

The heat is on: what's new for suppression of estrus in mares

D. K. Vanderwall

Department of Clinical Studies, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA, USA

Abstract

Therapeutic suppression of estrus in mares is routinely performed when exhibition of estrous behavior is deemed undesirable or it interferes with intended uses such as recreational and/or performance activities. Various modalities are available for suppression of estrus in mares, each with its own advantages, disadvantages and efficacy (or lack of). This paper will review currently available methods of suppressing estrous behavior in mares, which includes administration of exogenous progesterone/progestins, extending the duration of corpora luteal function, suppressing ovarian follicular activity and ovariectomy. Particular emphasis will be placed on recent studies supporting or refuting the effectiveness of these methods of blocking estrus, so the clinician can identify the most suitable and efficacious method of suppressing estrus for horse owners and their animals.

Keywords: Equine, mare, estrus, estrous behavior, therapeutics

Introduction

A relatively common complaint of horse owners and trainers is variable performance in mares related to the estrous cycle.¹ Similarly, approximately 90% of over 750 veterinarians

responding to a survey had the clinical impression the estrous cycle impacted the performance of mares, and the most frequently reported clinical sign associated with an effect of the reproductive cycle on performance was attitude change, while other signs included tail swishing, difficulty to train, squealing, “horsing”, excess urination, kicking, and a decrease in performance.² In some cases undesirable behavior has been associated with estrus and in other cases with diestrus.³ It is important to note that some problematic behaviors displayed by mares that are thought to be associated with estrus, are in fact not estrous behaviors; in particular, submissive behavior may be most easily confused with estrous behavior. Submissive behavior includes leaning away from perceived threats, swishing/ringing the tail and actively squirting urine, which collectively can give the impression of estrus.⁴ In contrast to submissive behavior, true estrous behavior includes leaning towards the stallion (or other stimulus), a relaxed lifting motion of the tail, stationary/squatting stance and passive urination (full stream or small amounts in spurts).⁴ In some mares the signs of true estrous behavior are so strong as to directly impair performance; for example, even under saddle some mares may “break down” and show estrus in response to being around other horses and/or other stimuli.⁴ In other mares, the condition may be much more subtle, simply causing owners and trainers to report the mare is less cooperative or attentive during estrus.¹

In an effort to evaluate the potential for an effect of stage of estrous cycle on the behavior of mares, Hedberg et al.⁵ conducted two behavior tests (novel object and isolation) on 12 mares once when they were in estrus and once during diestrus in a cross-over design. Five of the mares served as controls, while the other seven mares were classified as “problem” mares based on their owner’s perception of estrus-related behavioral problems. There were no significant

differences in the behavioral responses between estrus and diestrus within the control and “problem” groups, nor were there differences in the behavioral responses between the two groups of horses; however, as the authors note, the sample size was small and a cross-over study design may not have been the most appropriate experimental design. Therefore, further work in this area is warranted.

Ideally, a complaint of an estrous cycle-related behavior/performance problem in a mare should be systematically evaluated in order to determine if the problematic behavior is or is not related to a specific phase of the cycle (i.e., estrus or diestrus). A team approach to evaluating and addressing the problem involving behavioral and reproductive expertise may be beneficial.⁶ In situations where there is evidence that the problematic behavior is associated with estrus, suppression of estrus may be warranted. It is also common to suppress estrus in situations where the signs of estrous behavior are simply perceived to be associated with performance problems or to preemptively block the behavior to preclude the possibility of an adverse effect of estrus on performance. This paper will review currently available methods of suppressing estrous behavior in mares, with particular emphasis on recent data that support or refute various methods of blocking estrus. The following methods of suppressing estrous behavior will be discussed: 1) administration of exogenous progesterone/progestins, 2) extending the duration of corpora luteal (CL) function, 3) suppressing ovarian follicular activity and 4) ovariectomy.

Administration of exogenous progesterone/progestins

Progesterone

It was first demonstrated in the 1960's that daily intramuscular administration of 100 mg progesterone in oil (0.2 mg/kg) effectively suppressed signs of estrus in mares.⁷ Intramuscular

administration of 100 mg progesterone in oil to ovariectomized mares produced peak systemic blood levels of approximately 2 ng/mL, which then declined to 1 ng/mL or less 24 hours later.⁸ Progesterone in oil is available from several compounding pharmacies; however, the need for daily administration and the potential for soreness at the site of injection are limitations to its use. It has also been demonstrated that intramuscular administration of a compounded long-acting formulation of progesterone containing a total dose of 1.5 g progesterone will maintain blood levels of progesterone above 1.0 ng/mL for approximately 10 days,⁹ which is a sufficient level of progesterone to block estrous behavior;^{7,8} however, the potential for soreness at the injection site is a limitation to its use, particularly in performance horses.

