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EFFECTS OF PHOTOPERIOD, HOUSING AND DIET ON WINTER ANESTRUS IN MARES

By

Susan Lynn Woodley

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ABSTRACT

EFFECTS OF PHOTOPERIOD, HOUSING AND DIET
ON WINTER ANESTRUS IN MARES

By

Susan Lynn Woodley

In two experiments, mares were provided daily with a 16 hour light:8 hour dark period for four to seven months to determine factors controlling winter anestrus. Mares were housed outdoors. Serum progesterone was measured biweekly to estimate the time of ovulation. Body weight change, dietary grain supplementation and type of housing were also studied.

Experiment I, compared natural daylight (n = 6) to a 16 hour light period begun on September 16 (n = 6) or December 1 (n = 6). Mares in September and December light treated groups averaged more ($P < 0.05$) ovulations (4.0 and 3.2, respectively) than control mares (1.5) but the length of the anovulatory period was not significantly different.

Experiment II, included 40 mares in a factorially designed test of two levels each of photoperiod, dietary energy and housing beginning on December 17, 1976. Mares treated with a 16 hour photoperiod averaged 1.1 ovulations, whereas mares on natural daylength averaged 0.1 ovulations. Body weight changes and the interval to ovulation were not significantly effected by level of dietary energy. Group housed mares lost less weight (1.7%) than individually housed mares (7.3%) but did not ovulate sooner. At the end of the experiment, glucocorticoid concentrations were similar for individually (10.8 ng/ml) and group housed (10.4 ng/ml) mares.

This data indicates that 16 hour daily photoperiod treatment is a major factor in decreasing the winter anovulatory period in mares.

BIOGRAPHICAL SKETCH

I was born on July 16, 1955 in Ann Arbor, Michigan. I attended grade school in Trenton, Michigan and graduated from high school at the Academy of the Sacred Heart in Bloomfield Hills, Michigan in June, 1973. As a high school senior I attended another Sacred Heart school in London, England.

As an undergraduate I studied zoology at Michigan State University. I received a Bachelor of Science degree from the Honors College in December, 1976. In January, 1977, I began my studies toward the M.S. degree. During 1979, I worked as a graduate research assistant for Dr. P.A. Noden in the Department of Obstetrics an Gynecology at M.S.U. I matriculated in the M.S.U. College of Human Medicine in September, 1979. The publication of this thesis marks the culmination of invaluable experiences in research for me. My research exposure and the people with whom I have worked has left a lasting impression. I feel that they have given me useful tools with which I can further my understanding of basic and clinical sciences and more importantly, with which I can share such understanding with others.

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INTRODUCTION

Prior to 1833, the universal birthdate of registered horses was specified as May 1. Accordingly, each horse aged by one year on May 1 regardless of its biological birthdate. However, substantial numbers of foals are born between November and May. The May 1 birthdate caused difficulty at the track and in the show arena because several horses automatically changed age groups in the middle of the showing and racing season. In 1833, the English Jockey Association moved the birthdate to January 1 for Thoroughbreds and since then most other horse registry associations have adopted January 1 as the official birthdate. The legislated January 1 birthdate has created problems for breeders because many mares are seasonally anestrus from November through April each year.

The gestation period is eleven months in the mare. For foals to be born soon after January 1, conception must occur near February 15. The mare, however, is a seasonal breeder and less than 25% of mares are ovulating during the winter months. Long periods of estrus associated with ovulation failures are more frequent in mares during the period from January through April and this also lowers conception rates.

It has long been observed that increased conception rates in the mare coincide closely with increasing daylength. Attempts to improve the conception rates of mares during the early breeding season (December through March) have included hormonal stimulation

of ovarian function, nutritional supplementation of the diet and artifically lengthening daily light periods. The most successful attempts have focused on the use of an artifically extended daily light period.

The objective of these studies was to determine whether the type of ourdoor housing and the level of nutrition influenced the length of the winter anovulatory period in mares maintained on an artificial 16 hour light:8 hour dark photoperiod. Our major objective was to determine which combination of these factors would minimize the seasonal anovulatory period in mares maintained at outdoor temperatures during the winter when provided with an artificial 16 hour photoperiod.

REVIEW OF LITERATURE

Reproductive Patterns in the Mare

The Occurrence of Estrus, Anestrus and Ovulation

> "But it may equally plausibly be argued that monoestrum is simply decentralized polyoestrum. There are instances among domesticated animals of monoestrus animals with a tendency to polyoestrum (bitch), and of polyoestrus animals with a tendency to monoestrum (mare). So also among wild animals there are instances of animals which are monoestrus in one climate and apparently polyoestrus in another (Scurius vulgaris) (compare Bell, 1974 and Latarte, 1887)."

Walter Heape's analysis of the sexual season in mammals (1900) proved to be particularly insightful in regard to the mare. Throughout the 20th century, several surveys concerning the mare's breeding season have been conducted and although similar general patterns emerge, frequently one report conflicts with the next. Differences among reports are often so striking that one must conclude that the mares breeding season is a labile characteristic.

Küpfer (1928) observed large herds of donkey and horse mares on the open range and noted that the breeding season was confined within the South African spring and summer. The incidence of estrus and ovulation ranged between 1 and 3 times per year in individual mares and estrus occurred at irregular intervals (Küpfer, 1928). Satoh and Hoshi (1932) reported that the semi-wild Korean mare tended toward monoestrum during a spring and summer breeding season. When the first estrus of the year was observed in 78 cases over a five

year period in Japan, 58.9% occurred in April, 24.3% in March, 16.7% in May and in all cases, the mare was anestrous by the following December (Nishikawa, 1959).

Aitken (1927) and Trowbridge and Hett (1936) observed that draft mares maintained under ordinary farm conditions in the U.S. came into estrus throughout the year at approximately 22 day intervals. Similarly, Topp (1937) failed to find an anestrus period in 9 light weight mares observed in Germany. Berliner (1942) reported seeing very few cases of anestrus in Thoroughbred and other light weight mares. Between 63% and 100% of stabled Thoroughbred, light weight and Percheron mares in South Africa showed estrus during any given month of the year (Quinlan *et al.*, 1951). Analysis of individual animals revealed that none of them showed a regular rhythm throughout the year and that the length of the cycle was longer (35.9 *vs* 23.8) and more variable during the fall and winter months.

A similar pattern was observed by Van Niekerk (1967) who noted that the percentage of mares showing heat in any month varied between 53% and 100%. However, a more dramatic change from 20% to 100% occurred in the percentage of mares ovulating (Van Niekerk, 1967). Analysis of slaughter house specimens in England revealed that 16% to 63% of ovarian pairs had at least one functioning corpus luteum during the winter months (Arthur, 1958). Several thousand reproductive tracts collected in Australia revealed 18.5% mid-winter as opposed to 91.5% mid-summer specimens contained a functional corpus luteum (Osborne, 1966). Of four mares studied in California, only one cycled regularly throughout the year while the other three experienced 2 to 3.5 month anovulatory periods (Evans *et al.*, 1971). Ginther (1974) reported

that pony mares in Wisconsin had an anovulatory period which averaged 214 days.

