

MANUAL STIMULATION COLLECTION OF SEMEN FROM STALLIONS:
TRAINING TIME, SEXUAL BEHAVIOR AND SEMEN

S. M. McDonnell and C. C. Love

University of Pennsylvania School of Veterinary Medicine
Hofmann Center for Reproduction Research
New Bolton Center, Kennett Square, PA 19348

Received for publication: *October 25, 1989*

Accepted: *February 26, 1990*

ABSTRACT

In Experiment 1, 31 pony stallions of unknown breeding history were trained to either manual stimulation (n = 20) or traditional artificial vagina (n = 11) collection of semen. For manual stimulation, 45% achieved ejaculation on the ground during the initial training session. The remaining 55% achieved ejaculation by manual stimulation while mounted during the first or second training session. For artificial vagina training, 73% ejaculated during the first training session. The mean number of training sessions required was 1.15 for the manual stimulation technique and 1.18 for the traditional artificial vagina technique. Experiment 2 was conducted to compare sexual behavior and the semen obtained by manual stimulation and artificial vagina procedures. Six stallions which had been trained for manual stimulation collection of semen in Experiment 1 were used. Semen was collected from each stallion in two replicates of four collections per replicate, during which each stallion was exposed alternately on four successive days to manual stimulation or artificial vagina technique. There were no significant differences due to the method of collection in total collection time, number of mounts required, or mount to ejaculation latency. Manual stimulation required a mean of nine thrusts, while the artificial vagina method required only six. This difference was significant ($P < 0.0001$). There were no significant differences due to the method of collection for semen volume, gel volume, spermatozoa concentration, total number of spermatozoa per ejaculate, percent total motile spermatozoa or percent progressively motile spermatozoa. For pH, there was a slight, yet significant difference ($P < 0.01$) due to the collection method. The mean pH of semen was 7.51 for the artificial vagina method and 7.43 for the manual stimulation method. Manual stimulation was found to be a simple, efficient, and inexpensive method for collecting semen from stallions. The artificial vagina and manual stimulation methods required similar training time and yielded similar semen samples. Stallions maintained normal sexual behavior with manual stimulation and showed no difficulty alternating between manual stimulation and traditional artificial vagina methods of semen collection, or returning to natural service.

Key words: semen collection, manual stimulation, ejaculation, stallion

Acknowledgments: Nancy Diehl assisted with portions of these experiments.

INTRODUCTION

Crump and Crump (1) described a simple manual stimulation technique for collecting semen from stallions that are standing on the ground. Their technique involves placing a plastic bag as a collection vessel over the glans of the erect penis and simply manually stimulating the glans and shaft to elicit pelvic thrusting and ejaculation. The Crumps reported their successful use of this method with 16 different Arab and Akhal-Teke stallions since 1965 in their breeding and on-farm semen freezing program. Their work indicates that stallions can be readily trained to manual stimulation on the ground. The Crumps found that stallions trained to this method experienced no difficulty alternating from manual stimulation to artificial vagina or natural service methods. The Crumps had not detected any differences between ejaculates obtained by manual stimulation on the ground and those obtained using traditional artificial vagina methods.

This manual stimulation technique offers a simple and efficient alternative method for collecting semen from stallions. During the last three years, we have employed both the Crump manual stimulation technique as well as a modified version of the technique in which the stallion is allowed to mount a mare or dummy during manual stimulation for collecting semen from research pony stallions. We have also found the Crump technique of manual stimulation a clinically useful alternative collection method for some stallions that, due to physical injury or hind limb pain, experience difficulties in mounting a mare or dummy.

We report on the training of 20 pony stallions of unknown breeding history to manual stimulation semen collection, and for comparison, training of an additional 11 pony stallions to conventional artificial vagina methods. We also report the results of a study conducted 1) to compare ejaculates obtained by manual stimulation methods with those obtained by conventional artificial vagina semen collection methods, and 2) to evaluate the ability of stallions trained to manual stimulation to accept and to alternate between artificial vagina and manual stimulation collection methods.

MATERIALS AND METHODS

Experiment 1

Thirty-one pony stallions (175 to 225 kg; aged 2 to 18 yr) of unknown breeding history were acquired from local auctions or farms for use in a series of semen studies conducted between October 1986 and July 1988. Stallions were stabled in individual box stalls, with hay, pelleted complete feed, and fresh water provided to maintain good body condition.

Following acclimation (10-d minimum) to housing and routine handling, and before assignment to subsequent semen studies, each stallion was subjected to

training for manual stimulation ($n = 20$) or artificial vagina ($n = 11$) procedures. Three times weekly each animal was given a training session. Each session consisted of presenting the stallion under halter to a tethered stimulus pony mare (ovariectomized, estrogen-primed). The stallion was allowed to interact with the mare until erection was achieved. The stallion was then backed away from the mare for washing of the penis with clear warm water (45°C).

