ANALYSIS OF BEHAVIORAL SEXUAL RECEPTIVITY OF DOMESTIC HORSE AND PONY MARES (*EQUUS CABALLUS*) DURING ESTRUS IN RELATION TO OVULATION

By

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ABSTRACT

ANALYSIS OF BEHAVIORAL SEXUAL RECEPTIVITY OF DOMESTIC HORSE AND PONY MARES (EQUUS CABALLUS) DURING ESTRUS IN RELATION TO OVULATION

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Six horse mares and one pony stallion (Experiment 1) in 1975 and fourteen pony mares and four pony stallions (Experiment 2) in 1976 were observed during daily teasing sessions throughout a normal estrous cycle. Data were collected with a tape recorder and a stopwatch and consisted of frequencies of behaviors and latencies to tail raising, urination and squatting of the mares and mounting latencies of the stallions.

The hypothesis that sexual receptivity of mares increases prior to ovulation and decreases after ovulation was tested in two parts. The first part tested for an increase in sexual receptivity prior to ovulation (pre-ovulatory behavior). The second part tested the decrease of sexual receptivity after ovulation by examining behavior throughout estrus (peri-ovulatory) and comparing the results to the pre-ovulatory analyses. Ovulation was detected via palpation per rectum.

Pre-ovulatory tail raising latencies decreased significantly for horse (7.5 sec/day) and pony (2.9 sec/day) mares, supporting the hypothesis that sexual receptivity increased prior to ovulation. Further support for this hypothesis was

observed in the decreasing pre-ovulatory squatting latencies, which approached significance for horse (6.0 sec/day) and pony (2.9 sec/day) mares.

Only pre-ovulatory mounting latencies of the stallions in Experiment 2 decreased significantly (4.2 sec/day) suggesting that stallions perceived changes in the pre-ovulatory state of the pony mares.

Pre-ovulatory slopes and origins of tail raising, squatting and mounting latencies and the mean latencies (tail raising, urination and squatting) on the four days prior to ovulation were not different between Experiments 1 and 2. These results suggested that increasing sexual receptivity of horse and pony mares was alike prior to ovulation.

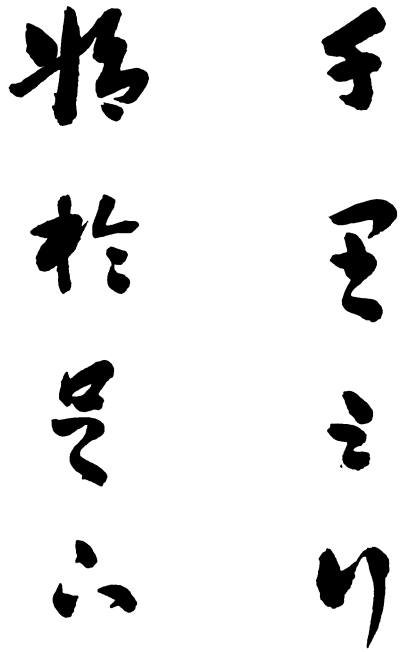
Peri-ovulatory tail raising latencies of horse mares decreased linearly 4.1 sec/day ($P < 0.05$), but also showed a slight increase ($P < 0.07$) beginning one day prior to ovulation. Peri-ovulatory tail raising latencies of pony mares also decreased significantly to a minimum at three days prior to ovulation and then increased for the remainder of estrus. The mean tail raising latencies on the four days prior to and on the day of ovulation were similar in horse and pony mares. These results generally support the hypothesis that sexual receptivity decreased after ovulation with the qualification that the decrease began before ovulation.

Peri-ovulatory mounting latencies of the single pony stallion in Experiment 1 significantly decreased 5.5 sec/day

even after ovulation. The variance of the mounting latencies of the stallions in Experiment 2 increased after ovulation and negated linear or curvilinear significance.

Oxender et al. (1977) conducted two experiments on horse mares in 1975. Experiment 3 (n = 4) tested dose effect of 0, 1, 2 and 5 mg of Gonadotropin Releasing Hormone (GnRH) following 5 mg Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) administration during four successive estrous cycles. Experiment 4 (n = 6) tested regimes of 0, 5 mg GnRH once and 5 mg GnRH daily (up to four times) following $PGF_{2\alpha}$ administration during three successive estrous cycles. The GnRH treatments did not affect the temporal decrease of tail raising latencies prior to ovulation. This agrees with the conclusion of Oxender et al. (1977) that GnRH treatments did not alter follicular maturation and the interval to ovulation.

To James C. Braddock, 1913-1978



The journey of a thousand miles
begins with one step.

Lao-tzu, 604?-?531 B.C.

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TABLE OF CONTENTS

LIST OF TABLES

Table | Page

LIST OF FIGURES

I. INTRODUCTION

Successful reproduction in mammals is contingent upon the synchronization of the copulatory patterns of both sexes. Behaviorists have quantitatively analyzed temporal changes in the sexual behavior of female rodents, canids and prosimians during estrus. However, only superficial analyses have been made on the sexual behavior of domestic equine mares throughout estrus in relation to ovulation. The purpose of this research was to refine the quantitative analyses on sexual behavior and receptivity of domestic mares in order to detect any changes in them during estrus in relation to ovulation.

The duration of estrus or the sexually receptive period of the domestic mare ranges from four to thirteen days and varies among individuals and among the seasons. Ovulation occurs spontaneously about twenty-four to forty-eight hours before estrus concludes (Hughes et al., 1972a). Breeding within three to four days prior to ovulation appears optimal for conception to occur (Hammond, 1938). However, the critical period for insemination cannot be readily predicted from the onset of estrus because ovulation, as detected by daily palpation of the ovaries, occurs at various times. As a consequence, the conception rate in domestic equids is low.

A precise analysis of behavior during estrus may enable development of prediction of the optimal period of fertility.

At the onset and termination of estrus the concert of hormonal and anatomical events that ensue are reflected in the sexual responses of the mare to the stallion. The hormonal, anatomical and behavioral events have been reported to change throughout the duration of estrus and are reviewed in the Literature Review of this dissertation.

Previous studies on the changes in sexual behavior of the mare throughout the duration of estrus in relation to ovulation have contributed little to predicting when mating would produce the highest incidence of conception. The concept that estrous behavior increases in 'intensity' from the onset of estrus up to ovulation has been reported in studies using subjective rating scales (Andrews and McKenzie, 1941; Ginther, 1978) and undefined behaviors (Nishikawa, 1959) which were analyzed with inappropriate statistical tests. These studies on 'intensity' of behavior have pervaded the literature as valid, and they have retarded further research efforts by trained behaviorists.

Animal behaviorists have quantified the sexual behavior of both sexes in several species. Female receptivity has been found to wax and wane throughout the duration of estrus among those species that have been studied quantitatively, mostly rodents and prosimians (Eaton et al., 1973; Hardy, 1972; Kuehn and Beach, 1963). These changes in sexual

receptivity may be correlated to ovulation, but because of the difficulty in monitoring ovulation in most mammalian species, this has not been demonstrated.

Two sets of studies were conducted: The first set tested the hypothesis that sexual receptivity increases prior to ovulation and decreases after ovulation with untreated horse mares (Experiment 1) and untreated pony mares (Experiment 2). The second set of studies tested the hypothesis that sexual receptivity could be temporally altered by hormonally treating horse mares, if the hormonal treatment also altered the rate of follicular maturation. The number of days of estrus before and after ovulation (as detected by ovarian palpation per rectum) functioned as the independent variable and the latencies of several behavior patterns as the dependent variables (Dennenberg and Banks, 1969), which were analyzed with regression and analysis of variance techniques.

Untreated horse mares (Experiment 1) and untreated pony mares (Experiment 2) were observed throughout an estrus period during teasing sessions with sexually active stallions. The objectives of the two studies were as follows:

1. Describe behavior patterns of mares in estrus and characterize differences in behavior patterns between horse and pony mares.

2. Describe the behavior patterns of stallions during teasing culminating with mounting of the mares.
3. Using ovulation as a referent point, quantify behavior patterns of the stallions and the mares in terms of frequency on each day of estrus.
4. Using ovulation as a referent point, quantify behavior in terms of latencies to behavior response of key behavior patterns (tail raising, squatting and urination of the mares and mounting by the stallions) throughout estrus.
5. Compare similarities in behavioral latencies of horse and pony mares.

In the second set of studies (Experiments 3 and 4) performed by Oxender et al. (1977), I observed horse mares during treatment regimes with Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and various doses and regime schedules of Gonadotrophin Releasing Hormone (GnRH). $PGF_{2\alpha}$ is a proven luteolytic agent which when administered to mares between days 5-9 of diestrus causes luteolysis of the corpus luteum and a return to estrus within two to four days, thus shortening the diestrous period of the estrous cycle. GnRH is a hypothalamic releasing factor which in other large domestic animals increases anterior pituitary release of luteinizing hormone (LH), the hormone which is thought to hasten follicular maturation and ovulation. The physiological objectives of Oxender et al.'s studies were to determine if treatment with GnRH (a) increased

serum LH levels in mares; and (b) decreased the time interval between $PGF_{2\alpha}$ induced luteolysis and ovulation, thus increasing follicular maturation rate and causing ovulation synchronization. The behavioral objectives of these studies were to:

1. Determine if behavior of horse mares prior to ovulation was altered during the treatments.
2. Determine if behavior of the horse mares was altered prior to ovulation by comparing regressions between untreated horse mares and mares under the different treatments.

II. LITERATURE REVIEW

A. Ethological Observations of Equids

Six species of wild Equidae are generally recognized (Klingel, 1971; Short, 1975; Simpson, 1951). These are: Equus przewalski (the wild horse or Przewalski's horse), E. assinus (the African wild ass), E. hemionus (the Asiatic wild ass), E. quagga (the plains, steppe or Burchell's zebra), E. zebra (the mountain zebra) and E. grevyi (Grevy's zebra). Klingel (1971) lists the subspecies and the common names of the zebra and ass species. Domesticated horse and pony breeds are a single species E. caballus, whose ancestors are extinct except E. przewalski, which is presently bred in captivity.

Observations of equine social behavior, activity patterns and population biology have been made. These observations encompass free-ranging horses (Stebbins, 1974; Zeeb, 1958 and 1961), feral horses (Feist, 1971; Feist and McCullough, 1975; Welsh, 1973 and 1975), feral asses (Moehlman, 1974), feral ponies (Keiper, 1976), semi-wild ponies (Tyler, 1972), several subspecies of plains zebras (Klingel, 1964, 1965, 1967, 1969a, and 1969b; Klingel and Klingel, 1966), several subspecies of mountain zebras (Klingel, 1968; Joubert, 1972) and Grevy's zebra (Zeeb and Kleinschmidt, 1963). Klingel (1971 and 1975) has reviewed

and compared patterns of reproduction and social organization among Equidae. Although some of the above studies describe reproductive behavior patterns, none has quantified the behavior patterns of the stallions and/or mares throughout estrus. This is difficult to do during field observation of free ranging, feral or wild equids because the observer cannot estimate ovulation time or even observe subjects every day (Moehlman, 1974). Knowledge of the sexual behavior patterns of natural bisexual populations is essential because domestic equids are generally sexually segregated prior to maturity (Willis, 1973). Distortion of sexual behavior patterns of equids in domestication have been observed (Rossdale, 1969). Ratner and Boice (1975) discussed the affects of domestication on physiological and behavioral response thresholds in many species. Therefore, descriptions of sexual behavior patterns of the various free-ranging, feral and wild species and breeds will be mentioned throughout this literature review as additions, and for comparison, to the literature on domestic horses and ponies.

Specific behavior patterns of equids, particularly facial expressions have been described by Schneider (1930), Trumler (1959) and Zeeb (1959). Vocalizations have been spectrographically analyzed by Waring (1971) and Ödberg (1974) for domestic horses. Perinatal behavior of the mare and newborn horse foal has been observed and described by Rossdale (1967, 1968a and 1968b) and Waring (1970c) with continued primary socialization described by Waring (1970a and 1970b).

B. The Equine Estrous Cycle

1. Phases of the Cycle

The normal estrous cycle of the equid is composed of two integral phases or periods.

a) Estrous or Heat Period

During estrus[1] or heat, ovarian follicles mature and ovulate, the external genitalia undergoes change in color and the mare is sexually receptive to a stallion (Asdell, 1964; Eckstein and Zuckerman, 1956b; Rossdale and Ricketts, 1974).

b) Diestrous Period

During diestrus the corpus luteum undergoes formation and regression in a non-pregnant mare. External genitalia remain pale and dry throughout diestrus and the mare is not sexually receptive to a stallion (Asdell, 1964; Eckstein and Zuckerman, 1956b; Rossdale and Ricketts, 1974).

2. Seasonal Variation

In the literature, there is a controversy over the patterns of estrous cycles throughout the year. Reviews of older literature by Asdell (1964) and Witherspoon (1971) maintain that there is a breeding season with

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[1]Grammatically Estrus is used as a noun and estrous is the adjective referring to the cycle, the period and behavior during the period (e.g. estrous cycle, estrous period and estrous behavior) (Morris, 1969). In this dissertation, the terms estrus, estrous period and estrous behavior all refer to the "in heat" period of the estrous cycle.

regular ovulatory cycles during the spring and summer months and periods of transition into and out of a winter non-reproductive or anestrous period. In the review by Andrews and McKenzie (1941) and in recent studies by Ginther (1974), Hughes et al. (1972a) and van Niekerk (1967) it was observed that some, but not all mares show periodic cycles year round. Ginther (1974) and Hughes et al. (1972a) noted that of the mares that did have estrous periods during the winter months, not all have accompanying ovulations [anovulatory estrus, see below and Section II.E.5.c)]. Moehlman (1974) observed year round natality in feral asses with peak natality from June through July indicating year round ovulation and estrus. Welsh (1975) similarly observed year round estrus and copulation in Sable Island horses with concentrations of foaling and mating during the late spring and early summer. This changes the concept of the mare as "seasonally polyestrus" (Asdell, 1964) to being 'polyovulatory' with some seasonal influence.

Latitude, which influences photoperiod length throughout the seasons, is probably the greatest influence on the regularity of ovulation and estrous cyclicity. The data of Hughes et al. (1972a) from latitude 38° 35' North reported 106 estrous periods between November and March for two subsequent years with only eight being anovulatory. From Ginther's data (1974) at latitude 43° 08' North, 68 estrous periods were recorded

from November through March with 65 of these being anovulatory. Experiments extending the number of daylight hours in the winter with artificial lighting to simulate spring and summer conditions by Burkhart (1947), Loy (1968), Nishikawa et al. (1952), Oxender and Noden (1976) and Sharp et al. (1975) have demonstrated that ovulatory estruses could be induced earlier in the year.

C. The Equine Ovary

1. Changes in the Ovary During the Estrous Cycle

Small follicles less than 1 cm in diameter can be felt via rectal palpation on the ovaries shortly after ovulation and throughout diestrus, thus indicating that proliferation of follicles happens early in diestrus (Evans and Irvine, 1975). Daily palpation via the rectum usually begins at the first signs of behavioral estrus in most experiments, when one or more follicles can be palpated. As estrus proceeds one follicle usually grows larger than the others and becomes the ovulatory follicle. The average size of an ovulatory follicle twenty-four hours prior to ovulation in horse mares was from 3.5 to 5.5 cm (Hughes et al., 1972a). Some follicles have been reported to soften prior to ovulation and others remain turgid until ovulation. Hughes et al. (1972b) maintain that there is no consistant pattern and some follicles that do soften become turgid again before ovulation. Witherspoon and Talbot (1970a) reported that 92% of the ovulations they

observed occurred between 11 p.m. and 7 a.m. Hughes et al. (1972a) observed 76% of the ovulations occurred between 4 p.m. and 8 a.m. Multiple ovulations were reported in 25.5% of horse mare cycles; 24.9% were twin ovulations and 0.6% were triple ovulations (Hughes et al., 1972a). The interval between twin ovulations averaged 24 hours. Ovulations have been reported to occur also during diestrus but without any signs of estrous behavior (Stabenfeldt et al., 1972; Hughes et al., 1972a).

Estrous behavior ceases about 24 to 48 hours after ovulation. Hughes et al. (1972a) observed considerable variation among mares in the duration of estrous behavior after ovulation.

The histological arrangement of the equine ovary differs from other mammalian ovaries in that ovulation occurs at a specific place on the ovary, the ovulatory fossa (Eckstein and Zuckerman, 1956a; Stabenfeldt et al., 1975). This has been confirmed by intraperitoneal and extraperitoneal cinematography (Witherspoon and Talbot, 1970b; Witherspoon, 1975).