The majority of authors have observed that more than half of all mares show estrus during the winter months (Trowbridge and Hett, 1936; Andrews and McKenzie, 1941; Quinlan *et al.*, 1951; Van Niekerk, 1967). Still, reports indicate that less than one fourth of all mares actually ovulate during this season (Arthur, 1958; Osborne, 1966; Van Niekerk, 1967; Evans *et al.*, 1971). This discrepancy between the fraction of mares showing estrus and the fraction which ovulate during the winter months may indicate that estrous periods are not always accompanied by ovulation as many authors have observed (Küpfer, 1928; Satoh and Hoshi, 1933; Nishikawa, 1959; Van Niekerk, 1967; Ginther *et al.*, 1972). Several authors have noted extended estrus periods, often in association with irregular cycles, decreased fertility and ovulatory failure in early spring as opposed to mid-summer (Satoh and Hoshi, 1933; Trowbridge and Hett, 1936; Caslick, 1937; Day, 1940; Andrews and McKenzie, 1941; Berliner, 1942; Quinlan *et al.*, 1951; Nishikawa, 1959; Van Niekerk, 1967; Ginther *et al.*, 1972). Trowbridge and Hett (1936) reported that estrus averaged 8.9 days but ranged from 2 to 40 days through the year with the longer periods associated with enlarged ovaries and infertility. Berliner (1942) noted that mares showed extended and irregular periods of estrus prior to recovery from wintering. Day (1940) observed periods of estrus ranging from 3 to 54 days in length. Periods observed in the early spring were usually 11 to 20 days as compared to 4 to 9 days in late summer (Day, 1940). Andrews and McKenzie (1941) observed the average length of the first four periods of the year to be similar

although the length of the first two cycles ranged from 1 to 37 days while that of the second two cycles decreased to between 2 and 10 days. Achneldt and Plas (1946) reported that the average duration of estrus decreased from 8.2 and 9.4 days in February and March to 4.1 days in July. The maximum number of disturbances in estrus and ovulation were seen in February and these disturbances disappeared by late spring and summer. Also, the highest conception rate was observed between May and July. The authors, therefore concluded that in Germany the physiological breeding season of the mare consists of May, June and July (Achneldt and Plas, 1946). Strikingly similar results were obtained when mares were studied in South Africa (Quinlan *et al.*, 1951). The duration of estrus decreased from 8.3 and 9.2 days in August and September to 4.6 during the mid-summer month of January. Also, the percentage of fertile services reached a maximum during the period from November through January (Quinlan *et al.*, 1951). Van Niekerk (1967) pointed out that this pattern could be reflective of shorter estrous periods and thus, a smaller total number of services rather than an actual increased conception rate. As many as 85% of the estrous periods observed in May, June and July were characterized by follicular growth accompanied by ovulatory failure and this percentage gradually decreased to zero by January (Van Niekerk, 1967). These figures may also explain, in part, the results obtained by Quinlan *et al.* (1951) where no conceptions occurred between April and July and breeding efficiency as measured by percentage fertile services was greatest in November and December.

In the United States, Caslick (1937) observed that 38 out of 52 barren and maiden mares were in heat for a total of 1562 out of a

possible 2130 days, or 71% of the time between February 14 and April 14. The majority of these mares went out of the "continuous estrus" late in March and began "normal cycles" (Caslick, 1937). Küpfer (1928) associated very long and very short estrous periods with ovulatory failure. Nishikawa (1959) reported that 41% of first estrous periods of the year were accompanied by ovulation. As few as 50% of all first estrous periods of the year were considered to be of normal length: 4 to 10 days in duration. However, while 61% of normal length first estrous periods were accompanied by ovulation, only 24% of abnormal length periods were associated with ovulation (Nishikawa, 1959). Pony mares in Wisconsin ovulated 7.2 times within a 12 month interval and 6.8 of these ovulations were associated with estrus (Ginther, 1974). These mares were observed to have 7.1 anovulatory estrous periods and 3.8 of these periods preceeded the breeding season (Ginther, 1974). Furthermore, there was a positive but non-significant correlation between the times of onset of an individual mare's ovulatory season in two consecutive years. A positive but non-significant correlation was also found between the beginning and end of the season within a mare. However, a negative and significant correlation ($r = -0.75$) existed between the time of onset of the ovulatory season and the total length of the season. These data suggest that the longer length of the breeding season is largely determined by the time of year that the first ovulation occurs (Ginther, 1974).

The information presented on the preceeding pages is not conclusive but it elucidates at least two important facts about reproductive patterns in the mare. First, the mare's breeding season

is certainly a labile characteristic under different managerial and environmental conditions and when different end points are measured. This is especially important when extended anovulatory estrous periods which occur in late winter and early spring are taken into account. Secondly, regardless of the inherent variation in conditions, a rhythmic pattern in the reproductive function of the mare emerges wherein a maximum and minimum in ovarian function is seen during the summer and winter months, respectively. The fall and spring may then be called transition periods wherein ovarian function is at a level intermediate between these two extremes.

The Estrous Cycle

Reproductive function in the majority of non-pregnant mares consists of two stages during the year; anestrus and polyestrus. Most non-pregnant mares are anestrous during the winter and polyestrous during the summer. The polyestrous season is preceded by a transitional anovulatory phase. Ovulation accompanies estrus behavior in the late spring and through the summer for most mares and has been called the physiological breeding season (Achneldt and Plas, 1946; Burkhart, 1947; Kenney *et al.*, 1975). In this thesis, the physiological breeding season will be referred to as the ovulatory season.

An average of 20 to 22 days was observed when the estrous cycle length was measured during the ovulatory season (Aitken, 1927; Satoh and Hoshi, 1933; Trowbridge and Hett, 1936; Asdell, 1946). The estrous cycle can be separated into two phases which are classically referred to as estrus and diestrus and have been

characterized with regard to the mare (Andrews and McKenzie, 1941; Van Niekerk, 1967; Noden *et al.*, 1974).

In most studies the estrous phase of the estrous cycle is characterized as the period when a mare will accept a stallion for copulation. During the ovulatory season, the average estrous period lasts 5.2 days for mares (Day, 1940; Andrews and McKenzie, 1941; Nishakawa, 1959; Van Niekerk, 1967; Ginther *et al.*, 1974; Noden *et al.*, 1974). Trum (1950) noted that periods of estrus ranged from 2 to 40 days in length when he observed 1500 Thoroughbred estrous cycles between March and July. As many as 83% of the estrous periods were between 2 and 7 days in length and none of the periods which occurred during June and July were greater than 7 days long (Trum, 1950). No appreciable differences in the duration of estrus have been observed among draft, Thoroughbred and other light weight mares (Andrews and McKenzie, 1941; Van Niekerk, 1967).

The diestrous phase of the estrous cycle is characterized by the refusal of a mare to accept a stallion for breeding (Andrews and McKenzie, 1941; Asdell, 1946). While diestrus cannot be distinguished from seasonal anestrus on a behavioral basis, it is associated with the interval between successive ovulatory estrous periods (Asdell, 1946; Ginther *et al.*, 1972). The diestrous phase of the estrous cycle averaged 15 days (Day, 1940; Andrews and McKenzie, 1941; Ginther *et al.*, 1972; Noden *et al.*, 1974). Although 54% of diestrous periods lasted between 14 and 18 days, as many as 30% were less than or equal to 13 days in length (Trum, 1950) and there was no correlation between the length of estrus and the subsequent diestrus. Day (1940) noted

that diestrous periods of less than 11 days in length occurred only when the preceding estrus was anovulatory.

Several authors have correlated the temporal pattern of progesterone concentrations in the peripheral circulation with events of the estrous cycle. Briefly, the highest levels of progesterone have been observed in the middle of diestrus, 7 to 10 days following the previous ovulation (Smith *et al.*, 1970; Sharp and Black, 1973; Noden *et al.*, 1974; Evans and Irvine, 1975). Progesterone levels declined rapidly during late diestrus and reach a nadir during estrus when concentrations were less than 1.0 ng/ml (Smith *et al.*, 1970; Sharp and Black, 1973; Noden *et al.*, 1974; Evans and Irvine, 1975; Plotka *et al.*, 1975). Peripheral plasma progesterone concentrations increased 100% by 24 hours after ovulation, concomitant with the end of estrus and continued increasing to mid-diestrus (Sharp and Black, 1973; Noden *et al.*, 1974; Evans and Irvine, 1975; Plotka *et al.*, 1975). Plotka *et al.* (1975) and Van Niekerk *et al.* (1975) described increasing serum progesterone levels corresponding to the formation of the corpus luteum after ovulation. Van Niekerk *et al.* (1975) also drew a correlation between temporal serum progesterone concentrations and progesterone concentrations in the corpus luteum.

In the non-pregnant mare, plasma progesterone concentrations remained low (< 1.0 ng/ml) during the winter anestrous period (Palmer and Jousset, 1975; Oxender *et al.*, 1977). With the onset of the ovulatory season, serum progesterone concentrations rise and fall in successive ovulatory cycles with subsequent corpus luteum formation and regression (Oxender *et al.*, 1977).