Altrenogest

Altrenogest (Regu-Mate®, Intervet/Schering-Plough Animal Health, Millsboro, DE, USA) is a synthetic progestin approved for use in horses for suppressing estrus, and is widely considered to be the “gold-standard” method of inhibiting estrous behavior. Daily oral administration of altrenogest at a dose of 0.044 mg/kg (1 mL per 110 lbs of body weight) is very efficacious for suppressing estrus in mares;^{10,11} however, the need for daily administration is a drawback to its use. It was recently reported that intramuscular administration of a compounded preparation containing 225 mg or 450 mg of altrenogest in a sustained-release vehicle blocked estrous behavior for approximately 12 and 15 days, respectively, while administration of 500 mg altrenogest in lactide-glycolide microparticles suppressed estrous behavior for approximately 30 days.¹²

Medroxyprogesterone acetate

Although there are anecdotal reports on the use of the synthetic progestin medroxyprogesterone acetate (MPA) for estrus suppression in mares, it was recently reported that intramuscular administration of an initial dose of 1,600 mg MPA followed by 400 mg once weekly for five weeks did not suppress estrous behavior in mares.¹³ Similarly, administration of 1,000 mg MPA in an aqueous suspension did not prolong the return to estrus compared to control mares.¹² On a related note, MPA was unable to maintain pregnancy in mares following induced luteolysis when 1,000 mg was administered intramuscularly every seven days.¹⁴ Therefore, the use of MPA for suppression of estrus cannot be advocated.

Hydroxyprogesterone caproate

Although it has not been tested for its efficacy for suppressing estrous behavior in mares, McKinnon et al. demonstrated that intramuscular administration of 500 mg hydroxyprogesterone caproate every other day was unable to maintain pregnancy in ovariectomized mares.¹⁵ Similarly, the same synthetic progestin, repackaged as hydroxyprogesterone hexanoate, though labeled for pregnancy maintenance was unable to maintain pregnancy in mares when 500 mg was administered intramuscularly every four days following induced luteolysis.¹⁴ Therefore, the use of hydroxyprogesterone caproate for suppression of estrus cannot be advocated.

Melengestrol acetate

Melengestrol acetate (MGA), a synthetic progestin, is labeled as a feed additive for estrus suppression in cattle. However, when fed to mares at 10 or 20 mg/day for up to 15 days, it failed to block estrus.⁷ It has been suggested higher doses (>100 mg/day) may be effective for suppression of estrus in mares,¹⁶ which may be plausible, since it was recently reported that oral administration of 100 mg or 150 mg MGA to mares during the spring transitional phase

significantly hastened the onset of ovulatory activity compared to control mares indicating apparent biological activity of higher doses of MGA.¹⁷ Therefore, further work to evaluate the effect of higher doses of MGA on estrous behavior is warranted.

Implants

A variety of implants containing various progestins, labeled and marketed for use in other species, have been used in mares with the intent of suppressing estrus. The most widely used implant has been Synovex S® (Fort Dodge Animal Health, Ft. Dodge, IA, USA), which contains 200 mg progesterone and 20 mg estradiol benzoate in each dose of eight pellets. McCue et al. were unable to suppress estrus or cyclicity in mares that received 80 Synovex S® pellets.¹⁸ Similarly, although not critically tested for its ability to suppress estrous behavior, Scheffrahn et al. reported that following subcutaneous placement of a implant containing 6.0 mg of the synthetic progestin norgestomet in six mares, two of the mares displayed estrous behavior two days before the implants were removed 10 days after placement.¹⁹ On a related note, placement of five subcutaneous implants containing a total of 15 mg norgestomet was unable to maintain pregnancy in mares following induced luteolysis.¹⁴ Therefore, at this time, there is no evidence that implants containing progesterone/progestins have efficacy for suppression of estrus in mares, so their use cannot be advocated.

