Review Report

Hormonal manipulation of the estrous cycle in mares

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Abstract

Hormonal manipulation of the estrous cycle is a mainstay of clinical equine reproduction practice. Purposes of hormonal manipulation include advancement of the breeding season, induction of estrus from anestrus or vernal transition, ovulation timing, estrus synchronization, and estrus suppression. This review summarizes methods and supporting research of hormonal manipulation of the estrous cycle utilized in mares in clinical equine reproduction practice.

Keywords: Mare, cyclicity, ovulation induction, synchronization, estrus suppression

Introduction

Mares are seasonally polyestrous with transitional periods occurring at the beginning and end of the period of normal cyclicity. Generally, the period of behavioral estrus can last up to a week and diestrus lasts ~ 14-17 days after ovulation. The high monetary value associated with equids and related activities has driven humans to investigate mechanisms for manipulating the normal cycle. For instance, racing industry birth date standards have prompted practitioners to find ways to induce earlier cyclicity in mares and the desire to continue training and competing with valuable mares has led to investigations into mechanisms for suppressing estrus and synchronizing ovulation for embryo transfer. This review focuses on the various hormonal manipulations studied, aiming to demonstrate that although reliable methods for achieving desired results exist, they are not without variability and dependence on multiple factors for success.

Induction of cyclicity

Photoperiod Manipulation

Although most mares experience a fall transition into winter anestrus, a proportion of mares continue to exhibit estrous cycles and ovulate throughout the year.[1,2] The transition to winter anestrus and subsequent onset of the spring transitional period are primarily dependent on photoperiod or day length. In mammals, the eye is the primary receptor of light for determination of photoperiod. Establishment of photoperiod does not occur through use of rod or cone receptors, but rather through photosensitive opsin proteins within retinal ganglion cells, which receive light signals and project these to the ventrolateral part of the suprachiasmatic nucleus (retinohypothalamic tract).[3] Changes in photoperiod affect circadian 'clock' genes within the suprachiasmatic nucleus that serve to maintain the homeostatic state appropriate for the current photoperiod.[4] Signals are relayed from the suprachiasmatic nucleus to the pineal gland, where neural signaling is then translated to endocrine signaling in the form of increased or decreased melatonin secretion in response to the photoperiod information received initially by the eyes.[5] Melatonin secretion increases with decreased day length and decreases with increased day length. These changes in melatonin concentrations affect reproductive cyclicity through gonadotropin-releasing hormone (GnRH) within the hypothalamus. Hypothalamic content of GnRH tended to be higher in the summer than during the winter in the northern hemisphere; mares treated with melatonin implants during the summer had lower content of GnRH than untreated mares.[6] These results are supportive of a relationship between GnRH and melatonin concentrations in the onset of the transitional period. GnRH secretion stimulates gonadotropins secretion from the anterior pituitary and is the first step in a cascade that results in follicular growth and eventually, ovulation.

Based on our understanding of the impact of photoperiod on the onset of cyclicity in the mare, photoperiod manipulation emerged as a popular method of attempting to initiate earlier cyclicity in mares, often in response to industry-imposed, standard birthdates. As the transition period takes weeks to months, the goal of introducing artificial lighting is not to shorten the transition period but

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rather to hasten its onset and completion, resulting in resumption of cyclicity. Generally, lighting programs are instituted in the late fall (around December 1 in the northern hemisphere). There was no beneficial effect of starting lighting programs on November 1 versus December 1; however, there was an advantage of starting December 1 versus January 1.[7] In general, a 100-200 W lightbulb in a 3.5 x 3.5 m box stall should provide sufficient supplementary light;[8] however, an array of lighting systems exists such as paddock lighting and light masks.[9] Low-level blue light (peak wavelength of 468 nm), directed at a single eye via a light mask inhibited melatonin release in Thoroughbred mares.[10] Several lighting schedules have been employed with success, though the classic model is ~ 14.5-16 hours of consistent light exposure, followed by 8-9.5 hours of darkness.[11] Adding only an additional 2.5 hours of light at sunset was successful at advancing onset of cyclicity and adding another few hours of light near sunrise did not have an additional significant beneficial effect.[12] Exposure of anestrous mares to a 1-hour pulse of artificial light 18.5 hours after dawn was beneficial.[11] This method was proposed to work by interrupting the normal nighttime secretion of melatonin, modulating the inhibitory effect of melatonin on GnRH.[13] Despite differences in timing, both mechanisms attempt to alter the equine circadian rhythm such that it resembles that of the longer days of the traditional breeding season.

Gonadotropin releasing hormone and agonists

GnRH is released from 2 regions within the female hypothalamus in differing patterns. The tonic center (ventromedial and arcuate nuclei) releases GnRH in a low amplitude, episodic manner, whereas the surge center (preoptic and suprachiasmatic nuclei and anterior hypothalamic area) releases GnRH with higher frequency and amplitude, resulting in a luteinizing hormone (LH) surge. Low, episodic secretion of GnRH appears to favor follicle stimulating hormone (FSH) secretion, whereas high amplitude pulses of GnRH favor LH secretion.[14] Initially during transition, LH secretion is limited due to low pituitary stores, and the amount of LH within the pituitary increases with increasing daylength.[15] Increases in GnRH during transition stimulate FSH release that results in follicular waves of steroidogenically incompetent follicles that will not ovulate but may produce enough estrogen to cause erratic estrous behavior.[16] Eventually these follicles gain the ability to produce enough estrogen to provide positive feedback on LH secretion, resulting in an LH surge and ovulation.

Studies attempting to use GnRH to advance ovulation and seasonal cyclicity have aimed to determine the route and frequency that provide the earliest onset of estrus. One of the first studies of the use of GnRH to induce ovulation in transitional mares used native GnRH twice daily during the transitional period at 10 day intervals for 3-5 injections, in combination with exogenous progesterone to simulate the luteal phase. The protocol was intended to simulate the normal hormonal secretion of cyclic mares and all 5 mares ovulated in response to treatment.[17] However, it must be considered with such a long treatment period (30-50 days) in transitional mares, whether the transitional period was completed before the end of the study and mares had naturally regained normal cyclicity.