The profile of circulating concentrations of estradiol 17β during the estrous cycle reciprocates that of progesterone. The lowest concentrations of estradiol 17β have been observed during diestrous (Pattison *et al.*, 1974; Noden *et al.*, 1975). Estradiol 17β concentrations increased gradually at the onset of estrus concurrent with decreased circulating progesterone (Pattison *et al.*, 1974; Noden *et al.*, 1975). Peak serum concentrations of estradiol occurred 2 days prior to ovulation and represented a 5-fold increase over diestrous concentrations. Thereafter, serum levels gradually decreased to basal levels within five days (Pattison *et al.*, 1974; Noden *et al.*, 1975).

During seasonal anestrus, serum concentrations of estradiol 17β remained low and averaged less then 2.5 pg/ml (Oxender *et al.*, 1977). However, prior to the onset of the ovulatory season, sporadic 10-fold increases in estradiol levels have been observed in many mares. Once ovulation had occurred, estradiol concentrations began a regular cyclic pattern (Oxender *et al.*, 1977).

Serum concentrations of luteinizing hormone (LH) followed a pattern similar to that of estradiol 17β during the estrous cycle. LH concentrations are lowest from 5 days after ovulation until the end of the diestrous period (Whitmore *et al.*, 1973; Noden *et al.*, 1974; Pattison *et al.*, 1974; Evans and Irvine, 1975). A 50% increase in serum LH concentrations occurred on the last day of diestrus (Whitmore *et al.*, 1973; Noden *et al.*, 1974). Thereafter, serum LH concentrations increased gradually until maximal levels occurred one day after ovulation, representing a 5-fold increase over basal diestrous levels (Whitmore *et al.*, 1973; Noden *et al.*,

1974; Pattison *et al.*, 1974; Evans and Irvine, 1975). Then, LH levels decreased gradually over the next four days and basal levels were reached at five days post-ovulation (Whitmore *et al.*, 1973; Noden *et al.*, 1974; Pattison *et al.*, 1974).

Although apparently normal serum LH levels occurred during the last ovulation of the ovulatory season, LH concentrations remained low as the mare entered the winter anovulatory season (Snyder *et al.*, 1979). A similar phenomenon is noted in the ovariectomized mare where, with the absence of gonadal feedback, serum LH concentrations were elevated and comparable to estrous levels throughout the ovulatory season but LH levels were low and nearer diestrus levels throughout the non-breeding season (Garcia and Ginther, 1976; Oxender *et al.*, 1977; Freedman *et al.*, 1979). As with estradiol 17β, sporadic increases in serum LH concentrations prior to the onset of the ovulatory season have been noted for some mares (Oxender *et al.*, 1977).

Serum concentrations of follicle stimulating hormone (FSH) were lowest during estrus and reach their maximum during the mid-diestrus in the mare (Evans and Irvine, 1975; Miller *et al.*, 1977). The mid-cycle elevation occurred 10 days prior to ovulation and represented a 4-fold increase over estrous concentrations (Evans and Irvine, 1975; Miller *et al.*, 1977). There is disagreement as to whether a secondary post-ovulation peak in serum FSH concentration occurs in the mare. Evans and Irvine (1975) reported post-ovulatory elevations of FSH levels of the same magnitude as the mid-cycle peak. Miller *et al.* (1977) reported that serum FSH

concentrations increased 2-fold at one day after ovulation and then fluctuated until a mid-cycle peak occurred at day 15.

Unlike LH, progesterone and estradiol, serum FSH concentrations are not lowest during winter anestrus in the mare (Turner-Hoffman *et al.*, 1978; Freedman *et al.*, 1979), although a significant increase in mid-month levels was reported in ovariectomized mares in the spring (Freedman *et al.*, 1979). Snyder *et al.* (1979) observed high serum levels of FSH 18 days after the last ovulation of the season as mares entered winter anestrus. Evans and Irvine (1975) noted similar increases in serum FSH concentrations at 10 and 20 days following conception and suggested that an inherent 10 day rhythmic pattern existed.

The ovulatory follicle is first palpable on the ovary an average of 9 days prior to ovulation and increases to a diameter of 40 to 50 mm prior to ovulation in the mare (Satoh and Hoshi, 1933; Woodley *et al.*, 1979). Stabenfeldt *et al.* (1972) observed that the follicular phase lasted 7 days in mares. As in other animals, several follicles grow during each estrous cycle but the majority of follicles become atretic and do not ovulate (Kenney *et al.*, 1979; Vandeplassche *et al.*, 1979). The largest population of follicles greater than 20 mm in diameter, the greatest diameter for the largest follicle and the smallest population of follicles less than 20 mm in diameter were observed during the ovulatory season (Turner-Hoffman *et al.*, 1978). The converse was true during the winter anovulatory season where the diameter of the largest follicle as well as the number of follicles greater than 20 mm in diameter was substantially decreased, but the number of follicles less than 20 mm in diameter

increased (Turner-Hoffman *et al.*, 1978). Freedman *et al.* (1979) reported that a decline in serum FSH levels occurred 16 days before the first ovulation of the ovulatory season and appeared to precede and parallel the decline in the number of small follicles. Furthermore, declining FSH levels were followed by increasing levels of LH which were temporally associated with the pre-ovulatory growth of the follicle (Freedman *et al.*, 1979).

Control of the Breeding Season

Photoperiod

The most successful attempts to stimulate ovulatory activity in mares early in the year for January and February breeding have involved the use of artifically extended photoperiods. Burkhardt (1947) subjected 4 previously barren mares to a gradually increasing photoperiod and noted ovarian recrudescence and estrus within 40 to 70 days compared to nearly 100 days in three control groups. Nishakawa (1959) found that mares came into heat approximately 75 days after the photoperiod was increased to 16 hours on November 12. This represented an average of 79 days earlier than estrus had occurred in previous years in the same mares (Nishakawa, 1959). There was no difference in effect when the photoperiod treatment was applied with a 200 watt or a 400 watt incandescent light bulb (Nishakawa, 1959). Furthermore, two of these mares which were exposed to a four hour daily photoperiod beginning in February, continued to cycle through the spring and artificial extension of the daily photoperiod beginning in August, did not prevent seasonal anestrus (Nishakawa, 1959).

Loy (1968) condensed the normally occurring changes in the natural photoperiod into a six month period and noted that mares ovulated from 60 to 90 days after the photoperiod was extended beginning in December. In two separate field trials involving several different farms, supplemental lighting was applied beginning in November and gradually increased until a 19 hour total daily photoperiod was obtained by May (Loy, 1968). In both of these trials, mares receiving 2 to 110 footcandles of supplemental light delivered more than 50% of their foals by late March in comparison to 20% for mares in control groups (Loy, 1958). In accordance with the "early breeding" rule adopted by the U.S. Trotting Association in 1969, Cooper (1972) applied additional daily lighting with a single 200 watt light bulb to 62 mares in gradual increments beginning on October 1. The author found that 13%, 32% and 24% of the mares conceived in December, January and February, respectively, in contrast to control groups wherein 30%, 30% and 6% conceived in May, June and July, respectively (Cooper, 1972). Overall breeding season conception rates were 66% and 69% for the group receiving supplemental lighting and the control group, respectively (Cooper, 1972).

Sharp and Ginther (1975) subjected two groups of pony mares to controlled environments simulating outdoor temperature, humidity and daily photoperiod at a light intensity of 600 footcandles, beginning in October. The control group was subjected to conditions simulating October through February while the "treated" group was exposed to conditions simulating March through July. Mares exposed to the spring and summer environment had more follicles greater than

10 to 20 mm in diameter and had larger sized follicles than the control mares by day 70 of the treatment period. The treated mares were in estrus an average of 96 days after the start of the experiment and 2 had ovulated by the end of the 120 day treatment regimen while no estrus behaviors or ovulations were observed in the controls (Sharp and Ginther, 1975). Kooistra and Ginther (1975) exposed pony mares to 9, 16 or 24 hours of light per day at an intensity of 13 to 43 footcandles between November and June. The photoperiod treatments in these three groups were applied abruptly without a gradual increment but a fourth group of mares was exposed to the natural length photoperiod which changes by approximately 2 minutes per day. Mares receiving 16 hours of light per day ovulated after 107 days of treatment. This was 49 days earlier than for mares subjected to the continuous photoperiod treatment and at least 85 days earlier than mares receiving either 9 hours of light per day or the natural length photoperiod (Kooistra and Ginther, 1975). Mares exposed to a 16 hour daily photoperiod also began shedding their hair in tufts after 56 days of treatment and acquired a smooth haircoat within 146 days. These figures were also significantly lower than for mares in the other 3 groups (Kooistra and Ginther, 1975). Oxender *et al.* (1977) reported that mares housed in a heated barn and exposed to artificial lighting at an intensity of 160 lux and a daily photoperiod which was gradually increased to 16 hours of light per day beginning in December, ovulated 59 days after the onset of the experiment. This was 50 days earlier than mares housed in the same building but exposed to the natural length photoperiod through windows ($\sim$ 30 lux) and 74 days earlier than two of the five mares that were housed outdoors and

exposed to the naturally occurring winter conditions (Oxender *et al.*, 1977). However, the mares receiving a 16 hour daily photoperiod, having their first ovulatory estrus period occur in February, stayed in estrus for 13 days; nearly twice as long as mares that ovulated later in the year (Oxender *et al.*, 1977).

