Oxytocin or prostaglandin failed to improve pharmacologically induced ejaculation in stallions

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Abstract

Pharmacologically induced ejaculation is utilized in stallions when traditional semen collection procedures are not appropriate or not effective. Most common protocol includes oral imipramine hydrochloride, with intravenous xylazine hydrochloride given 1 - 2 hours later. Goal was to document success rate from past clinical experience (historical data) and to determine whether addition of oxytocin along with xylazine or a prostaglandin analogue prior to xylazine treatment would enhance success rate in aged stallions in a clinical setting. Semen was successfully collected in 3 of 7 aged stallions using the standard protocol. In the modified protocol, 22 times (out of 50 attempts) semen was successfully collected from 12 stallions. However, addition of oxytocin or a prostaglandin analog had a deleterious effect on passive emission of semen.

Keywords: Stallion, pharmacologically induced ejaculation, imipramine, xylazine

Introduction

Semen is traditionally collected from stallions in an artificial vagina while stallions mount a mare or breeding phantom.[1-3] Traditional semen collection method may not be appropriate for stallions with severe musculoskeletal issues, neurologic defects, penile paralysis, or behavioral issues.[4-6] Alternatively, other techniques such as ground collection using an artificial vagina,[7-9] manual stimulation,[10,11] and pharmacologically induced ex copula ejaculation[12,13] have been developed for stallions.

Pharmacologically induced ex copula ejaculation ('chemical ejaculation') can be accomplished by administration of imipramine hydrochloride alone[14] or xylazine hydrochloride alone[15] or a combination of imipramine followed by xylazine.[12,16] Imipramine is a tricyclic antidepressant drug used in human medicine in the management of depression,[17] nocturnal enuresis,[18] retrograde ejaculation,[19] and premature ejaculation.[20] It was hypothesized that imipramine lowers the 'ejaculation threshold' of stallions.[4,6] Xylazine hydrochloride is an α_2 adrenergic receptor agonist used primarily for sedation in horses. Xylazine hydrochloride or other α_2 agonists such as detomidine hydrochloride[3,21] is occasionally associated with a side effect of inducing passive emission of semen during sedation. Other chemical ejaculation protocols have used various combinations of detomidine, butorphanol tartrate, imipramine, xylazine, clomipramine, and/or prostaglandin $F_{2\alpha}$.[4,6,16,21-23] Semen collected via chemical ejaculation was lower in volume and higher in sperm concentration;[6,12,16,23] however, it has been used for fresh insemination, cool-transport, and cryopreservation.[23,24] Mares have been successfully bred.[24,25]

Success rate of inducing ejaculation with imipramine and/or xylazine ranged from 33 to 68%,[12,13,16] and varied between stallions and between studies. Objectives were to: 1) document success rate of pharmacologically induced ejaculation of stallions from past clinical experience (historical data); and 2) determine if oxytocin treatment at the same time as xylazine or a prostaglandin analogue prior to xylazine would enhance ejaculation success rate of a standard imipramine-xylazine protocol in aged stallions.

Materials and methods

All procedures were approved by Institutional Animal Care and Use Committee. For the first part of the study, reproductive records of 12 stallions in which chemical ejaculation was attempted 50 times were reviewed. A standard protocol consisting of a single oral dose of imipramine hydrochloride (3.0 mg/kg) followed ~ 1 hour later by intravenous xylazine hydrochloride (0.33 - 0.5 mg/kg) was used. Semen parameters from 22 normal stallions collected without medication during the same time period were reviewed for comparative purposes.

For the second part of the study, 7 Quarter Horse stallions (17 - 27 years; mean 22.7 ± 3.5) were used. Stallions' weight ranged from 430 to 530 kg with a body condition score between 4 and 6 (based on score index of 1 [poor] - 9 [extremely fat]).[26] Three stallions were housed indoors in box stalls with daily turn out into a paddock and 4 stallions were housed outside in a paddock (brought inside into a box stall on treatment days). 'Cleanout' collections were performed on all stallions prior to experiment. The chemical ejaculation procedure was managed by only 1 person in 5 x 5 meter box stalls with minimal outside disturbance.

Standard chemical ejaculation

A standard protocol was used, consisting of a single oral dose of imipramine hydrochloride (3.0 mg/kg) followed 2 hours later with intravenous xylazine hydrochloride (0.5 mg/kg). Semen was collected in a funnel held beneath the stallion using a long pole. Funnel was lined with a disposable artificial vagina liner attached to a collection bottle fitted with a gel filter. Stallions were quietly observed ~ for 70 minutes for behavioral responses to treatment until either ejaculation occurred or until they recovered from sedation without ejaculation.

