Seasonality in mares

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Abstract

In this review, we have attempted to summarize, based on recent data obtained in our laboratory and elsewhere, our current understanding of the regulatory mechanisms of seasonality and discuss the implications with regard to treatment strategies to advance the onset of cyclic reproductive activity in the early spring. © 2000 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Animals have developed many strategies for seasonal breeding that ensure that their offspring is born at the appropriate time of the year. In the horse, as in many other species, the circannual rhythm of reproduction is cued primarily by photoperiod changes. This environmental signal is translated to an endocrine signal in the pineal gland, which secretes melatonin during the phase of darkness. In the mare, short daylength is associated with a decrease in gonadotropin secretion and consequently a decrease in ovarian activity. The mechanism whereby gonadotropin and presumably GnRH secretion is decreased during the anestrous period is not well understood in mares. It has been proposed that the absence of cyclic activity is the result of a lack of positive signals, e.g. long daylength, favorable climatic and nutritional conditions, the presence of a stallion, that stimulate GnRH and gonadotropin secretion during the breeding season. Recent data in horses and other species suggest that seasonal reproductive inactivity may be the result of a direct/active inhibition induced by signals such as short daylength, adverse climate, poor nutrition. The mare, as other seasonal breeders,

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has an endogenous circannual reproductive rhythm and the main role of seasonal clues appears to be to synchronize the endogenous rhythm to winter and summer.

The horse is a seasonal polyestrous species with onset of the breeding season occurring in spring, associated with increase in daylight, temperature and availability of food. The natural breeding season occurs from April to September in the Northern Hemisphere (Hughes et al., 1975). Regulations in many breeding associations have established as the official birth date of foals, January 1 in the northern hemisphere, and an official breeding season, from February to June, resulting in the same official age for all foals born the same season (Ginther, 1992). This results in economic pressure for the horse breeders to breed mares as early as possible in the year to have an age advantage over foals born later in the year. Horses that are born early in the year have an advantage and perform better than horses born later (Langlois and Blouin, 1996). This arbitrary birth date has stimulated researchers to understand the mechanisms of reproductive seasonality in mares and develop methods for induction of an early onset of the breeding season in mares. Most of the work has focussed on photoperiod and it has been demonstrated that artificial photoperiod, simulating long days, can be used to advance the time of the first ovulation of the year in mares (Burkhardt, 1947; Palmer and Guillaume, 1992). Even though this method has been widely used since at least 15 years it has remained the subject of active research. Recent findings have increased our understanding of the mechanisms and limitations of this important regulator in seasonal reproductive function and more effective methods using artificial photoperiod have been developed in recent years (Guillaume et al., 2000). Despite the efficacy of increased daylength for induction of cyclic ovarian activity in early spring, this method requires that artificial photoperiod treatment is started in December and there is considerable variation in the interval from start of treatment to first ovulation in individual mares. For these reasons, researchers have attempted to design pharmacological treatments that would at the same time decrease the treatment-to-first-ovulation interval and result in a more consistent and predictable response. In recent years, these studies have significantly increased our understanding of the hormonal (steroids and gonadotropins) and neuroendocrine (opioids, dopamine) control of seasonal reproduction in mares and have opened new avenues for the manipulation of the onset of reproductive activity in the spring.

2. Endogenous circannual rhythm of reproduction

The seasonal reproductive pattern is the result of a circannual endogenous rhythm that is entrained by external environmental factors such as photoperiod, temperature, nutrition and body condition. In ewes, Karsch et al. (1989) provided clear evidence for the existence of a circannual reproductive cycle. Ovariectomized, estrogen-implanted ewes maintained in constant lighting condition for 5 years maintain circannual changes in LH secretion. However, the period of elevated LH levels, representative for the breeding season, differed among individuals reflecting the lack of synchronizing environmental factors. The available data indicates that an endogenous reproductive rhythm exists also in horses. Mares maintained under constant long day photoperiod (16 h)
beginning at the summer solstice enter into anestrus and mares maintained under short day photoperiod (8.5 h light) beginning at the winter solstice resume cyclic ovarian activity (Kooistra and Ginther, 1975; Palmer and Driancourt, 1981; Palmer et al., 1982). Also, removal of the pineal gland or the ganglion cervicale superior does not result in the disappearance of seasonal reproductive activity but the reproductive rhythm of these animals can no longer be influenced by changes in photoperiod (Sharp et al., 1979; Grubaugh et al., 1982).