Freedman *et al.* (1979) measured increases in circulating levels of FSH and LH in ovariectomized pony mares receiving a 16 hour daily photoperiod that occurred 3 months earlier than parallel increases measured in contemporary control mares receiving the natural photoperiod. Standardizing the indices of follicular development and/or gonadotrophin profiles to the time of the first ovulation abolished all differences between the photoperiod treatment groups in both intact and ovariectomized groups of pony mares (Freedman *et al.*, 1979).

Sharp *et al.* (1979) obtained similar results between ganglionectomized mares, sham-operated and control mares. The authors postulated that the photoperiodic effect on sexual recrudescence in the mare is mediated via the pineal gland and that the primary nervous pathway for delivering photic information from the eyes to the pineal involves the superior cervical ganglia (Sharp *et al.*, 1979). Mares that were bilaterally ganglionectomized during the winter of 1975-76 began ovulatory cycles at the same time of year as the sham-operated and unoperated control mares during 1976 but in 1977 the same mares did not start ovulatory cycles until approximately 2 months after both groups of controls (Sharp *et al.*, 1979). During the first breeding season following the winter surgery, no differences were found in the indices of follicular growth or

haircoat length and firmness of hair attachment among the three groups. During the second breeding season (1977), follicular growth and typical changes in the length and firmness of attachment of the haircoat were also delayed by approximately 60 days in the ganglionectomized mares. However the differences among the groups were abolished when data were normalized to the time of the first ovulation (Sharp *et al.*, 1979). Across the three groups of mares, haircoat length displayed significant correlation of -0.686 and -0.663 with the number of follicles (> 20 mm) palpated and the diameter of the largest follicle, respectively (Sharp *et al.*, 1979). The firmness of attachment of the haircoat, as assessed on a four point scale, was also significantly but in this case positively correlated with the same traits (Sharp *et al.*, 1979). The three groups of mares became anestrus at the same time during the fall in both 1976 and 1977 so that ganglionectomy did not affect the cessation of ovulatory cycles (Sharp *et al.*, 1979).

Environmental Temperature

Effects of environmental temperature on serum levels of some anterior pituitary hormones have been reported by a number of workers in many species but the effect of temperature on the breeding season of the mare has not been tested. Serum levels of thyroid stimulating hormone (TSH) vary inversely with temperature in many species (Goodman and Van Middlesworth, 1974). However, Melrose *et al.* (1978) reported that serum levels of T_3 were elevated during the summer months in geldings but not in stallions. Other workers found that thyroidectomized mares conceived and delivered live foals and no major effect on reproductive function was noted even though the animals were sensitized to cold and exhibited a variety

of metabolic disorders (Lowe *et al.*, 1974). Tucker and Wetteman (1976) reported of a linear relationship between serum prolactin (PRL) levels and ambient temperatures from 4.5 C to 32 C in heifers on a 12 hour photoperiod. The amount of PRL released after thyrotropin releasing hormone (TRH) injection also decreased with decreasing temperatures and was completely inhibited at 4.5 C. Serum growth hormone (GH) concentrations were unchanged by the environmental temperatures to which the heifers were exposed. Sharp and Ginther (1975) adjusted ambient temperatures simultaneously with the photoperiod when pony mares were exposed to simulated seasonal climates. The spring-summer environment with temperature, photoperiod and humidity increased was effective in hastening the onset of the breeding season relative to the fall-winter environment (Sharp and Ginther, 1975). Oxender *et al.* (1977) found that of 10 mares exposed to the natural photoperiod during winter and spring, all 5 mares that were housed in a heated barn (10 to 15 C), as compared to 2 of 5 mares that were housed outdoors, had ovulated at least once by the end of the experiment on April 21. The five mares housed indoors averaged 1.4 ovulations by April and began ovulatory cycles an average of 24 days earlier than the 2 mares housed outdoors each of which ovulated only one time (Oxender *et al.*, 1977).

Nutrition

A number of authors have suggested that mares well fed and stabled tend to have a longer breeding season (Aitken, 1927; Topp, 1937; Day, 1939; Andrews and McKenzie, 1941). Others noted that the onset of regular estrous cycles correlates with the growth of

pastures and the availability of fresh grass (Küpfer, 1928; Berliner, 1942; Burkhardt, 1948). Bengtsson and Knudsen (1963) suggested that Standardbred mares in training had smaller ovaries and less ovarian activity when fed a ration of oats and hay compared to a higher energy diet of pelleted fed and hay. Van Niekerk and Van Heerden (1972) noted that 100% of mares fed hay and concentrate for 53 days, gained weight, ovulated and conceived while only 75% of mares in a second group on a maintenance ration showed behavioral estrus. The authors observed that in the maintenance group only the mares that gained weight ovulated. Thus, they speculated that the weight gain and not the actual diet was the cause of the increased frequency of ovulation. Ginther (1974) found that pony mares which gained an average of 5.5 kg ovulated 31 days earlier in the year than mares that lost weight. Although evidence is inconclusive, it appears that beyond an adequate plane of nutrition, supplemental feeding to stimulate weight gain may hasten the onset of the breeding season.

Glucocorticoids and Animal Management

Increased levels of nervous excitement in rats and rabbits, associated with various handling and restraint techniques have been correlated with adrenal cortical hypertrophy, increased serum levels of 17-OH-corticosteroids and leukopenia (Mason, 1968). Adrenal cortical hypertrophy has also been correlated with population density and crowding in mice, wild rats, wild deer and monkies (Mason, 1968). McKay *et al.* (1975) subjected rats to physical restraint on the afternoon of proestrus and observed a concomitant increase in serum corticosterone concentrations. Physical restraint also significantly reduced serum levels of LH and prolactin as well

as the percentage of animals which ovulated (McKay *et al.*, 1975). The decrease in LH and prolactin occurred regardless of whether the animals had been adrenalectomized. Riegle (1973) demonstrated that elevated levels of serum corticosterone induced by restraint or ether vapor, were not maintained when rats were repeatedly subjected to the treatment over a 20 day period. Although serum corticosterone levels remained higher than pretreatment levels and adrenal hypertrophy was apparent at the end of the treatment period, chronic restraint period induced increasingly lower serum levels of corticosterone (Riegle, 1973). Holley *et al.* (1975) reported that the increased serum cortisol concentration observed in sheep confined in cages subsided and did not differ from controls after six days of confinement. Serum corticoid levels under different managerial conditions have not been studied in the horse. Hoffsis *et al.* (1970) measured cortisol and corticosterone in 92 resting horses and found an average plasma concentration of 5.1 µg/100 ml with levels ranging from 1.1 to 8.8 µg/100 ml. No differences were found between age groups, sexes or pregnant and non-pregnant mares but a significant diurnal variation was indicated. Highest concentrations occurred at 8:00 a.m. (4.2 µg/100 ml) and lowest concentrations occurred at 4:00 p.m. (1.7 µg/100 ml). Plasma corticoid levels in 104 Standardbred race horses averaged 8.2 µg/100 ml when samples were collected after exercise in preparation for racing.

Exogenous Hormones

Several attempts to stimulate ovulation in the anovulatory mare with exogenous hormones have been made with minimal success (Nishakawa, 1972; Hoppe *et al.*, 1974; Lapin *et al.*, 1975; Bailey and Douglas, 1977;

Evans and Irvine, 1979). Perhaps the most intensive area of investigation has involved injections and oral feeding of progestogens as reviewed by Hoppe *et al.* (1974). Studies show that although most mares show heat and eventually ovulate following extended treatments, the ovulations were unsynchronized and low conception rates were reported even after several estrous periods (Hoppe *et al.*, 1974; Bailey and Douglas, 1977). Some authors reported cases in which estrus occurred during treatment while others reported that treatment with hormones induced cystic ovaries. Some of these results may be attributed to mares which were cycling or approaching the breeding season at the time the treatments were administered (Hoppe *et al.*, 1974).