Extending CL function

Intra-uterine glass marble

One alternative to the use of exogenous progesterone/progestins for estrus suppression is intrauterine insertion of a glass ball to extend CL function, which allows continued secretion of endogenous progesterone to block estrus. Nie et al. reported that placement of a 25 or 35 mm

sterile glass ball into the uterine body immediately following ovulation resulted in prolonged CL function in seven of 18 (39%) mares that retained the glass ball after insertion (six of 12 mares expelled the glass marble).²⁰ In mares that developed prolonged CL function following placement of the glass ball, CL function was maintained for approximately 90 days, during which time progesterone levels remained above 1.0 ng/mL and estrous behavior was not exhibited. In non-treated control mares, spontaneous prolongation of CL function occurred in four of 32 (13%) mares. Although placement of a glass ball appeared to be an efficacious means of blocking estrous behavior for an extended period of time, it should be noted that in addition to the 11 mares that retained the glass ball and never developed extended CL function (i.e., continued to cycle normally), three of the seven glass ball treated mares with extended CL function had one or two estrous cycles of normal duration after placement of the glass ball before CL function was prolonged. Therefore, on a “per-cycle” basis the incidence of prolonged CL function was only 11% (7/62 cycles) in the glass ball treated mares compared to 8% (4/50 cycles) in the non-treated control mares, which was not significantly different between groups. Because of its variable efficacy among mares, and the need to physically remove the glass ball when the resumption of cyclical reproductive activity is desired, placement of an intrauterine glass ball does not appear to be an optimal method of suppressing estrous behavior in mares.

In a more recent study, Rivera del Alamo et al. examined the effect of intrauterine placement of a 20 mm water-filled polypropylene ball on the duration of CL function in mares with the specific objective of investigating two potential mechanisms by which CL function is extended: 1) the intrauterine device induces mild endometrial inflammation that completely blocks or markedly attenuates prostaglandin (PG) F₂ α secretion (i.e., prevents high magnitude

luteolytic pulses) or 2) the physical presence of the device (movement and/or contact with the endometrium) directly mimics the inhibitory effect of a conceptus on PGF2 α secretion.²¹ Corpora luteal function was extended in nine of 12 mares (75%) with an average duration of 57 days compared to zero of 12 control mares in which the average duration of CL function was 16 days. In six of the nine mares with extended CL function, small accumulations of intrauterine fluid (≤ 10 mm x 20 mm) were identified during the luteal phase, but no neutrophils or bacteria were recovered on uterine swabs when they were examined during the subsequent estrus. In addition, changes in uterine biopsy scores for inflammation and glandular dilation pre- and post-treatment were similar for control and uterine device mares (with or without extended CL function); therefore, there was no evidence the intrauterine device induced an inflammatory response in the uterus. Based on intensive blood sampling and measurement of PGF2 α metabolite (PGFM) levels in the systemic circulation on days 11 to 16 post-ovulation in four control and eight uterine device mares, PGF2 α secretion was attenuated in mares with prolonged CL function, with the exception of two mares; one mare showed a single PGFM peak and another showed two isolated PGFM peaks. Because there was no evidence of inflammatory changes caused by the intrauterine device, the authors concluded the physical presence of the device in the uterine lumen somehow mimicked the effect of a conceptus by impairing endometrial secretion of PGF2 α ; however, the exact mechanism remains unknown.

Administration of exogenous oxytocin

In contrast to using an intrauterine device to extend CL function, administration of exogenous oxytocin during diestrus is an alternative method of blocking luteolysis to prolong CL function. Endogenous oxytocin secretion is involved in regulating prostaglandin PGF2 α

secretion from the endometrium during spontaneous luteolysis in the mare,^{22,23} and although administration of exogenous oxytocin to mares around the time of luteolysis (i.e., days 11 to 15 post-ovulation) stimulates an acute onset of PGF2 α secretion,²⁴⁻²⁶ when oxytocin is administered in the mid-luteal phase prior to the expected time of luteolysis (i.e., before day 10 post-ovulation) it does not induce PGF2 α secretion and often disrupts luteolysis causing prolonged CL function.²⁵