3. External factors entraining the annual reproductive rhythm

3.1. Photoperiod

It is well accepted that in the horse photoperiod is the most important external factor that influences the circannual endogenous reproductive rhythm (Ginther, 1992). Additional light exposure during winter and early spring stimulates ovarian activity in anestrous mares and is commonly used to advance the onset of the breeding season (Burkhardt, 1947). However, the correct response to this stimulatory photoperiod is subject to specific conditions and reproductive activity does not always follow exposure to long days. The ability to influence the circannual reproductive rhythm depends on several factors: (1) state of refractoriness to photoperiodic changes, (2) the photoperiodic history and (3) the existence of a photosensitive phase during the night.

When mares are maintained under constant lighting condition (long days or short days) they will resume their circannual rhythm despite the stimulatory or inhibitory lighting conditions (Kooistra and Ginther, 1975; Palmer et al., 1982; Scraba and Ginther, 1985). This condition is referred to as refractoriness and can best be described as an inability to continue to respond to the current type of photoperiod. For this reason, mares kept under 16L:8D starting in winter (Palmer et al., 1982) or summer (Kooistra and Ginther, 1975; Scraba and Ginther, 1985), still return to winter anestrous. The development of photorefractoriness can be prolonged or prevented by changing the photoperiod at regular intervals. In ewes, the photorefractory stage can be delayed by further decreasing daily light exposure (from 16 to 12 h of light then from 12 to 8 h of light) (Malpaux et al., 1988). This allows the reproductive system to maintain a constant sensitivity to light. In rams, permanent reproductive activity was observed if inhibitory and stimulatory photoperiods were applied with rapid, 1-month alternations (Pelletier and Almeida, 1987). A similar experiment has also been carried out in ovariectomized mares (Palmer and Guillaume, 1992). Animals were kept either under natural photoperiod or under rapid alteration of long and short day cycles (70LD:35SD and 35LD:35SD) for 25 months. The seasonal decrease in LH observed in control mares during winter was attenuated in groups under rapid photoperiodic alternation. However, in ovarian-intact mares (Guillaume, personal communication) and in pony stallions (Cox and Skidmore, 1991), the occurrence of seasonal anestrus could not be eliminated by such lighting schedule.

The photoperiodic history determines the response to a specific photoperiod. In ewes, 13-h daily light exposure is stimulatory if it follows a 16-h light photoperiod but is
inhibitory when preceded by a 10-h light period (Robinson and Karsch, 1987). No such study has been performed in horses.

Ovarian activity can be induced in seasonally anestrous mares by applying a 1- to 2-h light flash approximately 9.5–10 h after the abrupt beginning of darkness (Palmer and Driancourt, 1981; Palmer et al., 1982; Malinowski et al., 1985). These observations indicate that, similarly to other seasonal species, mares also have a photosensitive phase during the period of darkness. The presence or absence of light at 9.5 h after onset of darkness rather than the duration of light and darkness is important for the response. Exposure to 16L:8D, 14.5L:9.5D, 8L:9.5D:1L:5.5D or 4L:9.5D:1L:9.5D produces similar ovarian response, even though mares exposed to the last lighting protocol (4L:9.5D:1L:9.5D) received only 5 h of light per day (Palmer and Driancourt, 1981; Palmer et al., 1982). Under natural photoperiod conditions, there is a gradual change between the light and dark phase each day, the intensity of darkness may vary considerably and the length of each phase changes gradually. It is likely that under natural lighting conditions perception of sunrise and sunset is different from the above mentioned experimental situations and the place of the photosensitive phase during the circadian rhythm may vary.

3.2. Temperature

In a recent 10-year survey of breeding records of a thoroughbred farm in Australia, Guerin and Wang (1994) reported significant difference between years in the time of the first ovulation. The authors concluded that the onset of reproductive activity was closely related to minimum and maximum environmental temperatures. The minimum and maximum temperatures in the weeks immediately prior to the first ovulation were similar in all years of the study. Field data for thoroughbred mares in the UK suggest that the spring transition is slowed by cold weather (Allen, 1987). Thus it appears that under similar conditions of photoperiod, nutrition and management system, temperature plays a role in the timing of the circannual reproductive rhythm.