Evans and Irvine (1979) noted that repeated injections of progesterone caused an increase in serum FSH levels and an increased release of FSH in response to GnRH in anovulatory mares. Ginther and Wentworth (1974) demonstrated that the serum LH profile following GnRH injection in the anovulatory mare was similar to that observed in the ovulatory mare. If exogenous progesterone were to mimic the function of the corpus luteum in the ovulatory mare, then stimulation of pituitary function, folliculogenesis and ovulation would be expected (Evans and Irvine, 1979). However, although follicular growth occurred in most cases, ovulation occurred infrequently following progesterone withdrawal and was seldom followed by corpus luteum formation resulting from extended treatment with progesterone, GnRH or progesterone and GnRH (Evans and Irvine, 1979). Similar results were obtained when pony mares were injected with GnRH, progesterone or estradiol 17β and simultaneously exposed

to a 16 hour light:8 hour dark photoperiod (Bailey and Douglas, 1977). Most authors agree that the hormone treatments appear to be more effective when given very late in the non-breeding season but then it becomes difficult to determine whether or not the effect is actually due to the treatments (Hoppe *et al.*, 1974; Bailey and Douglas, 1977; Evans and Irvine, 1979). Nishakawa (1972) found that repeated injections of Pregnant Mare Serum (PMS) seemed to have a slight gonadotrophic effect by inducing growth of small follicles but failed to cause full follicular development or ovulation in anovulatory mares. However, Lapin *et al.*, 1975, injected anovulatory pony mares with equine pituitary ethanol extracts for a 14 day period and reported that 9 of 9 mares averaged 2.4 ovulations by 16 days after treatment was started. Thus, the homologous pituitary extracts appear to be the only treatment that has effectively induced ovulation in the anovulatory mare.

MATERIALS AND METHODS

Experimental Objectives

Two consecutive experiments were conducted during the winters of 1976 and 1977 as a continuation of the study of photoperiod and other factors which influence the initiation of the breeding season in mares. Experiment I was conducted during the 1975-1976 winter to determine whether a 16 hour photoperiod can be used to stimulate estrous cycles in mares housed at ambient outdoor temperatures in Michigan. A second objective of experiment I was to determine if estrous cycles could be maintained during the winter when mares are housed at outdoor ambient temperatures under a 16 hour photoperiod or if a decreasing photoperiod or a "dormant" period is necessary before a 16 hour photoperiod will stimulate estrous cycles in mares. Experiment II was conducted during the 1976-1977 winter to assess the effects of two levels each of photoperiod exposure and energy intake and two types of housing on the onset of the breeding season in mares maintained in outdoor temperatures during the winter in Michigan.

Experiment I

Experimental Design

This experiment was designed to include 3 photoperiod treatment groups consisting of 6 mares each. Depending upon their treatment group, mares were exposed either to the natural photoperiod throughout the experiment, a photoperiod which was extended to 16 hours throughout the experiment, or the natural photoperiod until December 1

at which time a 16 hour photoperiod was imposed for the remainder of the experimental period. Animals in the control group (n = 6) were housed outdoors in a thirty acre pasture with free access to a three-sided barn for the duration of the experiment (September 16, 1975 to May 1, 1976). The September light treated group (n = 6) were maintained in a 10 acre pasture and were group housed from 4:00 p.m. to about 11:00 p.m. daily in a 10 x 12 meter enclosure under an open sided pole barn in order to receive a supplemented photoperiod throughout the experimental period. The December light treated group (n = 6) were housed with the control group until December 1, 1975 and then with September light treated group until the end of the experiment.

The null hypothesis in this experiment was that no differences in the length of the anovulatory period would occur among the three groups. A significant difference between any of the groups would show that the particular photoperiod treatment altered the length of the anovulatory season when mares are group housed under ambient temperature conditions during the winter in Michigan.

Animals and Housing

Eighteen mares of mixed breeds, two to twenty years old, weighing 300 to 500 kg and with normal non-pregnant uteri and ovaries were randomly assigned to one of three treatment groups. All of the mares were treated for worms prior to the beginning of this study.

The mares were housed at the Veterinary Research facilities on the Michigan State University campus. Two barns, about 1 km apart, were used for the group(s) receiving either natural or

supplemental photoperiod so that the outdoor lighting would not affect the group(s) receiving the natural photoperiod. Mares in September and December light treated groups were confined in the open barn to receive supplementary lighting from 4:00 p.m. each evening until 12:00 a.m. Mares were turned loose in a single group at each housing unit for water, hay and exercise.

Lighting

Control mares were exposed to the natural photoperiod which ranged from nine to fourteen hours during the experimental period. The September light treated group were maintained on a 16 hour light: 8 hour dark photoperiod throughout the experimental period. Mares in the December light treated group were exposed to the natural photoperiod from September to December during which time the length of the photoperiod decreased from approximately 12 hours to 9 hours. On December 1, 1976 the December light treated mares were separated from the control mares and housed with the September light treated mares to maintain them on a 16 hour light:8 hour dark photoperiod. A time clock controlled the supplementary lighting which was supplied daily from 4:00 p.m. until eight hours prior to the next sunrise. The supplemental light was provided by white incandescent bulbs at an intensity which ranged from 55 to 160 lux at eye level throughout the exposure period.

Feeding

All mares were fed *ad libitum* a second cutting mixed timothy alfalfa hay. Hay was made available in a feed bunk in the center of the enclosure in the evening and additional hay was fed in the pasture in the morning.

Blood Samples

A 10 ml blood sample was collected from each mare by jugular venipuncture each Monday and Thursday afternoon throughout the experiment. Serum was separated and stored until progesterone was quantified.

Hormone Quantification

Serum progesterone was quantified by a single antibody radioimmunoassay as described by Noden *et al.* (1978).

Statistical Analysis

Data were examined by analysis of variance. Nonorthogonal contrasts involving Bonferonni t statistics were utilized in order to make specific comparisons among treatment means where analysis of variance indicated there were significant differences (Gill, 1978).

Experiment II

Experimental Design

The experimental design included eight treatment groups each containing five mares. Each group was assigned to one of two classes of photoperiod, diet and housing in a 2^3 factorial design. Each group of mares received either sixteen hours of light per day (SP) or the natural length photoperiod (NP) and was fed either a high energy (E) or a maintenance diet (e). Also, mares were housed and fed individually (I) in tie stalls or grouped (G) in four by six meter pens. Treatment continued from December 17, 1976 through April 16, 1977 for a period of 120 days. Although experimental treatments were stopped April 16 most mares were

palpated and had blood samples collected through May for a subsequent experiment from which we were able to determine the time of the first ovulation.

In this experiment the chief null hypothesis was that no differences would occur in the time of onset of the breeding season among groups which receive different levels of photoperiod, feed or type of housing confinement or as a result of the combination of different treatment factor levels.

Animals and Facilities

Forty mares of mixed breeds, two to twenty years old, weighing 300 to 500 kg and with normal non-pregnant uteri and ovaries were randomly assigned to one of eight treatment groups. All of the mares were treated for worms and the teeth were floated in mares having long teeth prior to the beginning of this study.

As in experiment I, mares were housed at the Veterinary Research facilities on the Michigan State University campus and the same two barns (∿ 1 km apart) were used. Ten tie stalls were built in each of two three sided barns for individual housing during confinement period. Two four by six meter pens were built near each set of tie stalls to confine treatment groups which were group housed.

The tie stalls were 1.3 meters wide and 3 meters in length. Neither the tie stalls nor the group pens had watering facilities. Mares were housed individually or in groups to receive treatments between 4:00 p.m. and midnight each day during the experimental period. From midnight to 4:00 p.m. each day mares were turned loose in a single group at each housing unit for water, hay and exercise.