Traditional semen ejaculation

Stallions were allowed a minimum of 2 days rest after chemical ejaculation attempt prior to traditional semen ejaculation. Stallions were teased to a mare in estrus and then allowed to mount a breeding phantom. Semen was collected in a Colorado model artificial vagina (Animal Reproduction Systems, Chino, CA) fitted with a collection bottle and disposable inline nylon micromesh gel filter (Animal Reproduction Systems).

Oxytocin added chemical ejaculation

Stallions were allowed a minimum of 2 days rest prior to chemical ejaculation attempt incorporating oxytocin in a standard protocol. Standardized dosages of imipramine hydrochloride and xylazine hydrochloride were used, with the addition of intravenous oxytocin (20 units) treatment immediately after xylazine hydrochloride.

Prostaglandin added chemical ejaculation

Stallions were rested for a minimum of 7 days prior to chemical ejaculation attempts incorporating prostaglandins in the protocol. Stallions were treated with standardized dose of imipramine hydrochloride and 2 hours later by either: a) 250 µg of cloprostenol sodium intramuscularly, followed 5 minutes later by 0.5 mg/kg xylazine intravenously (n = 2); or b) cloprostenol treatment without xylazine (n = 2).

Semen evaluation

Semen samples (traditional and chemical ejaculation) were evaluated for gel free volume and sperm concentration, motility, and morphology. Volume was measured in a warmed graduated cylinder. Sperm concentration was determined using a Densimeter 591B (Animal Reproduction Systems) or a NucleoCounter® SP-100™ (ChemoMetec, Allerod, Denmark). Total and progressive sperm motility were evaluated by computer assisted semen analysis (SpermVision®, Minitube of America, Inc., Verona, WI) attached to a ZeissAX10 microscope. Sperm morphology was evaluated using a differential interference contrast microscope (OlympusEX51) at 1,000 x magnification and high viscosity microscope immersion oil (Resolve™,Thermo Scientific, Waltham, MA).

Data analyses

Semen parameters (volume, concentration, total sperm number, total motility, and progressive motility) of chemical and standard collections were compared by ANOVA. Percentage of stallions that ejaculated using 3 protocols was compared by Chi square. Semen parameters (volume, concentration,

total motility, progressive motility, and percentage of morphologically normal sperm) were compared by ANOVA. Data are presented as mean ± SD. Values were considered significant at $p < 0.05$.

Results

A total of 50 attempts at chemical ejaculation were performed on a total of 12 client-owned stallions over a 6 year period (2013 - 2018). Semen was collected successfully in 22 (44%) attempts. Four stallions were presented with musculoskeletal issues, 4 presented for ejaculatory dysfunction, 2 had neurological issues, 1 had squamous cell carcinoma lesions on his penis and 1 had a history of hemospermia. Four of 12 stallions did not respond to chemical ejaculation; however, the remaining 8 stallions ejaculated at least once. Only 1 stallion ejaculated to every chemical ejaculation attempt. Semen parameters for ejaculates from the 22 successful traditional (without medication) and 22 medicated (imipramine-xylazine) collection methods are presented (Table 1). Total motility and progressive motility were the only semen parameters that were different ($p < 0.05$). Gel free volume, sperm concentration, and total sperm numbers were not statistically different.

Table 1. Semen parameters for ejaculates collected by traditional (without medication) and chemical (imipramine-xylazine) methods

Parameter	Without medication (n = 22) mean ± SD	Imipramine-xylazine (n = 22) mean ± SD	P Value
Interval from xylazine to emission of semen (minutes)	N/A	13.4 ± 10.6 (3 - 34)	N/A
Gel free volume (ml)	39.9 ± 20.6 (17 - 100)	37.7 ± 26.8 (8.2 - 130)	0.765
Sperm concentration (x 10^6/ml)	318.3 ± 282.3 (71 - 1270)	443.3 ± 401.7 (141 - 873)	0.239
Total sperm number	10,579 ± 7,718 (3,060 - 22,625)	12,512 ± 7,375 (3,240 - 33,150)	0.400
Total sperm motility (%)	74.1 ± 13.2 (46 - 92)	60.6 ± 22.3 (15 - 89)	0.019
Progressive sperm motility (%)	69.2 ± 14.5 (37 - 90)	52.8 ± 23.2 (10 - 84)	0.007

All stallions ejaculated when collected off a breeding phantom using an artificial vagina. Success rate of semen collection was different ($p < 0.05$) between traditional collection and chemical ejaculation procedures.