Hourly infusions of native GnRH to mares that had been kept under short-day length resulted in ovulation.[18,19] Similarly, deep anestrus mares treated with a continuous infusion of 0, 50, or 100 ng GnRH/kg/hour for 28 days via osmotic minipumps showed dose-dependent effects on ovulation rates, with the highest percentage of mares (70%) ovulating in the 100 ng GnRH/kg/hour treatment group. Mares in shallow anestrus that were treated with the 100 ng GnRH infusion ovulated at 18.6 days versus 41.9 days in untreated controls.[20]

Pulsatile treatment of native GnRH was more effective in inducing LH peak and ovulation. Thrice daily injections stimulated follicular growth; however, injection frequency needed to be increased to hourly to stimulate ovulation. Continuous release implants, in comparison, reduced labor and provided acceptable, increased ovulation rates compared to untreated control mares.[21] Anestrous pony and Thoroughbred mares were implanted with either 1 or 2 slow-release polymer implants impregnated with either 0.9 or 1.8 mg of the GnRH agonist, ICI 118 630/goserelin.[22] Implants delivered either 30 or 60 µg GnRH analogue, respectively, for 28 days; 76% of pony mares and 88% of Thoroughbred mares ovulated within 3-18 days after treatment. Seasonally anestrous mares (n = 45) were assigned to 3 groups: 1. control; 2. 40 µg of the intramuscular GnRH analogue, buserelin, twice daily for 28 days or until ovulation; and 3. subcutaneous implants that released 100 µg buserelin per day for 28 days.[23] None of the controls, 7 of 15 mares receiving 40 µg intramuscular buserelin, and 9 of 15 mares with subcutaneous implants ovulated within 30 days. Implants were more effective in inducing ovulation when implanted during late transition versus earlier in the year,[24] possibly due to larger follicles.[25] Although, there was an increase in follicle development after fitting anestrous mares with subcutaneous implants containing varying dosages of the GnRH analogue, goserelin, the largest follicular diameter achieved appeared to be more dependent on the largest follicular diameter that was present prior to treatment.[25] Authors also observed only a 30-35% increase in ovulation rate for the groups implanted with either a 3.6 or 5.4 mg GnRH implant. Ovulation rate was dependent on dosage, as lower-dose implants had significantly lower ovulation rates.[25]

As alluded to above, the effects of GnRH treatment on cyclicity and fertility appear to be dependent on the ovarian status and time of year during which treatment is initiated. Ovulation was induced in anestrous mares with twice-daily injections of a GnRH analogue starting either on December 23 or January 15.[26] Percentage of mares ovulating increased when treatment was started on January 15 versus December 23 and the percentage of mares ovulating also increased with the size of the largest follicle at the initiation of treatment, as observed earlier (100% ovulation rate when follicular diameter was > 25 mm at the initiation of treatment).[25] Ovulation was also induced in mares with inactive ovaries (follicles < 15 mm diameter) with 200 µg of a GnRH analog given twice daily; however, circulating progesterone and LH concentrations were lower and early embryo loss rates were much higher in treated mares versus controls. Though the authors induced ovulation and obtained fertilization, the previous anestrous state of the mares seemed to suppress luteal activity such that pregnancies were lost prior to development of the endometrial cups.[27]

Two schemes (increasing and constant dose of deslorelin) of GnRH treatment to anestrous and transitional mares were studied.[28] Both schemes increased ovulation rates over control, untreated mares, with no differences in ovulation rates between treatment groups. Systemic LH concentrations were similar between groups for the first 12 days and higher than controls, but after day 12, the constant-dosage group had

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higher LH concentrations than the increasing-dosage group, potentially due to decreasing pituitary sensitivity to GnRH with increasing GnRH dosage.[28]

Overall, the depth of anestrus and the GnRH preparation may explain differential responses to treatment with GnRH agonists for the purpose of inducing cyclicity[29] and mares induced to cycle out of deep anestrus may even return to anestrus after their first ovulation.[18,24-26] The corpus luteum (CL) that forms after GnRH treatment in anestrous or transitional mares is generally identical to that formed during the normal breeding season.[18,19,22,25] However, there are examples of failure to form a CL or failure of a CL to function correctly following GnRH treatment.[17,20,21,28]

Equine pituitary extract and gonadotropins

Stepping down the hypothalamic-pituitary-gonadal axis, the use of pituitary extracts (native and recombinant gonadotropins) aims to directly stimulate follicular growth and ovulation at the gonadal level. Gonadotropins are released from the anterior pituitary in response to gonadotropin releasing hormone (GnRH) and act directly on the gonads. The 2 main gonadotropins in the horse are FSH and LH, and these may be purified from pituitary extracts into different ratios. However, depending on the availability of equine pituitary tissue from slaughterhouses, pituitary extract and native gonadotropins may not be available in certain areas of the world. Thus, recombinant FSH, both equine and human preparations, would be useful where purification of natural gonadotropins is either not possible, too labor intensive, or cost prohibitive.[30,31]

Equine pituitary fractions were used to induce ovulation in seasonally anovulatory pony mares with follicles < 10 mm on each ovary. In this study, 87% of mares treated with 2 weeks of twice daily injections of pituitary extract ovulated by the end of the treatment period. Of the mares that ovulated, 58% had multiple ovulations per estrus.[32] Results were better in anestrous mares (follicles < 10 mm) treated with equine pituitary extract twice daily for 14 days; treated mares but none of the saline-treated controls ovulated.[33] Purified pituitary extract from equine pituitary glands obtained from slaughterhouses was used[34]; extract was injected into seasonally anovulatory mares when at least 1 follicle reached 25 mm. The extract was able to induce ovulation in 95% of mares and 64% of ovulations were multiple, as observed earlier.[32] As is the case with other agents discussed thus far (e.g. GnRH) and despite the apparent success of earlier studies, success of induction of ovulation was dependent on follicular size, as the pituitary extract used was less likely to induce ovulation when follicles were < 25 mm.[34] Deep anestrous (follicles < 15 mm) and transitional (at least 1 follicle > 25 mm) mares were treated with equine pituitary extract for a maximum of 15 days[35]; 22% of mares in deep anestrus ovulated within the treatment period in response to human chorionic gonadotropin (hCG) given once a follicle reached 35 mm. In comparison, 90% of mares in transition treated with equine pituitary extract ovulated within the treatment period in response to hCG once a follicle reached 35 mm. The mean interval from start of treatment to ovulation was 11.8 days.[35]

Growing follicles were supplemented with equine LH (eLH) from pituitary extracts during early transition to stimulate development of steroidogenically active dominant follicles with the ability to respond to an ovulatory stimulus.[36] Mares received intravenous eLH until a follicle reached 32 mm; they then received 3,000 IU of intravenous hCG to stimulate ovulation. Although eLH did stimulate follicle growth, it failed to generate steroidogenically active follicles responsive to hCG; eLH did not hasten the onset of the ovulatory season because the treated mares that did ovulate returned to an anovulatory transitional state.[36]

Equine FSH (eFSH) was given to transitional mares; Group 1 (n = 10) served as untreated controls, and Group 2 (n = 10) were given 12.5 mg of purified intramuscular eFSH (Bioniche Animal Health Athens, GA, USA) twice daily for a maximum of 15 consecutive days. Once 1 or more follicles > 35 mm were detected, eFSH treatment was discontinued and intravenous hCG was given. In 8 of 10 mares treated with eFSH, follicles developed, and ovulation occurred during the 15-day observation period, compared to 0 of 10 control mares. Interval from onset of treatment to ovulation was 7.6 ± 2.4 days for these 8 mares that were treated for an average of 5.2 ± 1.3 days with eFSH. Thus, the eFSH treatment was effective in advancing the first ovulation of the year in transitional mares.[37] However, decreased dosage and frequency of eFSH treatment produced lower ovulation rates than twice daily 12.5 mg eFSH treatment.[38]

