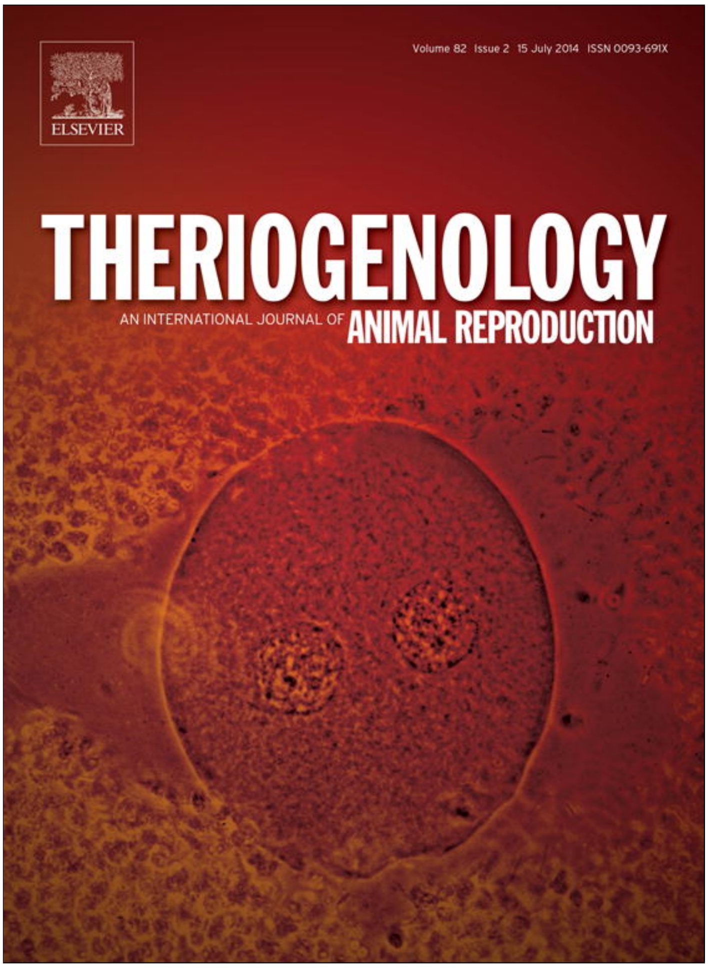
Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/authorsrights

Author's personal copy

Theriogenology 82 (2014) 189–194

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

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

A R T I C L E I N F O

Article history:
Received 30 October 2013
Received in revised form 18 March 2014
Accepted 20 March 2014

Keywords:
Horse
Estrous cycle
Cyclicity
Anestrus
Ovulation

A B S T R A C T

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.

© 2014 Elsevier Inc. All rights reserved.

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).

0093-691X/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2014.03.019

Author's personal copy

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. Materials 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).

Author's personal copy

2.5. Hormonal analysis

In the second year of the study, venous blood samples (Vacuette K3E K_3EDTA; Greiner bio-one, Kremsmünster, Austria) from all mares were collected three times per week, centrifuged for 15 minutes at 3000× *g* and derived plasma stored at −80 °C until further analysis. Analysis of progesterone was conducted in weekly samples until 4 weeks after the first ovulation with electrochemiluminescence immunoassay (Elecsys 2010; Roche Diagnostics, Basel, Switzerland). The detection limit of the assay was 0.03 ng/mL. Inter and intra-assay coefficients of variation for progesterone were 2.8% and 2.2%, respectively [39]. Serum or plasma progesterone values greater than 1 ng/mL indicate an endocrinologically active CL [40].

Three of the mares that had been used for the experiment in the first year and that were still available during the second year were also distributed to the two treatment groups in the second year. Their progesterone levels were included in the statistical analysis, whereas all other reproductive parameters in the second year were excluded from the statistics to avoid pseudoreplication.

2.6. Body condition score and parasitical analysis

The BCS and total body weight were determined for each animal before and on the second day of the study and then once per month. Mean values per mare were used for statistical analysis. Feces samples for parasite examination were collected rectally from each mare on the first day, then every 10 days, using palpation gloves. Samples were then refrigerated at 5 °C and analyzed within 4 days of collection. Fecal egg count was performed using a modified McMaster method with a detection limit of 50 eggs per gram of feces (EpG) [41].

