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Effects of ground semen collection on weight bearing on hindquarters, libido, and semen parameters in stallions

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ABSTRACT

Collection of semen on the ground from the standing stallion represents an alternative method to dummy mount semen collection and is of increasing popularity for sport stallions, males suffering from health problems, or in studs without a dummy or suitable mare at disposal. Our aim was to collect and compare spermatological and physiological data associated with traditional and ground semen collection. Twelve of 23 Franches-Montagnes stallions were selected to carry out semen collection on a dummy and while standing in a crossed experimental protocol. Semen quantity and quality parameters, weight bearing on hindquarters, and behavioral and libido data were recorded. Ground versus dummy mount semen collection was accompanied by lower seminal volume (15.9 ± 14.6 vs. 22.0 ± 13.3 mL; $P < 0.01$) and lower total sperm count ($4.913 \pm 2.721 \times 10^9$ vs. $6.544 \pm 2.856 \times 10^9$ sperm; $P < 0.001$). No significant differences were found concerning sperm motility and viability. Time to ejaculation was longer, and the number of attempts to ejaculation was higher ($P = 0.053$) in the standing position compared with the mount on the dummy. A higher ($P < 0.01$) amount of tail flagging was manifested by the stallions during ejaculation on the dummy compared to when standing. There was no difference in weight bearing on hindquarters when comparing dummy collection ($51.2 \pm 2.5\%$) and standing collection ($48.9 \pm 5.5\%$). Ground semen collection can be considered as a viable option for stallions that cannot mount a dummy or a mare. However, it requires training and may be not easily accepted by all stallions. Owners should be advised that ground semen collection is associated with significantly lower sperm numbers than with dummy mount semen collection.

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1. Introduction

The collection of semen on the ground in the standing stallion is an alternative method to dummy mount collection of semen and is increasing in popularity. Ground collection is particularly interesting for stallions suffering from health problems, e.g., musculoskeletal or neurologic, or in studs that do not have a dummy or suitable mare at their disposal. A general opinion appears to be that stallions place less stress on their hindquarters, pelvis, and back if the procedure is performed while standing rather than when mounting. This has made standing semen collection popular for stallions that are actively engaged in equestrian sports, although semen collection on a dummy is perceived as only a modest temporary stressor in sexually experienced and well-trained stallions [1].

However, there are currently only a few field studies that have examined ground semen collection using an artificial vagina [2,3] or manual stimulation [3–7] in the stallion, and several questions need further investigation such as potential differences in the weight bearing of hindquarters and back, variations in semen quality and quantity when

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collecting with the two different methods using an artificial vagina, accident risks for the stallions, and their acceptance toward the ground collection method along with the potential risks this could present toward the staff.

The aim of this study was to collect data on the acceptance, libido, and semen parameters as well as weight bearing on hindquarters associated with ground semen collection in the standing stallion to investigate these queries.

2. Materials and methods

2.1. Horses

Twenty-three clinically sound and lameness-free stallions aged between 3 and 18 years (mean age = 8.4 ± 4.7 years) with breeding experience and proven fertility, owned by the Swiss National Stud in Avenches, Switzerland, were at disposal for the study. All stallions were kept in individual boxes bedded with straw, were regularly and individually exercised (riding, carriage driving), and had daily access for approximately 1 hour to a paddock without any direct mare contact. They were fed three times daily on hay, oats, barley, corn, and pellets supplemented with minerals and had *ad libitum* access to water at all times. In two sessions of 15 minutes each on 2 days, early acceptance of the stallions for the ground semen collection method was evaluated, and selection of animals for further experimentation was based on (1) possibility to collect semen, (2) number of necessary collection attempts, (3) time to ejaculation, and (4) safety while collecting was performed. Twelve stallions aged between 3 and 15 years (7.8 ± 4.4 vs. 9.1 ± 5.1 years for nonselected stallions) with a mean height of 156.0 ± 1.7 versus 157.6 ± 1.0 cm for nonselected stallions were finally chosen to enter the study.

2.2. Study design

After a further training period for dummy (first 2 days) and ground (Days 3–5), semen collection on five consecutive days (Monday–Friday), which served also for minimizing extragonadal sperm reserves [8–10] and hence to allow for accurate determination of daily sperm output and other semen characteristics [11,12], the study was carried out using a crossed experimental protocol in the two following weeks. Stallions were randomly assigned into two groups of six stallions each, and semen was collected during two periods of five successive days (Monday–Friday) on a dummy and on ground while standing and *vice versa*. Semen samples used for this study were aliquots from semen collections performed for the artificial insemination program of the stud. Stallions were held according to national regulations and institutional animal care and use protocols.