After ovulation a "soft friable mass" occupying the ovulatory fossa can be felt (Rossdale and Ricketts, 1974). This becomes a firm pit within twenty-four hours. Hughes et al. (1972a) reported that the corpus luteum is palpable from twenty-four hours postovulation and for the next 7 to 10 days as a spongy to rubbery mass. In another study it was found that corpora lutea exhibited a mean

palpable lifespan of 12.4 ± 3.0 days which correlated with progesterone production in the mare (Stabenfeldt et al., 1972).

D. Hormone Levels During the Estrous Cycle

1. Overview of Equine Plasma Hormone Patterns

The recent advances in radio-immunoassay and other laboratory techniques for analyzing concentrations of biochemical substances in nanograms per ml (10^{-9} gm/ml) and picograms per ml (10^{-12} gm/ml) levels and the ease with which serial samples can be taken from equids has led to a flurry of research on the concentrations of the various cycling hormones in blood plasma, ovarian tissue and urine during the estrous cycle of the mare. Ginther (1978) has reviewed assay techniques and research on the concentrations of the various hormones in mares.

Figure 1 is a schematic representation of the patterns and relationships in plasma concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone and estradiol-17β in horse and pony mares as reported in the literature and reviewed by Ginther (1978). The values for each hormone are approximate due to the differences in reported values. Use of various assay techniques and standards by researchers account for the differing values of each hormone in the literature. It is noteworthy that the reported patterns of changing hormone levels in relation to follicular development,

maturation and ovulation as well as corpus luteum development and regression are fairly consistant in the literature.

It can be seen in Figure 1 that at the beginning of estrus, progesterone levels are low (below 1 ng/ml) and estradiol-17β levels followed by LH levels have begun to increase. As estrus proceeds, estradiol-17β levels peak one to three days before ovulation and LH levels peak at or 24 hours after ovulation when FSH levels are beginning to increase for the late estrus-early diestrus surge. Estradiol-17β levels decrease to diestrus levels by the end of estrus, but LH levels do not reach low levels until about five days after ovulation. Progesterone levels increase shortly after ovulation and remain high until about the fourteenth day of diestrus or three to four days before estrus begins again. It is the regression of the corpus luteum that results in a decrease in progesterone levels. The FSH surge during mid-diestrus, prior to the decrease in progesterone concentration, is hypothesized to induce the follicular growth that will result in the development of the ovulatory follicle (Evans and Irvine, 1975).

Plasma levels of two androgens, androstenedione (Noden et al., 1975) and dehydroepiandrosterone (Rance et al., 1976) have also been analyzed in the mare. Both appear to peak before or on the day of ovulation, then decrease and remain at lower levels during diestrus. The role that these androgens play during estrus is unknown.

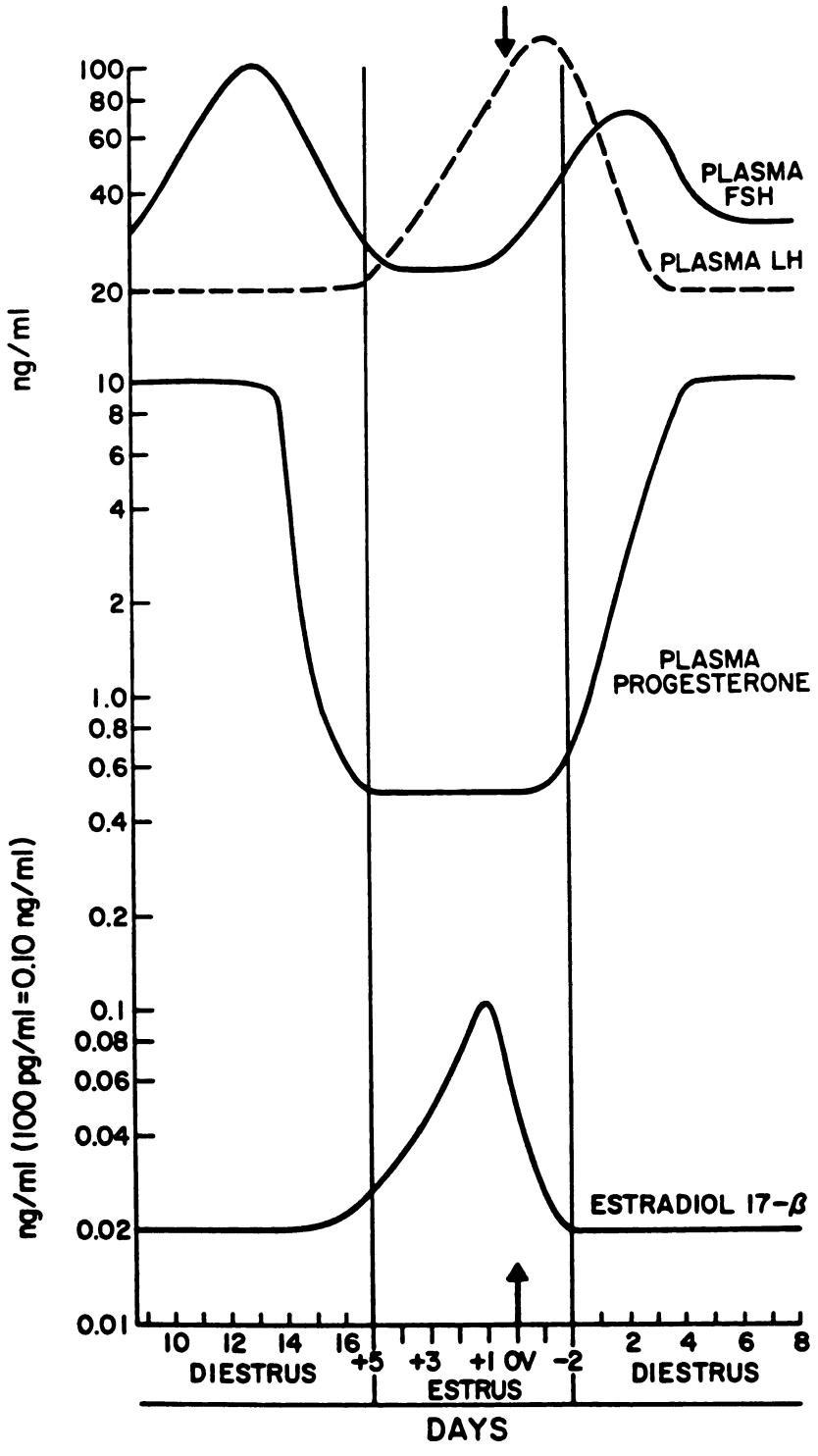

Figure 1. Plasma hormone pattern schematic of the equine estrous cycle.

Testosterone, the primary male androgen, which has been found in other female mammalians, has not been measured in equine plasma during the estrous cycle. Testosterone may function during estrus in relation to behavioral receptivity, as it can in rats and cats (Whalen and Hardy, 1970).

2. Comparison of Equine Plasma Hormone Patterns with Other Domestic and Laboratory Animals

Hansel and Echternkamp (1972) and Gay et al. (1970) reviewed research done on the plasma hormone patterns in the cow, ewe and sow. During the short period of estrus in the cow (18 hours with ovulation 11 hours after the end of estrus), ewe (28 hours with ovulation of several ova near the end of estrus) and sow (46 hours with multiple ovulations averaging 16.4 ova near the end of estrus) progesterone levels are below 1 ng/ml as in the mare. Progesterone increases rapidly during the first few days after estrus and ovulation to high diestrus levels (i.e. cow-day 5; ewe-day 4; sow-day 2; mare-day 5 of diestrus). LH increases at the onset of estrus in the cow, ewe and sow and quickly reaches peak levels which occur prior to ovulation in contrast to the LH peak in mares which occurs at or twenty-four hours after ovulation. Estrogens (estradiol-17β and estrone) have also been assayed in the cow, ewe and sow. Estradiol-17β levels increase before LH and the peak values occur before the onset of estrus

in the sow and at the onset of estrus in the cow and ewe. The main differences between the hormone patterns in the mare and those in the cow, ewe and sow are the durations of the estradiol-17β and LH peaks and the period of low values of progesterone. There is a temporal protraction of elevated levels of LH and estradiol-17β in the mare which slowly increase to peak levels and then slowly decrease after ovulation. Whereas, in the cow, ewe and sow the levels of LH and estradiol-17β increases rapidly, peak and decrease rapidly within 24 to 48 hours (the peaks resembline spikes). In the mare the period of progesterone decrease to nadir values is longer and the rate of increase to diestrus values is more abrupt than in cows, ewes and sows. FSH plasma concentrations apparently have not yet been quantified in all the species for appropriate comparison here.

Clemens and Christensen (1975), Davidson (1972), Schwartz (1969) and Short (1972) reviewed the hormonal patterns in the estrous cycle of the intact rat. Rats with four day estrous cycles showed estradiol-17β peak values on the morning of proestrus (the day prior to estrus). LH reaches peak values during the afternoon of the same day and ovulation occurs shortly after midnight. The appearance of sexual receptivity is either a few hours before ovulation or during the afternoon of pro-estrus (about 4 p.m.) and full receptivity is apparent early in the evening (about 7 p.m.) Rogers, 1970). Sexual

receptivity continues after ovulation and into the next day. Between the time of the LH peak and ovulation, progesterone reaches peak values. The progesterone peak, which follows the estrogen peak, has been reported as the stimulus for estrous behavior in the intact rat and other Rodentia, although sexual receptivity begins before progesterone reaches peak values (Rogers, 1970). Clemens and Christensen (1975) reviewed experiments on estrous behavior induction in ovariectomized female rodents. Estradiol benzoate administration followed by progesterone was found to be more effective than estradiol benzoate alone for induction of estrous behavior. Rats and most other rodents show a reversed estrogen, progesterone peak patterns in contrast to mares, cows, ewes and sows prior to ovulation. However, it is likely that the progesterone in rodents is of adrenal origin because corpora lutea only secrete progesterone if mating occurs during the previous estrus resulting in pregnancy or pseudopregnancy (Brown-Grant, 1971).

A prosimian, the thick-tailed bushbaby (Galago crassicaudatus crassicaudatus) most closely resembles the mare in duration of estrous behavior and patterns of plasma progesterone and estrogen concentrations. Eaton et al. (1973) found that the mean duration of estrus in the thick-tailed bushbaby was 5.8 days with mean progesterone levels decreasing prior to estrus and remaining at concentrations below 1 ng/ml until the end of estrus. Estradiol-17β levels increased at the onset of estrus and peaked one to

two days before the end of estrus. Peak estradiol-17β levels coincided with maximum behavioral receptivity and it was estimated from gestation lengths of pregnant females, that ovulation occurred on the day after the estradiol-17β peak as in the mare.

E. Equine Estrus

1. Primary Detection of Estrus

The entire behavior of a mare towards a stallion changes conspicuously during estrus. Detection of estrus or heat is usually accomplished by exposing a mare to a stallion, by a process known as "trying" (Mahaffey, 1950) or "teasing" (Ginther, 1974; Hughes et al., 1972a; Rossdale and Ricketts, 1974). Methods employed for teasing vary in the degrees of exposure of the mare to the stallion. Mares can be enclosed in a pen (Rossdale and Ricketts, 1974) or chute as a group (Back et al., 1974), be singly contained in a stall or chute (Andrews and McKenzie, 1941), behind a teasing bar or rail (Back et al., 1974; Rossdale and Ricketts, 1974) or be on a lead in the same enclosure with the stallion who is also on a lead (Ginther, 1974). The stallion used for teasing can be a normal, vasectomized or have a surgical retroversion of the penis (Rossdale and Ricketts, 1974). Pony stallions are commonly used for teasing because they are easier to handle than their horse counterparts and it is difficult for a pony stallion to accidentally copulate with a horse mare (Rossdale and Ricketts, 1974).

2. Confirmation of Estrus

Gynecological examinations by a veterinarian or trained technician are helpful in confirming estrus. Palpation of the ovaries via the rectum is used to detect the size of developing ovarian follicles in millimeters or centimeters. The position of the follicle on the ovary and its consistancy are also often noted (Rossdale and Ricketts, 1974). Voss et al. (1973) determined that rectal palpation did not affect the fertility of normally cycling mares. Examination of the vagina and cervix can be done using a speculum. During late estrus the vaginal mucosa is pink or red and moist and the cervix is relaxed, open and swollen. During diestrus the vaginal mucosa is pale and dry and the cervix is closed and smaller (Rossdale and Ricketts, 1974). However, examination of the vagina and cervix is not as reliable as ovarian palpation at the beginning of estrus (McDonald, 1969) or during estrus due to the wide variation among mares (Andrews and McKenzie, 1941). In addition, vaginal and cervical examinations can introduce bacteria with resulting uterine infections if not performed under septic conditions (Rossdale and Ricketts, 1974).

3. Duration of Estrus

As mentioned previously, the estrous cycle shows seasonal variations in its cyclicity. This is manifested in the changing length of the estrous cycle throughout the

year, with the duration of estrus varying more than the duration of diestrus (Ginther, 1974; van Niekerk, 1967). Estrus appears to be shortest during the months of summer and early fall (June to October in the Northern Hemisphere and November to May in the Southern Hemisphere). The results of several studies are shown in Table 1. It can be seen that the mean duration of estrus varies among the studies with the least variation seen from May to September (Northern Hemisphere equivalent months). The variation among studies can be attributed to many factors, some of which are latitude, breed differences of the subjects, sample size and difference in observation and experimental methods.

In other equids it has been reported that the duration of estrus for Przewalski mares in captivity is 2 to 4 days, rarely longer (Dobroruka, 1961). Average estrus duration of Hartmann's zebra mares (Equus zebra hartmannae) was reported as 2 to 3 days during which repeated copulations were observed at almost hourly intervals (Joubert, 1972). All horse mares observed during post-partum estrus on Sable Island (with the exception of one mare) were reported to be receptive for only 1 day (Welsh, 1975). These observations suggest that duration of estrus is affected by domestication and that in a normal free-ranging to wild herd environment, mares may be less sensitive to estrogen plasma concentration as the follicle matures and only display estrous behavior when

Table 1. Studies on monthly variation of duration of estrus in days.

Reference	Location	Breed(s)	Sample Size	Number of Cycles	Mean Duration of Estrus in Days by Month†											
					Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.
Arthur and Allen (1972)	London, England	Welsh Mountain Ponies	10	44	--	--	6.5	9.3	8.8	6.8	6.2	7.0	--	--	--	--
Ginther (1974)	Madison, Wisconsin	Large ponies 13 Riding horse 1	14	14 14				26.0	15.0	8.3	7.2	5.4	5.2	5.9	7.8	9.0
Hughes et al. (1972a)	Davis, California	Thoroughbreds 7 Quarter horse 4	11	293	7.23	5.06	6.69	8.38	7.76	5.72	4.47	4.70	4.75	4.44	4.53	5.20
van Niekerk (1967)	South Africa	Light Farm	8-26	8-21	18.7	--	19.2	10.4	10.6	5.9	5.7	5.1	5.1	6.9	9.3	19.3
			S. African Months		June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May

†Northern Hemisphere equivalent months

concentrations reach near ovulatory levels--thus copulation occurs at this time only; the probability of conception being greater at this time also.

4. Behavior of the Mare During Estrus and Courtship

Ginther (1974 and 1978) and Back et al. (1974) recognized that estrous behavior or receptivity in the mare during teasing is composed of several behavior patterns that can occur together or may be displayed in various combinations, rather than as a total on-off phenomenon implied by other researchers. Estrous behavior patterns are tail raising, squatting, winking and urination. These behavior patterns are fully described below.[2]

a) Tail Raising - Elevation of the tail, resembling that assumed for urination (Figure 2), but is maintained without movement (switching) for a longer period of time during teasing (Figure 3) (Ginther, 1978). Occasionally in addition to elevation a sideways deviation of the tail is seen in some mares (Figure 4). Height of tail raising varies among mares and breeds. Breed variation is probably

[2]Photographs of the behavior patterns were taken by the author with a 16 mm movie camera and a 35 mm camera. Photos illustrating some of the behavior patterns also appear in Ginther (1978) and Rossdale and Ricketts (1974).

Figure 2. Tail Raise during urination (rearview).

Figure 3. Tail Raise during teasing (rearview).

