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IMIPRAMINE AND XYLAZINE-INDUCED EX COPULA EJACULATION IN STALLIONS

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ABSTRACT

This study is a part of our ongoing work toward developing pharmacological methods for enhancing and inducing ejaculation in stallions with ejaculatory dysfunction. We evaluated a combination treatment of imipramine hydrochloride followed 10 minutes later by xylazine hydrochloride for the induction of ex copula ejaculation. Eight pony stallions each underwent 6 treatment trials conducted at 4-day intervals. The trials were conducted in the animals' stalls, where they were observed for 90 minutes following treatment. To evaluate the effect of pretreatment sexual stimulation on the rate of ejaculation for each of the 8 stallions, 3 of the 6 trials were preceded by exposure to a restrained ovariectomized pony mare. For 7 of the 8 stallions 1 to 4 of the 6 trials resulted in induced ejaculation, for a total of 16 ejaculations in the 48 trials. Six of the ejaculations occurred with imipramine treatment alone, before the administration of xylazine. All ejaculations were associated with erection and masturbation. Six of 24 trials' (24%) preceded by sexual stimulation resulted in ejaculation, while 10 of 24 trials (42%) without sexual prestimulation resulted in ejaculation. These proportions were not different ($P = 0.11$). Induced ejaculates were collected into a plastic bag positioned over the prepuce by a girth strap for the comparison of semen characteristics with 2 base line ejaculates obtained in copula from these stallions during the week preceding the series of induced ejaculation trials (with similar 4-day intervals from previous ejaculation). The induced ejaculates were of lower total volume, higher concentration, lower gel volume, higher total numbers of spermatozoa, and lower pH ($P < 0.05$) than the base line in copula ejaculates. Together, these semen characteristics suggest increased emission of the sperm-rich fraction and reduced emission of accessory gland fluids, probably resulting from imipramine treatment.

Key words: ejaculation, masturbation, semen, stallion, xylazine, imipramine

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INTRODUCTION

Xylazine, an alpha-Zadrenergic agonist, is commonly used for sedation and analgesia in horses (2). In previous work, limited success (ejaculation in 27% of trials) was achieved using treatment with xylazine to induce ejaculation ex copula in stallions (11). Subsequently, xylazine has been used clinically to induce ejaculation in stallions with neurologic or lameness problems that preclude normal copulation. In both research and clinical applications, the stallion typically fails to ejaculate unless he remains completely undisturbed during treatment. The requisite strict control of the environment is often impractical.

Imipramine, a tricyclic antidepressant, inhibits re-uptake of several neurotransmitters, including dopamine, norepinephrine and serotonin (1). Tricyclic antidepressants have been used in men for treatment of aspermia (6), premature ejaculation (5) and retrograde ejaculation (3,4). In horses, imipramine treatment typically induces a drowsy state, erection and masturbation (9). Ejaculation, both during masturbation and in copula, has been facilitated by treatment with imipramine (9).

The purpose of the present study was to evaluate the use of a combination treatment of imipramine followed by xylazine for inducing ejaculation ex copula in stallions.

MATERIALSAND METHODS

Subjects

Eight sexually-experienced pony stallions (2 to 22 years old; 150 to 250 kg) were used. The stallions were housed in individual, adjacent tie stalls in the Havemeyer Barn of Hofmann Center at New Bolton Center. The stallions were provided hay and water ad libitum to maintain good body condition. These stallions had been used as subjects of research on sexual behavior and semen during the previous 1 to 5 years at the same facility.

Trials

Each stallion was subjected to a total of 6 trials conducted at 4-day intervals. During the trials, the animal was tethered in either a tie stall or a box stall. Treatment consisted of intravenous injection of 2.0 mg/kg imipramine hydrochloride, followed 10 minutes later by intravenous injection of 0.3 mg/kg xylazine hydrochloride. These dosages were selected based on preliminary trials to evaluate 2 levels of xylazine (0.3 and 0.2 mg/kg i.v.) and 3 levels of imipramine (2.5, 2.0 and 1.5 mg/kg i.v.). During the trials, the stallions remained tethered in the stall for 90 minutes. Trials were videotaped as well as observed live. A prepared check sheet was used to record the following by minute: penis position and movement, ejaculation by number of jets, level of standing sedation by a 5 degree scale, respiration, general activity (e.g., standing quietly, eating hay, moving), urination, defecation and any unusual behavior.