2.3. Semen collection

All semen collections were carried out in the same room and always by the same person, using an artificial vagina (Avenches model, Switzerland) from the right side of the stallion and in the presence of an ovarectomized stimulus mare. The stallions were led by always the same handler using a bit in the mouth. For dummy mount collection, a commercially available dummy (Bouvry Sarl, Sées, France) was used. This had two adjustable legs at both front and back, was made of leather, positioned at a 12° angle from the vertical, 1.40-m high at the collection place, and 1.80-m in length with a large opening for collection (0.55-m wide). The mare was positioned in a stall perpendicular to the dummy with her head facing the stallion when the latter was collected on the dummy. For ground collection, a wall consisting of wood up to 1.50 m and a metal grill on the top with a round opening 1.60 m from the ground and 0.60 m diameter served to place the stallion behind the stimulus mare and permitting him to pass the head through the opening to take contact with the mare while avoiding the possibility of mounting her. The stallion was allowed to lower his head to balance whilst thrusting, and the semen collector was able to stabilize the stallion by light pressure with a hand on the right side shoulder as recommended by Forney and McDonnell [3]. Criteria for ejaculation confirmation (as described in Schumacher and Riddell [2]) included palpation of urethral pulsations, rhythmic tail flagging (the number observed and recorded, see Section Behavior and libido analysis), and semen present in the artificial vagina collection vial. Digital weighing scales were positioned on the floor at the collection points so that the hindquarters of the stallions were always placed onto it while collecting (see Section Weight bearing analysis).

2.4. Semen analysis

Immediately after semen collection, undiluted semen was placed in a water bath (37 °C). After filtering of the ejaculate, the volume was determined. Total sperm count (TSC) was calculated from ejaculate volume and sperm concentration as determined in a nucleocounter (SP-100; ChemoMetec, Allerød, Denmark), which served also for evaluation of viability. Sperm motility evaluation was assessed after dilution of the raw semen with INRA 96 (IMV, L'Aîgle, France) at a concentration of 30×10^6 sperm/mL with a computer-assisted sperm analyzer (HTM-IVOS, Version 12; Beverly, MA, USA). For that reason, diluted semen was placed in a standardized, prewarmed, 20-μm standard count analysis chamber (Standard Count Analysis Chambers SC 20-01-C; Leja, Nieuw-Vennep, the Netherlands) and assessed in 10 fields. Cells moving less (slower) than 10 μm/s were considered immotile, whereas cells moving greater (faster) than 25 μm/s were considered to be progressively motile.

2.5. Behavior and libido analysis

Breeding behavior was registered using a handycam, Sony DCR VX 2000 (Sony Electronics Inc., Park Ridge, NJ, USA) and measuring *via* video analysis the time that elapsed between the stallion entering the semen collection room and penile erection (erection time) and the ejaculation (ejaculation time), the number of times the stallion mounted the dummy per ejaculate or attempted to ejaculate in the standing position, and the number of tail flagging during ejaculation.

2.6. Weight bearing analysis

To measure the weight bearing borne by the stallions' hindquarters during semen collection for both methods, a digital scale (Bosche AS-300 cap 300 kg × 0.1 kg, BOSCHE GmbH & Co. KG, Germany) made of a rubber mat on a metal frame (2.53-m long × 1.24-m wide × 0.07-m high) was installed at floor level at the collection point where weight was exerted by the hindquarters. The weight was recorded (as a mean of all individual values registered while ejaculating) as a percentage of the total body weight.

2.7. Data analysis

Statistical analyses were performed using NCSS 2007 (Statistical Solutions, Saugus, MA, USA). Wilcoxon signed-rank tests were used for nonparametric data. Relationships between parameters were analyzed using linear regressions. Differences between the two methods were considered significant at P values of less than 0.01 and 0.001.

3. Results

Preselection of ground semen collection allowed for 12 out of 23 stallions to be selected for the study (Supplementary Material, Table S1). In 11 of 38 sessions (28.9%) and 9 of 23 stallions (39.1%), semen could not be collected. The average time to ejaculation was 335.8 ± 218.3 seconds in selected stallions, and an average of 2.1 ± 1.3 attempts to collect was observed compared to 747.5 ± 154.2 seconds and 4.3 ± 1.6 attempts in nonselected stallions. One stallion was deemed too dangerous for standing collection and was omitted for this reason.

The mean results of the parameters of the 12 selected stallions investigated over 5 days each in the standing versus dummy mount collection are summarized in Table 1. For health reasons (leucoma associated with severe oligospermia and oligozoospermia), shortly after the start of the study, one stallion had to be omitted from all spermatological (but not behavioral and weight bearing) statistical analyses. One stallion showed a very abnormal vertically instead of horizontally orientated technique when mounting the dummy, leading to extreme outlier weight bearing values. This horse was therefore omitted from these statistical analyses.