Experimentally, continuous infusion of oxytocin using a subcutaneous osmotic minipump from day eight to 20 post-ovulation blocked luteolysis in four of five mares, whereas luteolysis occurred at the expected time in all four control mares that received saline infusion.²⁷ Although it successfully induced prolonged CL function, continuous infusion of oxytocin to disrupt luteolysis would not be a practical method of long-term suppression of estrous behavior. More recently, Vanderwall et al. showed that twice daily intramuscular administration of 60 units of oxytocin on days seven to 14 post-ovulation was an efficacious method of disrupting luteolysis, since it caused prolonged CL function through day 30 post-ovulation in six treated mares (Figure 1), whereas six saline-treated control mares underwent luteolysis by day 16 post-ovulation (Figure 2).²⁸ Progesterone levels fell below 1.0 ng/mL between days 30 and 40 in two of the mares with prolonged CL function, while the other four mares maintained progesterone levels above 3.0 ng/mL through day 40 when blood sampling was discontinued. The cessation of CL function before day 40 in two mares may have reflected a seasonal effect on CL function, since the study was completed at the end of the physiological breeding season when gonadotropin secretion wanes, and CL function (i.e., progesterone secretion) is dependent upon adequate support from endogenous gonadotropin secretion.²⁹⁻³¹ A follow-up study (Vanderwall et al.,

unpublished) was then performed to compare use of the same dose of oxytocin (60 units) given twice daily compared to once daily on days seven to 14 post-ovulation; CL function was maintained for 50 days post-ovulation in five of seven mares treated twice daily, five of eight mares treated once daily and in one of seven untreated control mares. There was no difference ($P>0.05$) in the proportion of mares with extended CL function between once daily and twice daily administration of oxytocin, whereas collectively oxytocin treatment increased ($P<0.05$) the proportion of mares with extended CL function. An advantage of using exogenous oxytocin treatment to prolong CL function is that it can be readily reversed by the administration of a luteolytic dose of PGF2 α , in contrast to the need to physically remove an intrauterine device as described above.

Inducing late-diestrus ovulation

In 2006, Hedberg et al. described the results of a preliminary study in which their objective was to prolong the luteal phase in mares by using human chorionic gonadotropin (hCG) to induce a late-diestrus ovulation to produce a new CL that would be too immature to respond to the luteolytic effects of endogenous PGF2 α secretion at the end of diestrus (i.e., day 14 to 15 after the initial ovulation).³² Mares were randomly assigned to control (n=4) and experimental groups (n=5), and beginning on approximately day eight after ovulation (or last signs of estrous in three mares) their ovaries were examined with transrectal ultrasonography every other day to determine the size(s) of their diestrus follicles. When a diestrus follicle ≥ 30 mm was detected, control mares were treated with saline and the experimental mares were treated with 3,000 IU hCG IM. After treatment the mares were followed with transrectal ultrasonography for up to 72 hours or until ovulation was detected, and then once weekly for

three weeks. Beginning on the day of treatment, blood samples were collected twice weekly for at least one month and then once weekly for another two to four months for progesterone determination. If a mare did not develop a diestrus follicle ≥ 30 mm during the first diestrus period, they were monitored for a second, and if necessary a third diestrus period.

Three of the nine mares developed a follicle ≥ 30 mm during the first diestrus period, four mares during the second diestrus period and one mare in the third diestrus period. One experimental mare never developed a diestrus follicle that was ≥ 30 mm during the three diestrus periods that were monitored, and therefore, could not be treated with hCG. Overall, three out of the four (75%) experimental mares treated with hCG ovulated within 72 hours after treatment with hCG, which resulted in luteal phases that lasted for 58 to 82 days after treatment. None of the control mares ovulated during the luteal phase; however, one control mare had a spontaneously prolonged luteal phase during both a non-treated cycle in which she never developed a diestrus follicle ≥ 30 mm (CL function was terminated with exogenous PGF 2α) and during the subsequent cycle in which she was treated with saline when she had a large diestrus follicle (that did not ovulate).

Although, based on this study, the use of hCG to induce a late-diestrus ovulation looks promising for prolonging CL function, it is important to note that as described above, for some mares (five out of nine) it required multiple estrous cycles to develop a diestrus follicle ≥ 30 mm. In addition, one mare never developed a large diestrus follicle during the three cycles that were monitored, which precluded her from receiving the hCG treatment. Therefore, in addition to the effort (and expense) of monitoring mares in order to evaluate their suitability for treatment, the fact that some mares may not develop a large enough diestrus follicle to warrant treatment, the

use of hCG to induce a late-diestrus ovulation does not appear to be a reliable, “on-demand” method of blocking estrous behavior in mares. It is interesting to note that although the use of hCG was apparently efficacious for inducing ovulation of diestrus follicles ≥ 30 mm in diameter in this study by Hedberg et al.,³² previous work by Glazar et al.³³ demonstrated that administration of the GnRH agonist deslorelin acetate failed to induce ovulation and/or luteinization of diestrus follicles > 30 mm in diameter.