Figure 4. Tail Raise with side deviation during teasing (rearview).

associated with postural tonus (Kiley-Worthington, 1976) or breed confirmation. For example: tails of shetland ponies are set well below the croup, or top of the back, thus the degree of elevation from a normal resting position or tail down (Figure 5) to the raised position (Figure 6) is not as high as the elevation for a typical horse mare (Figure 7).

b) Urination - Fluid composed of urine, mucus and pheromonal secretions can be passed in small quantities or drops, a steady flow as in normal urination or in spurts (Ginther, 1978). The color and consistancy of the fluid varies from thin, clear yellow to thick, cloudy yellow (Ginther, 1978). The frequency of urination during teasing is far greater than the once every 4 to 4½ hours reported for adult New Forest Pony mares by Tyler (1972). Ginther (1978) reported that in 350 determinations of fluid discharge dripping, spurting, streaming and combinations of these were observed 39%, 2%, 13% and 10% of the times respectively (urination type was not observed 36% of the time).

c) Squatting - Hind legs are spread laterally with the stifles and hocks flexed posteriorly which tips the pelvis dorsally and posteriorly and this in turn lowers the perineal area (Ginther, 1978) (Figure 6).

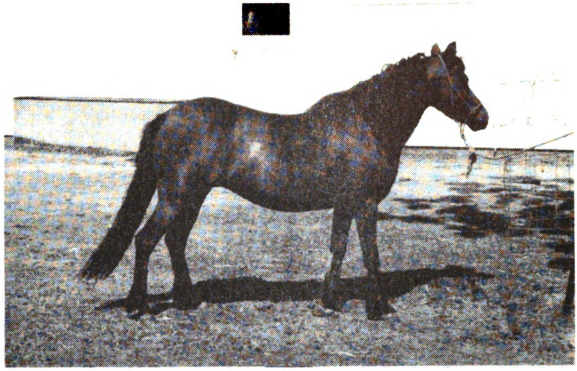

Figure 5. Pony mare tail down in resting position (sideview).

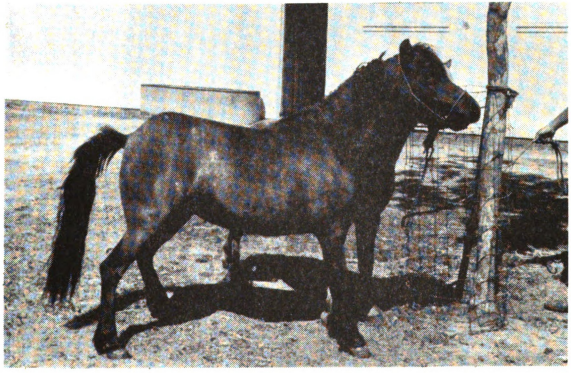

Figure 6. Pony mare tail raised during teasing (sideview).

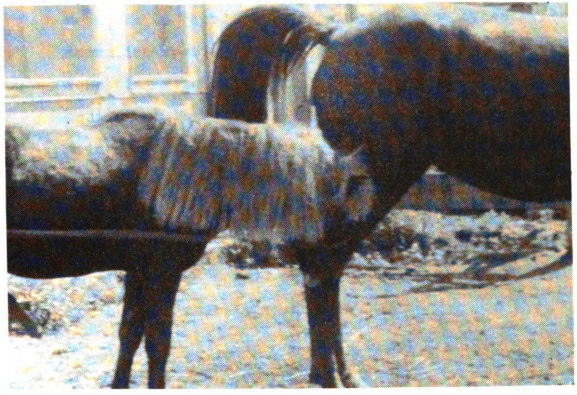

Figure 7. Horse mare tail raised during teasing (sideview).

Ocassionally one hind hoof is observed resting only on the tip, which led Ginther (1978) to believe that most of the mare's weight is on the front hooves. Tail raising and squatting combined are often referred to as a static posture (Tyler, 1972) or as posturing (Collery, 1974; Ginther, 1978). This corresponds to "lordosis" described in many other mammalian species, but whithout curvature of the back. Squatting resembles that observed for urination in equids but as with tail raising the posture is maintained for a long period of time. Squatting is not always seen in mares with leg and hoof ailments. Squatting is more subtle in pony mares than in horse mares because the legs are shorter. Therefore the legs cannot be spread as far apart at the hoof level nor bent as sharply at the knee as is seen in the horse mare.

d) Winking - Also called winking the clitoris, wink, eversion of the clitoris or clitoral flashing. Repeated muscular contractions which rhythmically evert the vulva and protrude or expose the clitoris (Ginther, 1978; Hughes et al., 1972a; Rossdale and Ricketts, 1974; Tyler, 1972). This can accompany dripping and spurting of urine or occur without urination. Normally a few arhythmic winks follow a normal urination.

Figures 8 and 9 show a pony mare with a relaxed vulva and during winking with the clitoris exposed. Most mares have highly pigmented vulvular and peroneal epithelium externally, thus when the vulva is everted the pink color of the inner vulva and clitoris (which is enhanced during estrus) creates a contrast against the surrounding vulvular and perineal area. Winking is difficult to observe in many mares when the tail is in the normal down position.

e) Other Behaviors - Other behavior patterns have been observed during estrus in equids although all are not exclusively estrous behavior patterns. Nuzzling (Ginther, 1978) or contact with the muzzle and lips (Tyler, 1972) also known as naso-nasal contact (Joubert, 1972) can be initiated by the mare or stallion prior to other estrous behavior patterns or during investigation by the stallion. Squealing and pawing of the fore feet of the mare was observed occasionally by Tyler (1972) while the stallion was investigating the genital area of the mare. Young mares in estrus have been observed moving away, snapping and kicking at the stallion and being generally fearful of the stallion's investigatory attentions (Tyler, 1972). Other young mares allow the stallion to mount and then move forward faster

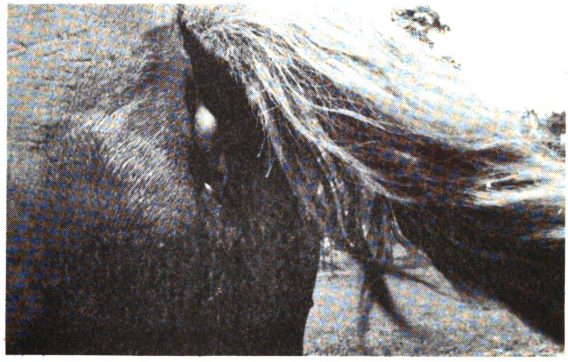

Figure 8. Pony mare vulva relaxed.

Figure 9. Pony mare winking vulva with clitoris exposed.

and faster until the stallion was dislodged (Tyler, 1972; Stebbins, personal communication).

Rossigkeitsgesicht or 'in heat' facial expression was described by Trumler (1959). It is characterized by the head stretched forward with the ears back or to the sides, the corners of the mouth are drawn back exposing the teeth and gums and there is occasional movement of the jaws. Rossigkeitsgesicht has been observed in plains zebras by Klingel (1969a) and Joubert (1972), Grevy's zebras by Zeeb and Kleinschmidt (1963), feral asses by Moehlman (1974) but is absent in New Forest Ponies (Tyler, 1972) and in true horses (Zeeb, 1959). This author has observed Rossigkeitsgesicht infrequently in ponies (on two occasions) and by one horse mare during four consecutive estruses. Figures 10 through 16 show Rossigkeitsgesicht in a Thoroughbred mare in a sequence of mouth openings and closings incompassing less than two seconds time.

It was noted by Klingel (1969a, and 1975) that adult plains zebra mares only show estrous behavior when being courted actively by the stallion. Whereas, in young mares the tail raise-squat posture is conspicuous and attracts other stallions which results in their abduction from the herd and ultimate breeding to a stallion other than their sire.

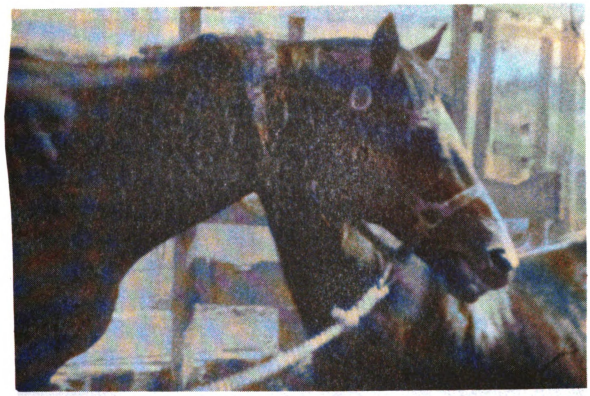

Figure 10. Rossigkeitsgesicht beginning, drawing back corners of mouth.

Figure 11. Rossigkeitsgesicht continued, opening mouth maximally, head stretched forward and to the side.

Figure 12. Rossigkeitsgesicht continued, closing mouth with teeth still exposed, head still stretched forward.

Figure 13. Rossigkeitsgesicht continued, opening mouth second time, head stretched forward.

Figure 14. Rossigkeitsgesicht continued, mouth closing second time with teeth still exposed, head stretched forward.

Figure 15. Rossigkeitsgesicht continued, mouth opening for third time.

Figure 16. Rossigkeitsgesicht continued, mouth closing for third time.

5. Behavioral and Physiological Anomalies Associated with Estrus

a) Silent Estrus - Also called quiet estrus or physiological estrus (Ginther, 1974; Andrews and McKenzie, 1941). An estrous period with normal ovarian development and ovulation without behavioral receptivity by the mare. Ginther (1974) reported a 6% frequency of silent estrus. This author calculated silent estrus frequencies of 7% and 5.6% for the data of Hughes et al. (1972a) and Andrews and McKenzie (1941) respectively.

b) Split Estrus - One or more days within estrus when behavioral receptivity is not shown although a follicle continues to develop. Frequences reported for split estrus were 12% (Ginther, 1974) and 4.8% (Hughes et al., 1972a). For the data of Andrews and McKenzie (1941) this author calculated a split estrus frequency of 14%.

c) Anovulatory Estrus - Behavioral receptivity without ovulation, although there may be some follicular development. Occurrence of anovulatory estrus during winter months was discussed in Section II.B.2. Frequency of anovulatory estrus year round was reported as 3.1% by Hughes et al. (1972a), with 0.34% occurring other than during the winter months. Thus it

can be assumed that anovulatory estrus occurs most frequently during the winter months.

d) Multiple Ovulation - More than one follicle maturing and ovulating during estrus. This has been commonly reported in horses, but rarely reported in ponies. Incidences reported were 25.5% in horses (Hughes et al., 1972a), 2% in pony-horse crosses (Ginther, 1974) and 0.8% in Welsh ponies (Arthur and Allen, 1972).

F. Behavior of the Mare During Diestrus

In the literature diestrous behavior is regarded as the antithesis of estrous behavior. There are few full descriptions of diestrous behavior in the literature. The combination of behavior patterns observed vary greatly among mares, moreso than during estrus. Behavior patterns that have been observed during diestrus are: kicking with one or both hind legs, striking with a forefoot, biting or attempting to bite the stallion, ear pinnae directed backwards, tail compressed tightly down, tail switching rapidly, squeal vocalizations, shaking the head and movement away from the stallion (Ginther, 1978; McKenzie and Andrews, 1937; Rossdale and Ricketts, 1974). On rare occasions any of these behavior patterns may be observed during estrus (Ginther, 1978).

G. Pre-Copulatory Behavior of the Stallion

This author defines pre-copulatory behavior of the stallion in an experimental situation as the behavior occurring during the interval of time between initial visual contact with the mare and mounting. In a non-experimental setting, i.e. field observation, pre-copulatory behavior is the behavior of the stallion towards the mare immediately prior to copulation (Tyler, 1972).

1. Observation and Description

Description of pre-copulatory behavior of stallions is sparse in the literature. It has been noted many times that the behavior of the stallion is more dynamic than that of the relatively static mare in estrus. The stallion performs investigative smelling of the muzzle, axilla, flank, groin and genital region (Tyler, 1972; Rossdale and Ricketts, 1974; Ginther, 1978). All of these areas of the mare contain histologically similar sebaceous glands described by Schaeffer (1940). The stallion also nibbles and licks the withers, forelegs, back, rump and hindlegs and occasionally bites the mare (McKenzie and Andrews, 1937; Tyler, 1972; Rossdale and Ricketts, 1974; Ginther, 1978). Flehmen or lipcurl by the stallion is displayed in response to smelling the genital area and the urine of the mare that are reported to contain pheromones (Rossdale and Ricketts, 1974). Although the chemical constitution of these pheromones

has not been elucidated for equids they have been identified for the rhesus monkey, Macaca mulatta (Michael et al., 1971). Flehmen was described by Schneider (1930 and 1931). It occurs in the males of many ungulate species and is most frequently observed when females are in estrus. During Flehmen the head is raised and stretched upward while the upper lip is curled back exposing the upper teeth and gums and in equids the external nares are closed and breath retained (Figure 17). It is suspected that the vomeronasal organ, a blind pouch connected to the nasal cavity (Sisson and Grossman, 1953) by the naso-palatine duct and innervated by the facial nerve is stimulated by sex pheromones during Flehmen (Eisenberg and Kleiman, 1972). Flehmen has also been observed in mares and immature equids of both sexes by Tyler (1972) and Stebbins (1974) in response to urine on the ground.

During the period of investigative smelling, licking, etc., the stallion vocalizes (whinnies and snorts) and the penis becomes erect. Protrusion and erection of the stallion's penis is slow. Erection is due to the gradual increasing tumescence of the organ which is vascular-muscular in nature (Walton, 1955) and is similar to man's. Walton (1955) and Wiersbowski and Hafez (1961) opined that continued courtship and stimuli reception is important for complete erection. Investigative behavior, which involves stimuli reception, and vocalization continues until the stallion mounts the mare (Tyler, 1972).

Figure 17. Pony stallion, Flehmen - head stretched forward and upward, upper lip curled back exposing teeth, external nares closed.

Free-ranging, semi-wild and wild equine stallions are depicted in the literature as ever ready to mate and it appears that in New Forest Ponies (Tyler, 1972) and in Plains Zebras (Klingel, 1969a) it is the mare who allows the stallion to copulate only at the height of estrus.

2. Experimentation

Experimentation of pre-copulatory behavior of the stallion is confined to the examination of stimuli for and latency to erection and mounting and the number of mounts per ejaculation. The parameters of other pre-copulatory behaviors have not been reported nor has the sequential pattern of pre-copulatory behaviors been examined for equids.

Wiersbowski (1959) found that the mean latency to erection and mounting for experienced stallions under normal breeding conditions was 119.2 seconds and 100.5 seconds respectively. Pickett and Voss (1972) and Pickett et al. (1970) measured the seasonal variation of reaction time (latency from visual contact until mounting and beginning of copulatory movements) in a group of stallions for two consecutive ejaculations. The yearly mean reaction time was 3.5 minutes for first ejaculates and 3.7 minutes for second ejaculates. Reaction time variated from 47 seconds to 10.8 minutes for first ejaculates and from 40 seconds to 15.9 minutes for second ejaculates and was significantly different among

stallions for both ejaculates ($P < 0.1$). The shortest reaction times occurred during the months from May to August for both ejaculates. This coincides with the breeding season.

Wiersbowski (1959) found that the mean number of mounts per ejaculation was 1.4 which compares with the mean of 1.8 reported by Pickett et al. (1970).

Experienced stallions display conditioning to the breeding situation (Wiersbowski and Hafez, 1961). Neither visual deprivation (via blindfold), olfactory suppression (nosemask soaked with trichloroethylene) or the use of a cow or dummy deterred the stallions from becoming erect or mounting (Weirsbowski, 1959). Whereas, young inexperienced stallions did not respond significantly in these unusual test situations.

In all of the above experiments the method of teasing and degrees of access to the mare by the stallion was not reported. In addition, the day of estrus of the mares used was not incorporated in any of the experiments and the possible ability of a stallion to detect impending ovulation in a mare has not yet concerned researchers.

H. Research on Estrus Induction and Ovulation Synchronization

For many years it was suspected that regression of the corpus luteum in cows, ewes and sows was governed by a uterine luteolytic agent (Anderson et al., 1969). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), a 20 carbon lipid, was found to be produced by the

uterus in cows and ewes at the time of corpus luteum regression (Anderson et al., 1969; Hansel and Echternkamp, 1972). It was then thought that $PGF_{2\alpha}$ was the luteolytic agent. In thirty anesthetized mares, Douglas and Ginther (1976) assayed Prostaglandins F in the uterine venous plasma. They found a significant increase of uterine venous plasma PGF levels occurred between day 10 and 14 of diestrus preceeding the rapid decrease of plasma progesterone levels (Section II.D.1.). In addition, a number of studies have shown that exogenous administration of as little as 1.25 mg of synthetic $PGF_{2\alpha}$ once between days 5 to 9 of diestrus caused plasma progesterone levels to decrease and estrus to ensue within two to four days in intact mares (Allen and Rossdale, 1973; Allen and Rowson, 1973; Allen et al., 1974; Douglas and Ginther, 1972, 1975a, 1975b and 1975c; Noden, 1975; Noden et al., 1974; Oxender et al., 1975; Palmer and Jousset, 1975; Spincemaille et al., 1975).