Table 1
Summary of mean results including standard deviation (SD) and statistical analysis for differences between dummy and ground semen collection of 12 stallions over 5 days each.

Parameters	On dummy (mean ± SD)	On ground (mean ± SD)
Semen volume (mL)	22.0 ± 13.3[a]	15.9 ± 14.6[b]
Semen density ($\times 10^6$ sperm/mL)	295 ± 88.5	288 ± 82.2
Total sperm count ($\times 10^9$ sperm/mL)	6.544 ± 2.856[a]	4.913 ± 2.721[b]
Progressive motility (%)	78 ± 11.8	77 ± 12.4
Viability (%)	86 ± 6.8	85 ± 6.4
Time to erection (s)	9 ± 64.3	95 ± 83.6
Number of attempts (n)	1.15 ± 0.5[a]	1.42 ± 0.8[b]
Time to ejaculation (s)	164 ± 117.0[a]	235 ± 194.1[b]
Number of ejaculatory jets (n tail flagging movements)	5.6 ± 1.6[a]	4.9 ± 1.2[b]

[a,b]Values with different superscript letters are significantly different for that parameter.

3.1. Semen quantity and quality

All stallions except for one gave a greater seminal volume over the 5 days with the dummy method with a mean of 22.0 ± 13.3 mL, which was approximately 5.0 mL larger than in the standing method (15.9 ± 14.6 mL; $P < 0.01$). Throughout each week, the volume decreased significantly in the dummy collections and tendentially in the standing position ($P = 0.075$). There was no significant difference between the average values for semen density between the dummy method and the standing method, which resulted in a significant decrease of 24.9% of the TSC in the standing method (4.913 ± 2.721 × 10^9 sperm) compared to the dummy method (6.544 ± 2.856 × 10^9 sperm; $P < 0.001$). Only one of 11 stallions showed a higher mean value in the standing method. All stallions showed in both methods a parallel tendency for a decrease in the TSC over the 5 collection days, and the difference of means between the two collection methods was significant on Days 1, 2, and 5 (Fig. 1A). No significant difference was found between the total and progressive motility parameters and viability of sperm in the dummy method compared to the standing method (Table 1).

3.2. Behavior and libido

No significant difference in time to erection between the dummy and the standing method was found, but stallions collected *via* the standing method showed a significantly longer ejaculation time (235.3 ± 194.1 seconds) when compared to the dummy (164.5 ± 117.0 seconds) collection method. Only on the dummy, a significant relationship between the variable times to erection and to ejaculation ($r^2 = 0.47$, $F(1,60) = 51.67$, $P < 0.001$) was found but not in the standing method. In addition, a significantly ($P < 0.01$) higher number of tail flagging during ejaculation was manifested for collection on the dummy compared to the standing method. Also this observed difference did not change over the 5-day period and results on Day 5 of both methods differed significantly (mean number of tail flagging in standing position: 4.73 and mean number of tail flagging on the dummy: 6.09). Influence of the number of ejaculatory jets on the volume and density of ejaculate as well as the TSC was also analyzed but no significant associations were observed in both methods. There was a difference ($P = 0.053$) between the number of attempts to ejaculate in the standing position (1.42 ± 0.83) and the number of attempts to mount (1.15 ± 0.52) the dummy per ejaculate (Table 1).

3.3. Weight bearing of hindquarters

There was no significant difference between the relative weight bearing of the hindquarters (percentage of individual body weight per stallion) in the dummy method where it was on average 51.2 ± 2.5% compared to 48.9 ± 5.5% in the standing method over the 5 days. The