Pregnancy

Pregnancy is another means of suspending cyclicity by taking advantage of the natural ability of the conceptus to block luteolysis and maintain CL function/progesterone secretion. Although efficacious, this method has obvious disadvantages that may make it undesirable for many horse owners. In addition to the time and expense necessary to establish pregnancy, is the need to eventually terminate pregnancy (unless an offspring is ultimately desired). Lefranc and Allen reported that manual transrectal rupture of the conceptus between days 16 and 22 of gestation in 11 mares resulted in continued CL function for at least 60 days in all of the mares, during which time they did not display estrous behavior.³⁴ Although efficacious, as noted above, terminating a normal, healthy pregnancy may be untenable to many horse owners.

Suppressing ovarian follicular activity

When considering the use of suppression of ovarian follicular activity as a method of blocking estrous behavior in mares, it is important to recognize that mares are unique among domestic animals, because in addition to the ovarian-derived estrogen-induced signs of estrous behavior that occur when progesterone is at a basal level, many seasonally anovulatory (and ovariectomized) mares exhibit paradoxical estrous behavior associated with hormone secretion

from the adrenal cortex.^{35,36} The intensity of this type of “unseasonable” estrous behavior was judged to be equivalent to the behavior intact cycling mares display during the initial and terminal days of estrus, but less intense than the behavior displayed near ovulation.³⁵ Such behavioral receptivity to a stallion outside the breeding/ovulatory season that is independent of ovarian estrogen secretion may have developed as a means of maintaining social bonds between a harem stallion and his mares.^{35,37} This phenomenon has important implications for the clinical management of estrous behavior in mares, since simply suppressing ovarian follicular activity (or removing the ovaries) and its attendant estrogen production may not ensure the elimination of estrous behavior.

Down-regulation of the hypothalamic-pituitary-ovarian axis with gonadotropin releasing hormone (GnRH) analogs

Although a subcutaneous implant containing the potent GnRH analog deslorelin acetate (Ovuplant®, Fort Dodge Animal Health) was initially developed and found to be efficacious for inducing timed ovulation for the breeding management of mares,^{38,39} it soon became evident the implant caused prolonged anovulatory intervals in some mares.⁴⁰⁻⁴² Subsequent work demonstrated the deslorelin implant caused reduced circulating FSH concentrations and absence of the mid-cycle FSH peak that was associated with a prolonged inter-ovulatory interval.⁴³ Although problematic for the breeding management of mares, the down-regulating effect of the deslorelin implant on pituitary function has been used clinically to suppress ovarian activity; for example, placement of two deslorelin implants suppressed follicular development in mares used as recipients for oocyte transfer.⁴⁴ Fitzgerald et al. reported suppressing ovulation for 30-90 days in 15 of 20 mares that were treated with a subcutaneous implant containing another GnRH

analog (goserelin acetate).⁴⁵ Collectively, these data indicate that treating mares with a potent GnRH analog may be efficacious for suspending cyclicity and suppressing estrus in mares for extended periods of time. However, as noted previously, since anovulatory mares can show estrous behavior, suppressing follicular activity may not ensure a complete absence of estrous behavior.

Immunologic

Immunizing a mare against GnRH would eliminate the stimulus for gonadotropin release from the pituitary resulting in suspension of cyclicity. Tshewang et al. reported successfully suspending ovarian activity and suppressing estrus for 25-30 weeks in mares after treating with a GnRH vaccine.⁴⁶ All of the vaccinated mares recovered from the effect of immunization with normal cyclicity and estrous behavior. Over the next two seasons, each mare also conceived and produced a normal foal. Subsequent studies have confirmed the efficacy of vaccination against GnRH for suppressing ovarian follicular activity; however, not surprisingly, some of the vaccinated mares continued to show estrous behavior in spite of their anovulatory state. It is important to note, that in one study⁴⁷ one vaccinated mare had not resumed normal ovarian activity two years after initial vaccination; therefore, although most mares appear to regain ovarian activity within a reasonable period of time post vaccination, some mares may not, which could be particularly problematic. Although a GnRH vaccine is not currently available in North America, commercial preparations are available in Europe and Australia (Equity®; Pfizer Australia Pty Ltd, West Ryde, NSW, Australia).