Prematurely shortening the duration of diestrus is only one of the objectives that is integral with the goal of ovulation synchronization in equids. Research using $PGF_{2\alpha}$ in cows and other large domestic species has proved more fruitful. Estrus follows ovulation in the cow, ewe and sow in a fixed short temporal pattern (Section II.D.2.). In these species shortening diestrus appears to insure ovulation at a constant time interval, so that administration of $PGF_{2\alpha}$ not only shortens diestrus, but also synchronizes ovulation. Whereas in equids, estrus length and the time interval from the

beginning of estrus to ovulation is variable (Section II.E.3.) and $PGF_{2\alpha}$ administration does not shorten this interval.

Human Chorionic Gonadotrophin (HGC), which resembles LH in structure and function (Baird, 1972), when given on the second day of estrus shortens the duration of estrus significantly ($P < 0.05$) and causes earlier ovulation in treated mares (Loy and Hughes, 1966; Voss et al., 1974; Webel et al., 1977). However, repeated administration of HGC during successive estruses has the opposite effect. By the third cycle, HGC significantly lengthens ($P < 0.05$) the duration of estrus and the interval to ovulation (Sullivan et al., 1973). It is thought that administration of HGC over time causes HGC-antibodies to develop in the mare.

Gonadotrophin Releasing Hormone (GnRH), a hypothalamic releasing factor, stimulates pituitary release of LH and FSH in many large domestic species (Convey, 1973). Studies show that a single dose of 1 mg synthetic GnRH on the second day of estrus causes a brief elevation in LH levels which shorten estrus duration but not the interval to ovulation in treated mares (Irvine et al., 1975). Further studies by Irvine et al. (1975) showed that a larger 2 mg dose of GnRH given daily until ovulation caused ovulation sooner in treated mares ($P < 0.05$).

Oxender et al. (1977), as previously mentioned in the introduction (Section I), combined $PGF_{2\alpha}$ treatment during diestrus (between days 5 and 7) followed 96 hours later by

GnRH administration in two experiments. The first experiment tested dose effect of 0, 1, 2 and 5 mg GnRH and found that thirty minutes after GnRH administration LH levels were elevated 0, 2, 2.5 and 2.5 fold, respectively. LH levels returned to near pre-treatment levels within 24 hours. Although LH levels increased briefly and the mean intervals from $PGF_{2\alpha}$ to ovulation and GnRH to ovulation appeared to decrease, the differences in the intervals were not statistically significant ($P > 0.05$) compared with the control. The second experiment tested the effectiveness of administration of 0, 5 mg once and 5 mg daily of GnRH till ovulation following $PGF_{2\alpha}$ administration. Again in the GnRH treated mares LH levels increased significantly ($P < 0.01$) within an hour of GnRH treatment but decreased to near pre-treatment levels within 24 hours. Interval from $PGF_{2\alpha}$ treatment to ovulation was not different between treatments and control ($P > 0.05$). Although the mean interval from GnRH to ovulation and the onset of estrus to ovulation as well as the duration of estrus were shorter due to the two GnRH treatments, the difference in comparison with the control was not statistically significant ($P > 0.05$).

Behavior, other than the combined responses of the mare to a stallion connoting estrus, was not measured during any of the single drug studies above. In many pharmacological studies today, researchers are as interested in the side effects (adverse behavioral changes produced by the drug) as they are in the effectiveness and proper dosage

of the drug. I observed behavior during Oxender et al.'s studies to determine if it was altered by the administration of $PGF_{2\alpha}$ and the various treatment regimes of GnRH. The results of the analyses are given in the results section of this dissertation.

I. Experimentation Quantifying Equine Estrous Behavior

Back et al. (1974) teased 35 and 54 horse mares on subsequent years to determine which behavior patterns were most indicative of equine estrus. They calculated coefficients of correlation for nine behavior patterns on the basis of presence or absence during estrus and diestrus. The behavior patterns were: tail raising, squatting, winking, urinating, kicking, ears back, squealing, fence pushing (pushing the fence of the teasing chute or bar) and striking. Winking had the highest coefficient of correlation for both years with +0.838 and +0.874 for 1971 and 1972 respectively. The rank order of the coefficients of correlation varied for both years for the other behaviors. Tail raising, urinating and squatting were all highly positively correlated with estrus during both years. The method of analysis to further specify the combination of behaviors most indicative of estrus was "multiple regression analysis through backward determination of procedures." The combination that the analysis produced was winking, squatting and the lack of kicking for both years. For the methods of teasing used in these experiments (single mares behind a rail in 1971 and up

to nine mares in a chute in 1972), the combination of behaviors specified were appropriate criteria for estrus determination because of the limited access to the mare by the stallion. There was no further correlation of estrous behavior with the day of estrus in relation to ovulation.

Ginther (1978) performed a similar experiment. He teased individual pony mares and stallions on leads, thus allowing mounting but not intromission by the stallion. Behaviors were scored during 581 teasing sessions when mares were confirmed to be in estrus and 2181 sessions when mares were in diestrus. Percent of occurrance based on presence of a behavior during a teasing session were computed for a list of behavior patterns over all days of estrus. The behaviors of the mare that were scored as present during teasing were: tail raising, urination, winking, remaining calm, nuzzling the stallion, posturing (squatting), mounted by the stallion (further qualified as standing with tail raised, or tail down, or not standing), not mounted by the stallion, kicking, biting, ears back, switching the tail, moving about, shaking the head, pawing, raising the front, raising the rear, vocal response, snorting and squealing. The behavior patterns with the highest percent of incidence during confirmed estrus were: mounted by the stallion and standing with tail raised (100%), raised tail (97.9%), remained calm (89.0%), winking (87.1%), posturing (72.3%) and urinating (53.9%). This study (Ginther, 1978) did not arrange the percentage of incidence

of the behavior patterns with the day of estrus of the mares or in relation to ovulation.

Sullivan (1972) calculated the percentage of 53 mares displaying estrous and non-estrous behavior patterns during 99 estrous cycles, in which the day of ovulation was determined. These behavior patterns were: kicking, ears back, tail raise, urination, winking and squatting. Tail raise, urination and winking were graphed as peaking at about 100% on the day of ovulation and declining thereafter. Squatting peaked at about 95% on the day before ovulation and subsequently declined. Kicking and ears back declined during estrus to lows of 5% and 10% respectively on the day of ovulation and increased quickly afterwards. There were no statistical analyses performed on the data, which like Ginther's (1978) was only percentage of incidence and not frequency data.

Andrews and McKenzie (1941) used a graded series of behavioral responses or "degrees of receptivity" that they described (McKenzie and Andrews, 1937) as ranging from I. Very Receptive to VIII. Very Actively Resistant during estrus and diestrus. They arrayed these graphically (Andrews and McKenzie, 1941) as I. = +3 to VIII. = -4, with a single value representing each day of teasing to determine the degree of receptivity during estrus (in relation to day of ovulation) and diestrus for each of 29 draft mares and 17 light mares observed for the breeding seasons of 1937 and 1938. The degrees of receptivity were described (McKenzie and Andrews,

1937) with a combination of criteria based upon objective and subjective terminology. In Table 2 this author separated the definitions into objective and subjective terminology for the degrees I to IV only. Despite the subjective terminology used in the definitions, they are remarkable for the year in which they were written.

Andrews and McKenzie (1941) concluded that during estrus 'maximum estrual response' (which this author interpreted to mean +3) was not reached until two days prior to ovulation. Following ovulation, receptivity decreased and estrus ceased within 24 to 48 hours. The data that the conclusions were based on were only presented graphically and no statistical analyses were performed on it by the authors.[3] However, they did recognize that even with a limited number of degrees of receptivity that there was individual variation among mares.

References to Andrews and McKenzie's work on sexual receptivity abound in the literature. A few researchers have stated that some of the mares they have observed show increased 'intensity' of sexual behavior up until ovulation but, there was generally a wide range of variation in display character and 'intensity' of behavior among all mares (Bain, 1957; Rossdale and Ricketts, 1974; Sullivan, 1972; Mahaffey,

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[3]This author was perplexed by the marks on the graphs that indicated intergrade values between the defined degrees of receptivity (i.e. ½, 1½ and 2½). These intergrade degrees of receptivity were not mentioned in McKenzie and Andrews' (1937) definitions, nor in the explanation or discussion of the addition, the intergrade degrees appear too numerous to be artist's mistakes.

Table 2. Degrees of receptivity from McKenzie and Andrews (1937) partitioned into objective and subjective behavioral terminology.

Degree of Receptivity	Objective Terminology	Subjective Terminology
I. Very Receptive	a. tail raising and winking as stallion approaches b. urination possible	a. mare definitely interested in stud b. mare follows stud's movements c. mare stands quietly as stud approaches d. mare is attentive to stud as he smells her head, neck e. mare stands firmly and waits for stud to mount.
II. Moderately Receptive	a. mare raises tail and winks as stud begins to tease (smelling and gently biting her over the withers and along the back) b. urination possible	a. mare stands quietly b. mare permits stallion to mount without difficulty.
III. Mildly Receptive	a. mare raises tail as stud approaches flank region when teasing b. some winking or no winking c. urination possible	a. mare stands quietly as stallion approaches b. mare appears little interested in stud.
IV. Phlegmatic	a. no signs of receptivity (no tail raise, no winking)	a. mare stands disinterestedly as a stallion approaches and teases b. mare allows stallion to tease vigorously even biting along the flank and back c. mare makes no attempts to repulse stallion.

1950). Unfortunately none of the above researchers provided data of any kind to support their statements.

Ginther (1978) examined Andrews and McKenzie's (1941) graphical data. Using their values of +3 to -4 for the degrees of receptivity he analyzed groups of estrous periods of varying lengths. Estrous periods were grouped according to the interval from first day of estrus, as defined by the first day of a +1 response, until ovulation.

Five groups of estrous periods with interval lengths of 4,5,6,7 or 8 days were analyzed for differences in 'degrees of receptivity' among days using an analysis of variance and a multiple range test. The degrees of receptivity were significantly different among days for the 4 day and 6 day interval estrous period groups only. For the 4 and 6 day interval groups the 'degree of receptivity' was significantly less on the day of ovulation than of the two days prior to ovulation. Ginther, however, concluded that there was "no statistical support for the hypothesis that maximum estrual response was not reached until one or two days preceding ovulation." Andrews and McKenzie (1941) did not claim that the degree of receptivity one or two days prior to ovulation was significantly different from the other days of estrus or on the day of ovulation. They only claimed that the maximum response was reached before ovulation, which indicates to this author a possible linear or curvilinear relationship between the degrees of receptivity and the days of estrus. The null

hypothesis that Ginther tested was not appropriate to his interpretation of the results.

Nishikawa (1959) used an undefined series of +'s ranging from + to +++ to indicate five stages of intensity of estrous behavior. He concluded that estrus became more intense until the day of ovulation and then decreased in intensity rapidly. A graph and table are given in the publication but there are no criteria stated for the stages or statistical analysis performed on the data.

Nishikawa's failure to describe the behavior of the mares in relation to the + values (i.e. behavioral criteria for his index), has inhibited further testing and quantification of behavioral phenomena because his research has permeated the literature. For example, reference to Nishikawa's results (and even his graph) appear in a respected veterinary endocrinology text (McDonald, 1969), again with the absence of criteria definitions in terms of behavioral descriptions.

Ginther (1978) noted that neither Nishikawa (1959) nor Andrews and McKenzie (1941) claimed that their observations on changes in estrus intensity could be used to predict ovulation time.

In attempting to show changes in intensity of estrous behavior more definitively Ginther (1978) examined 70 estrous periods of pony mares using an 'intensity index' that was a sum of weighted values of specific estrous behaviors. The individual values and their representative behaviors were: +3, standing for mounting with tail raised; +1, urinating;

+1, winking; +1, tail raising; 0, standing for mounting with tail down; -1, kicking; -1, tail switching; -1, ears back; -1, moving; -3, not standing for mounting. Ginther analyzed the first three days of estrus, midestrus, last three days of estrus and selected days of diestrus for mares with an estrous period of six or more days duration. This author presumed that an analysis of variance was used, although this was not stated. The results showed that the first and last days of estrus had significantly smaller mean intensity ($P < 0.5$) than did the other days of estrus. The mean intensity was not significantly different among the other days of estrus. The highest mean intensity was 5.2 ± 0.16 on the middle day of estrus. All of the estrus means were significantly higher than the diestrus means. With ovulation occurring between the third to last day of estrus and the last day of estrus in this particular data arrangement, there was no conclusion drawn about the intensity of estrus specifically in relation to ovulation. Ginther concluded that the results failed to indicate a gradual increase in intensity as estrus progressed to ovulation. However, neither the data arrangement nor testing significant differences among days was appropriate to demonstrate this conclusion.

Sokal and Rolf (1969) and Hutt and Hutt (1970) discussed the drawbacks of interpretation of analyses that use derived variables (ratios and indexes). Ginther's use of an index that is computed from the sums of positive and negative integers illustrates Sokal and Rolf's and Hutt and Hutt's

discussions. Only the values of +6 and -6 represent specific behavior patterns because there is only one way to compute each of these values. There are seven combinatorial ways of computing +5 or -5, twenty combinatorial ways of computing +4 or -4, and the possibilities increase exponentially for + or -3, + or -2, + or -1 and 0. Thus, the means of the indexes and furthermore the analysis of the means of the indexes cannot be interpreted behaviorally. Denenberg and Banks (1969), in their chapter on measurement of behavior, warns researchers that indexes should "be interpreted with great caution."

Intensity as a concept in behavior is "the dimension of magnitude or amplitude" of a behavior and is "the apparent level and form of the performance" (Denenberg and Banks, 1969). Intensity of response has been measured in objective quantities (e.g. time, speed, volume). Indexes are not true quantitative objective measurements. The dictionary definitions of intensity all express the measurement of a force or power in units objectively measured by machines or instruments (Morris, 1969). Thus this author does not think that intensity is a viable conceptual term for usage in sexual behavior at this time. Instead, the term receptivity that Andrews and McKenzie (1941) used and Beach (1976) equated with the female's readiness for copulation, is more suitable. Receptivity has been measured in other species in terms of specific parameters of behavior such as frequency, duration and latency.

Of all the equine researchers, this author believes that Andrews and McKenzie (1941) were on the right track. Their definitions of the degrees of receptivity (McKenzie and Andrews, 1937) although qualitative were based on a temporal occurance of the behavioral signs (i.e. before teasing began in I. and after teasing began in II. and III.--Table 2) during the teasing session. Temporal occurance of behavior is objectively measurable and quantifiable.

It is noteworthy that all of the researchers that are mentioned in this section are not behaviorists by training, this may explain some of the flaws in their research.

J. Quantification of Estrous Behavior of Other Mammalian Species

Sexual behavior of the female has been studied less frequently than that of the male in all mammalian species (Clemens and Christensen, 1975; Doty, 1974). With the discovery that estrus could be induced in guinea pigs with exogenous administration of a combination of estrogen and progesterone (Dempsey et al., 1936) research on estrous behavior and copulatory behavior increased using estrus induced females. Valuable information has been gathered about the hormonal control of estrous behavior (Eaton et al., 1975; Dixson et al., 1973), the form of copulatory behavior patterns (Diakow, 1975; Pfaff and Lewis, 1974), and the quantification of the components of copulatory behavior (Dewsbury, 1967) in various mammalian species. However, the

research on estrus induction was performed on ovariectomized females.

1. Measurement of Receptivity

Receptivity, in the literature, has been equated with the female's readiness to allow the male to copulate (Beach, 1976). Beach (1976) reviewed some of the measurements used in the research on sexual receptivity of females. Lordosis, which is analagous to tail raising and squatting of equids, is the behavior pattern most frequently measured in many laboratory species. Quantitative measurements consist of: frequency of lordosis or ratios involving the frequency of lordosis in relation to other behaviors (e.g. Lordosis Quotient in rats = $\frac{\text{number of lordosis responses by the female}}{\text{number of mounts by the male}} \times 100$), latency to lordosis and duration of lordosis. Other behavior patterns are also measured in terms of frequency, frequency ratios, duration and latency. Behavioral measurements appear to be species specific and are based on the copulatory patterns of the species in question.