3.3. Nutrition and body condition

The effect of nutrition and body condition on seasonal reproduction has been described by several authors. van Niekerk and van Heerden (1972) demonstrated that mares which receive supplementary diet of concentrates ovulate earlier after winter anestrus than control mares without supplementation also noted that the anovulatory period is shorter in mares which gain weight during early spring (Ginther, 1974). McDaniel et al. (1979) reported an additive effect of nutritional supplementation and artificially extended photoperiod on the onset of reproductive activity. Henneke et al. (1984) observed that the average interval to first ovulation was significantly longer in mares with condition score of less than 5.0 (scale from 1 to 9) compared to mares with condition score above 5.0. The interaction between energy intake and body condition in the reproductive performance of nonpregnant mares was evaluated by Kubiak et al. (1987). A high-energy intake shortens the interval to first ovulation in transitional mares with a low level of body fat but does not benefit mares in moderate or fat body
condition. Mares with a body fat content greater than 15% had shorter interval to first ovulation compared to those with a body fat content lower than 15%. The authors suggested that nonlactating mares should be brought into the breeding season with a body fat content above 15% and a body condition score above 5.0 and then maintained in a positive energy balance to obtain earlier onset of ovulation. Poor body condition not only affects the time of the transition phase and first spontaneous ovulation but also decreases the efficacy of administration of equine pituitary extract for the induction of ovulation (Bour et al., 1985). Not only energy intake but also the quality of dietary protein influences the onset of the breeding season. Animals receiving a high-quality protein diet exhibited increased FSH secretion and ovulated approximately 3–6 weeks earlier than mares on low-quality protein diet (van Niekerk and van Niekerk, 1997). The stimulatory effect of pasture grazing on the time of first ovulation has also been reported. First ovulation occurred over a large period of time in Thoroughbred mares that were housed inside at night and were allowed to eat grass on pasture for 4–6 h per day whereas pony mares that were kept in concrete yards during winter ovulated in synchrony after they were turned out to lush spring grass (Allen, 1987). Carnevale and Ginther (1997) demonstrated the beneficial effect of pasturing on the onset of cyclic ovarian activity. Anestrous mares pastured on green grass from early May ovulated sooner than mares housed on dry lot and fed hay. It is possible that the earlier onset of anestrus observed in young mares (Ginther, 1992) and the high incidence of anestrus in lactating mares (Palmer and Driancourt, 1983) are also related to nutritional factors. At present, an ongoing experiment in our laboratory clearly demonstrates the role of nutrition and body condition in influencing the onset of anestrus in mares. It is likely that studies related to the role of leptin will improve our understanding of the interaction between nutrition and seasonality.

4. Recent understanding of neuroendocrine control of seasonal reproduction

4.1. Role of melatonin

Over the last 20 years, melatonin has been the subject of many studies in horses and there is strong evidence that melatonin is one of the key elements in the control of seasonal reproduction. The role of melatonin in other species is beyond the scope of this review and has been reviewed elsewhere (Malpaux et al., 1999). It has been demonstrated that the pineal gland is implicated in the control mechanism of seasonal reproduction and translates the photoperiodic signals registered by the eye to endocrine signals. In mares, elevated melatonin concentrations are strongly associated with the dark phase. Melatonin secretion increases at the beginning of the dark phase and decreases rapidly at the end of the night. A short exposure to light during the dark phase results in an immediate decrease in melatonin concentration followed by a return to dark-phase melatonin concentrations when the light exposure is ended (Guillaume and Palmer, 1991; Palmer and Guillaume, 1992). During dark hours, melatonin secretion is stimulated by norepinephrine secreted by the postganglionic synaptic neurons of the ganglion cervicale superior. In the horse, this concept is supported by the observation
that isoproterenol, an α-adrenergic agonist, stimulates melatonin secretion (Sharp et al., 1980). Daily mean plasma melatonin concentrations are higher during autumn and winter than during spring and summer and HIOMT-activity, one of the key enzymes in melatonin synthesis, is highest during the anovulatory season and decreases significantly 2–3 months before the beginning of the ovulatory season (Wesson et al., 1979). The first direct evidence for a functional role of melatonin was provided by Grubaugh et al. (1982) using pinealectomized mares placed under extended photoperiod. In these mares, the onset of reproductive activity was not advanced by artificial photoperiod and winter pinealectomized mares resumed cyclic ovarian activity significantly later than controls during the second season after surgery. The role of melatonin in the transfer of the photoperiodic signal was further demonstrated by Guillaume and Palmer (1991) who reported that exogenous melatonin administered 4 h before the beginning of short nights (14.5L:9.5D) prevented the stimulatory effect of long days. Similarly, mares in seasonal anestrus placed under artificial photoperiod (14.5L:9.5D) do not respond to the stimulatory photoperiod when melatonin is administered every 2 h during a 12-h period that includes the 9.5-h dark period (Palmer and Guillaume, 1992). In these mares, the onset of darkness, marked by an increase in melatonin secretion, and the onset of daylight, marked by a decrease in melatonin secretion, were masked by the high levels of exogenous melatonin. Not only daily melatonin administration but also constant, long-term melatonin application influences seasonal reproduction in horses. Implants inserted near the shortest day of the year suppress the stimulatory effect of long days but does not prevent the occurrence of cyclic ovarian activity. Melatonin implants inserted near the summer solstice advanced the ovulatory season in the following year in adult mares, (Guillaume et al., 1995; Nagy and Huszenicza, personal communication). The likely explanation for the latter observation is a sequence of an early short-day perception, caused by the continuous high melatonin concentrations, followed by an early onset of the refractory state to short days. Under this refractory state, the annual reproductive cycle is phase advanced and is manifested in earlier occurrence of the ovulatory season next year. These observations support the concept that photoperiod and the rhythm of melatonin secretion entrain the endogenous circannual rhythm but do not influence reproductive activity directly. Although, the effect of photoperiod is well documented, the site of action of melatonin has not been studied in horses. We know from studies in other species that melatonin does not influence GnRH-secretion directly, but acts through a complex network of interneurons involving a number of different neurotransmitters and its target sites appear to be located within the hypothalamus (Malpaux et al., 1999). In horses, specific melatonin binding was found in the pars tuberalis, in the median eminence and in the suprachiasmatic nucleus (Stankov et al., 1991).

