Failure of Pharmacologically - Induced Ejaculation in Donkeys (*Equus Asinus*) Under Field Conditions: A Test of Two Different Treatment Protocols

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ABSTRACT

The aim of this study was to test pharmacologically-induced ejaculation in male donkeys under field conditions using two different protocols. Two methods were used: The first method was the administration of imipramine (3mg/kg, p.o.), and then 2 hours later, xylazine (0.66 mg/kg, i.v.). The second method was a single administration of a combination of butorphanol (0.02mg/kg, i.v.) and xylazine (0.33 mg/kg, i.v.). Nine male donkeys used in the study and both of the methods were applied to all of them. Ejaculation was not achieved in any of the donkeys using either method. Therefore the conclusion was made that these two methods are not satisfactory for ex-copula induction of ejaculation in male donkeys under the conditions of our study.

Keywords: Semen Collection; Xylazine; Imipramine; Butorphanol; Male Donkeys

INTRODUCTION

Pharmacologically-induced ejaculation is one of the methods used for semen collection which has been described for use in stallions (1-8), and also mentioned for use in male donkeys (9-11). The success of the protocols of this method in stallions differs considerably (25 – 84%), depending on the drugs used, their combinations and the route of administration and also there are even differences among individual animals (1-8). Additional protocols have also used in male donkeys (9-11). The protocol with a combination of imipramine and xylazine was tested in this study because it was found to be very successful in a previous study (9). However in another study using a similar protocol it was unsuccessful (10).

For the second protocol, a combination of butorphanol and xylazine was chosen as in stallions these drugs reached a satisfactory success rate (8) and, until now, there appears to be no research concerning the use of this combination in male donkeys.

MATERIAL AND METHODS

Nine mature male donkeys (5 – 16 years) were used in this field study. All the animals were without any clinical signs of disease. The male donkeys were housed in different locations and conditions and had completely different breeding histories (being kept with or completely without jennies or mares). Both treatment protocols were used in each of the nine male donkeys in the same order. The intervals between the protocols were different for each animal, depending on their availability, but there was at least 24 hours between the protocols.

The drugs were given without previous fasting of the animals. Assessment of the weight was done visually. The assessors were experienced at estimating the weight of donkey's from previous studies. A small percentage was added so as not to underestimate the bodyweight. After the administration of the sedatives, male donkeys were kept in a quiet place without access to food and observed until full recovery. Influence

of the sedation on ejaculation was categorized as follows: prolapse of penis, erection, masturbation, pre-ejaculatory secretion discharge and ejaculation (confirmed by microscopic examination). The donkeys were not interfered with in any way so as not to distract them. The prepuce was observed by two qualified veterinarians who were standing by with a plastic receptacle to catch any excretion. The results for each male donkey/protocol were recorded.

After complete recovery from sedation male donkeys were allowed to go back to their paddock without any other restriction.

Protocol 1

The first protocol involved a *per os* application 3 mg/kg of imipramin (Melipramin, EGIS Pharmaceuticals PLC, Hungary) and two hours later, an intravenous application 0.66mg/kg of xylazine (Xylazin Riemser 20mg/ml, Bioveta a. s., Czech Republic) (10).

After the imipramin application, the animals were left without any restriction and were not observed. Two hours later, xylazine was administered intravenously and the male donkeys were observed until complete recovery from sedation.

Protocol 2

The second protocol involved a single intravenous application of a combination of 0.33 mg/kg xylazine (Xylazin Riemser 20mg/ml, Bioveta a. s., Czech Republic) and 0.02 mg/kg butorphanol (Butomidor 10mg/ml, Richter Pharma AG, Austria) (8). After application of the drugs the animals were left without any restriction and observed the entire period from administration to complete recovery from sedation.

The study was conducted in accordance with ethical rules and procedures, and was approved by The Ministry of Education, Youth and Sports , (Certificate of Autorisation to Experiment on Living Animals MSMT-14648/2016-316-2016, The Local Ethical Committee of University of Veterinary and Pharmaceutical Sciences Brno N°16-2016).

RESULTS

The results of this study are summarized in Table 1 below. For each animal the reproduction history, age, current farming methods and also the response to each of the two treatment protocols are described because it was assumed that these facts could have an influence on the results. However during the whole experiment none of the 9 male donkeys ejaculated. No complications were observed during or after recovery from sedation. No adverse effects were observed during the study.

DISCUSSION

Neither ejaculation, nor pre-ejaculatory secretion discharge was observed in any of the 9 male donkeys in this study. .