For artificial vagina training, the stallion was then allowed to mount the stimulus mare, and a Missouri Model artificial vagina (NASCO, Fort Atkinson, WI 53538) was placed on the penis and held firmly against the mare for thrusting. An artificial vagina training session ended when the stallion ejaculated, failed to remount for 5 min, or mounted five times without ejaculation.

For manual stimulation training, a plastic bag (1 mil polyethylene mitts, cut to approximately 16 inches, NASCO, Fort Atkinson, WI) was placed over the glans penis, extending 6 to 8 inches up the shaft of the penis as well as 6 to 8 inches beyond the glans. The plastic bag was secured with a band of adhesive tape loosely wrapped around the penis near the edge of the bag (Figure 1). During a session, four variations of manual stimulation were done, twice each in a step-wise fashion in an effort to elicit thrusting and ejaculation. The first technique involved manual stimulation on the ground (Figure 2). With the stallion standing on the ground, the operator cupped the left hand firmly over the glans penis with the right hand firmly grasping the shaft approximately mid-way between the preputial fold and the base of the penis. The stallion was allowed to lower his head and step forward as necessary as described by Crumps (1988). The second technique involved manual stimulation on the ground with the addition of a warm (45°C) wet compress over the glans penis (Figure 3). The third technique was manual stimulation while mounted on pony mare (Figure 4). And the final technique involved manual stimulation while the stallion was mounted, with the addition of the compress.

A manual stimulation training session ended when ejaculation occurred or when the sequence was completed. Training was continued for six sessions over a 2-wk period. Subjects were then assigned to other experiments in which collection of semen was continued two to three times weekly for 4 to 12 wk depending on the protocol of the assigned study. We continued to monitor their response to manual stimulation during these studies. Following the completion of these studies, stallions were also bred naturally, to evaluate willingness and ability.

All training sessions were videotaped for subsequent derivation of the following information: 1) the number of training sessions and total attempts to achieve the first of three consecutive successful collections; 2) for manual stimulation, the manual stimulation technique of the first successful ejaculation; and 3) for manual stimulation, the regularly successful manual stimulation technique for collection of semen in subsequent experiments. Differences between methods were analyzed by independent t-tests.



Figure 1. Placement of plastic bag.



Figure 2. Manual stimulation while the stallion is standing.

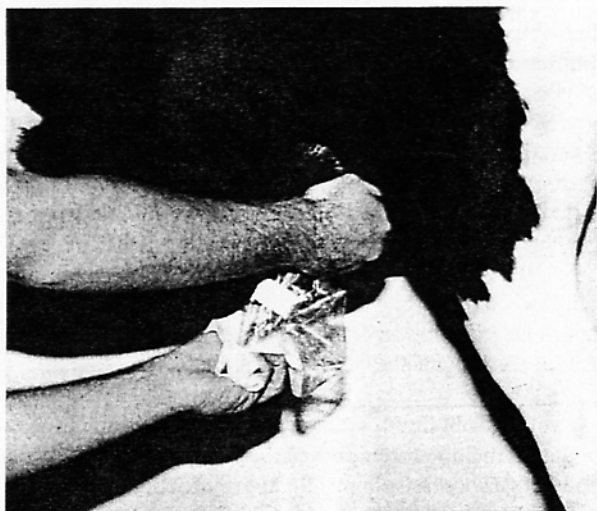


Figure 3. Manual stimulation with warm compress while the stallion is standing.

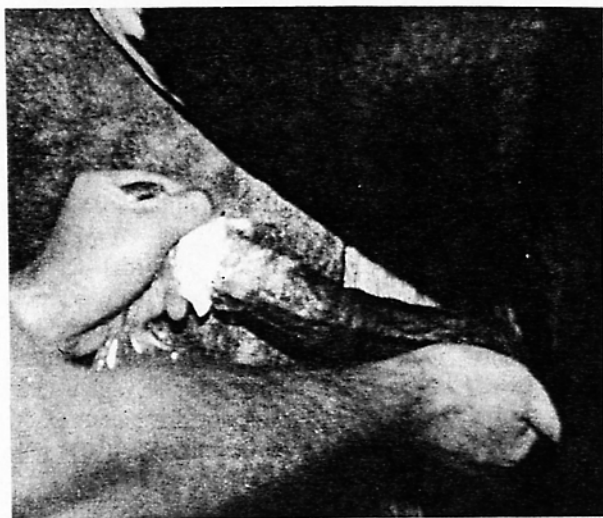


Figure 4. Manual stimulation with the stallion mounted.