Lighting

Mares subjected to the natural photoperiod was exposed to nine hours of daylight at the beginning of the experiment in December and fourteen hours of daylight by the end of the experiment in April. In Michigan, the natural photoperiod increases in length by two minutes per day between December 21 and June 21. Mares receiving supplemental lighting were maintained on a 16 hour light:8 hour dark photoperiod during the experimental period. A time clock controlled the supplementary lighting which was supplied daily from 4:00 p.m. until eight hours prior to the next sunrise. The supplementary light consisted of 25 lux of incandescent white light adjusted to provide a consistent intensity at eye level throughout the confinement areas.

Feeding

All mares were fed *ad libitum* a second cutting mixed timothy alfalfa hay. Each mare received 10 lbs of hay daily in the stall or group pen at 4:00 p.m. Hay and water was available when mares were turned into a single group at midnight on each farm. Mares on the high energy diet received 3 kg of oats daily at 4:00 p.m. Although feed was placed in separate areas of the group pens, there were no physical barriers between mares in the same group during feeding.

Body Weight

The body weight of each mare was recorded at the beginning and end of the treatment period. On December 11, 1976 water was withheld from the mares for approximately six hours prior to weighing. Mares were then hauled to a scale and weighed individually. Mares

were weighed again on April 16, 1977. This time mares were kept confined overnight and weighed the next morning so that water was withheld for at least sixteen hours when mares were weighed in April.

Haircoat Length

Beginning in February 1977, the degree of shedding and length of the haircoat were recorded on each mare at two week intervals. The degree of shedding was described in terms of whether or not chest hair was easily removed in tufts.

Temperature

The temperature in each of the housing conditions at both housing units was recorded daily throughout the experiment.

Blood Samples

A jugular blood sample was collected from each mare every Tuesday and Friday afternoon throughout the experiment. An additional sample was collected at 8:00 a.m. on three consecutive mornings in April. Serum was separated and stored at -20 C until hormones were quantified.

Hormone Quantification

Serum progesterone was quantified by a double antibody radioimmunoassay (Convey *et al.*, 1977). Duplicate 100 µl serum samples were diluted with 100 µl of 0.1% Knox gelatin in 0.05 M phosphate buffered saline (PBS-Knox) and the progesterone was extracted with 2 ml of redistilled benzene:hexane (1:2). The solvent was then evaporated from the samples in preparation for assay of the unknowns. Three sets of ten progesterone standards ranging from 0.05 to 1.00 ng were added to each assay (Sigma Chemical Co.).

Anti-progesterone produced in a rabbit (MSU #75) was diluted 1:2,000 in PBS-Knox containing 1:100 normal rabbit serum. Then 200 µl of the diluted anti-progesterone and 200 µl of ^{3}H-progesterone was added to each assay tube. Following a 24 hour incubation at 5 C, 400 µl of second antibody (sheep anti-rabbit gamma globulin diluted 1:10 in 0.1% PBS-Knox) was added to each tube. The assay was again incubated (48 hours) and then centrifuged for 30 minutes. The supernatant (0.5 ml) was aspirated and diluted with scintillation fluid (4.5 ml) in preparation for quantification of the radioactivie progesterone in a scintillation counter.

Total serum glucocorticoids were measured by a competitive protein binding assay which was previously validated in cow's milk and serum in our laboratory (Smith, Convey and Edgerton, 1972). Briefly, duplicate 200 µl aliquots of serum were used and the progestogens were extracted from these samples with iso-octane (2 ml). The steroids remaining after the progestogen extraction were then extracted with methylene chloride (2 ml). The solvent was evaporated from the samples in preparation for assay of the unknowns. Three sets of ten cortisol standards ranging from 0.05 to 2.50 ng were used in each assay (Sigma Chemical Co.). After 1.0 ml of ^{3}H-dog plasma, containing 40,000 CPM was added to each tube, each assay was incubated overnight at 5 C, then allowed to incubate in an ice bath for 15 minutes. Dextran coated charcoal suspended in double distilled water (0.5 ml) was added to each tube. The assay was immediately centrifuged for 15 minutes. A 0.5 ml aliquot of supernatant fluid was then diluted to 5.0 ml with scintillation fluid and the radioactivity counted in a scintillation counter.

Statistical Analysis

Data values reflecting the interval to the first ovulation, the number of ovulations and percent change in body weight were examined by factorial analysis of variance utilizing least squares.

The repeated measurements of the glucocorticoid concentrations were examined by split plot factorial analysis of variance using lease squares.

All data were examined for heterogeneous variance utilizing the Fmax test (Gill, 1979). Variances among treatment groups approached heterogeity for the data values reflecting the percent body weight change during the experimental period ($p \sim 0.25$). These values were examined both before and after square root transformation which reduces the heterogeneity of the variances but did not change the inferences from the analysis.

RESULTS

Experiment I

In experiment I, the length, the time of onset and the end of the winter anovulatory period was examined. Previous experiments indicated that quantification of serum progesterone twice weekly provided accurate data for predicting when ovulation had occurred in mares (Oxender *et al.*, 1977). The total number of estrous cycles during the experimental period and the number of ovulations from September 16 to December 1 and from December 1 to May 1 were calculated for each group based on changes in concentrations of serum progesterone (Figure 1). Two mares were eliminated from the data for the September light treated group; one mare fractured her skull by fighting while penned in the barn area and the other died in December, leaving 4 mares in this group. One mare in the December light treated group died in February, leaving 5 mares in the group. The length of the anovulatory period as determined by the number of days with consecutive biweekly serum progesterone concentrations less than 1.0 ng/ml averaged 164, 87 and 136 for mares in the control, September light treated and December light treated groups, respectively (Table 1). Although the difference among groups in the length of the anovulatory period appeared large it was not statistically significant ($P \sim 0.10$). Furthermore, the data also demonstrate the failure of the September light treatment for prevention of the seasonal anovulatory period in these mares.

Figure 1: Serum progesterone in mares during a 16 hour photoperiod initiated on September 16 or December 1, 1975.

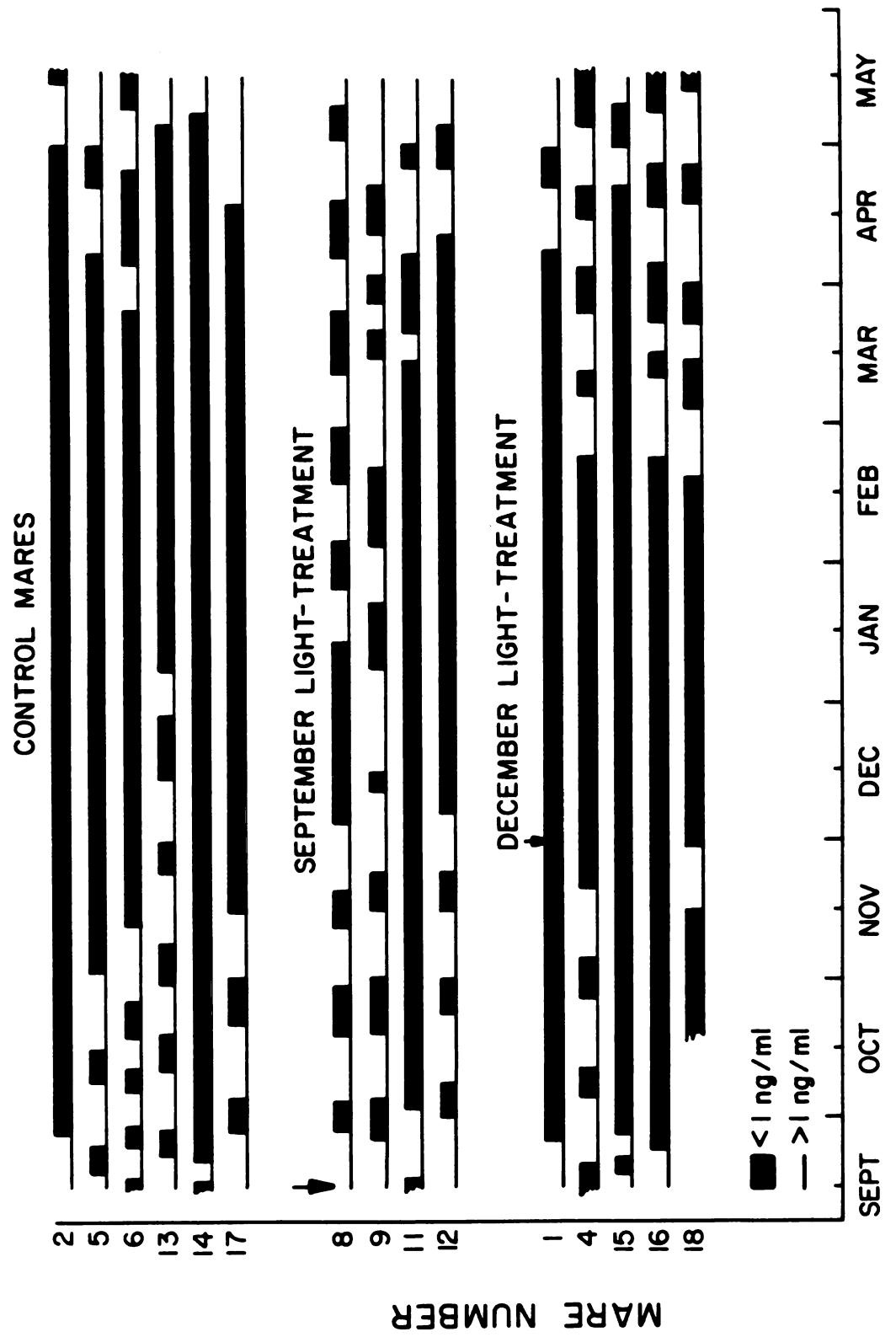
CONTROL MARES
SEPTEMBER LIGHT-TREATMENT
DECEMBER LIGHT-TREATMENT
MARE NUMBER
2
5
6
13
14
17
8
9
11
12
1
4
15
16
18
<1 ng/ml
>1 ng/ml
SEPT
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
MONTH