2. Changes in Sexual Receptivity During the Estrous Cycle

Research on the change of sexual receptivity of intact females during a normal estrous cycle is limited to a few species. These are: ringtailed lemurs (Evans and Goy, 1968), talapoin monkeys (Scrunton and Herbert, 1970), pigtailed macaques (Bullock et al., 1972; Eaton

and Resko, 1974), rhesus monkeys (Michael and Welegalla, 1968; Michael and Zumpe, 1970; Michael et al., 1966 and 1967; Keverne, 1976) and dogs (Christie and Bell, 1972). Only two of these studies concluded from the quantified behavioral data that females were most receptive during estrus (Christie and Bell, 1972) or the follicular phase (Bullock et al., 1972) of the cycle. Unfortunately critical examination of the change of behavior of the female within estrus or the follicular phase was not done for either of the studies.

Bullock et al. (1972), did observe that quantified parameters of male pigtailed macaque behavior (i.e. latency to ejaculation, rate of intromission and ejaculation, and rate of pelvic thrusting) varied with the changes in perineal sex skin swelling of the female during the follicular phase. Laparotomies on several female pigtailed macaques revealed that ovulation occurred between the day of peak sex skin swelling and the first day of detumescence of the sex skin when male behavior patterns quantitatively peaked. Michael and Zumpe (1970) also found that mean ejaculation rate of male rhesus monkeys peaked during the late part of the follicular phase. Although these studies suggest that male primates are sensitive to the follicular state of the female, statistical analyses on the quantified behavior patterns of the male (other than calculation of means) were not performed.

3. Changes in Sexual Receptivity During Estrus

Research examining changes of receptivity during the estrous period is meager. This section focuses on the few experiments that have measured change in quantified behavior patterns of intact mammalian females during natural estrus.

Kuehn and Beach (1963) studied sexual receptivity of 32 virgin female rats for one estrus by testing them at hourly intervals with sexually experienced vasectomized males. Receptivity was measured as a "sensitivity index" (SI) which was the same as the lordosis quotient defined in Section II.J.1. The mean duration of sexual receptivity of the group of females was 19.7 hours. The total period of receptivity for each female was divided into fifths. Median SI's were calculated for each fifth of the receptive period. All possible pairs of medians were compared with a sign test. Kuehn and Beach concluded that readiness increased to its highest during the second fifth of the receptive period, which had an SI median significantly higher than that of the other fifths. Readiness or receptivity then gradually declined by the fifth period. Duration of lordosis was also measured to the nearest second. The researchers determined that the duration of lordosis was dependent upon the degree of sexual contact by the male (e.g. mount only, mount with intromission, and mount with intromission and ejaculation). Mounts with intromission and ejaculation produced the

longest duration of lordosis. No marked variation of lordosis duration was observed during the fifths of the receptive period, although the data to support this were not presented. Time of probable ovulation was not discussed.

Hardy (1972) studied sexual behavior in 10 continuously cycling female rats under a reversed light cycle regime (12 hours light, 12 hours dark). She measured the changes in vaginal histology along with the changes in behavior. Tests during 17 heat periods were performed every three hours. The receptive periods ranged in duration from 15 to 21 hours. The females were equipped with vaginal masks, a piece of masking tape which prevented intromission and thus coital stimulation. Hardy and DeBold (1972) had shown that coital stimulation in comparison with no stimulation decreased the probability of subsequent lordosis and increased rejection of the male. Hardy (1972) measured the lordosis quotient (LQ) and rejection quotient

$$(RQ = \frac{\text{number of rejections of male}}{\text{number of mount or mount attempts by male}} \times 100).$$

She found that during the first six hours of the receptive period (mid six hours of light) the LQ began to increase while the RQ began to decrease. The highest level of the LQ and the lowest level of the RQ was reached during the mid six hours of the dark cycle. By the beginning of the next light cycle females were decreasing the frequency of lordosis and increasing the frequency of rejection.

LQ decreased slower than RQ increased during the twelve hours of this light cycle. Day 1 of the vaginal estrous cycle or Proestrus-Estrus, was considered to be from mid light cycle to mid dark cycle of the LQ increase and peak and the RQ decrease and nadir which was the major portion of the receptive period. Hardy's results were similar to those of Kuehn and Beach (1963). The time of ovulation was not recorded.

Eaton et al. (1973) tested 23 female thick-tailed bush babies (Galago crassicaudatus crassicaudatus), a prosimian, for fifteen minutes daily during the estrous cycle. Behavioral estrus, the days on which males achieved intromission, had a mean duration of 5.8 ± 1.7 days. Frequencies per minute of a list of behaviors of males and females were compared during vaginal estrus, behavioral estrus and diestrus using a one-way analysis of variance. Frequencies per minute of sniffing and licking by the male, contact by the male, mounting by the male, gentle biting by the male, grooming by the male and grooming by the female were significantly higher during behavioral estrus than during any other part of the estrous cycle. Latency to intromission by the male was the measurement used for female sexual receptivity during behavioral estrus. The mean latency to intromission decreased to its lowest value on day 3 of behavioral estrus. Mean latencies to intromission on days 2 and 3 were significantly less than means on the other days but

were not significantly different from each other. Eaton et al. (1973) concluded that females were more receptive on the second and third day of behavioral estrus than on any other days on which they allowed the male to copulate. Vaginal smears were taken and plasma levels of estradiol and progesterone were measured for the females in the study. Progesterone levels were below 2 ng/ml during behavioral estrus. Plasma levels of estradiol rose shortly before the beginning of behavioral estrus and peaked (mean = 519 ng/ml) and declined sharply. Females remained receptive for one or 2 additional days after the estradiol peak. Estradiol was at diestrus levels on the last day of behavioral estrus. Vaginal smears containing cornified cells were observed during the period of maximal receptivity (days 2 and 3) and the estradiol peak. From gestation periods of pregnant females and known breeding on specific days of estrus it was presumed that ovulation coincided with days 2 and 3 of estrus, the days of maximal receptivity. Eaton et al. (1973) proposed that ovulation occurred on the day after the estradiol peak.

Although in the above mentioned experiments the researchers did not correlate changes in sexual receptivity of females with known ovulation time, they did show that sexual receptivity, as measured, does change during estrus.

III. MATERIALS AND METHODS

A. General Methods

Three experiments were conducted during the spring and summer of 1975, and one experiment during the spring of 1976. The locations and the animals used were different for the two years, but the procedure for teasing and data collection were duplicated as much as possible. Each year is described separately.

B. Experiments 1, 3 and 4 - 1975

In 1975, three experiments (Experiments 1, 3 and 4) were conducted in conjunction with Drs. Wayne Oxender, Patricia Noden and Manley Pratt (Oxender, et al. 1977) of the College of Veterinary Medicine and the Departments of Dairy Science and Anatomy. The physiological portions of the experiments were funded by the American Quarter Horse Association.

1. Location

The experiments were conducted at the Animal Disease Barn No. 3, otherwise known as Bennett Farm. Figure 18, is a schematic drawing of the barn area, corrals, palpation chutes, ramp and gates at the farm.

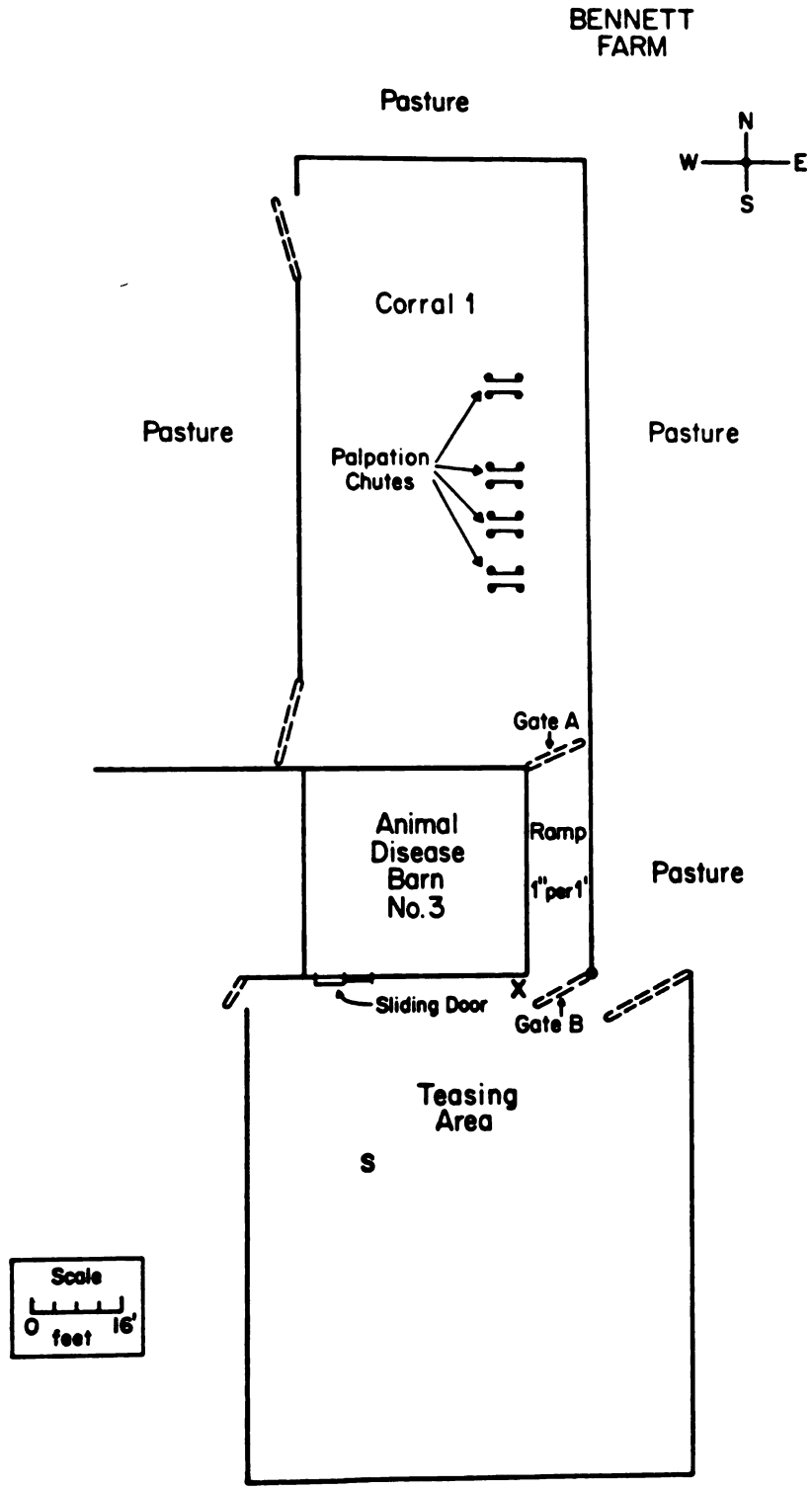

Figure 18. Bennett Farm - schematic drawing.

2. Subjects

Eleven horse mares of mixed breeds, ages 3-22 years, weighing 300-550 kg were used in the three experiments. There were, however, from fourteen to twenty mares pastured together in the pasture surrounding the barn and corral area. The one stallion, a shetland pony, was used for teasing. He was kept in a box stall within the barn isolated from the mares except during teasing sessions.

3. Procedures

The mares were somewhat conditioned to come into corral 1 (Figure 18) when whistled for at 3 p.m. each day. They were rewarded with grain after entering the corral. Then the mares were singly tied to the main posts of the palpation chutes (Figure 18) in a random order. Blood samples (20 cc) were then taken by jugular venipuncture from mares in Experiments 3 and 4. All of the mares had been in a previous winter study, so that the procedure for blood sampling was not new to them and apparently did not disturb the mares. Later for Experiments 3 and 4 progesterone and luteinizing hormone (LH) were quantified from the serum using radioimmunoassay techniques (Oxender et al. 1977).

4. Teasing and Data Collection

At 4 p.m. each day, the stallion was placed on a ten foot lead by a handler and brought out of the barn into the teasing area (Figure 18). The stallion was positioned about

forty feet from Gate B at the point labeled S out of the line of vision of anyone coming down the ramp (Figure 18) until they passed through Gate B. For teasing, a mare in Corral 1 was placed on a ten foot lead at the top of the ramp at Gate A by a handler. The handler called out the mare's number and passed through Gate A and down the ramp. The observer (myself) was positioned beside Gate B in the teasing area at X when a mare's number was called.

I started the tape recorder and spoke the mare's number. As the mare's head passed through the barrier (i.e. Gate B), the stop watch was started, recording an audible click on the tape. The mare was led toward the stallion, who advanced in the mare's direction, and behavioral interactions were begun. The mare and stallion remained held on the leads; both were permitted movement in a ten foot radius. The behavioral actions that ensued were recorded by voice.

The watch was stopped, again recording an audible click, when a mare in estrus raised her tail fully. The latency to tail raising was reported onto the tape when the teasing session ended and the mare was being led up the ramp back to Corral 1. The criterion for ending a teasing session was determination of either estrus or diestrus (see Materials and Methods Section III.D.). If a mare was in estrus (i.e. met the criteria of Section III.D.), she was detained for rectal palpation in a chute in Corral 1 (Figure 18). The size and position of follicle(s) were recorded daily. Treatments for

Experiments 3 and 4 were given after mares were teased [see Experimental Design Section III.B.5.b)].

5. Experimental Design

a) Experiment 1

Experiment 1 took place from May 8 to June 17, 1975. Essentially, this was the pre-experiment or base-line study for Experiments 3 and 4. Experiment 1 was used by Drs. Oxender et al. (1977) to ascertain the ovulation date for each mare within an untreated estrous period. Mares with known date of previous ovulation and those in estrus on May 8th were shunted directly into Experiments 3 and 4. Behavioral information was gathered for n = 7 mares in Experiment 1 (mare nos. 1,2,5,7,9,10 and 11). The mares in Experiment 1 will be referred to as untreated horse mares.

b) Experiments 3 and 4

Experiments 3 and 4 took place from May 8 to August 2, 1975. These experiments were designed to determine if treatment with Gonadotropin Releasing Hormone (GnRH), increased LH levels and decreased the time interval between luteolysis induced by prostaglandin $F_{2\alpha}$ and ovulation in mares (Oxender et al., 1977). The physiological objectives and results of Experiments 3 and 4 were discussed in Section IV.D.3.

(1) Experiment 3

Experiment 3 began five to seven days after ovulation by mares 1,5,9 and 11 from Experiment 1. A 5 mg dose of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was injected subcutaneously (sc) on day 5 to 7 post-ovulation. Various doses (0 = saline, 1, 2 or 5 mg) of GnRH sc were given one time 96 hours after $PGF_{2\alpha}$. This experiment using four mares was originally set up as a 4×4 Latin Square Design so that residual effects of previous treatments could be detected. But because of the delay in shipment of the experimental synthetic GnRH from the drug manufacturer, the Latin Square Design was not carried out. Table 3 shows the sequence of treatments received by mares in successive estrous cycles.

Table 3. Arrangement of treatments received by horse mares in Experiment 3.

Mare No.	1	9	11	5
Cycle 1	Saline	1mg GnRH	2mg GnRH	5mg GnRH
Cycle 2	1mg GnRH	5mg GnRH	Saline	2mg GnRH
Cycle 3	2mg GnRH	Saline	1mg GnRH	1mg GnRH
Cycle 4	5mg GnRH	2mg GnRH	None	Saline

Data on serum LH, progesterone and ovulation (via rectal palpation) was gathered by Drs. Oxender, Noden and Pratt (Oxender et al., 1977). The physiological

data were analyzed as a Randomized Complete Block Design with mares as blocks (Oxender et al., 1977).

(2) Experiment 4

Experiment 4 was begun on May 8, 1975, for mares with date of last ovulation less than 6 days previous and those in estrus on May 8 (nos. 3,4,6,8). This experiment began later for mares 2 and 10. Mares were injected with a 5 mg dose of $PGF_{2\alpha}$ sc five to seven days post-ovulation and 96 hours later injected sc with either 0 = Saline, 5 mg GnRH once or 5 mg GnRH daily (up to four times) until ovulation. This experiment was originally designed as two 3×3 Latin Squares but, because of the delay in shipment of the experimental synthetic GnRH, the design was not fully carried out. Table 4 shows the sequence of the treatments that were received by the mares in successive estrous cycles.