2.7. Statistical analysis

Group comparisons, within-subject changes, and correlations were analyzed with ANOVAs, paired *t*-tests, and the Pearson correlation coefficient r, respectively. Permutation tests (5000 runs each) were added if graphical inspection of the data suggested violations of the model assumptions for ANOVAs, such as high prevalence of tied data or unequal variances. All P values are two tailed.

2.8. Ethical note

Ethical clearance for this work was granted by the committee for animal experiments of the canton Vaud (N° 2409.0, 2409.2). Daily experimental handling time of mares and stallions was minimized to avoid causing significant stress. No manipulations resulted in injuries. One mare, in 2011, developed chronic diarrhea toward the end of the experiment because of later histologically confirmed inflammatory bowel disease. This mare was included in statistical analysis as her general health condition during the experiment was good, and no other abnormalities were observed.

3. Results

3.1. Treatment effects

Mares exposed to a stallion ovulated earlier than mares with no stallion exposure (Fig. 1; $F_{1,20} = 5.67$; $P = 0.03$; permutation test: $P = 0.03$). Exposure to a stallion also led to more ovulation cycles during the observational period (Fig. 2; $F_{1,20} = 6.58$; $P = 0.02$; permutation test: $P = 0.03$). The experimental treatment had, however, no significant effect on the time interval between consecutive ovulations among mares with multiple estrus cycles (permutation tests, first interval: $P = 0.24$, second interval: $P = 0.27$). Both stallions had similar effects on the timing of first ovulation and the number of ovulations ($F_{1,20}$ always ≤ 0.3; $P \geq 0.59$).

3.2. Effects of follicle size at start of experiment

Mares assigned to category 1 (largest follicle ≤ 20 mm at the beginning of the project) ovulated for the first time 39.8 (CI: 27.2–52.4) and mares in category 2 (largest follicle 21–30 mm) 30.8 (CI: 13.4–48.2) days after the study began, respectively. There was no significant difference between mares of category 1 and 2 ($F_{1,20} = 0.92$; $P = 0.35$). The two categories of mares did also not significantly differ with respect to number of ovulatory cycles ($F_{1,20} = 1.1$; $P = 0.31$) or time between the first and second ovulation ($F_{1,17} = 2.0$; $P = 0.18$).

3.3. Progesterone, BCS, age, and parasites

The mean progesterone values before the first ovulation were 0.031 (CI: 0.028–0.034) ng/mL in the exposed group

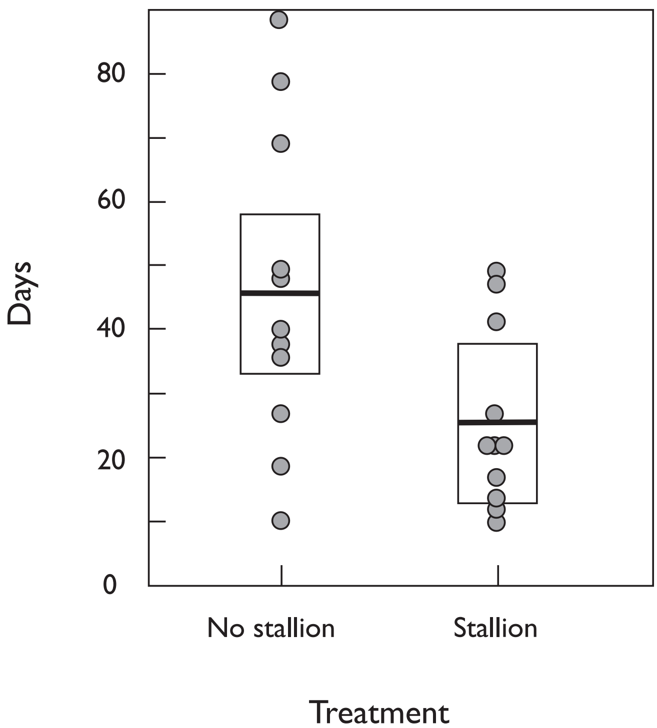


Fig. 1. Time to first ovulation after start of experiment in mares exposed or not exposed to a stallion. The boxes give the means and the 95% confidence intervals. See text for statistics.