Ovariectomy

Bilateral ovariectomy may be warranted in some circumstances for permanently eliminating ovarian activity in mares. Although the ovaries can be removed through a ventral abdominal or flank laparotomy, laparoscopy or a colpotomy, at this time the latter two methods are most commonly utilized.^{48,49} In an initial report, Hooper et al. described the outcome of the use of bilateral ovariectomy for treatment of objectionable behavior during estrus in 17 mares; they found that after surgery owners reported that behavior was no longer a problem in 14 mares (82%), while the remaining three mares continued to show estrous behavior.⁴⁸ Of the 23 mares in that study that underwent bilateral ovariectomy for any reason, eight (35%) continued to show estrous behavior after surgery. In a recent report, Kamm and Hendrickson evaluated clients' perspectives on the outcome following the use of laparoscopic ovariectomy for behavioral and medical problems in mares.⁴⁹ Overall, client satisfaction (rated as very satisfied or satisfied) with bilateral ovariectomy as a treatment for behavioral problems was 78% (18 of 23 of mares). Assessment of outcomes for specific behavioral problems showed that aggression problems improved in 86% of cases; general disagreeable demeanor improved in 81%; excitability improved in 75%; kicking and biting at other horses improved in 73%; problems in training improved in 72%; frequent urination improved in 64%; and problems with other horses improved in 64% of cases. The most common source of dissatisfaction for owners of patients with behavioral problems was lack of behavioral change after surgery, including continued signs of estrous behavior. Although ovariectomy offers the advantage of being a potentially permanent solution to a cycle-related behavior/performance problem, there are significant disadvantages as well. The procedure is relatively expensive, though when compared to repeated hormonal treatments, the cost may, in fact, be comparable. There are also risks associated with

the surgical procedure, though newer laparoscopic procedures have significantly reduced them.^{48,49} The permanency of the procedure can also be a significant disadvantage because all possibility for future reproduction is eliminated. And as noted above, because of the potential for paradoxical estrous behavior, removing the ovaries may not ensure cessation of estrous behavior. Therefore, this option should be weighed carefully before proceeding. One way of evaluating the potential effectiveness of ovariectomy for a behavioral problem is to evaluate the mare's behavior during either a natural (i.e., seasonal) or induced anovulatory state (e.g., down-regulation with a GnRH agonist); if the problem is not resolved or at least significantly improved during the anovulatory state, it is unlikely ovariectomy will be any more efficacious.

Summary

The primary indication for suppressing estrous behavior in mares is cycle-related behavior/performance problems during estrus. When evaluating an owner/trainer complaint of an estrous cycle-related problem in a mare, it should first be determined if the problematic behavior is or is not related to a specific phase of the estrous cycle. In order to thoroughly evaluate the mare, additional expertise may be needed in the form of consultation with or referrals to behavior and/or reproduction experts. Once a behavior/performance problem is confidently defined as being related to estrus, the previously discussed methods (with proven efficacy) of suppressing estrous behavior can be considered for use. Each method has advantages and potential disadvantages, that should be weighed for each individual animal/owner/trainer.

References

1. McDonnell S: Estrus cycle-related performance problems. *J Equine Vet Sci* 1997;17:196.
2. Jorgensen JS, Vivrette S, Correa M, et al: Significance of the estrous cycle on athletic performance in mares. *Proc Annu Conv Am Assoc Equine Practnr* 1996;42:98-100.
3. McDonnell SM: Evaluation and modification of mare behavior problems. *Proc Annu Mtg Soc Therio* 1993; p. 185-189.
4. McDonnell S: Performance problems in mares. *The Horse*; April 2000:61-70.
5. Hedberg Y, Dalin AM, Ohagen P, et al: Effect of oestrous-cycle stage on the response of mares in a novel object test and isolation test. *Reprod Domest Anim* 2005;40:480-488.
6. McDonnell SM: Is it psychological, physical, or both? *Proc Annu Conv Am Assoc Equine Pract* 2005;51:231-238.
7. Loy RG, Swan SM: Effects of exogenous progestogens on reproductive phenomena in mares. *J Anim Sci* 1966;25:821-826.
8. Hawkins DL, Neely DP, Stabenfeldt GH: Plasma progesterone concentrations derived from the administration of exogenous progesterone to ovariectomized mares. *J Reprod Fertil* 1979 Suppl 27;211-216.
9. Vanderwall DK, Marquardt JL, Woods GL: Use of a compounded long-acting progesterone formulation for equine pregnancy maintenance. *J Equine Vet Sci* 2007;27:62-66.
10. Squires EL, Stevens WB, McGlothlin DE, et al: Effect of an oral progestin on the estrous cycle and fertility of mares. *J Anim Sci* 1979;49:729-735.