Table 4. Arrangement of treatments received by horse mares in Experiment 4.†

Mare No.	6	4	8	2	10	3
Cycle 1	5mg 1X	Saline	Saline	5mg d.	5mg 1X	5mg 1X
Cycle 2	5mg d.	5mg d.	5mg 1X	Saline	Saline	5mg d.
Cycle 3	Saline	5mg 1X	5mg d.	5mg 1X	5mg d.	Saline

†5mg 1X = 5 mg GnRH 1X
5mg d. = 5 mg GnRH daily

Data on serum LH, progesterone and ovulation (via rectal palpation) was gathered by Drs. Oxender, Noden and Pratt. The physiological data were analyzed as a Randomized Complete Block Design (Oxender et al., 1977). The experiments for 1975 ended on August 2.

C. Experiment 2 - 1976

In 1976, one experiment was conducted. The purpose of this experiment was to establish a base line of estrous behavior in untreated pony mares for comparison with the untreated horse mares of Experiment 1, conducted in 1975. Experiment 2 was conducted from May 8 to June 30, 1976.

1. Location

The experiment was conducted at the Endocrine Research Unit (ERU) on College Road south of the main campus of Michigan State University. Figure 19 is a schematic drawing of the building, pens and pastures of the ERU that were used in this experiment.

2. Subjects

Twenty-four pony mares and four pony stallions weighing 115 to 300 kg were available for this experiment courtesy of Dr. Robert Douglas. Because of the difficulty of handling so many animals, the mares were divided randomly into three groups of eight (A, B and C). Table 5 shows the group to which mares were assigned.

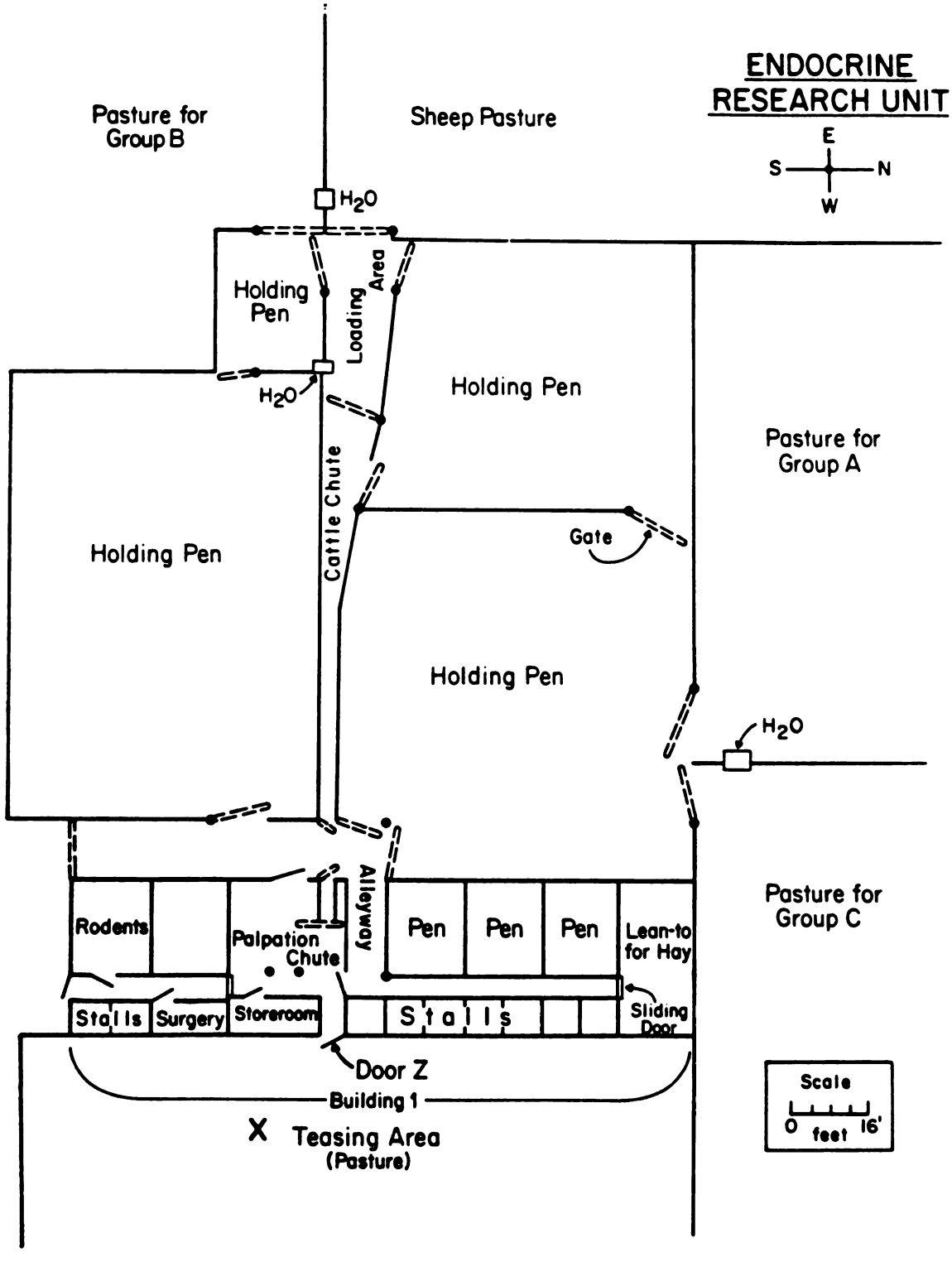

Figure 19. Endocrine Research Unit - schematic drawing.

Table 5. Pony Mare Groups A, B and C, randomly assigned.

Pony Mare Group	A	B	C	Mare Number†
Mares by Number	14	12	6	1
	16	15	10	2
	23	17	18	3
	24	22	19	4
	26	25	21	5
	466	34	35	6
	757	36	725	7
	770	759	790	8

†For coordination with computer print-out; applies to every group.

3. Procedures

A computer program designed by Dr. Hal Grossman of Lyman Briggs College listed a random series of 100 sets of the numbers 1 to 8 arranged in random order. Each day three marbles labeled A, B and C (corresponding to the pony mare groups) were picked randomly out of a cup by a student worker. This established the group order for the next day. The daily order was written on successive lines of the computer print-out of the randomly ordered sets of random numbers. Table 6 shows a simulated example of this.

The groups of mares were kept in separate pastures (Figure 19) and were brought into holding pens and given grain and hay every day at 4:30 p.m. The groups were then loaded into the cattle chute (Figure 19) in the group order of the day. Each group was randomized into the order prescribed by the computer print-out, as the mares came out of the cattle chute. This was done by tying each mare in the daily order

Table 6. Example of computer print-out of random sets of randomly ordered numbers 1-8 coordinated with a date and group of pony mares.

DATE	RANDOM ORDER OF MARES								GROUP
5/15	4	2	8	3	6	5	7	1	A
	2	3	6	4	5	8	1	7	C
	7	2	4	3	5	1	6	8	B
5/16	1	4	8	2	3	5	6	7	A
	4	6	2	1	5	3	8	7	B
	6	4	3	2	1	8	5	7	C

within the alleyway and spare stalls (Figure 19). Physical randomization was done by student workers and myself.

Four stallions (Olaf, Topper, Painter and Major) were used for teasing. They were alternated in a set pattern so that one stallion would tease the 24 mares every fourth day. The stallion order was kept constant except when a stallion was injured and then he was not used until the injuries healed. Painter was injured during the experiment and was absent from the teasing order from May 9 to May 17 and from June 10 to June 15, 1976. Although Olaf was injured during the experiment, his injuries were healed in four days, so he was never absent from the teasing order. The stallions were kept in stalls in Building 1 (Figure 19) when not teasing, with the exception that the stallion used for teasing was left in the teasing pasture to graze until the next day.

4. Teasing and Data Collection

The teasing procedure was similar to that for Experiments 1, 3 and 4. Student workers and volunteers were used as handlers for the teaser stallion and the mares. The teaser stallion of the day was put on a ten foot lead and stationed in the teasing area (Figure 19) about 25 feet from Door Z at X (Figure 19) and out of the line of vision of anyone in Building 1 until they came through Door Z (i.e. the barrier) into the Teasing Area. This observer stood beside Door Z in the pasture with a list of the daily order of the mares, a tape recorder, microphone and stop watch. The tape recorder was started when hoof steps were heard within the building approaching the barrier. A mare's number was reported into the neck microphone before she was led through the barrier (i.e. Door Z). When a mare's head came through the barrier, the stop watch was started close to the microphone. This recorded an audible click. The mare was led in the direction of the stallion. Frequently the stallion simultaneously approached the mare. The mare and stallion remained held on the leads; both had an individual ten foot radius of movement available. The behavioral actions that ensued were orally reported onto the tape. The watch was stopped when a mare raised her tail fully. The latency to tail raising was reported into the tape after the teasing session ended and the mare was being returned to Building 1. The criterion for ending a teasing session was determination of either estrus or diestrus (Materials and Methods Section III.D.). Mares in

estrus were detained for palpation. Palpation was performed in the palpation chute within Building 1 (Figure 19). The position and the size of follicle(s) was recorded into the daily log book kept by Dr. Douglas. If a mare was in diestrus she was either detained for palpation every third day or she was returned to a holding pen to be turned out to pasture after the other mares in her group were palpated.

D. Behavioral Criteria

1. Criteria for Diestrus

Diestrus was characterized by one or more of the following sets of behaviors:

a) Passive Resistance - a minimum of two minutes of behavior characterized by little or no response by the mare to the stallion, sometimes only the mare's ear pinnae were directed caudally and occasionally barely audible squeal vocalizations were produced.

b) Intention of Active Resistance - a minimum of two minutes of behavior characterized by:

   (1) moving away from the stallion quickly with ear pinnae directed caudally. This was sometimes accompanied by tail switching and squeal vocalizations;

   (2) little or no response by the mare to the stallion accompanied by tail switching, squeal vocalizations and ear pinnae directed caudally.

c) Active Resistance - (no minimum time requirement because of possible injury to the subjects):

   (1) kicking with rear legs;

   (2) striking with forelegs;

   (3) both (1) and (2) accompanied by ear pinnae directed caudally and frequently squeal

vocalizations (occasionally biting of the stallion by the mare was observed).

Many of the horse mares in Experiments 1, 3 and 4 were donated to the College of Veterinary Medicine for research purposes because of chronic leg and/or hoof ailments which produced lameness and made them no longer useful as riding horses to their previous owners. These leg and hoof ailments sometimes made it painful for the mares to walk normally much less strike or kick, thus the absence of some active resistance behaviors may have been due mainly to inability.[1] The pony mares in Experiment 2 had better legs and hooves so the full behavioral repertoire could be and was performed during diestrus. Diestrous behavior was so consistant within mares that both the observer and the handlers knew what to expect from a particularly active resistant mare and they acted accordingly to protect their own persons.

When a mare was determined to be in the diestrous portion of the estrous cycle, she was taken from the teasing area. Diestrus horse mares in Experiments 1, 3 and 4 were not detained for palpation. Diestrus pony mares in Experiment 2 were detained for palpation every third day.

2. Criteria for Estrus

Estrus was characterized by tail raising plus any of the following behaviors:

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[1]It might be noted that many mares used exclusively for breeding also have these ailments and are successfully bred, conceive and are adequate dams.

a) Winking
b) Squatting[2]
c) Urinating
d) Being mounted by the stallion.[3]

If a mare did not display any or only one of the above behaviors, plus tail raising, and the log book showed that she was in estrus on the previous day, the mare was then palpated. If palpation determined that the mare had not yet ovulated, she was considered to be in estrus, but having a 'split estrus'.[4] (In all cases of 'split estrus' that were observed, the mares in question displayed estrous behavior on the following day.) Figure 20 shows a symbolic representation of the estrous behaviors of all pony mares on all 'in estrus' days in Experiment 2. When a mare was determined to be in estrus, she was detained for ovarian palpation, per rectum, and the size of the follicle was reported. Figure 21 shows Dr. Pratt palpating Mare No. 2 at Bennett Farm in 1975.

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[2]Squatting was not often seen in horse mares that were lame.

[3]Standing still for mounting with the tail raised often has been used by other researchers who use the same teasing techniques (Ginther, 1974 and 1978) as one of the main criterion for estrus. However, it was suspected by this observer that mares were becoming conditioned to being pulled away from the mounted stud (to prevent intromission, especially the pony mares). In addition, if standing for mounting, or even mounting per se, were a main criterion, much of the data would have been unusable in Experiment 2. See Figure 20 (e.g. mare #12 day +2, mare #23 days +3 and +2, mare #757 day +3).

[4]Split estrus is defined in the literature review, Section II.E.5.b).

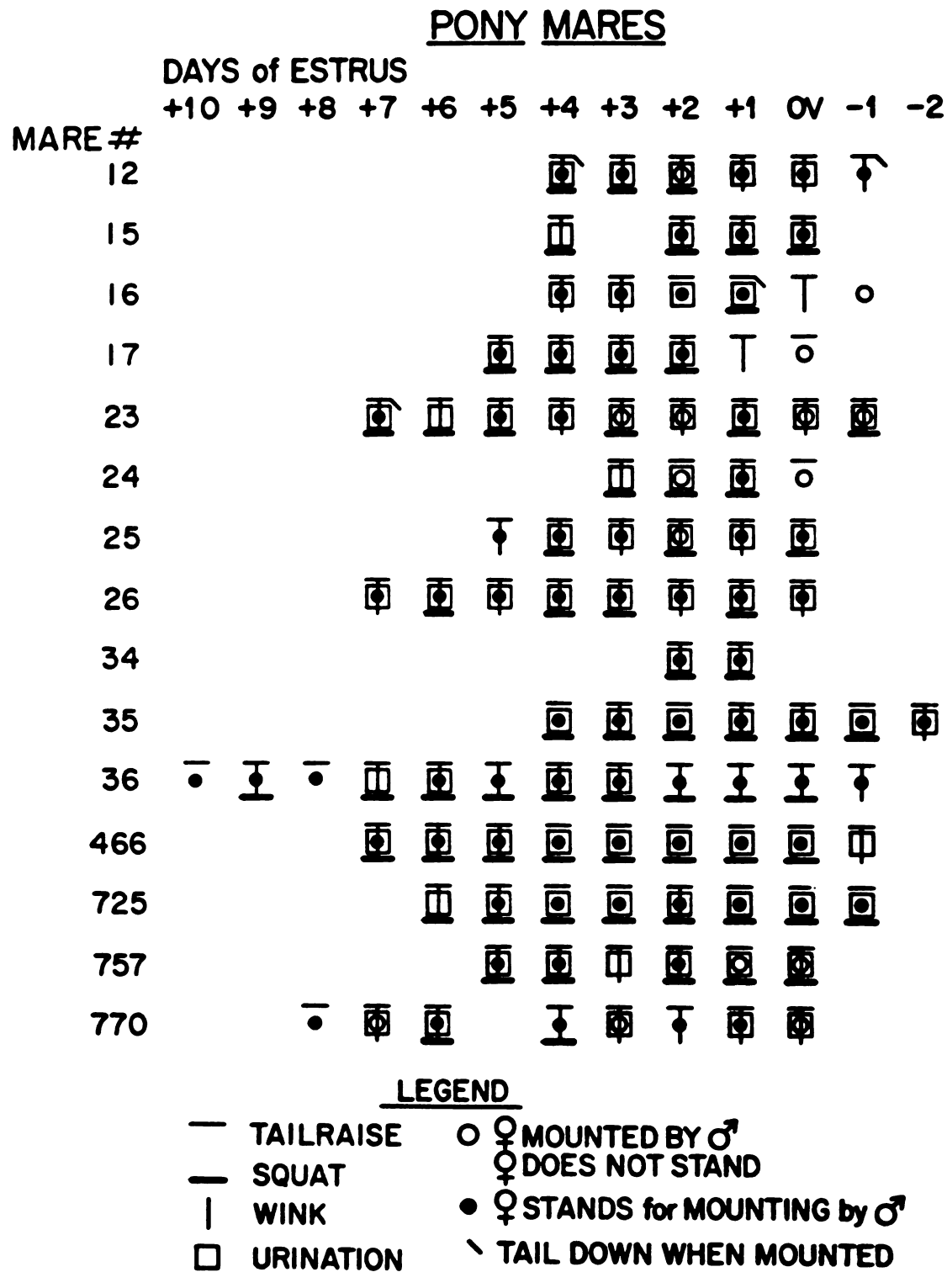

Figure 20. Symbolic representation of all estrous behaviors on all days of estrus for pony mares in Experiment 2.