Author's personal copy

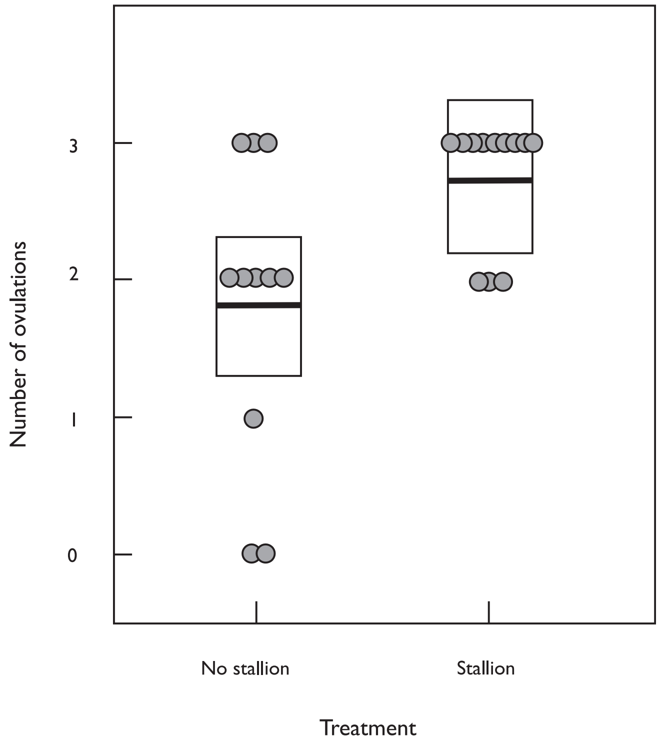


Fig. 2. Number of ovulations per mare of the two treatment groups. The boxes give the means and the 95% confidence intervals.

and 0.039 (CI: 0.026–0.052) ng/mL in the control group, respectively ($F_{1,9} = 2.2$; $P = 0.17$). After the first ovulation and during the rest of the experimental period, the mean progesterone levels were 8.95 (CI: 1.91–15.98) ng/mL in the exposed group, and 8.95 (CI: 3.76–14.13) ng/mL in the control group ($F_{1,8} < 0.1$; $P = 1.0$).

No significant effects of mare age, BCS (mean = 5.9; CI: 5.5–6.3), or parasite load (mean = 23.6 EpG; CI: 0.008–47.2) could be found on timing of the first ovulation or the number of ovulations within the experimental periods (r always ≤ 0.28; $n = 22$; P always > 0.20). Average direct contact time per mare was 14.4 s/h (CI: 5.8–23.0) and did not significantly increase during estrus (paired *t*-test, $t = -0.02$; $P = 0.99$). Average direct contact time neither correlated with the time of the first ovulation ($r = -0.14$; $P = 0.67$) nor was it a statistically significant predictor of the number of ovulations during the observational period ($F_{1,9} = 1.0$; $P = 0.33$).

4. Discussion

Management of mares in transition from winter anestrus to cyclic ovarian activity can be a challenge for breeders and veterinarians alike. Estrous behavior and ovulations are sometimes difficult to recognize, highly unpredictable, and anovulatory estruses are not uncommon. Light programs have been shown to be effective in shifting the beginning of the ovulatory season in mares [12,42], but they typically have to start running from the beginning of December in the Northern hemisphere. Hormonal therapies have also proven to be mostly unsatisfying, as they can be expensive and time consuming (e.g., daily or twice daily treatments) [18,22,43–45] while often not very effective [23,26,45].

We found that the proximity of a stallion significantly shortened the time to the first ovulation in mares. Those exposed to a stallion therefore ovulated more often during the observational period. This opens the possibility of new management methods for anestrous and transitional mares. Even if no stallions are available as a social stimulus, the possibility exists that male stimuli such as urinary or fecal odors may be sufficient to stimulate ovulation (see the following).