Similar success was achieved in treating donor mares for embryo transfer during the transitional period with eFSH.[39] Mares were housed under ambient conditions and had transrectal ultrasonography performed starting on January 30. Once a follicle reached 25 mm, twice daily eFSH treatment was initiated until a follicle reached 35 mm. At that point, eFSH treatment was discontinued and hCG was given after 36 hours. Mares were then inseminated every 48 hours until ovulation occurred; mean interval from initiation of treatment to ovulation was 7.9 days. Treated mares were noted to have more preovulatory follicles and more ovulations than mares in untreated cycles; however, embryo recovery rates were similar and embryos from the treated mares were noted to have more morphologic abnormalities. Additionally, approximately half of the treated mares were noted to have a prolonged inter-ovulatory interval between the first (eFSH treated) and second (untreated) cycles of the season.[39] Twice daily eFSH were compared to twice daily injections of 63 µg deslorelin acetate for hastening first ovulation of the year in transitional mares.[40] Interval from onset of treatment to first ovulation was similar; however, the numbers of ovulations and embryos recovered were higher in the eFSH-treated mares than in the deslorelin-treated mares. Additionally, no differences in embryo quality were noted between treatment groups. More recently, mares in deep anestrus under natural photoperiod were treated with recombinant FSH (reFSH) or a combination of reFSH/reLH and compared to mares maintained under artificial photoperiod. Despite lacking the effects of photoperiod manipulation, mares given reFSH and reFSH/reLH developed preovulatory follicles significantly faster than control mares and all treated mares ovulated within 10 days after beginning treatment. Importantly, treated mares that conceived and had pregnancies terminated with prostaglandin at 25 days continued to cycle normally for the season after pregnancy termination.[41] However, reFSH is not currently commercially available. Its arrival to market is much anticipated as it appears to be a viable option for induction of cyclicity, even without photoperiod manipulation.

Progestin supplementation

For induction of cyclicity, natural progesterone or synthetic progestins are used to suppress estrus for a period, allowing synchronization of estrus when the progestin is withdrawn.

Altrenogest given by mouth or mixed in feed at a dose of 0.044 mg/kg may be used for this purpose. Additionally, both short- and long-acting preparations of injectable progesterone exist. Short-acting intramuscular progesterone is given at a dose of 150 mg per day, whereas long-acting progesterone is given at a dose of 1.5 g every 7-10 days.[42] Follicular activity is not fully suppressed with the use of progesterone or synthetic progestins alone. For example, although LH secretion is generally reduced with the use of altrenogest, ovulation sometimes still occurred.[43,44]

For best results using progestins to induce cyclicity, mares must be in mid to late transition with follicles of at least 20-25 mm in each ovary.[45-47] Typically, mares are treated for 12-14 days and then the exogenous progestin is withdrawn. On the contrary, other studies refute this finding with results that neither progesterone in oil nor altrenogest advanced first ovulation in mares, regardless of depth of anestrus/transition and follicular size during treatment.[15,48] Progestin therapy, specifically with altrenogest, was effective at inducing estrus and first ovulation when combined with photoperiod manipulation.[45] Researchers exposed mares to a 16-hour daily photoperiod for 2 months prior to daily altrenogest treatment for 12 days. More progestin-treated mares had signs of estrus and ovulated within 12 days after treatment than control mares.

Other forms of progesterone that have been studied include estrogen/progesterone combinations and long-acting progesterone formulations. In anovulatory mares, long-acting progesterone formulations designed to elevate progesterone for 10-12 days were successful in elevating progesterone for 10 days. Additionally, noncycling mares that had not responded to photoperiod manipulation were treated with the long-acting progesterone formulation, LA300 (BET Pharmacy, Lexington, Kentucky, USA), and 57% of these mares ovulated within 4 weeks. This was in comparison to a control group of mares in which only 7% of mares ovulated.[49] Long-acting progesterone was utilized to program mares and advance ovulation; however, it was only significant in late transitional mares (follicles 20-25 mm).[50] Treatment had no effect on the early transition mares but resulted in ovulation in 10-24 days in 10 of 12 mares treated in late transition versus only 3 of 12 control mares during the same period.[50] The combination of injectable progesterone and estrogen provides more effective follicular activity suppression than when using a progestin alone; whereas both methods may be useful for inducing cyclicity in late-transition mares, interval to estrus and ovulation was longer for combined progesterone/estrogen therapy than for altrenogest alone.[51] The additional follicular activity suppression in the progesterone/estrogen combination stems from estrogen's suppressive effects on FSH secretion, as FSH drives follicular growth and development. Such a progesterone/estrogen protocol was used; 150 mg intramuscular progesterone and 10 mg estradiol-17β were given daily for 15 days, with 10 mg of $PGF_{2\alpha}$ given on the last day of steroid treatment. In 2 trials within this study, mares ovulated 8-14 and 9-16 days after finishing treatment with conception rates of 77 and 62%, respectively. Authors concluded that this combination provided satisfactory control of ovulation in mares early in the breeding season, with no adverse effects on fertility.[52]

Other sources of exogenous progesterone include intravaginal devices such as controlled internal drug release (CIDR), used extensively to synchronize ruminant reproductive cycles. Mares were inserted with CIDR-B device (1.9 g of progesterone) for 12 days; when a follicle reached 35 mm, the device was removed and a deslorelin implant was placed.[53] Follicular diameter at the end of progesterone treatment was larger in mares treated with CIDR compared to untreated control mares, with 77% of treated mares having follicles > 35 mm compared to 8% of untreated controls. In treated mares, 80% with follicles > 35 mm ovulated within 24-48 hours in response to implantation of GnRH device.[53] Similar, positive results have been demonstrated in multiple studies using both transitional mares and truly anestrous mares.[54-56] However, vaginitis of varying degrees was noted in mares and thus may make this method of induction of cyclicity less desirable, despite the ease of use.[54,55,57]

Transvaginal aspiration of follicles

In a similar vein to the ovarian 'reset' provided by progesterone and estrogen combination therapies, transvaginal aspiration (TVA) may be used to remove all follicles, such that a synchronized follicular wave emerges following aspiration. In transitional mares, hCG was given when 1 follicle reached 30 mm and TVA (follicles > 10 mm) was applied to 8 mares (treatment group). Mares (control and treatment) received prostaglandin on day 7 after hCG; 3 of 8 (38%) mares that had TVA formed luteal tissue with systemic progesterone concentrations > 1 ng/ml. These mares continued to cycle normally; however, only 1 mare (1/6; 16%) in the control group ovulated in response to hCG and this mare did not continue to cycle after prostaglandin treatment. Although the ovulation rate obtained in this experiment was not very high, treated mares that ovulated formed functional corpora lutea and continued to cycle normally after prostaglandin treatment.[58]

In transitional mares, follicles > 8 mm were aspirated at 3-4 days intervals for 4 total aspirations. These mares ovulated, on average, 9.8 days after the last transitional stage aspiration. However, there were no control mares for comparison in this study nor were any deep anestrous mares included.[59] Similarly, luteal tissue formed in 9 of 11 mares 8 days after TVA was performed;[60] however, researchers in this study were only aspirating the dominant follicle in late transitional mares. The remaining 2 mares in the treatment group in this study formed luteal tissue following a second aspiration procedure. Again, deep anestrous mares were not studied.