Figure 21. Rectal palpation performed by Dr. Manley Pratt at Bennett Farm.

E. Quantitative Methods

1. Tape Transcription

Cassette tapes were transcribed for Experiment 1 in July and August of 1975 and for Experiment 2 in July of 1976. I missed one day of data collection (June 23, 1975) because of illness. Information was transcribed onto lined 5" x 8" cards, one card per mare per daily teasing session. The following information was recorded on the cards for Experiments 1, 3 and 4. Date, Mare Number, Day of Estrus in Relation to Ovulation,[5] Latency Time to Tail Raising, and Sequence of Behavioral Interactions Between the Stallion and the Mare. In addition, the following information was also recorded on the cards for Experiment 2: Stallion Used for the Teasing Session, Latency to Squatting, First Urination and Mounting. The latency times to squatting, first urination and mounting were obtained with a stop watch while listening to the tape, from the sound of the stop watch starting (an audible click) until the behavior was just beginning to be recorded. This required replaying each tape segment many times. Some of these latency times may be off by ± 0.5 to 1.5 seconds on the tape itself, because of the delay in speaking a behavior into a tape after it was perceived. The tapes for Experiment 1 were examined again in September of 1976 to time and record the latencies to squatting, first urination and mounting as described for Experiment 2.

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[5]See next section for Data Arrangement.

The information on the cards for Experiments 1 and 2 was transferred to large sheets, one sheet per mare. Each line on the sheet represented a teasing session and each column represented a single behavior. There were ninety-two (92) columns. The sheets were designed for recording the frequencies of the various behavior patterns for each teasing session. Frequencies of individual behavior patterns were then transferred to a series of tables, each representing a single behavior pattern with frequencies arranged by mare per day of estrus (Results, Section IV.B.).

2. Data Arrangement

Sections III.B.4., III.C.4., and III.D.2. mentioned that mares in all experiments were rectally palpated once each day when they were in estrus. Ovarian palpation per rectum is the most direct way of establishing the date of ovulation by detecting the absence of a previously recorded follicle. Anatomical changes in the ovaries that can be perceived by palpation (via rectum) during estrus and diestrus are described in Section II.C.1.

The day on which ovulation was detected was considered as the day of ovulation (Ov.); in reality, ovulation may have occurred at any time within the 24 (± 1.5) hours since the previous palpation on what was considered day +1. Despite problems with accuracy with the commonly practiced estimation of ovulation, all the latency and frequency data for each estrus of each mare were arranged in relation to the day of

ovulation detection (Ov.). For example, arrangement of latency to tail raising for mare Number 5 in Experiment 1 is given below:

Day	+4	+3	+2	+1	Ov.	-1
Latency to Tail Raising in Seconds	24.5	10.8	5.7	8.0	10.7	37.3

This made the data uniform (as possible) for all mares. By using this arrangement, it was possible to analyze preovulatory behavior (until day +1) as a separate phenomenon from the entire estrus (i.e. peri-ovulatory behavior) part of the estrous cycle.

As estrus continues only for 24 to 48 hours post-ovulation (days Ov., -1 and -2), post-ovulatory data were few for most mares, especially those who displayed estrus only until day +1 or day Ov. Thus, analysis of post-ovulatory behavior as a separate phenomenon was impossible. Instead, post-ovulatory behavior was analyzed in the context of the entire estrus (peri-ovulatory) in comparison to pre-ovulatory behavior.

The bulk of the statistical analyses were performed on Olivetti-Underwood Programmas 101 and P 602, and a Hewlett Packard 2825a, with and without statistical programs recorded on magnetic cards and tapes. The paper tapes on which input and output were recorded were kept and checked, so that errors were corrected.

3. Data Adjustment

Two seconds were added to every latency time data point for each estrous behavior pattern in both experiments. This was initially done to eliminate 0 (zero) seconds latency times to tail raising which caused miscalculations with some of the programs used for the Olivetti-Underwood Programma 101 calculator. To create uniformity, two seconds was also added to each data point for all of the other behavioral latency times (i.e. urination, squatting and mounting). This maintained the original relationships with the latency time to tail raising. No adjustment was made on the frequency data.

IV. RESULTS

A. Behavior Patterns Observed and Recorded

Many behavior patterns by the mares and stallions were observed during teasing when mares were in estrus. Behaviors can be categorized into several arbitrary categories: Social behaviors, Investigatory behaviors, Gender behaviors, Withdrawal behaviors and Aggressive behaviors. The names of the categories were subjective and used only in this experimental context.

1. Social Behaviors

Social behaviors were displayed primarily at the beginning of a teasing session, but were observed ocassionally at other times during teasing.

a) Naso-Nasal - Upon approaching head to head, the stallion and the mare touched nostrils and/or muzzles with smelling of each other observed on many occasions. This behavior has been called nuzzling by Tyler (1972) and Ginther (1978) and is part of greeting. Figure 22 shows naso-nasal.

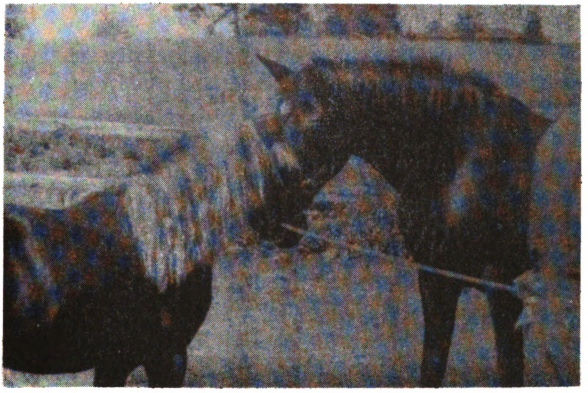

Figure 22. Naso-Nasal between pony stallion and horse mare.

b) Whinney or Neigh - A long high pitched pulsing vocalization heard most frequently being produced by the stallion as the mare approached, after naso-nasal and also infrequently during teasing. According to Tyler (1972) whinnies are produced in many contextual situations by all sexes and ages.

2. Investigatory Behaviors

Investigatory behaviors followed the Social behaviors during the teasing session and were performed mainly by the stallion, although they were observed rarely by the mare as well. Investigatory behaviors constituted the major portion of most teasing sessions. This author presumed that the function of investigation of the mare by the stallion was to ascertain if she were in estrus and if she would allow copulation. Investigatory behaviors consist of smelling, licking, nibbling, rubbing with the forehead or chin, pushing with the head, exertion of pressure with the head by the placement of the head upon the body and knee contact while pawing with the foreleg. These will be further categorized as to region of the body to which the behavior is directed.

a) Smelling - The nose is directed at an area and the nostrils and the skin above the nostrils can be observed moving more rapidly and distinctly during the inhalations than during normal breathing.

Smelling was quantified in bouts as one or more sniffs directed to an area. Smelling can further be classified according to the place where it was directed and whether it was performed by the stallion (s) or the mare (m). Classifications of smelling behavior were:

(1) smell chin (s and m)
(2) smell jaw (s)
(3) smell neck (s and m)
(4) smell shoulder (s and m)
(5) smell neck-chest juncture (s)
(6) smell chest (s)
(7) smell behind fore leg (s) or the axilla
(8) smell side (s and m)
(9) smell abdomen (s and m)
(10) smell flank (s) or iguinal area
(11) smell mammaries (s)
(12) smell hind leg (s)
(13) smell inside hind leg (s)
(14) smell tail hairs (s)
(15) smell rump (s) or buttock (Figure 23)
(16) smell under the tail head (s)
(17) smell vulvular area (s) (Figure 24)
(18) smell penis (m)
(19) smell urine flow (s)
(20) smell urine on the ground (s)

b) Licking - Repeated protrusion of the tongue contacting an area of the other subject, often accompanied by slurping sounds. Licks were quantified in bouts of one or more directed to an area. Further classification of licking was the place at which tongue contact was made. No instances of licking by a mare were observed, thus all licking was performed by the stallions. Classifications of licking behavior were:

Figure 23. Pony stallion smelling rump of horse mare.

Figure 24. Pony stallion smelling vulvular region of horse mare. (Note: Stallion with full erection.)

(1) lick chin
(2) lick jaw
(3) lick chest
(4) lick neck (Figure 25)
(5) lick shoulder
(6) lick behind fore leg, the axilla
(7) lick side
(8) lick abdomen
(9) lick flank, inguinal area
(10) lick mammaries
(11) lick hind leg
(12) lick down hind leg (Figure 26)
(13) lick inside hind legs
(14) lick rump
(15) lick tail hairs
(16) lick under tail head (Figure 27)

c) Nibbling or Gentle Biting - One individual opens and closes the teeth while in contact with the fur of the other subject. Nibbling was not observed in Experiment 1, but was observed by all stallions in Experiment 2, albeit rarely. Regions of the body nibbled were the same as regions licked with addition of the face and mane. Nibbling was only performed by the stallion and was the same behavior pattern seen in mutual grooming (Tyler, 1972), but alas was performed by only one subject.

d) Rubbing - Repeated sideways or up and down movements of the head with the forehead or muzzle in contact with an area of the other subject. Rubbing was performed only by the stallions. Classifications of rubbing behavior were:

(1) rub jaw
(2) rub neck

Figure 25. Pony stallion licking neck of horse mare.

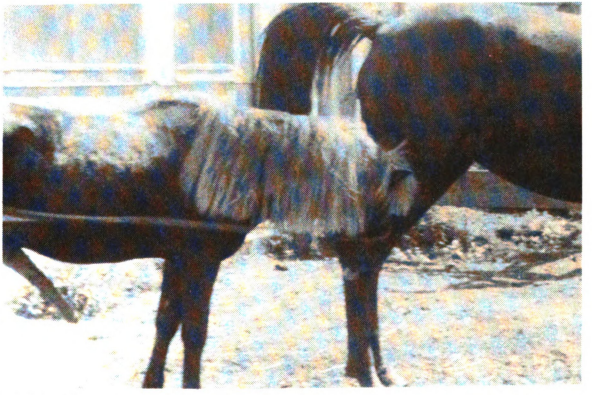

Figure 26. Pony stallion licking down hind leg of horse mare.

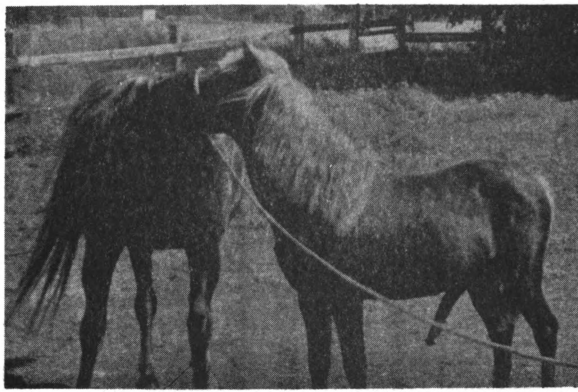

Figure 27. Pony stallion licking under tail head of horse mare.

(3) rub chest
(4) rub shoulder
(5) rub whithers
(6) rub behind fore leg, the axilla
(7) rub side
(8) rub flank, the inguinal area
(9) rub rump

e) Pushing - The fore part of the head was pushed into areas of the other subject for repeated sustained durations. Pushing was performed only by the stallion. Classification of the areas of the mare's body that was pushed into were:

(1) push neck
(2) push behind fore leg, the axilla
(3) push flank, the inguinal area
(4) push rump

f) Pawing the Fore Leg - The fore leg is lifted and pawed with the knee contacting body areas of the other subject. Pawing the fore leg was only performed by the stallion. Areas pawed against were:

(1) paw fore leg against chest
(2) paw fore leg against abdomen

g) Head Resting - The head exerted pressure by resting it on an area of the other subject's body. Head resting was observed only for stallions in Experiment 2 (the stallion in Experiment 1 was too short to perform this behavior with the horse mares). Classification of head resting behaviors were:

(1) head resting on the neck
(2) head resting on the withers
(3) head resting on the back
(4) head resting on the rump

3. Gender Behaviors

Gender behaviors were those behavior patterns that were displayed exclusively by either sex and associated with the facilitation of and accomplishment of mounting and one would assume copulation also, if it were permitted.

a) Behavior Patterns Displayed by Mares

Some were described in Section II.E.4. of the Literature Review. They are presented here with further classification where it applied.

(1) Tail Raising
  (a) full tail raise - highest level of tail characteristic of the mare
  (b) slight to half tail raise - usually followed by a full tail raise, but this level was sustained for a noticable duration.
(2) Squatting
(3) Urination
(4) Winking (frequency was computed in bouts of one or more)
  (a) regular winking - rhythm regular
  (b) irregular winking - rhythm irregular (rarely observed).
(5) Ear Positions - observed and recorded only for mares. Equids are capable of rapid independant ear movements in two almost perpendicular planes (Wallach, unpublished). Only a few possible positions were recorded.
  (a) ears forward - pinnae upright with openings directed forward
  (b) ears to side - pinnae out to side with openings directed downward
  (c) ears back - pinnae directed caudally with openings directed downward

associated with aggression [Section IV.A.5.h)].

(6) Head Low - holding the neck forward with the head directed downward during investigation or mounting by the stallion (rarely observed).

(7) Rossigkeitsgesicht or in heat expression - described in Section II.E.4.e), Figures 10-16.

(8) Bellow - a vocalization displayed by only one mare in Experiment 1. It is of low pitch which varies, long duration and loud resembling the call of a bull elephant. Following this vocalization the mare would often turn and present (see below) rapidly.

(9) Turn and Present - a turning of the whole body so that the rump or buttocks of the mare is directly in front of the stallion's head.

(10) Turn to Look - turning head to look at the stallion while he is investigating the rear portion of the body.

(11) Pivot Rear - pivoting on the forelegs so that only the rear part of the body turns towards the stallion's head.

(12) Backs Up - walking backwards several steps and pushing the rump into the stallion's head or chest.

(13) Stands for Mounting - stands still while stallion mounts.

(14) Not Stand for Mounting - moves away from the stallion when mounted or kicks or attempts to kick the mounting or mounted stallion.

(15) Not Mounted - not mounted by the stallion during the teasing session.

b) Behavior Patterns Displayed by the Stallions

(1) Snort - a sound, considered a vocalization by Tyler (1972), is nasal in character and of short duration. It is produced most frequently before a stallion mounted and rarely at other times during teasing.

(2) Erection - The fully erect penis of the pony stallion is about two feet in length. Erection is a slow process in stallions because of the vascular-muscular nature of the organ (Section II.G.1.). Several subjective stages of erection were classified.

(a) full erection - full expansion and tumescence.

(b) almost full erection - full expansion, but not completely turgid, the penis at this stage is slightly curved with the glands drooping.
(c) half erect - half expanded and lacking full turgidity.
(c) becoming erect - protrusion of the penis still covered by prepuce foldings.
(e) losing erection - detumescence of the penis.
(f) lost erection - completely detumesced and retracted.

(3) Mounting - the stallion is on the hind legs with the fore legs on the rump or back of the mare. Mounting is prior to thrusting of the pelvis to facilitate intromission. Several categories of mounting were classified.
(a) mounting with erection - half erect to full erect (Figure 28).
(b) mounting without erection.
(c) attempted mount - incompleted mount, frequently with orientation at the side or flank of the mare.

(4) Flehmen - facial expression after smelling the mare's genital region and/or urine, described in Section II.G.1., Figure 17.

4. Withdrawal Behaviors

Behavior patterns in this category were more frequently observed during diestrus. Occasionally, however, they were observed during estrus and that is why they are mentioned here. Designation of the performer of the behavior (s) or (m) is indicated below.

a) Walk Past (s and m) - continuing to walk past the other subject during the approach at the beginning of the teasing session, thus eliminating contact.

b) Walk Away (s and m) - walking away from other subject during active teasing, often the

Figure 28. Pony stallion with erection mounting horse mare.

non-withdrawing subject would again approach the one that had withdrawn.

c) Walk Forward (s and m) - one or two steps taken forward during teasing.

d) Avoid (m) - movement faster than a walk away from the stallion as he approached before teasing, often accompanied by squealing and turn away.

e) Turn Away (m) - turning the head away from the approaching stallion with or without movement away.

f) Look Away (s) - turns head away from mare during teasing and focuses attention (visual and auditory) on the handlers or other animals outside the teasing area.

g) Eats Grass (s) - in the midst of teasing the stallion would begin grazing.

