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# Exposure to stallion accelerates the onset of mares' cyclicity

B. Wespi [a], H. Sieme [b], C. Wedekind [c], D. Burger [a,*]

[a] *Swiss Institute of Equine Medicine, Agroscope and University of Berne, Avenches, Switzerland*
[b] *Clinic for Horses - Unit for Reproductive Medicine, University of Veterinary Medicine, Hannover, Germany*
[c] *Department of Ecology and Evolution, Biophore, University of Lausanne, Lausanne, Switzerland*

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ABSTRACT

Horses (*Equus caballus*) belong to the group of seasonally polyestrous mammals. Estrous cycles typically start with increasing daylight length after winter, but mares can differ greatly in the timing of onset of regular estrus cycles. Here, we test whether spatial proximity to a stallion also plays a role. Twenty-two anestrous mares were either exposed to one of two stallions (without direct physical contact) or not exposed (controls) under experimental conditions during two consecutive springs (February to April). Ovarian activity was monitored via transrectal ultrasound and stallion's direct contact time with each mare was determined three times per week for one hour each. We found that mares exposed to a stallion ovulated earlier and more often during the observational period than mares that were not exposed to stallions. Neither stallion identity nor direct contact time, mare age, body condition, size of her largest follicle at the onset of the experiment, or parasite burden significantly affected the onset of cyclicity. In conclusion, the timing of estrous cycles and cycle frequency, i.e., crucial aspects of female reproductive strategy, strongly depend on how the mares perceive their social environment. Exposing mares to the proximity of a stallion can therefore be an alternative to, for example, light programs or elaborated hormonal therapies to start the breeding season earlier and increase the number of estrous cycles in horses.



## 1. Introduction

The objective of our study was to test whether spatial proximity to a stallion plays a role in the onset of mares' cyclicity after winter anestrus. Domestic horses (*Equus caballus*) can be classified as seasonally polyestrous, even if approximately one-third of mares within riding and racing breeds show little seasonality and can be cyclic throughout the whole year if kept under optimized conditions [1]. In less domesticated horse breeds, ovulatory estrous cycles typically occur between spring and autumn [1]. In general, onsets of winter anestrus and the subsequent spring transition are believed to primarily depend on day length [2] and can be further influenced by nutrition [3,4], body condition and metabolism [5], age [3,6], environmental temperature [7], and breed [8]. The biological start of the breeding season, defined as the first ovulation of the year, can be well synchronized among mares within a herd, whereas the timing of the autumnal transition is often highly variable [9,10].

For some breeds, especially in those competing in sport events at a relatively young age, there is a high interest and economic pressure to breed mares as early as possible in the year [11]. Earlier cyclicity is therefore often induced by shortening the winter anestrus or the transition period, usually by manipulating the photoperiod [12–16] or by using various schemes of administering GnRH or GnRH analogues [17–21], dopamine antagonists [22,23], recombinant prolactin [24,25], progesterone [26–30], estrogen [31], melatonin [6], clenbuterol and melatonin [32], and follicular aspiration [33].

* Corresponding author. Tel.: +41 26 676 63 00; fax: +41 26 676 63 04.
*E-mail address:* dominik.burger@vetsuisse.unibe.ch (D. Burger).

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Life-history theory predicts that females adjust their reproductive strategy not only to their own physiological conditions but also to how they perceive their environment, including their social situation [34,35]. Feral horses live in herds with constant interactions between stallions and mares throughout the year, also during anestrus [36]. Under domesticated conditions, however, mares and stallions are typically kept separated. Therefore, the aim of our study was to test whether contact with a stallion had an influence on the onset of seasonal cycle activity in mares, and if so, whether the strength of such an effect could be predicted by factors such as body condition, age, parasite infection, follicle size at the start of the experiments, or stallion contact time.