11. Webel SK, Squires EL: Control of the oestrous cycle in mares with altrenogest. *J Reprod Fertil* 1982 Suppl 32;193-198.
12. Storer WA, Thompson DL, Jr., Gilley RM, et al: Evaluation of injectable sustained release progestin formulations for suppression of estrus and ovulation in mares. *J Equine Vet Sci* 2009;29:33-36.
13. Gee EK, McCue PM, DeLuca CA, et al: Efficacy of medroxyprogesterone acetate in suppression of estrous behavior and follicular activity in cycling mares. *Theriogenology* 2008;70:588 (abstract).
14. McKinnon AO, Lescun TB, Walker JH, et al: The inability of some synthetic progestagens to maintain pregnancy in the mare. *Equine Vet J* 2000;32:83-85.
15. McKinnon AO, Tarrida Del Marmol Figueroa S, Nobelius AM, et al: Failure of hydroxyprogesterone caproate to maintain pregnancy in ovariectomized mares. *Equine Vet J* 1993;25:158-160.
16. Neely DP: Progesterone/progestin therapy in the broodmare. *Proc Annu Conv Am Assoc Equine Pract* 1988;34:203-218.
17. Lopez-Bayghen C, Zozaya H, Ocampo L, et al: Melengestrol acetate as a tool for inducing early ovulation in transitional mares. *Acta Vet Hung* 2008;56:125-131.
18. McCue PM, Lemons SS, Squires EL, et al: Efficacy of Synovex-S implants in suppression of estrus in the mare. *J Equine Vet Sci* 1997;17:327-329.
19. Scheffrahn NS, Wiseman BS, Vincent DL, et al: Reproductive hormone secretions in pony mares subsequent to ovulation control during late winter. *Theriogenology* 1982;17:571-585.

20. Nie GJ, Johnson KE, Braden TD, et al: Use of an intra-uterine glass ball protocol to extend luteal function in mares. *J Equine Vet Sci* 2003;23:266-273.
21. Rivera Del Alamo MM, Reilas T, Kindahl H, et al: Mechanisms behind intrauterine device-induced luteal persistence in mares. *Anim Reprod Sci* 2008;107:94-106.
22. Vanderwall DK, Silvia WJ, Fitzgerald BP: Concentrations of oxytocin in the intercavernous sinus of mares during luteolysis: temporal relationship with concentrations of 13,14-dihydro-15-keto-prostaglandin F_{2a}. *J Reprod Fertil* 1998;112:337-346.
23. Shand N, Irvine CHG, Turner JE, et al.: A detailed study of hormonal profiles in mares at luteolysis. *J Reprod Fertil* 2000 Suppl 56;271-279.
24. Betteridge KJ, Renard A, Goff AK: Uterine prostaglandin release relative to embryo collection, transfer procedures and maintenance of the corpus luteum. *Equine Vet J* 1985 Suppl 3;25-33.
25. Goff AK, Pontbriand D, Sirois J: Oxytocin stimulation of plasma 15-keto-13,14- dihydro prostaglandin F-2a during the oestrous cycle and early pregnancy in the mare. *J Reprod Fertil* 1987 Suppl 35;253-260.
26. Starbuck GR, Stout TA, Lamming GE, et al: Endometrial oxytocin receptor and uterine prostaglandin secretion in mares during the oestrous cycle and early pregnancy. *J Reprod Fertil* 1998;113:173-179.
27. Stout TAE, Lamming GE, Allen W.: Oxytocin administration prolongs luteal function in cyclic mares. *J Reprod Fertil* 1999;116:315-320.