Melatonin concentrations do not always appear to reflect actual lighting conditions. Under natural photoperiod, the nighttime rise in circulating melatonin concentrations are sometimes difficult to distinguish at the beginning of the dark phase and can even be absent during the entire dark period (Fitzgerald and Schmidt, 1995; Nagy and Huszenicza, personal communication). It is uncertain if these mares lack a circadian rhythm or if the available methods for measuring melatonin concentrations in peripheral blood are inadequate to measure very slight changes in melatonin secretion between day and night. In sheep, it has been demonstrated that melatonin concentrations are several-fold higher...
in the third ventricle than in peripheral blood and it is postulated that in horses, as in sheep, circulating melatonin concentrations are a poor reflection of melatonin secretion (Skinner and Malpaux, 1999).

4.2. Role of neurotransmitters

Similarly to other seasonal breeders, the available data in mares suggest that the absence of reproductive activity during anestrus is the result of a suppression of GnRH secretion induced by several inhibitory neuronal systems within the hypothalamus. These neuronal systems mediate the effect of internal and external factors such as the endogenous rhythm, photoperiod, nutrition, and temperature, each perhaps acting through a partly independent pathway. It was recently suggested that a changing interaction exists between these inhibitory systems and that their relative contributions to the suppression of reproductive activity vary during the anestrous period (Kao et al., 1992; Bertrand et al., 1998). In horses, two different experimental approaches have been used to study the involvement of neurotransmitters in the control of GnRH and/or gonadotropin secretion. The role of neurotransmitters has been examined by determining the acute effect of agonists and antagonists on gonadotropin secretion (Fitzgerald and Mellbye, 1988; Irvine et al., 1994; Aurich et al., 2000). Antagonists and agonists have also been administered for prolonged periods of time to study the effect on ovarian activity and time of first ovulation in the spring (Besognet et al., 1996, 1997; Brendemuehl and Cross, 2000).

4.2.1. Opioids

In seasonally anestrous mares, administration of an opioid antagonist, naloxone, results in an immediate increase in LH secretion (Aurich et al., 1994). Irvine et al. (1994) reported that the naloxone-induced increase in gonadotropin secretion is dose dependent and the response curve is bell shaped. This may explain in part why Sharp et al. (1985) using high dose of naloxone (2 mg/BW kg) did not observe an effect of opioids on LH-secretion in anestrous mares. In addition, inhibition is increased during the anovulatory season and the occurrence of cyclic ovarian activity during the non-breeding season is associated with reduced opioidergic inhibition of the hypothalamic-pituitary axis (Turner et al., 1995; Davison et al., 1998).