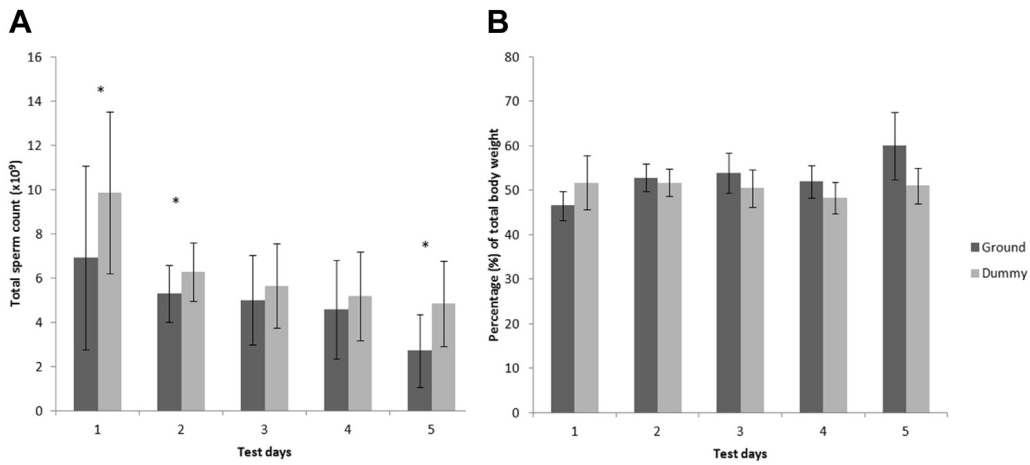

Fig. 1. (A) Effect of semen collection method on sperm output determined as total sperm count ($\times 10^9$ sperm/ejaculate) in the stallion and (B) differences between weight bearing of the hindquarters (in percentage of the total body weight) during dummy and ground semen collection. Means ± standard deviations were determined from 11 stallions tested in a crossed experimental protocol during two periods of 5 successive days on a dummy and while standing. Values with the symbol * added as superscript differ significantly between the methods of semen collection.

weight bearing did not vary significantly over the 5 days (Fig. 1B). There was no correlation between the proportional dummy height to stallion height and the weight bearing measured ($r = 0.007$, degrees of freedom = 34).

4. Discussion

This study represents a first account of the significant spermatological and physiological differences in stallions between semen collection with an artificial vagina on a dummy and while standing. Not observing any differences regarding traditional semen quality parameters such as motility and viability, in contrast, a strong reduction in the TSC in the standing collection method was recorded. This observation of approximately 25% fewer sperm coincides with results in human beings, where also lower sperm counts are found in ejaculates after masturbation into a vial for diagnostic purposes compared to such after coitus (reviewed by [13]). On the other hand, our findings contrast somehow to those of McDonnell and Love [5] who found equal spermatological values in conditions on the dummy and while standing. However, another study design was applied in this latter study [5] and ground semen collection was performed by manual stimulation in comparison to the use of an artificial vagina at dummy collection.

In the light of an evolutionary biological approach, our findings could reflect the phenomenon of “strategic ejaculation” observed in males of various species. Sperm number and certain parameters of sperm quality are known to have evolved under sperm competition (reviewed by Pizzari and Parker [14]), suggesting plasticity of these key ejaculate traits. Strategic ejaculation theory and empirical studies (e.g., [15,16]) suggest an adjustment of sperm investment in relation to sperm competition, social interactions, recently also shown in stallions [17], or female quality, e.g., males are expected to invest more sperm in good-quality females [18–20]. Conditions of ground semen collection have to be judged as rather unnatural, representing a severely modified position of the stallion and limitations of social interaction between the stallion and the mare, and may be only compared with periodic spontaneous erection and penile movements (“masturbation”) in stallions [21]. However, observations of McDonnell [22] clearly show that this frequently manifested behavior is rarely associated with ejaculation, occurring in less than 1% of observed episodes.

In human beings, possible physiological explanations for mechanisms underlying the difference between results in coitus and masturbation conditions are thought to be the considerable control at the cerebral level over the emission phase [23] and the sexual stimulation of men by their partner during the ejaculation process [24]. Also in our study, beside the sperm counts and ejaculate volumes, significant differences in relation to the ejaculatory activity could be revealed between ground and standing semen collection. Ejaculation is observed in mounting stallions as a characteristic downward movement of the tail termed “tail flagging”, signaling the polyphasic delivery of semen in 5 to 10 successive forceful jets of which the first three are sperm rich (76%–80% of the TSC), followed by poor sperm fractions consisting of mainly secretions from the accessory glands [25,26]. Ejaculatory dysfunctions in stallion are manifold and have multiple origins. Failure of ejaculation, one of the most common disorders, is often associated with musculoskeletal deficits and psychogenic components (pain, anxiety, distraction); these two conditions often not easily distinguishable from each other [27]. The slightly but significantly reduced number of ejaculatory jets observed in our study at ground semen collection suggests the hypothesis that there might exist a certain degree of mechanical or psychogenic discomfort experienced by the animals in the standing method, although all horses were deemed as sound and lameness free on entering the study. This is supported by the significantly longer time until ejaculation registered compared to semen collection on the dummy. However, the ejaculation time is influenced by various factors including the process of collection and the collecting technician himself.