Semen Evaluation

Induced ejaculates were collected into a plastic bag held by a plastic ring positioned over the prepuce by a girth strap, as described by McDonnell and Love (11) and were then immediately evaluated according to Kenney et al. (7). In the event that a stallion did not ejaculate ex copula, semen was obtained using manual

stimulation on the dummy mount, as described by McDonnell and Love (8). This was done to maintain a 4-day ejaculation interval between induced ejaculation trials as well as to evaluate effects of the agents on in copula ejaculates. In addition, during the week preceding commencement of the ex copula trials, 2 in copula semen samples were obtained from each stallion and evaluated as described above (each with a 4-day interval from the previous ejaculation) for comparison with in copula samples obtained following treatment. Semen handling and evaluation techniques were the same for all semen samples.

Sexual Prestimulation

In previous work with xylazine, we found that sexual prestimulation significantly increased the percentage of successful trials (11). To evaluate possible effects of sexual prestimulation on ex copula ejaculation in the present study, 3 of the 6 trials for each stallion were preceded by exposure to a restrained ovariectomized pony mare. Exposure consisted of either 6 mounts without intromission or a maximum of 10 minutes of interaction. The stallion was then returned to the stall and was left to stand for 10 minutes prior to the start of treatment.

Evaluation of Possible Side Effects

Prior to the first induced ejaculation trial and again after the last trial, jugular blood samples were obtained from 4 stallions for evaluation of total chemistry and total bilirubin concentration. During preliminary trials, some hemolysis was evident in the plasma and urine samples. Accordingly, immediately before each trial and 90 minutes after treatment, plasma samples were obtained and graded on a gross color scale of 0 to 10 (0 = normal color, 10 = dark red). Urine was obtained during or following each induced ejaculation trial for notation of hematuria. In addition, behavior, appetite and stool were monitored daily.

RESULTS

Ejaculation

Ejaculation occurred in 16 of 48 trials (33%). Figure 1 shows the 16 ejaculations in relation to time of imipramine and xylazine treatment. Six of the 16 ejaculations occurred during the initial 10 minutes, before injection of xylazine. Seven of the eight stallions ejaculated at least once during six trials (3 stallions 1 of 6 trials; 1 stallion 2 of 6 trials; 1 stallion 3 of 6 trials; 2 stallions 4 of 6 trails). The remaining stallion had ejaculated on this dose once during preliminary trials.

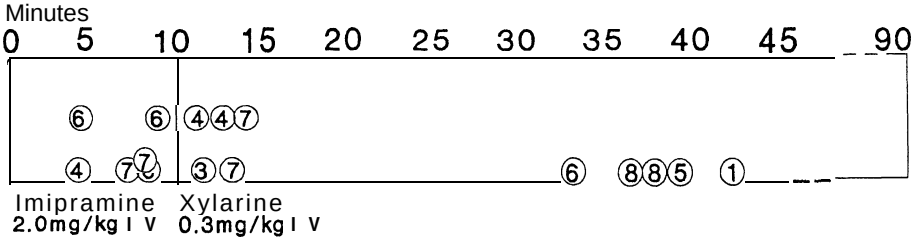

Figure 1. Representation of 16 induced ejaculations during a total of 48 trials (8 animals, 6 trials each). Numbers within circles represent animal identifications.

Erection occurred in 34 of 48 trials (71%), and masturbation occurred in 25 of 48 trials (52%). All 16 ejaculations occurred during masturbation. Ejaculation occurred as 4 to 8 jets (x = 5.25, mode = 4). Most ejaculations occurred either as the animal was becoming sedated or as it was recovering from sedation.

Ejaculation occurred in 6 of 24 (25%) trials with sexual prestimulation and in 10 of 24 (42%) trials without sexual prestimulation. These proportions were not significantly different ($z = 1.24$; $P = 0.11$); however, there was a tendency for sexual prestimulation to reduce the likelihood of ejaculation using this treatment.

Semen

Semen characteristics for ejaculates are shown in Table 1. These include base line ejaculates (n = 16), pharmacologically-induced ex copula ejaculates (n = 16), and ejaculates obtained 90 minutes after unsuccessful induction attempts (n = 32). Compared with base line in copula ejaculates, induced ex copula ejaculates had significantly higher concentrations of spermatozoa, lower total volume, lower gel volume, higher total numbers of spermatozoa, and lower pH ($P < 0.05$). Compared with in copula ejaculates obtained following unsuccessful ex copula induction trials, induced ex copula ejaculates had higher concentrations of spermatozoa and lower pH. Progressive and total motilities as well as gel-free semen volumes were similar for all 3 classes of ejaculate ($P > 0.05$). For induced ejaculates, the number of jets was significantly correlated with volume (Pearson's $r = 0.78$, $P < 0.05$) as well as with total number of sperm (Pearson's $r = 0.51$, $P < 0.05$). Volume and total number of sperm were significantly correlated (Pearson's $r = 0.62$, $P < 0.05$).