Overall, TVA appears to be an effective means of inducing ovulation in late transitional mares. However, there are inherent risks to TVA. Development of adhesions, internal hemorrhage, and ovarian abscesses have infrequently been reported,[61,62] and transient rectal bleeding in 16% of TVA sessions was also reported.[63] The equipment, skill, and risks associated with the procedure, when the success rates of other induction methods used in transitional mares are considered, may make this option less desirable.

Dopamine antagonists/prolactin manipulation

Dopamine has a role in regulating reproduction through synapses that are present between dopaminergic and GnRH neurons in the median eminence. Inhibition of dopamine D2 receptors increased LH secretion during anestrus, suggesting an inhibitory role of dopamine on

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cyclicity.[64] Additionally, dopamine inhibits prolactin secretion. Although dopamine concentrations in cerebrospinal fluid of mares were higher during the anovulatory period,[65] plasma concentrations of prolactin were higher during the breeding season,[66] suggesting an inverse relationship. Prolactin treatment to anestrous mares stimulated ovarian activity and induced ovulation in seasonally anovulatory mares. Recombinant porcine prolactin given to mares (starting mid-January) resulted in ovulation, an average of 5 weeks earlier than placebo-treated mares (February 6 versus March 14).[67]

Dopamine D2 receptors are not only present within the median eminence, but also in theca cells, granulosa cells, luteal tissue, and ovarian cortex tissue,[68,69] suggesting an influence on follicular growth. This may be why the use of dopamine D2 antagonists (e.g. sulpiride and domperidone), have a stimulatory effect on follicular development in anestrous mares.[70] Domperidone is a dopamine D2 and D3 receptor antagonist, whereas sulpiride is a selective dopamine D2 receptor antagonist; sulpiride can cross blood brain barrier but domperidone cannot. Both have been used to attempt to shorten the transitional period and hasten the first ovulation of the year.

Intramuscular sulpiride (1 mg/kg) given daily (starting in late January) and continued until ovulation[71] or 200 mg given daily (starting early February) and continued until ovulation or for a maximum of 58 days[72] advanced the first ovulation of the year by 21 days and 33 days, respectively. These studies were performed in seasonally anestrous mares maintained under natural photoperiod. Similar results were observed in mares exposed to an artificial photoperiod (starting in January, 2 weeks prior to onset of sulpiride treatment)[73] and in transitional mares (follicular diameter > 25 mm).[74] However, attempts at beginning the sulpiride protocol earlier in January without photoperiod manipulation failed to affect ovarian activity or ovulation, despite increased circulating prolactin concentrations.[75]

Oral domperidone (1.1 mg/kg) treatment to anestrous mares (starting on January 15) resulted in follicular development within 14 days and advanced the average date of ovulation by 78 days; 6 out of 8 mares continued to cycle after the first ovulation, whereas the remaining 2 experienced prolonged intervals to second ovulation (mean = 67 days). Domperidone treatment increased prolactin secretion and concentrations of LH and estrogen conjugates; FSH was not affected as with sulpiride treatment.[76] In contrast, there was no positive effect on shortening the transitional period in anestrous mares (natural photoperiod) treated with domperidone for 60 days, or transitional mares (artificial photoperiod) treated for 30 days.[77] Authors proposed that differences in ambient temperatures and climates between the 2 study locations could have had a role in the opposing results.[77] Similarly, sulpiride was effective in advancing ovulation in a majority (80%) of the mares studied within 38 days of beginning treatment, whereas domperidone was only effective in advancing ovulation in a small subset (20%) of mares over control mares.[78]

One potential explanation for the failure of dopamine antagonists to stimulate follicular activity is a lack of estrogen in seasonally anestrous mares. Estrogen exposure increased pituitary prolactin storage,[79] and thus studies have been performed that have added estradiol priming to their dopamine antagonist protocols. Seasonally anestrous mares received 10 injections of intramuscular estradiol benzoate (11 mg once every 2 days) with subcutaneous sulpiride (250 mg once daily) beginning on day 11 (day 0 = beginning of estradiol treatment);[80] treatment was continued until ovulation or until 35 days of sulpiride treatment. The control group was given only sulpiride. Mares that received estradiol benzoate prior to sulpiride ovulated 45 days earlier than mares receiving sulpiride alone. Pretreatment with estrogen enhanced the prolactin response to daily treatment with sulpiride, causing both increased concentrations and increased duration of elevated secretion compared to controls. Pretreatment with estradiol also significantly increased LH concentrations to concentrations comparable to the preovulatory LH surge. In another experiment (performed in cyclic mares during the summer) by these authors, a similar hormonal response was observed with 1 injection of domperidone microparticles (3 g) after estradiol benzoate treatment.[80] A few years later, the combination of domperidone microparticles (3 g) and estradiol (estradiol cypionate [ECP]) was compared to combinations of these 2 medications with long-acting progesterone in anestrous mares; mares receiving estradiol cypionate and domperidone ovulated within the study period of 35 days unlike those not receiving treatment with the combination of these 2 medications. Addition of progesterone to ECP and domperidone did not affect the date of first ovulation over mares just receiving ECP and domperidone. An additional experiment within this study showed a positive effect of ECP on advancing ovulation in domperidone-treated mares, regardless of the dose of ECP or domperidone used.[81] Treatment with domperidone (1.5 g microparticles) beginning 1 day after ECP has also been compared with treatment with either 0.75 g or 1.5 g sulpiride 1, 6, and 11 days after treatment with ECP. Using this experimental protocol, 7 of 9 mares treated with 1.5 g sulpiride ovulated earlier than controls. Mares receiving domperidone or 0.75 g sulpiride did not ovulate earlier than controls.[82] Previous studies seem to suggest that sulpiride may be more efficacious than domperidone in inducing cyclicity, though adding estradiol to protocols for either dopamine antagonist has additional positive effects.