4.2.2. Catecholamines

In addition to the opioid system, catecholaminergic neurons have been implicated in the seasonal suppression of GnRH-secretion. Xylazine, an α-adrenergic agonist, increases both FSH and LH pulse frequency in seasonally anestrous mares. The absence of the xylazine-induced increase in cyclic mares and mares exposed to extended daylength for 27 days indicates that the activity of this inhibitory catecholaminergic system decreases towards the breeding season (Fitzgerald and Mellbye, 1988).

The role of dopamine in reproduction has been the subject of extensive studies in many species. In ewes, dopamine mediates the estradiol negative feedback in seasonal
anestrous ewes (Havern et al., 1994). Gonadotropin secretion during anestrus appears to be inhibited in part by dopaminergic neurons that act directly on the GnRH secreting neurons (Pau et al., 1982; Kao et al., 1992; Le Corre and Chemineau, 1993; Havern et al., 1994). Systemic administration of dopamine antagonist to ovary-intact and ovariec-
tomized estrogen-implanted ewes or to rams, stimulates LH pulse frequency during the non breeding season but not during the breeding season (Meyer and Goodman, 1985; Meyer and Goodman, 1986; Le Corre and Chemineau, 1993; Tortorese and Lincoln, 1994). Similarly, gonadotropin secretion is increased when dopamine antagonist im-
plants are placed directly into the median eminence, dopamine pathways are destroyed by surgical or chemical intervention or after blockage of dopamine synthesis in the median eminence (Pau et al., 1982; Thiery et al., 1989; Havern et al., 1991; Bertrand et al., 1998; Vigué et al., 1998).

In the mare, dopamine concentration in the cerebrospinal fluid (CSF) is higher during the anovulatory period than during the breeding season (Melrose et al., 1990) and long-term treatment with dopamine D2-antagonists (sulpiride, perphenazine, domperi-
done) induces cyclic ovarian activity in seasonally anestrous mares (Besognet et al., 1996, 1997; Brendemuehl and Cross, 1998; Bennett-Wimbush et al., 1998). However, the effect of dopamine antagonist on the onset of reproductive activity is highly variable between experiments and individual mares and raises questions on the relative role of dopamine in the regulation of seasonal reproduction. In an ongoing study, we have observed that twice daily administration of dopamine D2 antagonist for 28 days success fully advances follicular development and the onset of cyclic ovarian activity in mares that had previously been under extended photoperiod for 28 days but did not result in early resumption of cyclic reproductive activity in mares that had not previously been exposed to long days and were presumed to be in deep anestrus (Nagy et al., 1999). These latter observations indicate that dopamine D2 antagonists only induce ovulation when mares have entered the transition from deep anestrus to cyclic ovarian activity and may explain the large variability in interval from the start of the treatment to first ovulation among mares and studies, and the failure of treatment in mares kept under unfavorable environmental conditions (Nequin et al., 1993; Besognet et al., 1996, 1997; Brendemuehl and Cross, 1998; Bennett-Wimbush et al., 1998; Daels et al., 2000).

The mechanism of action of dopamine in the control of seasonal reproduction is not clear in the horse. Recent data on gonadotropin secretion after dopamine antagonist administration questions the central/hypothalamic site of action of dopamine (Nequin et al., 1993; Daels et al., 2000). Administration of dopamine D2-antagonists to mares during anestrus did not induce acute changes in LH and FSH secretion (Nequin et al., 1993; Aurich et al., 2000). Long-term administration of a dopamine antagonist may have influenced FSH secretion and mean LH and FSH levels were significantly higher during the 7-day period preceding the first ovulatory LH peak in treated mares (Besognet et al., 1997). However, Brendemuehl and Cross (2000) found no effect on FSH secretion after domperidone treatment but both LH and estrogen conjugate levels were significantly increased by 28 days of treatment. Mean plasma FSH concentrations 30 days prior to ovulation were not different in perphenazine-treated and control mares (Bennett-Wimbush et al., 1998). Finally, FSH secretion parameters during a 24-h period of intensive
sampling remained unchanged in anestrous mares administered a dopamine antagonist, sulpiride (Daels et al., 2000). In the ram, sulpiride alone has minor effect on LH secretion but significantly enhances the stimulatory effect of naloxone during inhibitory photoperiod (Tortonese, 1999). This suggests an active interaction between the dopaminergic and the opioidergic systems in the control of seasonal reproduction and indicates that the effect of dopamine on GnRH secretion may not be inhibitory but rather permissive. Dopaminergic and opioidergic interactions have also been observed in the rat but researchers have not been able to demonstrate this interaction in the horse (Callahan et al., 1996; Aurich et al., 2000).