## 2. Material and methods

### 2.1. Animals, stables, and management

A total of 22 mares and two stallions were available for this 2-year study. Mares were on average 12-year old at the time of the experiments (range 7–19) and of various breeds (Warmblood, Franches-Montagnes, Thoroughbred, and Standardbred horses). They varied in their reproductive history and were maiden (n = 4), barren (n = 5), rested (n = 7) or foaling in the previous year, and rested (n = 6). The body condition score (BCS) [37] and total body weight of the mares before the study was 4 to 8 (mean = 6 ± 0.93, on a scale from 1 to 9) and 496 to 687 kg (mean = 582.1 ± 55.7 kg), respectively. One stallion was available in each year. Both were of the Franches-Montagnes breed, 8- and 18-year old (in 2011 and 2012, respectively), and both had breeding experience.

Two identical and specifically designed stables were used. These consisted of eight boxes of 12 $m^2$ separated from each other by a 147-cm-high wooden wall and a 200-cm-high metal grill above it, allowing visual, olfactory but limited body contact. The stable corridors were 290-cm wide and 12-m length with rubber flooring. The stallion occupied his own box at one end of one of the two stables. The analogous box in the control stable was kept empty. A camera (Digital Handycam DCR-TRV17E, Sony, Tokyo, Japan) for video monitoring was installed at the end of the corridor for a 1-hour monitoring period three times a week. The mares' genital tracts were examined by means of transrectal 7.5 MHz ultrasonography with a 60-mm linear array probe (Aquila Pro VET, Esaote, Genoa, Italy) three times a week.

### 2.2. Experimental design

The experiment lasted from the 16th of February until the 30th of April in both years. All mares except one had previously been kept at the stud in a group-keeping system without stallion contact since the beginning of December in the previous year. From December, the mares were regularly examined via ultrasound to ensure that they had not ovulated and that no follicle larger than 30 mm in diameter was present before the start of the experiment. In mid-February, the mares were assigned to two categories depending on maximal size of follicles (category 1: largest follicle in diameter ≤20 mm; category 2: largest follicle in diameter between 21 and 30 mm). Mares from both categories were then assigned to the two treatment groups and to the boxes within the stables ensuring that breed, age, reproductive history, BCS, and total body weight of the mares was on average equal in the different groups. Assignment to boxes was not changed during an experimental period.

The stallion was allowed to move freely within the stable corridor, the door to his box staying open except for 3 hours every morning when all horses followed their daily routine exercise program. In stable 2, mares were kept analogous to stable 1 but had no stallion contact over the whole duration of the study. Mares were turned out daily in groups for 3 hours in paddocks without stallion contact. For this, mares from stables 1 and 2 were mixed and box neighbors were not in the same group. The stallions were exercised daily in a horse walker. All boxes were bedded with straw, with the standardized feeding consisting of hay and cereals without additives. The natural and artificial lighting in the two stables was identical.

### 2.3. Monitoring of ovarian activity

All mares were examined three times per week between 1.30 and 5.00 PM, first clinically (temperature, heart rate, respiratory rate), then transrectally by ultrasonography to monitor ovarian activity. The number and diameter of all follicles larger than 1 cm in diameter were recorded and at each scanning the ovaries measured in two perpendicular dimensions. The presence of a CL was also noted. The degree of edema in the uterine wall was scored as 1 (homogeneous in appearance, not edematous), 2 (mildly heterogeneous, slightly edematous), or 3 ("wagon wheel" appearance, highly edematous) [38]. The date of an ovulation was defined as the date of detection of a new CL. In the event of a double ovulation, the day of the first ovulation was taken into account for the statistics. One mare from each year did not ovulate during the experimental period but shortly afterward when stabled in a group-keeping system (where mares were still regularly examined). Both mares belonged to the nonexposed control group, and the date of their first ovulation was included in the respective comparison.

### 2.4. Behavioral analysis

Three times weekly, i.e., every Monday, Wednesday, and Friday, after the daily routine exercise program of all horses in the study, the stallion was led into the stable and presented head to head to each mare for 15 seconds, allowing nostril contact, and then set free in the middle of the corridor. The behavior of the stallion and interactions between the stallion and the mares were then filmed for one hour and the stallion's contact time with each mare was determined. Contact time per mare (in seconds) was defined as the accumulated time over 1 hour that a stallion stood in front of a mare's box with his head pointing toward the mare. (The mare often reacted by nostril nuzzling through the metal grill or by presenting her hindquarters).