Other dopamine antagonists (receptor blockers), such as fluphenazine and perphenazine, have also been studied. Intramuscular fluphenazine (178.6 µg/kg daily for 3 weeks) treatment to anestrous mares resulted in follicular growth but did not advance first ovulation.[83] Perphenazine, however, advanced follicular growth and first ovulation of the year by ~ 30 days but required ~ 4 weeks of daily treatment.[84]

Prostaglandin therapy

Intercavernous sinuses (pituitary venous outflow) of late anestrous or transitional mares were cannulated to determine GnRH, LH, and FSH concentrations in response to systemic prostaglandin analogue (luprostiol) treatment.[85] An increase in GnRH was noted 20-25 minutes after luprostiol treatment; surprisingly, LH and FSH increased prior to GnRH. Pulses of all 3 hormones were noted to be longer in duration but lower in amplitude than those in normally cyclic mares. Authors also reported the results of a field study in which mares were treated with alfaprostol, dinoprost, or cloprostenol; 35% of treated mares came into estrus and 68% of mares ovulated within 10 days after prostaglandin treatment.[85] However, the authors cited 2 earlier

studies in their discussion in which alfaprostol failed to produce estrus and ovulation in transitional and anestrous mares.[86,87]

As reviewed,[38] prostaglandins have been used to initiate cyclicity; however, the duration of anestrus experienced prior to treatment and/or depth of anestrus were unclear from these studies, so exactly how and when prostaglandin treatment induces cyclicity was not well-defined. Overall, although prostaglandins may have some ability to stimulate gonadotropin release and ovulation, they have not emerged as a popular choice for induction of cyclicity and first ovulation of the year.

Manipulation of the cycling mare

Prostaglandin $F_{2\alpha}$ – luteolysis, antiluteogenesis, and estrus synchronization

Prostaglandins are lipid compounds (eicosanoids) with diverse effects throughout the body. These compounds are produced from arachidonic acid via the cyclooxygenase pathway. Arachidonic acid is converted to endoperoxide PGH2, a precursor of all prostaglandins, by the enzyme prostaglandin-endoperoxide synthase 2 (PTGS2). From there, prostaglandin synthases are responsible for producing the various specific prostaglandins (e.g. prostaglandin $F_{2\alpha}$ [$PGF_{2\alpha}$]). $PGF_{2\alpha}$'s effect on the estrous cycle was first described in 1972;[88] authors reported that $PGF_{2\alpha}$ caused lysis of the CL, with return to estrus 3-4 days after treatment.[88] The main source of $PGF_{2\alpha}$ was suggested to be the uterus, as a study performed the prior year[89] had demonstrated that hysterectomized mares experienced prolonged CL function.

Mare's CL is highly sensitive to the effects of prostaglandin; it responds to a minimally effective dose (9 µg/kg) of 1 injection of free acid $PGF_{2\alpha}$, significantly lower than the luteolytic dose in cows.[90,91] Additionally, $PGF_{2\alpha}$ metabolism is primarily in the lungs and occurs more slowly in mares compared to cows.[92] The combination of increased sensitivity of the mare CL to $PGF_{2\alpha}$ and decreased rate of metabolism is necessary, as the mare lacks the utero-ovarian countercurrent exchange mechanism present in other species. Thus, $PGF_{2\alpha}$ produced by the uterus must first circulate systemically prior to reaching the ovary, making the concentration that reaches the ovary much lower than in species with a countercurrent exchange mechanism.

Since the initial report of its effectiveness in shortening diestrus, $PGF_{2\alpha}$ has become a key tool for veterinarians seeking to manipulate estrous cycle lengths, synchronize mares, and terminate pregnancies, among other uses. The most used prostaglandins are naturally occurring $PGF_{2\alpha}$, dinoprost tromethamine (Lutalyse®, Zoetis, Parsipanny, NJ, USA), and the synthetic prostaglandin analogue, cloprostenol (Estrumate®, Merck and Co, Inc, Rahway, NJ, USA).[93] Recommended dosage for dinoprost tromethamine is 0.01-0.02 mg/kg (5-10 mg or 1-2 ml per 500 kg horse) and for cloprostenol is 0.55 µg/kg (~ 250 mg or 1 ml per 500 kg horse).[93] $PGF_{2\alpha}$ can be used in a variety of ways to produce luteolysis;[94,95] however, intramuscular treatment is generally preferred due to ease and decreased severity of side effects. Side effects include increased gastrointestinal motility, abdominal discomfort, hyperthermia, sweating, and potentially diarrhea, labored breathing, and ataxia. These side effects appear to be dose dependent.[96] As some horses may be perceived as being distressed by the side effects occurring at standard doses of $PGF_{2\alpha}$ for luteolysis, researchers have attempted to determine if lower doses of prostaglandin may be used. Intramuscular cloprostenol (25 µg; 10th of the recommended dose) given to a standard-sized horse, effectively induced luteolysis.[97] Similarly, studies using intramuscular dinoprost tromethamine have had success using only 1 dose of 1.25 mg or 2 doses of 0.5 mg given 24 hours apart.[98-100] The lower dosages used in these studies were associated with no visible side effects when these were recorded as part of the study protocol.[97-99]

The CL has been noted to be refractory to lysis until day 5 after ovulation. However, a subset of mares may respond to luteolytic agents earlier than this.[91,101] CL regressed with 10 mg dinoprost given on day 3 after ovulation; however, functional resurgence of the CL, evidenced by increased blood progesterone concentrations, was detected in 75% of mares 3 days after treatment and initial regression. On average, the mares in the day 3 prostaglandin group ovulated significantly earlier than mares injected with prostaglandin on day 10.[102] Other researchers have also investigated the effects of prostaglandin given prior to day 5 after ovulation; however, those studies utilized multi-day protocols with various doses (e.g. 2.5 versus 10 mg dinoprost). Although some regression/resurgence of corpora lutea was observed, most mares experienced luteal regression and ovulated as early as 7-9 days after treatment.[103,104] Branching off these earlier studies, specific research into antiluteogenesis, or the complete prevention of CL formation, has been performed. In addition to dinoprost (10 mg) given on days 0, 1, and 2 of the estrous cycle (ovulation = day 0), 2 additional doses of 10 mg $PGF_{2\alpha}$ were given on days 3 and 4 after the initial 3 days of twice-daily treatment; there was lack of CL formation in 10 of 10 mares treated.[105] They also observed a 5 day shorter inter-ovulatory interval in the mares treated with the antiluteogenic protocol and these mares had equal pregnancy rates to controls when bred on the next cycle.[105] Protocols involving early (days 0-2) prostaglandin treatment have had various results with regards to effects on same-cycle pregnancy rates, with some studies having decreased pregnancy rates with these protocols[106,107] and with no effect on pregnancy rates.[108] The potential for reduced same-cycle pregnancy rates is important to consider when using prostaglandins for their ecbolic effects postbreeding. However, prostaglandins may be preferred over oxytocin due to their ability to produce lower amplitude, longer lasting contractions of the myometrium and assist in lymphatic flow.[109,110] Indeed, the use of prostaglandins in the periovulatory period may be beneficial in mares predisposed to developing mating-induced endometritis (due to cervical issues, poor conformation, bred using frozen semen, etc.) or in mares with suspected issues with lymphatic drainages (i.e. mares with endometrial cysts).