Table 1. Summary of semen characteristics of base line in copula, pharmacologically-induced ex copula, and post-treatment in copula ejaculates of 8 pony stallions. Values were calculated from the mean of 2 ejaculates from each stallion (except that 1 stallion had no induced ejaculates and 2 stallions had only 1 induced ejaculate). Across rows, means with similar superscripts were not significantly different.

	Base line in copula	Induced ex copula	Post treatment in copula
Concentration (x 10^6)	194.3 (25.5)^a	409.9 (36.22)^b	240.1 (22.26)^a
Total volume (ml)	84.9 (17.62)^a	36.4 (8.28)^b	63.1 (9.90)^{ab}
Gel-ffee vôlume (ml)	33.3 (3.29)^a	27.8 (3.93)^a	34.8 (2.17)^a
Gel volume (ml)	52.0 (17.07)^a	8.6 (5.13)^b	28.3 (9.75)^{ab}
Total number sperm (x 10^9)	6.7 (1.05)^a	10.3 (1.24)^b	8.0 (0.76)^{ab}
PH	7.0 (0.04)^a	6.7 (0.06)^b	7.0 (0.04)^a
Total motility	65.0 (3.29)^a	68.4 (3.78)^a	67.0 (2.79)^a
Progressive motility	53.1 (3.70)^a	56.6 (3.92)^a	52.8 (3.22)^a

a,b Across rows, means with similar superscripts are not significantly different.

Side Effects

Treatment with imipramine generally resulted in mild sedation or no sedation before xylazine treatment. With the addition of xylazine, sedation typically occurred within 1 to 3 minutes, reaching mild to heavy standing sedation, with decreased respiratory rate typical of xylazine anesthesia. Animals typically resumed normal activity within 50 minutes (range, 5 to 60 minutes) after xylazine treatment.

Serum chemistry and total bilirubin concentration remained within normal limits. Some hemolysis was evident in all the serum samples, typically with a level of 3 (slightly red-tinged) on our gross color scale of 0 to 10. Mildly to severely discolored urine was noted during or following most trials (43 of 47 trials). In 4 animals for which serum samples were again obtained at 24 hours after treatment, the color had returned to level 0 (no evidence of hemolysis) on our scale. Appetite, stool, and general behavior remained normal throughout the study.

DISCUSSION

The combination of imipramine followed by xylazine resulted in ex copula ejaculation in 16 of 48 trials (33%). This rate is slightly higher than that achieved with our previously reported xylazine protocol (27%; 11). In the previous study, xylazine-induced ex copula ejaculates were similar to in copula ejaculates (11). In the present study, ejaculates obtained with the combined imipramine and xylazine protocol had significantly greater concentrations of spermatozoa, higher total numbers of spermatozoa, lower gel volumes and lower total volumes than base line in copula ejaculates (8). There were also fewer jets than is typical for stallions (8). The pH of induced ex copula ejaculates was significantly lower than that of base line in copula ejaculates (8). Together these observations suggest that the imipramine-xylazine protocol results in reduced contribution to the ejaculate from the accessory glands. Six ejaculates that occurred with imipramine alone (before xylazine could be administered) were extreme in these characteristics; therefore, the differences in semen characteristics were probably related to the imipramine treatment. In subsequent studies in our laboratory, this dosage of imipramine administered without xylazine (unpublished) has yielded ejaculates of similarly extremely low volume (5 to 12 ml) of semen, and high concentration of spermatozoa (800-1300 x 10^6 per ml).

Compared with the results of the previously reported xylazine protocol, this protocol may have some practical advantages. First, inadvertent disturbances of the animal did not seem to interfere with ejaculation. Second, the low-volume / high sperm-concentration ejaculate may be useful for cryopreservation. Higher total numbers of spermatozoa per ejaculate may be advantageous in clinical cases in which ejaculation frequency must be reduced or for stallions with typically low number of spermatozoa per ejaculate.

This study also addressed the effects of treatment with imipramine followed by xylazine on in copula ejaculates. Although the differences were not significant, there was a tendency for in copula ejaculates obtained 90 minutes after treatment to have higher spermatozoa concentrations, lower gel and total volumes, and a greater total number of spermatozoa than base line in copula ejaculates. This treatment protocol may have clinical application for obtaining a higher number of spermatozoa per ejaculate in copula. Additional research is needed to further investigate in copula treatment regimens.

In this study, no effect of sexual prestimulation on the frequency of ex co pula ejaculation was found. This is in contrast to our previous study using xylazine alone (11) in which sexual prestimulation increased the likelihood of ex copula ejaculation. One salient difference in the 2 studies was the interval from sexual prestimulation to treatment. The interval was 30 minutes in the initial xylazine study, and it was only 10 minutes in our present imipramine-xyiazine study.

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