Oral treatment of 3.0 mg/kg of imipramine hydrochloride, followed 2 hours later by intravenous treatment of 0.5 mg/kg of xylazine hydrochloride resulted in passive emission of semen in 3 of the 7 stallions (42.9%) after only 1 attempt. Average interval between xylazine treatment and ejaculation was 33.1 minutes (range; 31 - 35 minutes). Stallions' ages for which chemical ejaculation was successful were 22, 22, and 27 years, whereas stallions' ages for which chemical ejaculation was not successful were 17, 22, 22, and 27 years. None of the stallions, that were treated with standard imipramine/xylazine protocol, ejaculated when 20 units of oxytocin was given concurrently with xylazine hydrochloride. Stallions did exhibit a greater degree of spasmodic muscle contractions in the dorsal scrotal region when oxytocin was included in the protocol compared to standard protocol.

None of the stallions, previously treated with imipramine-xylazine, ejaculated when treated with a prostaglandin analogue. All stallions exhibited muscular contractions in the dorsal scrotal region and dropped their penis and gained an erection within 5 minutes after prostaglandin treatment. Sweating and mild diarrhea were observed in 2 stallions.

There were no differences ($p > 0.05$) in gel free volume, sperm concentration, total sperm motility, progressive sperm motility, and total sperm number between ejaculates collected from 3 stallions using imipramine-xylazine protocol and ejaculates collected from all 7 stallions without medication (Table 2). There were also no differences ($p > 0.05$) in semen parameters when only comparing ejaculates from the same 3 stallions collected using the imipramine xylazine protocol or without medication.

Table 2. Semen parameters for ejaculates collected by traditional (without medication) and chemical (imipramine-xylazine) methods

Parameter	Without medication (n = 7) mean ± SD	Imipramine-xylazine (n = 3) mean ± SD	P Value
Gel free volume (ml)	41.1 ± 18.8	44 ± 40.7	0.92
Sperm concentration (x 10^6/ml)	342.2 ± 253.4	795 ± 617.4	0.26
Total sperm motility (%)	66.9 ± 22.5	89.7 ± 2.1	0.28
Progressive sperm motility (%)	59.9 ± 23.8	85 ± 4.6	0.29
Total sperm number (x 10^9)	11.2 ± 8.9	19.7 ± 7.2	0.97

Discussion

Success rate of chemical ejaculation in our clinical program (44%) was similar to what has been reported (33 - 68%).[12,13,16] Success for individual stallions ranged from 100% (i.e. 5 for 5 in 1 stallion) to 0% (a combined 0 for 9 attempts for 4 stallions). The rationale for the prospective part of the study was to determine if addition of oxytocin or prostaglandins to standard protocol would improve success of chemical ejaculation in a population of aged stallions.

The standard imipramine-xylazine protocol success rate for aged stallions in the current study (42.9%) was in the same range as in our clinical practice. Specific titrated doses modified for each individual stallion significantly improved the success rate of chemical ejaculation. [15] Limited anecdotal data have been reported on the effects of oxytocin or prostaglandins on pharmacologically induced emission of semen in stallions.[4]

Oxytocin is a peptide hormone synthesized in the paraventricular nuclei of the hypothalamus and secreted from the posterior pituitary gland. Oxytocin administration to stallions stimulates contraction of smooth muscles of the ductus deferens and epididymis and has been used clinically to alleviate blockage of plugged ampullae.[4] It was hypothesized in the current study that oxytocin would enhance the success of imipramine-xylazine induced emission of semen by stimulation of smooth muscle contraction. However, none of the stallions ejaculated when oxytocin was added to the protocol.

Prostaglandin $F_{2\alpha}$ is a fatty acid hormone which can induce smooth muscle contraction. A previous report noted that spontaneous ejaculations were observed within 10 minutes after intramuscular treatment of an analogue of prostaglandin $F_{2\alpha}$.[4] In the current study, none of the stallions ejaculated when a dose of prostaglandin was incorporated in the protocol. Type of prostaglandin, dose used, and timing of treatment could all have affected the outcome.

Semen collected via chemical ejaculation had lower volume and higher sperm concentration compared to traditional ejaculates.[16] However, in the present study, all semen parameters were similar between the 7 traditional ejaculates and all 3 chemically induced ejaculates in the aged research stallions.

Passive emission of semen with chemical ejaculation, if successful at all, usually occurs within 3 - 5 minutes after xylazine treatment as the stallion is becoming sedated or less commonly when the stallion is recovering from sedation.[12,27] In the present study, passive emission of semen occurred in all 3 stallions ~ 30 minutes after xylazine treatment. None of the stallions emitted semen in the early period after xylazine treatment.

Conclusion

Chemical ejaculation was successful in aged stallions using a standard protocol of imipramine and xylazine. Addition of oxytocin or cloprostenol to the standard protocol at the dosages and times used apparently had deleterious effects on passive emission of semen in aged stallions, as all attempts were unsuccessful.

Conflict of interest

None to declare.

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