Timing of ovulation is 2-15 days (average 8-10 days) after 1 prostaglandin treatment.[88,91,111,112] Interval from prostaglandin to ovulation depends on the follicular size and dynamics at treatment. Specifically, ovulation may occur within 2-3 days if there is a large, growing follicle on 1 of the ovaries (near the end of the growth phase of a follicular wave) at treatment. Conversely, if treated during atresia of previously growing follicles or when the follicles on the ovary are quite small, interval to ovulation will be longer.[112,113] Endometrial edema may aid in determining whether a large follicle (> 30 mm) present at prostaglandin treatment is

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destined to ovulate or become atretic. Large follicles that will ovulate may be preceded by edema that forms within 24 hours after treatment, whereas treatment when a large atretic follicle is present will not result in endometrial edema for 3-5 days.[114] However, some mares with large follicles may not exhibit signs of estrus and may never develop signs associated with rising estrogen (endometrial edema, cervical softening) prior to ovulation.[112,115] Care must be taken to closely observe other indicators of estrous cycle status (endometrial edema, cervical softening, etc.) when using $PGF_{2\alpha}$ to 'short cycle' mares so that timing of breeding coincides with observed or expected ovulation. The variability in interval from treatment to ovulation makes the sole use of prostaglandin for such purposes as estrus synchronization for embryo transfer less than ideal, even when 2 injections (14 days apart) are used. As described above, depending on the follicular status at prostaglandin treatment, ovulation may potentially occur anywhere in a wide range of days after 2 injections of prostaglandin.[116] Thus it was concluded that, with this variability, at least 10 recipient mares would be needed to have an 80% chance that at least 1 would ovulate within 24 hours of the donor mare using this technique.[117] The use of transrectal ultrasonography may improve success; however, the application of additional hormonal manipulations, as will be described in upcoming sections, will also improve success and reduce the number of recipients needed per donor.

Human chorionic gonadotropin - induction of ovulation

Human chorionic gonadotropin is a glycoprotein synthesized by the chorionic villi of the human placenta beginning very early in pregnancy and excreted in the urine. Its secretion starts only a few days after conception and continues throughout the first part of human pregnancy. Human chorionic gonadotrophin supports the CL, which is responsible for maintaining pregnancy during this time. Secretion declines once the placenta has developed enough to take over maintenance of pregnancy, a scenario that is very similar to the secretion of equine chorionic gonadotropin (eCG) during the first 40 to at least 100 days of pregnancy in the mare. Use of hCG to induce ovulation was first suggested in 1939.[118] Human chorionic gonadotropin has luteinizing-hormone activity in the mare; thus, it promotes CL formation and progesterone production.[119] It has become widely used by equine practitioners for this purpose. Broodmares bred by live cover often have prescheduled appointments with specific stallions, making more precise timing and control of ovulation necessary. This is especially true during the busiest part of the breeding season, when a mare may not be allowed multiple visits to the stallion in a single estrous cycle. Additionally, practitioners breeding mares via artificial insemination must have some control of timing of ovulation as cooled semen, and especially frozen semen, has a limited lifespan. Practitioners should aim to inseminate mares with cooled, transported semen within 24 hours before ovulation and within 6-8 hours before or after ovulation for frozen semen.

Human chorionic gonadotrophin is given to advance ovulation of a follicle or follicles destined for this fate. Generally, these follicles are identified using a combination of palpable and ultrasonographic characteristics, in addition to potential known history about the timing of previous cycles. Some large follicles may be destined for atresia and thus will not respond to hCG with ovulation. This is the challenge confronting practitioners, especially when close monitoring of the cycle has not been performed up to that point. Multiple doses and routes of treatment have been described for hCG, with variable efficacy. Differences in response to hCG treatment may be observed across breeds, as some breeds (e.g. Friesians) tend to ovulate larger follicles and thus a follicle of a diameter appropriate for ovulation induction in one breed may not have achieved dominance in another breed. In general, dosages ranging from < 1,000 up to 6,000 IU have been used with success across various treatment routes.

Follicles induced to ovulate must be steroidogenically competent in addition to achieving a larger diameter. During the transitional period, large follicles may appear that are not competent to ovulate and may, as a result, regress. These follicles may not respond to hCG treatment; however, some researchers have had success in treating transitional follicles and causing ovulation earlier than in untreated mares,[120] especially when progesterone priming was used.[121] Human chorionic gonadotrophin has been more effective than GnRH agonists in transitional mares in some studies[122] but less effective in others.[123]

During normal cyclicity, hCG causes ovulation within 48 hours in most mares. Intravenous hCG (3,000 IU) treatment to mares with follicles > 30 mm resulted in ovulation (~ 45 hours after treatment).[124] A 2,500 IU hCG dose resulted in ovulations occurring earlier than in the previous study (36 hours); however, both studies documented that ovulation resulting from hCG treatment occurred earlier than those resulting from deslorelin.[124,125] Though hCG may cause ovulation earlier than deslorelin, it has been suggested that it is less reliable, as only 83.3% of hCG-treated mares ovulated within 48 hours versus all mares receiving subcutaneous deslorelin acetate (Ovuplant™) in one study.[126] Ovulations occurred 25-48 hours in 75% of mares after 2,000 IU of hCG treatment.[127] Various doses of hCG have been used by practitioners and researchers, prompting investigation of potential response differences among doses. Ovulation and pregnancy rates among mares treated with 1,500, 3,000, or 6,000 IU doses of hCG were not different.[128]

In addition to accelerating ovulation, hCG has also been observed to increase the synchronicity of multiple ovulations.[129] Increased synchronicity results in ovulations occurring < 12 hours apart, as opposed to up to 2-3 days apart.[130] This may increase chances of pregnancy or multiple pregnancies; however, this effect was not observed in control mares, despite an increase in synchronicity of multiple ovulations.[130]

It is important to note that response to hCG, even during the peak breeding season and not during the transitional period, may be more unpredictable in older mares.[127,131] One of the suggested reasons for this observation is antibody formation as a result of treatment with hCG. Some studies suggest that mares must receive 4 or 5 treatments with hCG during the breeding season before loss of efficacy due to antibody formation,[132,133] whereas others noticed reductions in efficacy as early as the second or third treatment.[134,135] Loss of efficacy is evident as failure of hCG to advance ovulation and failure of hCG to induce predictable ovulations. In contrast, loss of ovulatory efficacy was not observed despite the development of hCG antibodies with half-lives from 1 to multiple months.[136] More recently, the same pattern of decreased 48-hour ovulation rates was observed by the third treatment with hCG, but

also noted an increase in ovulatory failures in mares > 15 years of age versus mares < 15 years of age.[137] Similarly, follicular dynamics and oocyte maturation in mares positive for hCG antibodies was investigated.[138] Antibody-positive mares had less blood flow within the follicular wall and fewer mature oocytes on aspiration at 30 hours after hCG, suggesting an effect of hCG antibodies on oocyte maturation and ovulation.[138] It is important to note that both intramuscular and intravenous routes of treatment have been investigated in the aforementioned studies. Although hCG has been proven efficacious in advancing ovulation, the risk of antibody formation and diminished or unpredictable response has prompted investigation[139] and use of other compounds that may be more reliable across multiple cycles.