The controversial data on the role of dopamine in the control of gonadotropin secretion in mares leads us to suggest that dopamine antagonists may act at a different place of the reproductive axis, possibly the ovary. Dopamine antagonist administration results in important increase in prolactin secretion. Prolactin could exert its effect on the ovary by increasing the number of gonadotropin receptors and thus mediating the effect of circulating gonadotropins, as has been demonstrated in the male golden hamster and the female prepubertal rat (Advis and Ojeda, 1978; Advis et al., 1981; Klemcke et al., 1981, 1984). While prolactin receptors have been demonstrated on granulosa cells in pigs and hamsters (Oxberry and Greenwald, 1982; Bevers et al., 1988), no information is available on the presence of prolactin receptors in the equine ovary. However, administration of recombinant porcine prolactin hastened the date of first ovulation in seasonally anestrous mares (Thompson et al., 1997). Bromocryptin-induced decrease in prolactin secretion did not have an effect on follicular growth, steroidogenesis and ovulation in postpartum mares (Neuschaefer et al., 1991) but appeared to delay the growth of preovulatory size follicles without affecting the time of ovulation in seasonally anestrous mares (Bennett-Wimbush et al., 1998).

In conclusion, the available data suggest strongly that dopamine antagonists hasten the onset of cyclic ovarian activity in vernal transition mares but the treatment may be ineffective during deep anestrus. This finding suggests but does not completely confirm the hypothesis that dopamine, similarly to other neurotransmitters, exerts a tonic inhibition at the hypothalamic level in the control of seasonal reproduction in mares. It remains possible that dopamine antagonists exert their effect on ovarian activity by increasing the responsiveness of the ovary to circulating gonadotropins through a prolactin-induced increase in LH and/or FSH receptors.

4.2.3. Neuro-excitatory amino acids and serotonin

It was recently suggested that the cessation of reproductive activity during anestrus could be partly due to decreased stimulatory neuronal activity. In a preliminary study, N-methyl-DL-aspartic acid (NMA), an agonist of neuro-excitatory amino acid receptors, increased gonadotropin secretion both in intact and ovariectomized mares during anestrus (Fitzgerald, 1996). However, a role in the regulation of seasonal reproduction could not be demonstrated, since NMA induced similar increases in anestrous mares and mares exhibiting cyclic ovarian activity during the nonbreeding season (Fitzgerald and Davison, 1997). The effect of serotonin, involved in the steroid-independent suppression of GnRH-secretion in the anestrous ewe, has not been investigated in horses (Meyer and Goodman, 1986; Le Corre and Chemineau, 1993).
4.3. Role of thyroid hormones

In birds and in several mammalian species, the role of thyroid hormones in controlling seasonal reproduction has been firmly established (Nicholls et al., 1988; Moenter et al., 1991). In ewes, thyroidectomy during the anestrous period blocks the transition from the breeding season into anestrus. The effect of thyroidectomy can be fully prevented by the administration of exogenous thyroxine during the breeding season (Webster et al., 1991). In mares, thyroidectomy failed to alter the onset of anestrus (Porter et al., 1995) but plasma thyroid levels are significantly lower in anestrous mares compared to those that continue to exhibit estrous cycles during the anovulatory season (Fitzgerald and Davison, 1998; Huszenicza et al., 2000). It is suggested that T₃ and T₄ levels and reproductive activity are regulated by similar hypothalamic control mechanisms. Compared to ewes, thyroid hormones do not seem to play an important role in the control of seasonal reproduction in mares.

5. Practical problems associated with the management and study of the anestrous mare

The transition in and out of the anestrous period is a gradual process in mares and presents us with several challenges with regard to the clinical management and the study of anestrous in mares. These challenges include (1) prediction of the occurrence and onset of anestrus, (2) diagnosis of anestrus, (3) estimation of the stage/depth of anestrus, (4) prediction of the time of spontaneous return to cyclic ovarian activity and (5) the appropriate time to start a treatment to stimulate the return to cyclic activity.

At present, there is no method to predict the onset of anestrus. The comparison of anestrous mares and those that continue to exhibit cyclic ovarian activity during winter might lead us to understand better why and when anestrus occurs. In advance, we can only estimate the probability of anestrus in a population based on reproductive history. Young mares and those that foaled and nursed during the previous breeding season have the highest probability to enter seasonal anestrus. In contrast, well-fed barren mares kept indoors have a much lower probability to enter anestrus (Palmer and Driancourt, 1983).