In feral herds, so-called harems usually consist of a stallion and one to –eight mares, which typically remains unchanged during the nonbreeding period [36,46]. Our stallions seemed to accept the experimental procedure well. They appeared excited at the beginning (e.g., trotting through the stable, sweating, frequent penile erection), but soon habituated to the situation showing behaviors comparable to those of harem stallions (e.g., patrolling through the stable to check mares cycle status and odors, frequent marking with excrement). Our experimental set-up may therefore be a closer representation of a natural situation than the commonly used separate housing system of mares and stallions, and the late onset of estrous cycles in mares without contact to stallions can be interpreted as a consequence of an unnatural social environment.

In sheep (*Ovis aries*) and goats (*Capra aegagrus*), exposure of seasonally anestrous females to sexually active males resulted in synchronized ovulation and activation of LH secretion [47]. O'Callaghan, et al. [48] showed that the onset of the breeding season was earlier in ewes maintained with rams, compared with ewes isolated from rams. The presence of rams not only decreased anestrous time but also lengthened the breeding season. Ungerfeld, et al. [49] showed that adult rams induced a greater response in ewes as compared with yearling rams. However, serum testosterone concentrations and behavior of the males were comparable between adult and yearling rams. The two stallions we used did not differ in the effects they had on mares. This could be either because, in horses, the induced onset of estrous cycles is independent of stallion characteristics, or because the two stallions we used were similar in key characteristics. Both were mature, healthy, and had many years of breeding experience, i.e., they were similar in factors that may play an important role.

Claus, et al. [50] suggested that, in goats, buck hairs contain pheromones that may stimulate the resumption of cyclic activity. Ungerfeld, et al. [49] showed that when ewes were stimulated with masks containing wool, a higher number of them showed estrous behavior and had more ovulations when put in contact with wool from an adult ram compared with wool from a yearling. These results in other seasonal polyestrous species suggest that male-specific odors may be important. Our study design does not permit differentiation if stallion presence *per se*, odor, or voice exhibited the most important stimulatory effects. It could be economically interesting to test if only a simulated presence would induce enough stimulation.

Few studies describe the potential stimulating effects of stallions on mares. Ginther [51] reported on the sexual behavior of mares after the introduction of a stallion into a group of mares and speculated that teasing may affect estrus cycles during the breeding season. He showed that

Author's personal copy

introducing a stallion into a group of cyclic mares resulted in good estrus detection in most of the mares (96% of mares with stallion in group vs. 60% at individual teasing), but stallion presence did not affect the time of ovulation or the length of the interovulatory interval in these mares. Pickerel, et al. [52] showed that mares during estrus exhibited preferences for certain stallions. In their study, the mare had to initiate contact in order to be taken into account. The number of stallion vocalizations per minute, interpreted as courtship behavior of the stallion, was found to have a positive effect on mare preference. It is therefore possible that vocalizations also influence aspects of female reproductive strategies such as the onset of estrous cycles. Janson and McDonnell [53] examined estrus detection in mares using stallion vocalization playback with or without stallion scent. In most of the cases, estrus was more often detected when the olfactory stimulus was added. Considering the hormonal status, Pantke [54] found the relative bioactivity of the plasma LH in one transitional mare to be increased by 2.5 times when she was exposed to a teasing stallion. This observation indicates that contact with a teasing male may induce short-term hormone releases from the pituitary gland.

We could not find any influence of the mare's body condition or age on the timing of the first ovulation of the year. However, our animals did not exhibit extreme values: all mares were middle aged, had a medium to good BCS, and received the same nutrition. Previous studies found age to have an impact only in very young or very old mares, and only extreme body conditions to influence the duration of the anestrous phase [3,6,55]. Low body condition tends to prolong the anovulatory phase: the average interval to the first ovulation was found to be significantly longer in mares with BCS less than5 compared with mares having BCS greater than5 [56]. In our study, all mares met the latter condition. In addition, they could also be classified as "well-fed" after Salazar-Ortiz, et al. [5] who determined the impact of food restriction and found that winter ovarian inactivity is defined by metabolic hormones.

In female mice, parasitic infections have been shown to influence female sexual behavior, estrous cycles, and estradiol levels [57]. Parasite control is important to ensure general health of the domesticated horse population and deworming is recommended at a level above 100 EpG [58]. Mean EpG levels in our horses were all less than 100 EpG and showed no significant influence on estrous cycles.