Gonadotropin releasing hormone and analogues – induction of ovulation

GnRH is a small peptide released from the hypothalamus in specific patterns throughout the estrous cycle, as described previously in this review. Its release stimulates gonadotropins (FSH and LH) release from the anterior pituitary, affecting the gonads (ovaries). The use of GnRH and its analogues in inducing cyclicity has been described above. GnRH analogues, such as deslorelin or buserelin, are widely used among practitioners. Past and current formulations of deslorelin include a 2.1 mg, slow-release, subcutaneous implant called Ovuplant™, and Sucromate® (Dechra Veterinary Products, Overland Park, KS, USA), an FDA-approved intramuscular injection containing 1.8 mg deslorelin acetate.

Short-term subcutaneous implants of deslorelin acetate to mares when a follicle reached 40 mm resulted in 93% of mares ovulating within 48 hours compared to only 7% in controls.[140] Additionally, 63% of ovulations occurred between 36-48 hours, which was a tighter window of ovulation when compared to various doses of hCG.[140] Additional studies have corroborated these findings using the same short-term release implant.[141,142] Buserelin has also been described to elicit high ovulation rates, with one study citing that 100% of draft mares ovulated in response to 40 µg of intramuscular buserelin given once a follicle had reached 45 mm and endometrial edema was present; average interval to ovulation was 29 hours in treated mares versus 59 hours in controls.[143] Though draft mares do tend to ovulate larger follicles, it must be considered whether, with follicles that large, ovulation was imminent regardless of treatment with buserelin as an ovulation-inducing agent. Buserelin has been compared to hCG for its effectiveness in inducing ovulation in multiple studies. Results were variable, with some studies having a similar efficacy[144,145] and some had reduced efficacy compared to hCG.[146]

In comparison to injectable forms of GnRH analogues, the slow-release device Ovuplant™ (2.1 mg deslorelin) was developed as a means of releasing a GnRH analogue over multiple days with a single implant. Various doses of deslorelin in implant form have been evaluated and although all doses investigated were successful in inducing ovulation earlier than controls, only the 2.2 mg-containing implant was as successful as a 3,000 IU intravenous dose of hCG.[124] As has been previously mentioned with injectable deslorelin, Ovuplant™ has a tight window of ovulation, with 94% of mares ovulating between 38-42 hours after implantation.[147] Due to the success in causing ovulation within a narrow time frame, the authors suggested that overnight checks, when employing Ovuplant™ for the purpose of frozen semen breeding, were unnecessary.[147] Unfortunately, no control group was included in this study and Ovuplant™ was implanted once a follicle reached 42 mm; therefore, it must be considered whether control mares would have naturally ovulated within a similar time frame with this size follicle. Just as age and seasonal effects have been reported for hCG, effects of these 2 factors on ovulation rates in response to Ovuplant™ were studied.[148] Higher ovulation rate was observed in mares aged 10-14 years than in other age groups, with the lowest ovulation rate being in mares aged > 20 years. Additionally, Ovuplant™ was more effective in inducing ovulation in July-October than in March and April.

Although implanted deslorelin was demonstrated to have clear benefits, a prolonged inter-ovulatory interval was noted in mares receiving Ovuplant™ implants.[149-152] Investigation into this issue resulted in the discovery that the implant depressed early FSH secretion after ovulation, resulting in delayed emergence of the next follicular wave during early diestrus.[150-152] These effects are not observed, in comparison, when hCG is used to induce ovulation.[153] It was then demonstrated that removal of the implant 1-2 days after ovulation prevented FSH depression and prolonged inter-ovulatory intervals.[152,154] Thus, it was recommended to implant Ovuplant™ into the lips of the vulva and remove the device 1-2 days after ovulation, if possible.[155]

Efficacy of deslorelin acetate (Sucromate®) on induction of ovulation was evaluated.[156] Deslorelin acetate (138 mares; 168 estrous cycles) was compared to hCG (118 mares; 136 estrous cycles); treatment was given when mares were in estrus with a ≥ 35 mm follicle. Deslorelin acetate yielded an 89.9% ovulation rate compared to an 82.8% ovulation rate for hCG within 48 hours after treatment.[156] Additionally, unlike with Ovuplant™ implants, prolonged return to estrus was not observed with deslorelin acetate.

Recombinant luteinizing hormone – induction of ovulation

Recombinant equine luteinizing hormone (reLH) has been studied and is a reliable option for induction of ovulation in mares. Once a follicle reached 35-39 mm, mares (3-20 years) were treated with either 1 of 4 doses (0.3, 0.6, 0.75, 0.9 mg) of intravenous reLH or hCG (2,500 IU). Increasing doses of reLH had increasing effectiveness at inducing ovulation within 48 hours after treatment. Similar 48-hour ovulation rates were obtained using the 2 highest doses of reLH (0.75 and 0.9 mg) compared to hCG; 90 and 80% ovulated within 48 hours in the 0.75 mg and 0.9 mg reLH groups, respectively, compared to 85.7% in the hCG group. Both hormonal profiles and inter-ovulatory intervals appeared to be similar between control and reLH-treated cycles.[157] In addition to induction of ovulation, reLH increased the number of ovulations, while reducing the number of anovulatory follicles, when combined with reFSH for the purpose of embryo recovery in superovulated mares.[158]

Progesterone and progestins +/- estradiol – use in estrus synchronization

Among their many other applications, progesterone and progestins, of which the most popular is altrenogest, can be used to

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synchronize estrus in mares. This may be desired when there is limited availability of a particular stallion, when it is desired to concentrate breeding times and thus foaling times, or in embryo transfer programs in which recipient mares' cycles and ovulation must closely match a donor mare. Commonly employed protocols involve 10-14 days of oral altrenogest (0.044 mg/kg) or 10 days of intramuscular progesterone (150 mg) and estradiol (10 mg).[159] The addition of estradiol, as discussed in previous sections, provides inhibition of FSH, causing more complete ovarian suppression than is achieved with natural progesterone or synthetic progestins alone. Both treatment strategies are followed on the last day with an injection of $PGF_{2\alpha}$. Depending on the size of follicle present when progestin therapy is discontinued, interval to ovulation may be anywhere from 3 to 11 days. With the addition of estradiol, which allows for a more uniform population of follicles once therapy is discontinued, most mares will ovulate within 18-23 days after the beginning of steroid treatment.[159]