The diagnosis of seasonal anestrus can be done either by serial ovarian examination or by progesterone determination and is part of routine management programs at the beginning of the breeding season. Repeated (three to four times) plasma progesterone determination is recommended at weekly intervals with mare-side qualitative or quantitative immunoassays (Allen and Sanderson, 1987; Nagy et al., 1998a,b). Care should be taken to determine the reproductive status close to the time of breeding as some mares may enter anestrus late in January or February (Fitzgerald and Schmidt, 1995; Nagy et al., 1998a,b).

Endocrine and ovarian changes are well defined during the anestrous period, but the different phases are not precisely described. Clinical determination of the stage of anestrus is usually based on palpation per rectum, ultrasonography and calendar date. Mares with small, firm ovary and follicles smaller than 15 mm during January and early February are considered to be in deep anestrus. Mares with larger ovaries and follicles of
20–35 mm in late February and March are thought to be in transitional phase. However, follicular activity does not necessarily represent hypothalamic-pituitary status or depth of anestrus. In a recent study, we observed that FSH-secretion parameters and interval to first ovulation varied significantly between mares with similar ovarian activity (Daels et al., 2000). Some mares develop several anovulatory follicular waves before the first ovulation of the year while others do not show significant follicular growth before the ovulatory follicular wave (Ginther, 1990).

Clinicians are frequently asked to predict the time of first spontaneous ovulation or determine the appropriate time for induction ovulation. It has been demonstrated that the steroidogenic capacity of the preovulatory follicles increases from early transition to late transition (Seamans and Sharp, 1982; Davis and Sharp, 1991). In a recent study, Roser et al. (1997) demonstrated that urinary estrogen conjugates (≥46–50 ng/mg Cr) and plasma estradiol (≥10 pg/ml) appear to be the most reliable indicators of first ovulation of the year. It is a common belief that increased uterine echotexture, reflecting estrogen production by the developing follicle(s), indicates impending ovulation in transitional mares. We have observed that anestrous pony mares, ovulating at the end of April, may have preovulatory size follicles in January accompanied by uterine changes similar to those observed during normal estrus. Thus it appears that the presence of edema is not a reliable sign of impending ovulation in anestrous mares. Other parameters such as follicle size, uterine tone and behaviour are erratic and poor predictors of impending ovulation (Roser et al., 1997). In conclusion, breeders and veterinarians do not have a reliable parameter to estimate the time of first ovulation of the year.

6. Methods available to induce ovulation, cyclic ovarian activity early in the season

Owing to the economical pressure, there has always been an interest in treatment protocols for the induction of ovulation in anestrous mares. The most common and reliable method for induction of cyclic ovarian activity remains artificial photoperiod but other techniques are available and could also be useful in clinical practice especially when combined with artificial photoperiod.

6.1. Light treatment

Since the original report of Burkhardt (1947), many studies have demonstrated the beneficial effect of extended photoperiod on the onset of ovarian activity in early spring (Ginther, 1992). At present, this is the most reliable method to bring mares from deep anestrus into vernal transition. Extended daylight treatment (14.5–16 h daily light exposure, 100 lx) should be applied starting at the winter solstice and may be applied without a gradual transition from short to long days. Follicular activity is significantly increased after 2 weeks of artificial photoperiod, and ovulation occurs approximately 6–12 weeks after the beginning of treatment.

If the artificial photoperiod is started later (January), mares may not ovulate earlier than mares under natural photoperiod (Scraba and Ginther, 1985). Interestingly, daily exposure to 20 h of light does not result in maximal stimulation indicating that minimum
amount of dark phase is also necessary (Palmer et al., 1982). Alternatively, one can take
advantage of the photosensitive phase of the mare by applying 1–2 h of light 9.5 h after
the beginning of darkness. In practice conditions, the disadvantage of this technique is
that the time of the flash must be adjusted to the changing onset of darkness or the onset
of darkness must be controlled artificially.

Traditionally, the recommended intensity of the light source is around 100 lx and the
treatment is continued beyond the first ovulation. Recently, we have shown that either a
35-day light exposure (100 lx, 14.5 h per day) or low light intensity (10 lx continued
beyond the first ovulation) are sufficient to hasten the onset of cyclic ovarian activity
(Guillaume et al., 2000).

6.2. Pituitary extracts

Multiple ovulations can be induced in seasonally anestrous mares using equine
pituitary extracts containing eFSH and eLH but such products are not available
commercially and for this reason, are not used in practice (Lapin and Ginther, 1977;
Hofferer et al., 1991).