In conclusion, the onset of the estrous cycle in horses is a plastic trait that depends on how the mares perceive their social environment. Exposing mares to a mature stallion during the transitional phase between the anestrous and estrous period increases the mares' fecundity and hence the number of possible breeding attempts during a reproductive season. This method offers an alternative to light and hormone treatments. However, it remains to be tested whether stallions can be replaced by sound or odor stimuli to further extend management options.

Acknowledgments

This work was supported by *ISMEquine Research*. We thank S. Thomas and the vet team of the Swiss Institute of Equine Medicine for assistance in the field, G. Cosentino and F. Flahaut (Dr Risch, Liebefeld, Switzerland) for hormonal analysis, and V. Carcangiu and an anonymous for comments on the manuscript.

Competing interests

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

References

[1] Aurich C. Reproductive cycles of horses. Anim Reprod Sci 2011;124:220–8.
[2] Hart PJ, Squires EL, Imel KJ, Nett TM. Seasonal-variation in hypothalamic content of gonadotropin-releasing hormone (GnRH), pituitary receptors for GnRH, and pituitary content of luteinizing hormone and follicle-stimulating hormone in the mare. Biol Reprod 1984;30:1055–62.
[3] Carnevale EM, Hermenet MJ, Ginther OJ. Age and pasture effects on vernal transition in mares. Theriogenology 1997;47:1009–18.
[4] Kubiak JR, Crawford BH, Squires EL, Wrigley RH, Ward GM. The influence of energy intake and percentage of body fat on the reproductive performance of nonpregnant mares. Theriogenology 1987;28:587–98.
[5] Salazar-Ortiz J, Camous S, Briant C, Lardic L, Chesneau D, Guillaume D. Effects of nutritional cues on the duration of the winter anovulatory phase and on associated hormone levels in adult female Welsh pony horses (Equus caballus). Reprod Biol Endocrinol 2011;9:130.
[6] Fitzgerald BP, McManus CJ. Photoperiodic versus metabolic signals as determinants of seasonal anestrus in the mare. Biol Reprod 2000;63:335–40.
[7] Guerin MV, Wang XJ. Environmental temperature has an influence on timing of the first ovulation of seasonal estrus in the mare. Theriogenology 1994;42:1053–60.
[8] Sharp DC. Vernal transition into the breeding season. In: McKinnon AO, Squires EL, Vaala WE, Varner DD, editors. Equine reproduction. Second edition. West Sussex: Wiley-Blackwell; 2011. p. 1704–15.
[9] Ginther OJ. Reproductive biology of the mare: basic and applied aspects. Second edition. Wisconsin: Equiservices; 1992.
[10] Sharp DC, Kooistra L, Ginther OJ. Effects of artificial light on oestrous cycle of mare. J Reprod Fertil Suppl 1975;23:241–6.
[11] Nagy P, Guillaume D, Daels P. Seasonality in mares. Anim Reprod Sci 2000;60-61:245–62.
[12] Burkhardt J. Transition from anoestrus in the mare and the effects of artificial lighting. J Agr Sci 1947;37:64–8.
[13] Kooistra LH, Ginther OJ. Effect of photoperiod on reproductive activity and hair in mares. Am J Vet Res 1975;36:1413–9.
[14] Oxender WD, Noden PA, Hafs HD. Estrus, ovulation, and serum progesterone, estradiol, and LH concentrations in mares after an increased photoperiod during winter. Am J Vet Res 1977;38:203–7.
[15] Palmer E, Driancourt M, Ortavant R. Photoperiodic stimulation of the mare during winter anoestrus. J Reprod Fertil Suppl 1982;32:275.
[16] Sharp DC. Environmental influences on reproduction in horses. Vet Clin North Am Large Anim Pract 1980;2:207–23.
[17] Allen WR, Sanderson MW, Greenwood RE, Ellis DR, Crowhurst JS, Simpson DJ, et al. Induction of ovulation in anoestrous mares with a slow-release implant of a GnRH analogue. J Reprod Fertil Suppl 1987;35:469–78.
[18] Harrison LA, Squires EL, Nett TM, McKinnon AO. Use of gonadotropin-releasing hormone for hastening ovulation in transitional mares. J Anim Sci 1990;68:690–9.
[19] Hyland JH, Wright PJ, Clarke IJ, Carson RS, Langsford DA, Jeffcott LB. Infusion of gonadotrophin-releasing hormone (GnRH) induces ovulation and fertile oestrus in mares during seasonal anoestrus. J Reprod Fertil Suppl 1987;35:211–20.
[20] McKinnon AO, Vasey JR, Lescun TB, Trigg TE. Repeated use of a GnRH analogue deslorelin (Ovuplant) for hastening ovulation in the transitional mare. Equine Vet J 1996;29:153–5.
[21] Turner JE, Irvine CHG. The effect of various gonadotrophin-releasing hormone regimens on gonadotrophins, follicular growth and