Experiment 2

Semen was collected from six stallions in two replicates of four collections per replicate, during which each stallion was exposed alternately on four successive days to manual stimulation or artificial vagina technique. These stallions had been trained to manual stimulation in Experiment 1, and most had been used previously for natural service. The first replicate was begun three days after the end of manual stimulation training and the second replicate was conducted one month later, following four weeks of manual stimulation collection conducted three times weekly as a part of another study. For each 4-day collection period, each stallion was randomly assigned to a sequence of alternating artificial vagina and manual stimulation. All collections were videotaped for subsequent derivation of the number of mounts to achieve ejaculation, the number of thrusts on the ejaculatory mount, the mount to ejaculation latency, and the total collection time (interval from completion of washing of the penis to ejaculation).

All semen samples obtained were evaluated according to Kenney et al. (2). Endpoints evaluated included semen volume, gel volume, percent total motile spermatozoa, percent progressively motile spermatozoa, concentration (million per milliliter), total number spermatozoa, and pH of semen. An analysis of variance for repeated measures was used to evaluate the effect of technique for each semen and behavioral variable. One semen sample for each animal from each collection method was evaluated for bacterial contaminants. Immediately following collection of semen, a sterile swab was dipped into the semen sample and plated on blood agar and McConkey's media for incubation for 48 hrs incubation at 37° C.

RESULTS

Results of manual stimulation and artificial vagina training are summarized in Table 1. Nine of the twenty stallions (45%) achieved ejaculation during their first session with manual stimulation on the ground. Three of these required the addition of a warm compress. The remaining 11 stallions (55%) achieved their first ejaculation with manual stimulation while mounted. Two of these ejaculated without the warm compress and nine with the compress. Nine of the eleven stallions trained to the artificial vagina achieved their first ejaculate during the first session, with one to three mounts. The remaining two required two sessions, with three and seven mounts. The mean number of sessions required for manual stimulation training was 1.15 (+/- 0.36), while artificial vagina training required a mean of 1.18 (+/- 0.40). This difference was not significant ($P > 0.05$). Manual stimulation training, however, required significantly ($P < 0.01$) greater number of attempts (5.75 +/- 3.8) than artificial vagina training (2.18 +/- 1.77). These subjects continued to ejaculate by this method in subsequent experiments, and all subjects exhibited normal copulatory behavior when allowed to breed by natural service.

Table 1. Summary of training of pony stallions for semen collection by manual stimulation and artificial vagina

Manual stimulation				Artificial vagina	
Animal ID	No. of training sessions (attempts)	Manual stimulation technique at 1st ejaculation	Regularly successful technique	Animal ID	No. of training sessions (mounts)
1	1 (2)	G	M+	1	1 (2)
2	1 (1)	G	G	2	1 (1)
3	1 (2)	G+	G	3	2 (7)
4	1 (2)	G	G+	4	1 (2)
5	1 (3)	G+	G+	5	1 (1)
6	1 (1)	G	M+	6	1 (1)
7	1 (7)	M+	*	7	1 (3)
8	1 (2)	G	G	8	1 (2)
9	2 (15)	M+	M+	9	1 (1)
10	1 (8)	M+	M+	10	1 (1)
11	1 (7)	G	M	11	2 (3)
12	1 (3)	G+	G+		
13	1 (8)	M+	M+	\bar{X}	1.18 (2.18)
14	1 (7)	M+	M+		
15	1 (7)	M+	M+		
16	1 (5)	M	M		
17	2 (7)	M+	M+		
18	2 (13)	M	M		
19	1 (8)	M+	M+		
20	1 (7)	M+	M+		
\bar{X}	1.15 (5.75)				

G = manual stimulation on the ground; G+ = manual stimulation on the ground plus a warm compress; M = manual stimulation while mounted; M+ = manual stimulation while mounted plus a warm compress.

* This stallion did not achieve erection regularly. When erection occurred, M+ was successful.

THERIOGENOLOGY

Results of Experiment 2 are summarized in Table 2. Differences between the two methods in the number of mounts required for ejaculation and the mount to ejaculation latency were not significant ($P < 0.05$). The mean number of thrusts required for ejaculation was significantly greater for the manual stimulation method. The pH of artificial vagina collected samples of was 7.51 (+/- 0.16), while it was 7.43 (+/- 0.15) for manual stimulation collected samples. This difference, though slight, was significant ($P < 0.01$). For all other semen endpoints, there were no significant differences due to technique. Both methods yielded heavy growth of a variety of commensal organisms, with colonies too numerous to quantitate, and no obvious difference between samples obtained by manual stimulation or artificial vagina method.