In addition to oral and short-term injectable forms, certain sustained release formulations of altrenogest may be useful in estrus synchronization.[160] In this study, 225 and 450 mg slow-release altrenogest (BioRelease LA 150, BET Pharm LLC, Lexington, Kentucky, USA) groups had short-term suppression of estrus. However, although ovulation was delayed compared to controls, when individuals in these groups did ovulate, they tended to ovulate within a tight time frame without the use of an ovulation-inducing agent, which may prove useful for purposes of estrus synchronization.[160] Another sustained-release formulation, the intravaginal device, CIDR® was investigated.[161] Mares received an intravaginal device containing 1.9 gram progesterone (CIDR® B, Zoetis South Africa, Sandton, South Africa) during various points in their cycles. Devices were removed after 12 days and mares were treated with $PGF_{2\alpha}$. Mares were implanted with a short-term deslorelin implant once a follicle reached at least 40 mm in diameter. Onset of estrus was synchronized following removal of the CIDR®-B devices; however, ovulation was not, due to a lack of synchronization of follicular development during the period of estrus suppression. Silent ovulations were noted to occur in 20% of mares while the devices were in place. Additionally, as previously noted with intravaginal devices, vaginitis of some degree was noted in all treated mares.[161] However, there was no difference in pregnancy rates in mares treated with CIDR versus controls despite development of mild vaginitis.[162] Although progesterone alone can synchronize the onset of estrus, additional agents must be utilized to obtain the tight synchronization of ovulation that is necessary between donor and recipient mares.

Transvaginal aspiration of follicles – use in estrus synchronization

An alternative to steroid-induced (i.e. progesterone and estrogen) synchronization of ovulation is TVA of follicles.[163,164] Follicles > 10 mm were aspirated on day 0 and $PGF_{2\alpha}$ was given 4 days later and hCG was used to induce ovulation, either 6 days after that or when a follicle reached at least 30 mm. This protocol was 96% successful in inducing ovulation within 48 hours after hCG treatment.[163] This protocol was then compared to traditional progesterone and estrogen suppression of ovarian activity. Ovulation synchronization was similar between the 2 groups, but TVA of follicles resulted in a shorter interval from both the start of the study and prostaglandin treatment to ovulation.[164] Although TVA of follicles may not be practical in all settings and requires specialized training, it remains a viable alternative to daily steroid injections for suppression of estrus in the interest of synchronizing ovulation in donor and recipient mares.

Suppression of estrus

Suppression of estrous behavior and contraception in the mare are related, but not equivalent concepts. Estrus may be suppressed without disrupting a mare's ability to conceive and removing a mare's ability to conceive may not remove 'mareish' behaviors if, in fact, they are not due to reproductive hormonal cycling. Reviews on suppression of estrus were published in this journal,[165,166] so the methods discussed in those reviews will not be repeated here. However, new additions to the arsenal of estrus suppression are presented below. The key point to remember, as discussed in the above-mentioned review papers, is that estrous behaviors arise from low or lack of progesterone, hence mares in anestrus and transition with no functional corpora lutea will have variable signs of estrus despite their inability to ovulate during these time frames. Therefore, a majority of estrus suppression techniques supplement progestins or aim to prolong luteal function.

Prolonging the luteal phase – slow-release oxytocin

Use of specifically timed (days 7-14 after ovulation) oxytocin treatment to prolong the luteal phase was reviewed.[165] It was noted that this method requires daily ultrasonography prior to ovulation to determine the exact day of ovulation. It also requires daily injections, albeit intramuscular, making it a potentially cumbersome treatment option. Intramuscular oxytocin (60 IU) given once daily from days 7-14 after ovulation (day 0) prolonged the luteal phase for 2 months in 67% of treated mares.[167]

Recently, the use of a proprietary slow-release oxytocin formulation in the suppression of estrus in mares was evaluated.[168] Initially, 1 ml of intramuscular slow-release oxytocin (2,400 IU) given on days 7 and 10 after ovulation prolonged luteal function in 75% of treated mares. The group then evaluated a single injection of slow-release oxytocin (4,800 IU) given on either day 8 or 9 after ovulation. This single, higher dose did prolong luteal function over the 40-day progesterone-sampling period in 4/8 treated mares; however, they did not achieve the 70% estrus suppression rate that was expected. The authors noted that the small sample size used in the study may have limited their ability to find statistical significance in CL lifespan prolongation between treated and untreated mares.[168]

Intrauterine devices – iUPODs®

The iUPOD® (Pearl Pod LLC, Amherst, MA, USA) intrauterine device system is comprised of 3, 12 x 26 mm, elliptical, magnetic devices that are inserted independently. Once free of the insertion tube, the 3 pods self-assemble into a low-energy ring or tripod formation. Like the intrauterine marble, the device aims to mimic pregnancy by providing mechanical stimulation to the endometrium, similar to embryo (prior to fixation on days 16-17). When inserted during estrus, average length of diestrus was 73.4 days. In a second experiment, the iUPODs® were inserted at any stage of the estrous cycle, producing an average prolonged diestrus length of 51.3 days. Endometrial scores (obtained before and after device insertion from mares in the first experiment) were not affected by

presence of the iUPODs®. Eight mares were randomly selected for breeding in the season after devices removal and 100% of these mares became pregnant.[169]

Results were similar in another study that used a device with the same configuration but larger size (16 x 38 and 16 x 40 mm).[170] Devices were implanted in 6 mares regardless of estrous cycle stage and mares were placed in a paddock with a known-fertile stallion. None of the mares became pregnant during the 3-month study period and all mares retained the devices. Authors did note that fluid was observed on 77% of ultrasonography examinations during the study period, suggesting that the combination of the device and breeding had caused a mating induced endometritis. Prolongation of the luteal phase (37 and 91 days) was observed in 2 mares, but for the other mares the average length of diestrus was shortened at 7.8 days, potentially due to uterine inflammation and related premature release of prostaglandin.[170] A similar study was conducted in feral equids, wherein devices were implanted in 8 mares in various stages of the estrous cycle. These mares were housed with a fertile stallion for 120 days after being fitted with the device. None of the mares became pregnant during the study period, and 2 of 4 mares conceived within 30 days after device removal; 2 mares were noted to have abundant biofilm on the devices at removal.[171] Although the above-mentioned intrauterine devices appear to be effective at preventing pregnancy, their presence within the uterus may be associated with intrauterine inflammation and biofilm formation in a subset of mares.

Conclusion

Great advances have been made in our ability to manipulate the mare's estrous cycle, however more research is needed in multiple fields (e.g. estrus synchronization). Recombinant hormone products have promise for the future and as their availability increases, we will surely know more about how successful they can be compared to traditional hormonal manipulations. In the meantime, progestins, GnRH analogues, hCG, and prostaglandins have received attention across the decades that have elucidated some of the idiosyncrasies associated with their use.

Conflict of interest

Authors disclose no conflicts of interest.

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