have the practical advantage of not requiring centrifugation or fractionation to yield concentrations suitable for current equine semen cryopreservation protocols.

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