In Protocol 1, a combination of orally administrated imipramine and intravenously applied xylazine was chosen because we found two studies with similar treatment pro-

Table 1

No.	Age (years)	History of reproduction	Current farming method	Protocol 1 Melipramine, Xylazine categorized effect	Protocol 2 Xylazine, Butorphenol categorized effect
1	6 years	Breeding experience	Together with jennies	Weakly sedated/ Without prolapse	Deeply sedated/ Significant prolapse
2	16 years	Breeding experience	Together with jennie	Deeply sedated/ Without prolapse	Middle sedated/ Without prolapse
3	5 years	Breeding experience	Alone	Weakly sedated/ Without prolapse	Deeply sedated/ Significant prolapse
4	11 years	Breeding experience	Free housing, together with another donkey male	Deeply sedated/ Without prolapse	Middle sedated/ Significant prolapse
5	7 years	Breeding experience	Free housing, together with another donkey male	Middle sedated/ Without prolapse	Deeply sedated/ Partial prolapse
6	15 years	Breeding experience	Free housing with jennie (without posterity)	Deeply sedated/ Without prolapse	Deeply sedated/ Significant prolapse
7	9 years	Without breeding experience	Alone	Deeply sedated/ Partial prolapse	Deeply sedated/ Partial prolapse
8	14 years	Breeding experience	Together with jennies	Deeply sedated/ Partial prolapse	Deeply sedated/ Significant prolapse
9	10 years	Breeding experience	Together with mares	Middle sedated/ Partial prolapse	Weakly sedated/ Without prolapse

tocols and completely different results in donkeys. The first study by Sghiri *et al.* (9) was focused mostly on changes of behavior, especially sexual behavior. They studied combinations of different imipramine dosages and different time intervals between imipramine and xylazine administration. Ejaculation was observed in only one of the 55 male donkeys. In contrast, the Naoman and Ali's (10) study achieved a 96.6% success rate of ejaculation with the same protocol on animals housed at the clinic.

This trial was only made on a small number of animals (n=5), so we decided to use the same dosage of drugs and the same time interval to verify their results. Considering our results, it was concluded that this protocol is not suitable for pharmacologically induced ejaculation in donkeys in field conditions. This contrasts with the results obtained by Naoman and Ali (10) and could be due to the large variability of responsiveness of individual male donkeys. Also, in the Naoman and Ali (10) study, smaller and younger animals which were housed in the same place in the animal house of the College of Veterinary Medicine were used. In our study and in the study by Sghiri *et al.* (9), larger and older animals were used. The animals in our study were donkeys from private owners, so we did not determine the dose or the manipulation with animals before the trial, as was done in the Naoman and Ali's (10) study. Unfortunately, we have no information about the origin and housing of the animals in the study by Sghiri *et al.* (9). Another important factor which is mentioned in a study for the successful semen collection from stallions – a quiet and calm place (4) is sometimes difficult to find and arrange in field conditions and also the manipulation with donkeys may be stressful for them.

The second protocol involved a butorphanol and xylazine application. When only xylazine was used for pharmacologically-induced ejaculation in stallions, the success rate was 14% (3) but it is possible to improve the success rate by teasing the stallion to estrous mares. When teasing was used, the success rate increased to 39% (3). Josson and Whitacre (8) tried to improve the result by the addition of butorphanol. Their outcome was an 84% success rate in two severely injured stallions, but in six healthy stallions the success rate was only 15%.

To the best of our knowledge, this protocol has not been used in donkeys. We have had relatively good experience with this combination for pharmacologically induced ejaculation in healthy stallions. So, we expected at least some success, but the outcome was that even this protocol is not suitable for pharmacologically induced ejaculation in male donkeys. The failure of pharmacologically induced ejaculation in male donkeys could be explained by the different reaction of donkeys to the administered drugs. This has been documented in previous studies (12). More studies are needed to evaluate the influence of the dosage level on the success rate of pharmacologically-induced ejaculation in male donkeys.

The increasing number of donkeys kept as pet animals increases the demand for artificial insemination. However semen collection in male donkeys without a jenny on heat is very problematic not only under field conditions. Finding some alternative methods of collecting ejaculate from male donkeys for preparing and freezing insemination doses could greatly facilitate this situation. It has been shown that stallion semen collected by pharmacologically-induced ejaculation can be successfully used for artificial insemination (1). Therefore, we assumed that this method could be similarly successful for male donkeys.

CONCLUSION

Our results indicate that using a combination of *per os* imipramine application and intravenous xylazine administration or an intravenous administration of butorphanol and xylazine combination is not suitable for pharmacologically-induced ejaculation in male donkeys under field conditions. Although some works of other authors show positive results with this treatment protocols in horses and in donkeys, we suggest that further research is needed in this field.

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