Figure 1

Table 1: The ovulatory activity of mares on different artificial 16L:8D photoperiods.

Event	Photoperiod		
	Control	Sept 16 light-treated	Dec 1 light-treated
	(n=6)	(n=4)	(n=5)
Length of anovulatory period	164 ± 23	87 ± 24	136 ± 25
Interval from Sept 16 to onset of the anovulatory period	48 ± 16	71 ± 19	32 ± 17
Interval from Dec 1 to start of ovulatory season	136 ± 13	82 ± 17	103 ± 15
Number of ovulations during the experiment	4.2 ± 0.9	7.3 ± 1.1	4.8 ± 1.1*
Number of ovulations from Sept 16 to Dec 1	2.7 ± 0.6	3.3 ± 0.7	1.8 ± 0.7*
Number of ovulations after Dec 1	1.5 ± 0.5[a]	4.0 ± 0.6[b]	3.2 ± 0.5[b]

* n = 4

[a,b] means with different superscripts are significantly different ($P < .05$)

The interval from September 16 to the onset of the anovulatory period averaged 48, 71 and 32 days for the control, September light treated and December light treated groups, respectively, and was not significantly different among the groups (Table 1). The first ovulation following the anovulatory period indicated the initiation of estrous cycles for the breeding season and occurred 136, 82 and 103 days after December 1 for control, September light treated and December light treated groups, respectively. The difference among groups approached traditional statistical significant ($P \sim 0.07$) however specific comparisons confirmed that this interval (December 1-ovulation) was not significantly different between control and light treated mares or between the two light treatment groups.

During the experimental period, the control, September light treated and December light treated groups averaged a total of 4.2, 7.3 and 4.8 ovulations per mare, respectively which tended to be different only at the $P \sim 0.10$ level. There were no differences among the groups in the number of ovulations which occurred between September 16 and December 1 (Table 1). Between December 1 and May 1, mares averaged 1.5, 4.0 and 3.2 ovulations in the control, September light treated and December light treated groups, respectively which was different at the $P < .05$ level. Specific comparisons showed that mares in the two light treated groups averaged more ovulations between December 1 and May 1 than the control group ($P < .05$) but that the light treated groups did not differ significantly from each other.

The results of this experiment showed that mares housed outdoors receiving a 16L:8D photoperiod from either September 16 or

December 1 until May 1 had more than twice the number of ovulations between December 1 and May 1 as mares receiving the natural photoperiod. Additionally, one mare in the September light treated group ovulated at lease once each month of the experiment. This phenomenon has been reported to occur in 10-20% of normal populations (Quinlan *et al.*, 1951; Osborne, 1966) and may or may not have been potentiated by the photoperiod treatment. In either case, the photoperiod treatments in this experiment, without heated housing, did not appear to be as effective in hastening the onset of the ovulatory season as it had been the previous year (Oxender *et al.*, 1977). This may be at least partially attributable to the loss of 3 out of the 12 mares and thus smaller sample size in the light treated groups.

Experiment II

In this experiment, serum glucocorticoid and progesterone concentrations were measured in order to detect endocrine differences and physiological changes among the treatment combinations during the experiment. Body weight change and firmness of attachment of the haircoat were also observed. Four mares have been omitted from all of the data either because they continued to cycle through the winter (n = 2), or because of illness (n = 2).

Based on the assumption that glucocorticoids might serve as an index of nervous excitement or some level of hypothalamic activity related to the type of housing confinement, serum glucocorticoid concentrations were measured by a competitive protein binding assay. Statistical analysis indicated that glucocorticoid concentrations taken from each mare at 8:00 a.m. on 3 consecutive days were not

significantly different; therefore the values were pooled. The glucocorticoid concentrations averaged 10.8 ng/ml for mares housed individually compared with 10.4 ng/ml for mares housed in groups and were not significantly different (Table 2). Additionally, analysis of the data indicated glucocorticoids were also unchanged by the photoperiod and diet treatments and that no significant interactions had occurred among the treatment factors.

Table 2: The effect of housing on serum glucocorticoid concentrations in mares.

Housing	# of Mares	Glucocorticoids (ng/ml)
Individual	18	10.8 ± 0.4
Group	18	10.4 ± 0.4

The body weight of each mare was recorded at the beginning and end of the experimental period. Mares fed the high energy diet lost an average of 3.7% of their body weight over the experimental period while mares fed the maintenance diet lost 5.3% (Table 3). These values were not significantly different indicating the dietary intake was inadequate to maintain body weight even for mares receiving oats in addition to hay. The failure of either diet to maintain body weight may have been partially caused by the extreme weather. Michigan experienced the most severe winter conditions of the past 100 years during this experiment.

Table 3: The effect of energy intake on body weight in mares.

Diet	# of Mares	% Weight Change
High Energy	18	-3.7 ± 1.4
Maintenance	18	-5.3 ± 1.4

Mares that were individually housed (I) lost significantly more body weight (7.3%) during the experimental period than did mares housed in groups (G) (1.7%) (Table 4). This represents an average weight loss of 29 and 8 kg per mare for I and G mares, respectively. We had expected feeding conditions would be better for individually housed mares and thus these mares would have been better able to maintain body weight; however, this was not the case in this experiment. Also, individual body weight changes were not significantly correlated ($r = 0.10$) with the length of time to the first ovulation, indicating that the body weight losses may not have been large enough to interfere with the initiation of the ovulatory season.

Table 4: The effect of housing on body weight in mares.

Housing	# of Mares	% Weight Change
Individual	18	-7.3 ± 1.4
Group	18	-1.7 ± 1.4

The firmness of attachment of the haircoat was assessed and recorded every other week so that it would be compared with the onset of the ovulatory season. Shedding of the haircoat was not significantly correlated with the first ovulation of the year ($r = 0.27$) in the 28 mares for which dates of the first ovulation was determined. This figure is similar to that determined by Kooistra and Ginther (1975).

The date of the first ovulation of the breeding season was determined in 29 of the 36 mares on the project. Seven mares were sold after the end of the experimental treatment period but before an ovulation had occurred. Only one of these 7 mares had received the supplementary photoperiod treatment. The loss of data from these animals made it more difficult to determine the effect of photoperiod on ovulation. Statistical analysis indicated that no significant interactions were present and that neither the type of diet nor housing influenced the interval to the first ovulation. Thus the data were pooled in Table 5.

Table 5: The effect of an artificial 16L:8D photoperiod on the number of mares ovulating during the experiment.

Photoperiod	# of Mares	% Ovulating			# of Ovulations per mare*
		Feb	Mar	Apr	
16L:8D	18	11	33	56	1.1 ± 0.71
Natural	18	0	0	11	0.1 ± 0.71

* mean ± S.E.M.