6.3. GnRH

Following the original work of Evans and Irvine (1977), several studies demonstrated
that GnRH or its analogues can induce ovulation in seasonal anoestrous mares (Palmer
and Quellier, 1988). GnRH should be administered for 2–3 weeks and both pulsatile and
continuous administration was found to be successful (Hyland, 1993). GnRH can be
administered by repeated daily intravenous injections, by external pulsatile-release
pumps, by subcutaneously implanted continuous-release osmotic minipumps or by
low-dose, slow-release subcutaneous implants. From the available data, it appears that
the efficacy of GnRH treatment depends in part on the stage of anestrus at the onset of
treatment and in part on the mode of administration. Mares in deep anestrous may fail to
respond to continuous administration but pulsatile systems appear as effective during the
early and the late spring (Hyland, 1993). Due to its cost and the preferred modes of
administration, GnRH and its analogues do not have an important place in the manage-
ment of anestrous mares in practice. McKinnon et al. (1997) have recently demonstrated
that ovulation can be induced in transitional mares by repeated administration of a
short-term, slow-release subcutaneous implant containing GnRH analogue, deslorelin. It
is possible that this commercially available preparation developed for cyclic mares may
become of value for vernal transition mares in the future.

6.4. Progestogens

Progestogens have been widely used to hasten the onset of cyclic ovarian activity in
seasonally anoestrous mares (Squires, 1993). There is still a debate on the effectiveness
of prolonged (8–15 days) progestogen administration in anestrous mares. Some authors
claim that the treatment does not affect the mean time of ovulation but serves to
synchronize the onset of ovarian activity (Alexander and Irvine, 1991). Similarly to
GnRH, the outcome of the treatment depends on the stage of anestrus and ovarian activity at the beginning of treatment. Daily progestogen administration does not induce ovulation in deep anestrus but ovulation occurs within 15 days after the end of treatment when applied during vernal transition (Squires, 1993; Nagy et al., 1998a,b). Mares may ovulate during treatment. For this reason, it is recommended to administer prostaglandin at the end of treatment. Progestogens have also been combined with estradiol 17-β but this combination does not seem more effective in inducing ovulation in anestrous mares than progestogens alone (Wiepz et al., 1988).

6.5. Dopamine antagonists

As has been discussed above, dopamine D2-antagonists may offer a practical alternative to hasten the onset of cyclic ovarian activity in seasonally anestrous mares. Daily administration of domperidone (1.1 mg/kg PO) or sulpiride (0.5 mg/kg IM) results in earlier ovulation in treated compared to control mares (Besognet et al., 1996, 1997). The period of treatment to induce ovulation varies depending on the stage of anestrus before treatment. In vernal transition mares, ovulation is induced after 12–22 days of treatment, however, in deep anestrous mares a prolonged period of treatment (50–60 days) is required (Daels et al., 2000). For this reason, it is recommended to apply dopamine antagonists in vernal transition mares or include a period of artificial photoperiod before the period of dopamine antagonist administration.

6.6. Combinations

The reproductive management of anoestrous mares can be made more efficient when combining some of the abovementioned techniques. The combination strategy that is already in use is based on a classic light treatment to induce cyclic ovarian activity, combined with a period of progestogen with or without estradiol treatment to synchronize or time ovulation at the end of the light treatment. This method has allowed the reduction of the variable internal from start of light treatment to first ovulation. The second combination strategy emerges from the studies on the role of dopamine in anestrus. Experiments over several years suggest that the interval from start of dopamine antagonist treatment to first ovulation is significantly shorter when mares are placed under extended daylight (Nagy et al., 1999; Daels et al., 2000). In these experiments, the mean interval from the beginning of the treatment to first ovulation has ranged from 15 to 18 days. These observations lead us to conclude that a combination of photoperiod and dopamine antagonist treatment may result in a shorter total treatment period (photoperiod plus dopamine antagonist) and a more predictable time of first ovulation. Experiments are currently underway to test this hypothesis.

7. Conclusion

In recent years our understanding of the regulation of seasonal regulation of reproduction in mares has increased significantly. This new insight will undoubtedly lead to
better treatment strategies for the induction of cyclic ovarian activity in the early spring. Hopefully, in the coming years we will also have a better understanding of mechanisms controlling the development of anestrus. With the continued pressure of the equine industry to develop methods to extend the breeding season, it appears that continued research in this area remains fully justified.

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