- [8. Vanderwall DK, Rasmussen DM, Woods GL: Effect of repeated administration of oxytocin during diestrus on duration of function of corpora lutea in mares. *J Am Vet Med Assoc* 2007;231:1864-1867.
29. Pineda MH, Ginther OJ, McShan WH: Regression of corpus luteum in mares treated with an antiserum against an equine pituitary fraction. *Am J Vet Res* 1972;33:1767-1773.
30. Pineda MH, Garcia MC, Ginther OJ: Effect of antiserum against an equine pituitary fraction on corpus luteum and follicles in mares during diestrus. *Am J Vet Res* 1973;34:181-183.
31. Bergfelt DR, Ginther OJ: Embryo loss following GnRH-induced ovulation in anovulatory mares. *Theriogenology* 1992;38:33-43.
32. Hedberg Y, Dalin AM, Santesson M, et al: A preliminary study on the induction of dioestrous ovulation in the mare--a possible method for inducing prolonged luteal phase. *Acta Vet Scand* 2006;48:12.
33. Glazar BS, McCue PM, Bruemmer JE, et al: Deslorelin on Day 8 or 12 postovulation does not luteinize follicles during an artificially maintained diestrous phase in the mare. *Theriogenology* 2004;62:57-64.
34. Lefranc AC, Allen WR: Nonpharmacological suppression of oestrus in the mare. *Equine Vet J* 2004;36:183-185.
35. Asa CS, Goldfoot DA, Garcia MC, et al: Sexual behavior in ovariectomized and seasonally anovulatory pony mares (*Equus caballus*). *Horm Behav* 1980;14:46-54.
36. Asa CS, Goldfoot DA, Carcia MC, et al: Dexamethasone suppression of sexual behavior in the ovariectomized mare. *Horm Behav* 1980;14:55-64.

37. Crowell-Davis SL: Sexual behavior of mares. *Horm Behav* 2007;52:12-17.
38. Meinert C, Silva JFS, Kroetz I, et al: Advancing the time of ovulation in the mare with a short-term implant releasing the GnRH analogue deslorelin. *Equine Vet J* 1993;25:65-68.
39. McKinnon AO, Nobelius AM, Tarrida Del Marmol Figueroa S, et al: Predictable ovulation in mares treated with an implant of the GnRH analogue deslorelin. *Equine Vet J* 1993;25:321-323.
40. Johnson CA, Thompson DL, Jr., Kulinski KM, et al: Prolonged interovulatory interval and hormonal changes in mares following the use of OvuplantTM to hasten ovulation. *J Equine Vet Sci* 2000;20:331-336.
41. Morehead JA, Blanchard TL. Clinical experience with deslorelin (OvuplantTM) in a Kentucky Thoroughbred broodmare practice (1999). *J Equine Vet Sci* 2000;20:358-402.
42. Vanderwall DK, Juergens TD, Woods GL. Reproductive performance of commercial broodmares after induction of ovulation with HCG or OvuplantTM (Deslorelin). *J Equine Vet Sci* 2001;21:539-542.
43. Farquhar VJ, McCue PM, Nett TM, et al: Effect of deslorelin acetate on gonadotropin secretion and ovarian follicle development in cycling mares. *J Am Vet Med Assoc* 2001;218:749-752.
44. Carnevale EM, Checure CH, Coutinho da Silva MA, et al: Use of deslorelin acetate to suppress follicular activity in mares used as recipients for oocyte transfer. *Theriogenology* 2001;55:358 (abstract).
45. Fitzgerald BP, Peterson KD, Silvia PJ. Effect of constant administration of a gonadotropin-releasing hormone agonist on reproductive activity in mares: preliminary

- evidence on suppression of ovulation during the breeding season. *Am J Vet Res* 1993;54:1746-1751.
46. Tshewang U, Dowsett KF, Knott LM, et al: Preliminary study of ovarian activity in fillies treated with a GnRH vaccine. *Aust Vet J* 1997;75:663-667.
 47. Imboden I, Janett F, Burger D, et al: Influence of immunization against GnRH on reproductive cyclicity and estrous behavior in the mare. *Theriogenology* 2006;66:1866-1875.
 48. Hooper RN, Taylor TS, Varner DD, et al: Effects of bilateral ovariectomy via colpotomy in mares: 23 cases (1984-1990). *J Am Vet Med Assoc* 1993;203:1043-1046.
 49. Kamm JL, Hendrickson DA. Client's perspectives on the effects of laparoscopic ovariectomy on equine behavioral and medical problems. *J Equine Vet Sci* 2007;27:435-438.

Figure 1. Serum progesterone concentrations from the day of ovulation (day 0) through day 40 after ovulation in six mares treated with 60 units oxytocin intramuscularly twice daily on days seven to 14 after ovulation (reprinted with permission from JAVMA 2007;231:1864-1867).

Figure 2. Serum progesterone concentrations from the day of ovulation (day 0) through day 40 after ovulation in six mares treated with 3 mL sterile saline intramuscularly twice daily on days seven to 14 after ovulation (reprinted with permission from JAVMA 2007;231:1864-1867).