The effect of photoperiod on the percentage of mares ovulating during each of the last three months of the experiment and the average number of ovulations during the treatment period in the two photoperiod treatment groups is presented in Table 5. Eleven, 33 and 56 percent of the mares receiving a daily 16L:8D photoperiod (SP) had ovulated at least once by the end of February, March and April, respectively. Conversely, none of the mares on the natural photoperiod (NP) ovulated during February or March and by the end of April only 11% had ovulated. Increasing the photoperiod to 16 hours per day significantly increased the number of mares cycling by late April ($P < .025$). Additionally, looking only at those mares that began cycling by late April, mares receiving supplementary lighting averaged 1.1 ± 0.71 cycles while mares kept on the natural photoperiod ovulated an average of 0.1 ± 0.71 times. This difference was also significant ($P < .005$). Thus, during the experimental period, more mares began ovulating in the SP group than in the NP group.

The average interval (in days) from the start of the experiment on December 17 until the first ovulation for each treatment combination is shown in Table 6. The numbers in parentheses represent the number of mares represented by each mean. The interval to the first ovulation was significantly ($P < 0.025$) reduced from an average of 145 days in 12 of the NP mares to 120 days in 17 of the SP mares. However, the interval to the first ovulation was not changed by added dietary energy or by the type of outdoor housing. Thus, photoperiod is the only factor of the three factors tested which significantly influenced the onset of the ovulatory season in mares housed outdoors during the winter in Michigan. In this experiment,

the two types of housing conditions and levels of energy intake did not differentially effect the onset of the breeding season in anovulatory mares either directly or through interaction with the photoperiod treatments.

Table 6: The interval from December 17 to the first ovulation in mares.

Photoperiod	Diet	Housing	
		Individual	Group
		Days	
16L:8D	High Energy	124 (4)*	106 (4)
	Maintenance	114 (5)	139 (4)
Natural	High Energy	151 (2)	142 (4)
	Maintenance	148 (4)	141 (2)

* numbers in () indicate number of mares. The standard error of each mean is 12, 13 or 19 for 5, 4 or 2 mares, respectively.

DISCUSSION

In the first experiment, treating mares with a photoperiod supplemented to 16L:8D beginning in September did not prevent or significantly delay the anovulatory season. The natural day length begins to shorten in June and it is possible that the photoperiod extension was initiated too late to be effective. However, another study indicated that extending the photoperiod from either June or October delayed the onset of the anovulatory season in pony mares relative to controls but there was no difference between the two photoperiod treatments (Kooistra and Ginther, 1975). Just as for the September initiated artificial light in our study, neither treatment prevented the anovulatory period in mares (Kooistra and Ginther, 1975). It appears that there may be an inherent period of gonadal regression in the mare as has been cited in other animals (Reiter, 1974).

A previous study in the same laboratory demonstrated that mares stabled in a barn 10 to 13 C ovulated after an average of 59 days of supplemental light exposure corresponding to 39 days after January 1 (Oxender *et al.*, 1977). Indoor controls ovulated an average of 90 days after January 1 and 2 of 5 outdoor controls ovulated an average of 121 days after January 1 in the same study. In experiment I, the 6 outdoor control mares ovulated an average of 105 days after January 1, while the September and December light treated groups ovulated an average of 51 and 72 days after January 1.

Although data were collected on more mares in the present study and the averages appear comparable, we failed to show a statistically significant advantage for the photoperiod treatment. In fact, the average first opportunity to breed a mare in the December light treated group occurred in mid-March as opposed to early February in the previous study. In comparing the two studies, it seems that there was an increase in the number of uncontrolled variables such as temperature and the availability of food which may have interacted with or in some way influenced the effect of the photoperiod treatments in this study (Experiment I). It was noted that the mares housed indoors gained an appreciable amount of weight over the winter whereas mares in this experiment may have lost weight. Body weights were not measured in either of the experiments so differences that might have occurred among the groups within either experiment due to body weight changes could not be quantified. Temperature is another factor which may have influenced the response to treatments in the two experiments. These factors might have influenced the onset of the breeding season through interaction with the response to the artificially supplemented photoperiod or a direct effect on the onset of the breeding season.

In experiment II, an effort was made to control some of the variables associated with experiment I as an effort to improve the efficacy of an artificial 16L:8D photoperiod for mares maintained at outdoor temperatures. Underweight mares were fed a high energy diet for 2 weeks preceding the experimental period in an attempt to get the mares into a more uniform condition. However, mares still entered the experiment with varying degrees of body condition.

Although all mares received high quality hay *ad libitum* and some gained weight, even the high energy diet (supplemented by oats) was inadequate to maintain weight in all of the mares in these groups. Since the onset of the ovulatory season was not affected by the type of diet or correlated with the body weight change, it seems that either the differences in body weight changes observed here were not of sufficient magnitude to have an effect or that intake beyond severely deficient levels does not have a positive effect on the onset of the mare's breeding season. Another possibility is that a substantial weight gain is a prerequisite to a positive influence of nutrition on the onset of the breeding season in the mare.

In contrast to our hypothesis, mares that were group housed lost significantly less weight than individually housed mares and there was no interaction between the type of housing and the level of energy intake. It is possible that increased competition for feed may have increased consumption. More likely, the decrease in weight loss was due to the decrease loss of body heat resulting from its reflection off of adjacent animals within the groups. This may have been of substantial importance considering the severity of the weather as ambient temperatures and windchill factors were often well below zero. In any case, energy intake and body weight changes were monitored in this experiment so that they did not serve to be a confounding factor. In this experiment, the advancement of the onset of the breeding season by the supplemental photoperiod despite difference in body weight changes, as well as the lack of significant interaction between

the photoperiod treatments and type of housing, indicate that in experiment II changes in body weight did not affect the onset of the breeding season.

Riegle (1973) noted that the glucocorticoid elevation in response to restraint was diminished after chronic treatment. The failure to detect any difference in serum glucocorticoid concentrations among the treatment combinations may be due to a similar adaptation because samples measured were taken after 100 days of treatment and the mares may have adjusted to the housing. However, the time of sampling corresponds closely to the time of the expected onset of the ovulatory season when differences in serum glucocorticoid levels should correlate most closely with events which could interfere with ovulation (McKay *et al.*, 1975). It appears from the present experiment that glucocorticoid concentrations in mares probably are not correlated with stabling methods and ovarian function.

In experiment II, mares in the supplemental photoperiod treated group ovulated an average of 106 days after January 1 whereas 12 mares in the natural photoperiod group ovulated an average of 131 days after January 1. This average may have been even larger if ovulation dates could have been obtained for six remaining mares in the NP group. In either case, the outdoor supplemental photoperiod hastened the onset of the breeding season by approximately 25 days. A similar advantage was obtained by the outdoor supplemented photoperiod treatment in experiment I. Although this would be of some advantage to horse breeders, the failure of the outdoor 16L:8D exposure to provide a 2 to 3 estrous cycle advantage as indoor trials have done (Sharp *et al.*, 1975; Kooistra and Ginther, 1975; Oxender

et al., 1977) raises questions concerning the effect of ambient temperatures, body weight changes and their possible interactions with photoperiodic induction of the breeding season in the mare. The effect of ambient temperature on the time of first ovulation remains to be critically tested. If ambient temperatures prove to be an important factor the results of outdoor photoperiod supplementation may be influenced by geographical location.

SUMMARY

The results of experiment I indicated that treating mares with a 16L:8D photoperiod from either September or December increased the number of ovulations between December 1 and May 1 but did not change the average day of onset for the ovulatory season. The seasonal anovulatory period was not prevented by placing mares on a 16L:8D photoperiod from September to April.

In experiment II, supplementary lighting to 16 hours per day increased the number of mares ovulating as well as the number of ovulations per mare during the winter and early spring in mares housed at outdoor temperatures. Added dietary energy in the form of 3 kg of oats per day did not significantly reduce the percentage of body weight lost during the winter. However, group housed mares lost significantly less weight than mares that were individually housed. Serum glucocorticoid concentrations were not changed by different types of housing. Neither added dietary energy nor type of outdoor housing caused a significant difference in the number of mares ovulating during the experiment.

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