In the literature regarding ground semen collection so far, a good acceptance of this method is generally described [4,5], most stallions necessitating a training period of less than three 15-minute sessions with an experienced technician and handler, and within five sessions with novice staff [3], with the exception of the study by Schumacher and Riddell [2], who found limitations of the method such as the possibility of incomplete ejaculates or safety considerations depending on stallion temperament and manageability. Also in our study, not all 23 stallions seemed to tolerate the standing semen collection in the same manner and their age and breeding experience did not seem to influence how well they adapted to the new condition. Therefore, in our study, a preselection of stallions took place, followed by a standing collection training phase of 3 days. Further studies with a prolonged training period might lead to less variation of behavioral results. However and interestingly, throughout the 5-day experimental phase, no changes nor improving in trend were found when analyzing the spermatological and behavioral parameters in both collection conditions on the dummy and while standing.

Our observed decrease of ejaculatory activity seems also not to be related to less libido or precoital stimulation in stallions at ground collection, having not observed any difference of times to erection, an indirect measure of libido in stallions, in our study: Despite individual variations in arousal and closeness to the teasing mare, all experimental stallions reported a good libido in both methods, the mean erection times lying within 1–2 minutes, representing normal precopulatory behavior of domestic stallions [28]. However, all semen collections took place in the same room, and some degree of conditioning of the stallions before our study might have had occurred as some were sexually excited even before reaching the collection barn. Finally, the decreased ejaculatory activity seems to have contributed to the findings of decreased volume of ground collection ejaculates, but not the total sperm number, as the first three sperm-rich ejaculatory jets were observed in all collections in the study.

Nonetheless, also in our study, standing semen collection is shown to be an interesting alternative to the traditional dummy-mounting method for stallions e.g., suffering from neurological or musculoskeletal problems. However and contrary to empirical assumptions, we found that the standing method did not provide a lower burden on the hindquarters than the dummy collection method. It has to be considered that the chosen height and position of the dummy, kept constant throughout in our study, can also have alterations on the amount of weight that is exerted on the stallion's hindquarters. In addition, our study does not take into account the stretched position on the dummy and associated physical strains e.g., on the back as well as degree of pelvic flexion and local forces on the articulations and tendinous structures e.g., the suspensory ligament. However, the focus was set primarily on the efficiency or loss of semen output when collecting on the ground, and weight exerted on hindquarters was additionally measured to address a specific major concern of the horse industry.

In conclusion, our results suggest that ground semen collection is a viable option for stallions that cannot mount a dummy or a mare. However, it was found that there is a variable acceptance of stallions to this method and subsequent training may be necessary. Further research on biomechanical consequences is needed, and stallion owners should be advised that ground semen collection is associated with significantly lower sperm numbers than with dummy mount semen collection.

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Competing Interests

The authors declare that they have no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.theriogenology.2015.04.029.

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Table S1
Early acceptance evaluation of ground semen collection procedure in two sessions of maximum 15 minutes each in 12 selected stallions for further experimentation and 11 nonselected stallions: age (years), number of attempts (n), successful collection (yes or no, Y/N), and time until ejaculation (s).

Stallion	Age (y)	Session 1			Session 2		
		Attempts (n)	Success (Y/N)	Time (s)	Attempts (n)	Success (Y/N)	Time (s)
Selected							
1	15	5	Y	910	2	Y	320
2	9	2	Y	265	1	Y	90
3	6	2	Y	235	4	Y	600
4	3	1	Y	130	1	Y	90
5	3	2	Y	490	1	Y	180
6	11	1	Y	180	1	Y	90
7	5	2	Y	260	1	Y	145
8	3	3	Y	530	5	N	560
9	6	2	Y	560	2	Y	390
10	15	2	Y	310	1	Y	195
11	11	4	Y	720	2	Y	350
12	7	2	Y	305	1	Y	155
Mean ± SD	7.8 ± 4.4	2.3 ± 1.2		407.9 ± 236.6	1.8 ± 1.3		263.8 ± 179.8
Nonselected							
13	3	5	N	740	/	/	/
14	7	6	N	785	/	/	/
15	4	6	N	900	/	/	/
16	6	3	Y	520	6	N	890
17	8	6	N	690	/	/	/
18	8	5	N	700	/	/	/
19[a]	7	3	Y	740	1	Y	405
20	18	3	N	810	/	/	/
21	13	6	N	840	/	/	/
22	18	4	Y	1020	3	N	720
23	8	3	N	705	/	/	/
Mean ± SD	9.1 ± 5.1	4.5 ± 1.4		768.2 ± 128.8	3.3 ± 2.5		671.7 ± 246.1

Means are given with standard deviation (SD) for age, number of attempts, and time until ejaculation for both selected and nonselected in both sessions.
[a] Dangerous behavior toward semen collector.