Author's personal copy

ovulation in deeply anestrous mares. J Reprod Fertil Suppl 1991;44:213–25.
[22] Besognet B, Hansen BS, Daels PF. Dopaminergic regulation of gonadotrophin secretion in seasonally anoestrous mares. J Reprod Fertil 1996;108:55–61.
[23] Donadeu FX, Thompson DL. Administration of sulpiride to anovulatory mares in winter: effects on prolactin and gonadotropin concentrations, ovarian activity, ovulation and hair shedding. Theriogenology 2002;57:963–76.
[24] Nequin LG, King SS, Johnson AL, Gow GM, Ferreira-Dias GM. Prolactin may play a role in stimulating the equine ovary during the spring reproductive transition. J Equine Vet Sci 1993;13:631–5.
[25] Thompson DL, Hoffman R, Depew CL. Prolactin administration to seasonally anestrous mares: reproductive, metabolic, and hair-shedding responses. J Anim Sci 1997;75:1092–9.
[26] Alexander SL, Irvine CHG. Control of onset of breeding season in the mare and its artificial regulation by progesterone treatment. J Reprod Fertil Suppl 1991;44:307–18.
[27] Evans MJ, Irvine CHG. Induction of follicular development and ovulation in seasonally acyclic mares using gonadotrophin-releasing hormones and progesterone. J Reprod Fertil Suppl 1979;27:113–21.
[28] Hanlon D, Firth E. The reproductive performance of Thoroughbred mares treated with intravaginal progesterone at the start of the breeding season. Theriogenology 2012;77:952–8.
[29] Squires EL, Heesemann CP, Webel SK, Shideler RK, Voss JL. Relationship of altrenogest to ovarian activity, hormone concentrations and fertility of mares. J Anim Sci 1983;56:901–10.
[30] Staempfli SA, Clavier S, Thompson DL, Burns PJ, Lyle SK, McKinnon AO. Effect of a single injection of long-acting progesterone on the first ovulation in early and late spring transitional mares. J Equine Vet Sci 2011;31:744–8.
[31] Mumford EL, Squires EL, Jasko DJ, Nett TM. Use of gonadotropin-releasing hormone, estrogen, or a combination to increase releasable pituitary luteinizing hormone in early transitional mares. J Anim Sci 1994;72:174–7.
[32] McManus CJ, Fitzgerald BP. Effect of daily clenbuterol and exogenous melatonin treatment on body fat, serum leptin and the expression of seasonal anestrus in the mare. Anim Reprod Sci 2003;76:217–30.
[33] Alvarenga MA, McCue PM, Franz LC. Effect of follicular aspiration on ovarian function in transitional mares. Theriogenology 1999;51:431.
[34] Piersma T, van Gils JA. The flexible phenotype: a body-centred integration of ecology, physiology, and behaviour. Oxford: Oxford University Press; 2011.
[35] Stearns SC. The evolution of life histories. Oxford: Oxford University Press; 1992.
[36] McDonnell SM. Reproductive behavior of stallions and mares: comparison of free-running and domestic in-hand breeding. Anim Reprod Sci 2000;60:211–9.
[37] Henneke DR, Potter GD, Kreider JL, Yeates BF. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet J 1983;15:371–2.
[38] Ginther OJ. Ultrasonic imaging and reproductive events in the mare. Wisconsin: Equiservices; 1986.
[39] Imboden I, Janett F, Burger D, Crowe M, Haessig M, Thun R. Influence of immunization against GnRH on reproductive cyclicity and estrous behavior in the mare. Theriogenology 2006;66:1866–75.