Table 2. Summary of sexual behavior and semen using artificial vagina and manual stimulation collection methods

	AV method		MS method		
	\bar{X}	SD	\bar{X}	SD	
Behavior					
Mounts to successful collection	1.5	(1.5)	1.9	(1.5)	NS
Thrusts on ejaculatory mount	6.6	(1.5)	9.3	(2.2)	$P < 0.0001$
Mount to ejaculation latency (sec)	18.6	(8.5)	21.4	(6.3)	NS
Collection time (sec)	185.5	(128.0)	175.0	(140.0)	NS
Semen					
Semen volume (ml)	25.2	(15.5)	24.8	(12.2)	NS
Gel volume (ml)	3.1	(5.0)	3.9	(12.0)	NS
Concentration (million/ml)	111.4	(86.2)	95.0	(56.1)	NS
Total number sperm (billion)	22.44	(14.7)	21.77	(15.0)	NS
Percent total motile sperm	68.0	(18.9)	66.6	(23.8)	NS
Percent progressively motile sperm	61.7	(18.9)	60.8	(25.0)	NS
pH	7.51	(0.16)	7.43	(0.15)	$P < 0.01$

AV = artificial vagina; MS = manual stimulation; NS = not significant, $P > 0.05$.

DISCUSSION

Training for manual stimulation was relatively simple, with no difference from traditional artificial vagina in the number of sessions required. The greater number of attempts to train to manual stimulation did not represent additional time compared to the artificial vagina method. In our continued work, we find that

increased operator experience with manual stimulation techniques seems to reduce the time required to train an animal.

These two techniques yielded similar semen and behavior results. The only behavioral difference involved a greater number of thrusts required to achieve ejaculation with manual stimulation (nine thrusts) than with the artificial vagina (six thrusts). However, the number of thrusts as well as the total mount time required to achieve ejaculation was within the range for normal stallions during artificial vagina procedures (3). Of the semen endpoints evaluated, the only significant difference involved a slight difference in pH. Again, both techniques yielded values within the normal range, and the difference may have been due to the different substances contacted by semen in the two methods. Finally, bacterial contamination of semen was high for both techniques.

The stallions showed no difficulty in alternating between artificial vagina and manual stimulation techniques. Subsequent similar work in our lab and clinic, together with the Crumps' observations, confirms this remarkable flexibility of the horse. Stallions readily learned these techniques and responded to associated events with appropriate anticipatory postures for manual stimulation or artificial vagina techniques. It was clear to us that the artificial vagina stimulated stallions to approach the mare, while the plastic bag elicited standing for placement and then ground thrusting. These and other stallions trained to manual stimulation maintained excellent libido for several months of ground collection. As with the horses described by the Crumps, the ponies in our study readily became conditioned to become sexually aroused by the presentation of the plastic bag and related cues that collection was about to commence. Some did not require the presence of a stimulus mare for successful semen collection.

We have used and recommended manual stimulation on the ground for collection of semen from horses with back and rear limb injuries that interfere with mounting ability. We have also found that manual and thermal stimulation of the base of the penis is a useful adjunct to the standard artificial vagina method for horses with ejaculatory dysfunction in association with poor coupling or thrusting as well as in stallions that couple and thrust adequately yet fail to ejaculate.

In this work we have found that erection is not necessary for ejaculation. In animals we have trained to manual stimulation we have been able to induce pelvic thrusting and ejaculation by manual stimulation of the base of the penis before the animal achieves full erection of the shaft (McDonnell, unpublished observations). Following those observations, we have used manual stimulation to induce ejaculation in a horse that, as a sequela to priapism, was unable to achieve erection. The stallion was teased to a high level of arousal and allowed to mount the mare. The extended, flaccid penis was grasped at the glans and base. Warm compresses applied to the base of the penis reliably induced strong pelvic thrusting and ejaculation.

In conclusion, we found manual stimulation to be a simple, efficient method for collecting semen from stallions, yielding semen samples similar to those obtained by artificial vagina. Stallions are readily trained to manual stimulation and exhibit no apparent difficulties alternating between manual stimulation and traditional artificial vagina methods of semen collection, or returning to natural service. This technique is inexpensive and efficient, and compared with the artificial vagina, it requires less preparation and care of equipment.

REFERENCES

1. Crump, J. and Crump, J. Stallion ejaculation by manual stimulation of the penis. *Theriogenology* 31: 341-346 (1988).
2. Kenney, R. M., Hurtgen, J. P., Pierson, R., Witherspoon, D. and Simons, J. Clinical fertility evaluation of the stallion. Hastings, NE: Society for Theriogenology (1983).
3. McDonnell, S. M. Reproductive behavior of the stallion. In: Crowell-Davis, S. and Houpt, K. (eds.), *Veterinary Clinics of North America: Equine Practice* 2. W. B. Saunders, Philadelphia, 1986, pp. 535-555.