[40] Terblanche H, Maree L. Plasma progesterone levels in the mare during the oestrous cycle and pregnancy. J S Afr Vet Assoc 1981;52:181–5.
[41] Mines JJ. Modifications of the McMaster worm egg counting method. Aust Vet J 1977;53:342–3.
[42] Guillaume D, Duchamp G, Nagy P, Palmer E. Determination of minimum light treatment required for photostimulation of winter anoestrous mares. J Reprod Fertil Suppl 2000;56:205–16.
[43] Niswender KD, McCue PM, Squires EL. Effect of purified equine follicle-stimulating hormone on follicular development and ovulation in transitional mares. J Equine Vet Sci 2004;24:37–9.
[44] Panzani D, Zicchino I, Taras A, Marmorini P, Crisci A, Rota A, et al. Clinical use of dopamine antagonist sulpiride to advance first ovulation in transitional mares. Theriogenology 2011;75:138–43.
[45] Schauer S, Briant C, Ottogalli M, Decourt C, Handel I, Donadeu FX. Supplementation of equine early spring transitional follicles with luteinizing hormone stimulates follicle growth but does not restore steroidogenic activity. Theriogenology 2011;75:1076–84.
[46] Klingel H. Social organization of feral horses. J Reprod Fertil Suppl 1982;32:89–95.
[47] Gelez H, Fabre-Nys C. The “male effect” in sheep and goats: a review of the respective roles of the two olfactory systems. Horm Behav 2004;46:257–71.
[48] O’Callaghan D, Donovan A, Sunderland SJ, Boland MP, Roche JF. Effect of the presence of male and female flockmates on reproductive activity in ewes. J Reprod Fertil 1994;100:497–503.
[49] Ungerfeld R, Ramos M, Gonzalez-Pensado S. Ram effect: adult rams induce a greater reproductive response in anestrous ewes than yearling rams. Anim Reprod Sci 2008;103:271–7.
[50] Claus R, Over R, Dehnhard M. Effect of male odor on LH secretion and the induction of ovulation in seasonally anestrous goats. Anim Reprod Sci 1990;22:27–38.
[51] Ginther OJ. Sexual behavior following introduction of a stallion into a group of mares. Theriogenology 1983;19:877–86.
[52] Pickerel TM, Crowell-Davis SL, Caudle AB, Estep DQ. Sexual preference of mares (Equus caballus) for individual stallions. Appl Anim Behav Sci 1993;38:1–13.
[53] Janson KM, McDonnell SM. Estrus detection in mares using contextually congruent stallion vocalization playback with and without stallion scent. Anim Reprod Sci 2010;121:123–5.
[54] Pantke P. Characterization of short-term patterns of LH and FSH secretion in the pituitary venous effluent of the mare. University of Hannover; 1990. Dissertation.
[55] Gentry LR, Thompson DL, Gentry GT, Davis KA, Godke RA, Cartmill JA. The relationship between body condition, leptin, and reproductive and hormonal characteristics of mares during the seasonal anovulatory period. J Anim Sci 2002;80:2695–703.
[56] Henneke DR, Potter GD, Kreider JL. Body condition during pregnancy and lactation and reproductive efficiency of mares. Theriogenology 1984;21:897–909.
[57] Arteaga-Silva M, Vargas-Villavicencio JA, Vigueras-Villasenor RM, Rodriguez-Dorantes M, Morales-Montor J. Taenia crassiceps infection disrupts estrous cycle and reproductive behavior in BALB/c female mice. Acta Trop 2009;109:141–5.
[58] Lyons ET, Ionita M, Tolliver SC. Important gastrointestinal parasites. In: McKinnon AO, Squires EL, Vaala WE, Varner DD, editors. Equine reproduction. Second edition. West Sussex: Wiley-Blackwell; 2011. p. 292–301.