5. Aggressive Behaviors

The behavior patterns in this category were also most frequently observed during diestrus and were rarely seen during estrus. Designation of performer (m or s) is indicated below.

a) Bite (s) - grabbing of the mare's flesh with the teeth and pulling it. May also be considered under the class of investigatory behavior.

b) Kick (s and m) - one or both hind legs kicking in the direction of the other subject, accompanied by ears back.

c) Attempt Kick (m) - raising one hind leg and tensing musculature for a kick, accompanied by ears back.

d) Strike (s and m) - lifting a fore leg and bringing it down or towards the other subject quickly, sometimes accompanied by squeal and ears back [resembles pawing the fore leg, IV.A.2.f), but is more rapid, and knee contact is not made].

e) Rear (s and m) - raising both fore legs and the fore body off the ground, directing fore legs towards the other subject, sometimes accompanied by squealing.

f) Squeal (s and m) - a high pitched vocalization of varying loudness and short duration (Waring, 1971; Ödberg, 1974), resembling a scream.

g) Tail Switch (m) - rapid movement of the tail up and down or sideways producing a clipped swishing noise, accompanied by ears back.

h) Ears Back (s and m) - recorded only for mares, see Section IV.A.3.a)(5)(c) for description.

B. Frequency Data

1. Means and Standard Deviations of Frequency Data

Frequencies of behavior patterns classified in Section IV.A. were taken from individual frequency sheets for each untreated mare (described in Section III.E.1.) in Experiments 1 and 2. Frequencies for each behavior were arranged in tabular form by subject and day of estrus

without regard for the length of the individual teasing session. For Experiment 1 two series of tables were constructed; one for the frequency of the behaviors observed for the mares (per mare per day of estrus) and the other series for the frequencies of the behaviors observed for the stallion (per mare per day of estrus). For Experiment 2 two series of tables were also constructed; one series for the behavior frequencies observed for the mares (per mare per day of estrus) and the second series for the frequencies of behaviors observed for the stallions (per mare per day of estrus). The myriad of frequency tables is not given in this dissertation.

It was found that many behavior patterns had rare frequencies of occurance. Frequencies of related behavior patterns were combined. Licking and smelling behavior frequencies were each combined for body regions; the fore body (from chin to behind fore leg), the rear body (from the side and abdomen backwards) and the total body (addition of the fore and rear body frequencies). Frequencies of the Withdrawal and Aggressive behaviors for each sex, and particular Gender behaviors (e.g. almost erect and full erection) for the stallions were also combined.

After looking at the frequencies of individual behaviors and groups of behaviors, either in tabular or graphic form, it was decided that patterns of frequency change in this form throughout estrus were not discernable.

In addition variation among mares per day of estrus was great as was the length of the teasing sessions. It was therefore decided that only means and standard deviations (Sokal and Rolf, 1969) of the frequencies would be calculated per mare per day of estrus. These were done on a Hewlett Packard 9825A Calculator, with a program designed by Dr. Robert Boling of the Department of Zoology.

Tables A15, A16, A17 and A18 in Appendix A show the means and standard deviations per mare per day of estrus for frequencies of individual or groups of behavior patterns which occurred on more than 25% of the total number of days mares were in estrus for at least one of the two experiments. It can be seen that the means of the frequencies are low per mare per day of estrus and many of the standard deviations are quite large in comparison to the means. No further calculation of the means with regard to length of teasing sessions or analysis of the present frequency data was performed [see Discussion, Section V.C.2.a)].

2. Percent of Incidence of Key Gender Behaviors

a) Experiment 1

Tail raising was the first estrous behavior observed in 82% of the teasing sessions when horse mares were in estrus (n = 50 sessions). Winking was observed first in 12% of the estrous teasing sessions when the tail was only partially raised. It is

possible that winking preceeds tail raising in estrous mares, but it cannot be readily observed when the tail is in the normal or down position. Latency to winking was not measured because of the inability of the observer to determine when winking began. Thus, tail raising was the first readily observable estrous behavior and its latency was the principal behavioral sign of the estrus condition. In addition, tail raising was nearly always observed (98%) during the teasing sessions when mares were in estrus. (This included the single session of split estrus, when behavior signs were not shown but the mare had been in estrus on previous days and had not yet ovulated.) Winking, urination, mounting and squatting were observed in 72%, 70%, 70% and 50% of the teasing sessions, respectively, when mares were in estrus. Latencies to urination, squatting and mounting were also measured and analyses are presented in Section IV.D.

b) Experiment 2

Tail raising was calculated as the first estrous behavior observed in 88.2% of the teasing sessions (n = 102 sessions) when pony mares were in estrus. Winking was observed first in 8.8% of the estrous teasing sessions when the tail was only partially raised. Tail raising was observed during 98% of the

teasing sessions when pony mares were in estrus. (This included the two sessions of split estrus when behavior signs of estrus were not shown.) Mounting, urination, winking and squatting were observed in 89.6%, 81.3%, 72% and 63.7% of the teasing sessions, respectively, when mares were in estrus. Tail raising was the most frequent estrous behavior observed, in addition to being predominantly the first estrous behavior observed. Latencies to tail raising, urination, squatting and mounting were measured and the analyses of these are presented in Section IV.D.

C. Sequence of Behavior During Teasing Sessions

In order to discern if behavior from the beginning of estrous teasing sessions to the culmination of the sessions followed a sequential pattern, behavior patterns were assigned numbers (even for the stallions and odd for the mares). The numbers were then arranged in the order of appearance of the behaviors during each teasing session for all of the experiments. It was observed from the arrangements that there was a beginning phase, central phase and culminating phase for each teasing session.

The beginning phase consisted of a greeting with vocalization by the stallion (whinney) and often naso-nasal between the stallion and mare (earlier characterized as social behaviors).

The central phase of the teasing session varied among the sessions. The behavior of the mares consisted of a smaller repertiore than that of the stallions and appeared to be more stereotyped in sequence because of the smaller number of behaviors in the repertiore. Mares raised their tails before squatting, winking and urination. The latter behaviors occurred in a variety of sequence permutations and frequency of individual behaviors and combinations. Other behaviors by the mares such as the categories of withdrawal, aggression and investigation were infrequently observed and did not follow a set sequence when they occurred. The behavior of the stallions during the central phase consisted of a large repertoire of investigative behaviors, a smaller repertoire of gender, withdrawal and aggressive behaviors displayed in a wide range of combinations, permutations and frequencies. This diversity of behavior displayed by the stallions varied among the teasing sessions on each day and on every day during estrus of individual mares. Although behavior during the central phase of teasing appeared ritualized, the sequence of the behaviors did not follow a rigid pattern.

The culmination phase of the teasing sessions consisted of either mounting by the stallion or a decision by the experimenter that more than two minutes had elapsed without signs of intended mounting by the stallion. Mounting by the stallion was frequently preceeded by a snort vocalization. The behavior of the mare prior to mounting was no different

from that of the central phase and characterized by tail raise, often squatting accompanied by rhythmic winking and frequent urination.

A sequence of behavior was indicated only during the beginning phase of teasing for both mare and stallion and the culmination phase for the stallion. These phases were relatively brief during the teasing sessions. No sequence of behavior was observed for the central phase or the major portion of each teasing session. Further analysis of the sequence of behaviors and their probabilities was not performed because preliminary examination did not indicate any orderly change in the sequence of behavior during the days of estrus in relation to ovulation.

D. Latency Data

1. Collected Data and Estimation of Missing Data

Collected latency data from all experiments are in Appendix A. Missing data in Experiments 1 and 2 for latencies to urination, squatting and mounting on days +4, +3, +2 and +1 of estrus were estimated for each mare separately using procedures recommended by Gill (1978). For Experiment 1, Table A11 and for Experiment 2, Table A14 show the data for all mares and the estimated values for missing data where possible. Estimations of missing data for latency to tail raising on days +4, +3, +2 and +1 in Experiments 3 and 4 were also made (Gill, 1978). Tables A12 and A13 show the data with the estimated values for

missing data where possible for Experiments 3 and 4 respectively.

2. Behavior of Untreated Mares

The hypothesis that sexual receptivity of normal mares increases before ovulation and decreases after ovulation, was tested in two parts. The first part tested sexual receptivity increase before ovulation (pre-ovulatory behavior). The second part tested the decrease of sexual receptivity after ovulation and is in sections entitled peri-ovulatory behavior.

a) Experiment 1 - Horse Mares

(1) Pre-Ovulatory Behavior - Intramare Linear Regressions

Intramare linear regressions (ILRs) (Kirk, 1978; Gill, 1978 and personal communication) were performed on the latencies to tail raising, urination, squatting and mounting on pre-ovulatory days of estrus to determine if sexual receptivity increased prior to ovulation. Data for each ILR came from Tables A1, A2, A3 and A4 in Appendix A with estimated values of missing data incorporated from Table A11.

A linear regression program was used to calculate the ILRs.[1] Discussion of this form of regression and the formulae used are given in Appendix B (Section A.1.).

Tail raising latencies of untreated horse mares decreased[2] significantly (Figure 29, Table 7) from 56.6 seconds on day +7, at a rate of 7.5 seconds per day to day +1 and the extrapolated origin on the day of ovulation (b_o = 4.0 seconds). Squatting latencies of untreated horse mares decreased[2] (Figure 30, Table 7) from 68.2 seconds on day +7, at a rate of 6.0 seconds per day to +1 and the origin on day Ov (b_o = 26.5 seconds). The slope of squatting latencies approached significance ($P < 0.07$), but the slopes to urination and mounting[3] were not significantly different from zero (Table 7).

---

[1]Data from a mare was used if it had at least three data points which provided one degree of freedom per mare (d.f. = n - 2). This eliminated at least one mare from the ILRs on latencies to urination, squatting and mounting.

[2]Although the values of the slopes in Table 7 are positive indicating an increase in latency times from day +1 to day +7, the figures are in reverse projection in time, such that day +7 is the onset of estrus and the slopes decrease as estrus procedes.

[3]Mounting horse mares appeared to be physically awkward for the pony stallion. This may have increased the variation of latency to mounting in Experiment 1, thus causing the value of the slope to be below significance.

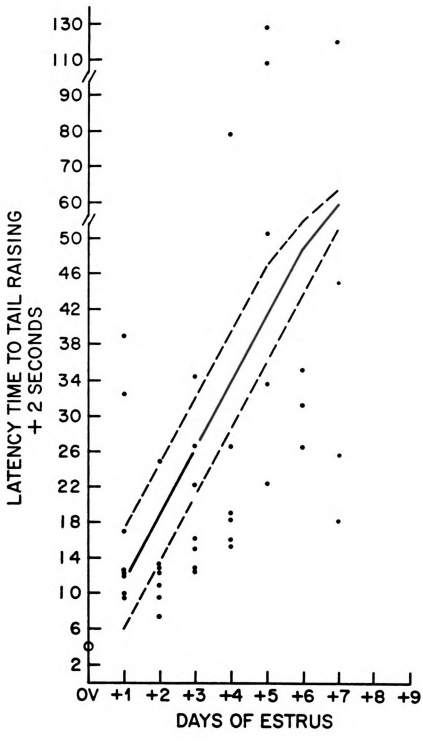

Figure 29. Data, slope and 95% C.I. estimate around the slope of the pre-ovulatory ILR on tail raising latencies of untreated horse mares in Experiment 1.

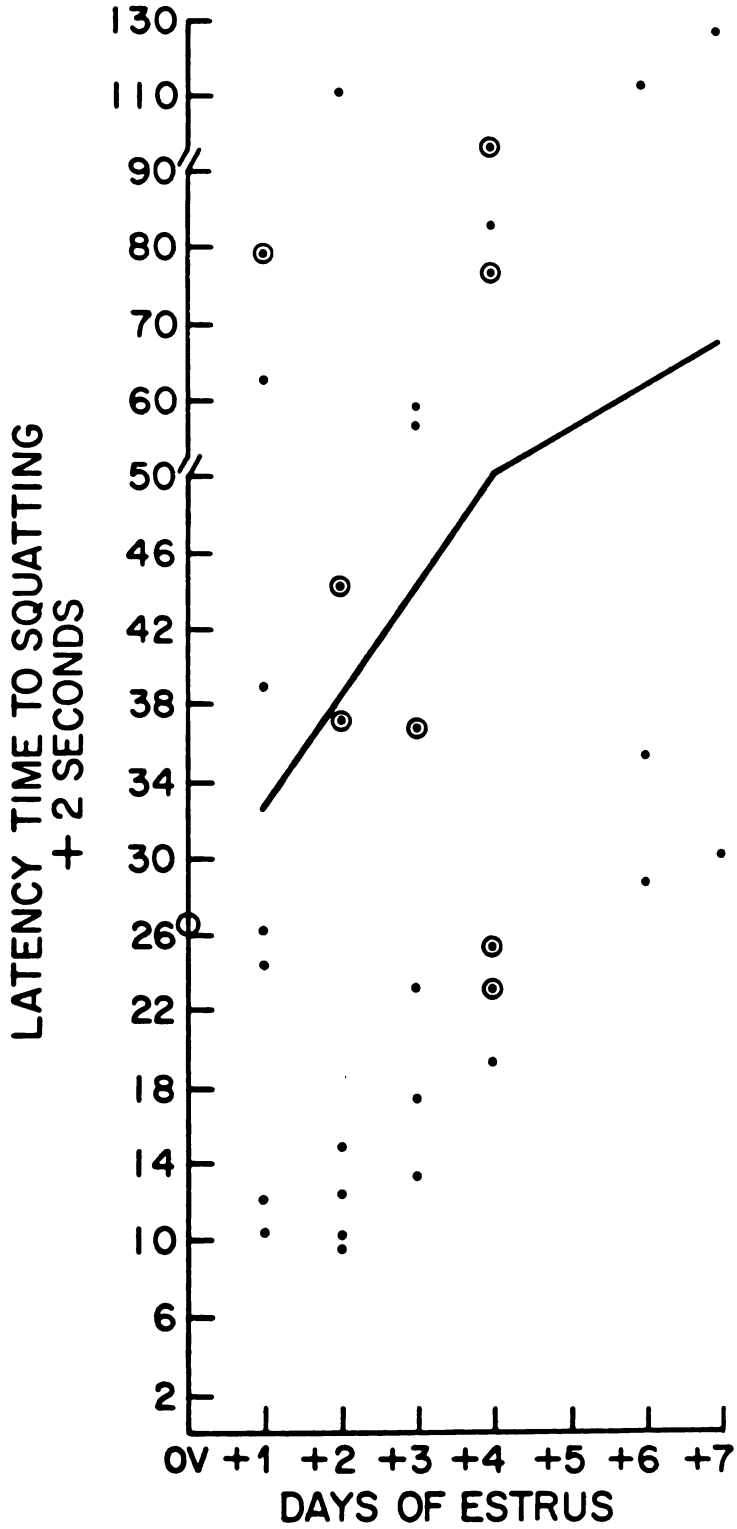

Figure 30. Data and slope of the pre-ovulatory ILR on squatting latencies of untreated horse mares in Experiment 1.†

†Circled data points = values of estimated missing data.

Table 7. Results of pre-ovulatory ILRs - Experiment 1.

	Latency to Tail Raising (+2 sec)	Latency to Urination (+2 sec)	Latency to Squatting (+2 sec)	Latency to Mounting (+2 sec)
Mares	1,2,5,7,9 10 and 11	1,2,5,7,9 and 10	1,2,5,7,9 and 10	1,5,7,9,10 and 11
ν = d.f	25	19	17	15
b_1(slope)	7.5	4.1	6.0	2.8
b_o(origin)	4.0	21.6	26.5	54.1
H:$\beta_1 = 0$				
H:$\beta_1 \neq 0$				
t =	2.9**	$1.7^{n.s.}$	1.9*	$0.7^{n.s.}$
95% C.I. estimate				
Upper	+12.89		+12.59	
Lower	+ 2.11		- 0.65	

*(P < 0.07)
**(P < 0.05)
n.s.(P > 0.1)

Only the results of the significantly decreasing tail raising latencies support the hypothesis that sexual receptivity increases in untreated horse mares prior to ovulation.

(2) Peri-Ovulatory Behavior

Because estrous behavior is displayed for only 24-48 hours (1 to 2 days) post-ovulation and with great variation in duration among mares, the post-ovulatory data (from days Ov to -2) alone was not sufficient for analysis. Therefore, the whole peri-ovulatory period (or period of estrus) had to be examined and the results compared to the analyses on pre-ovulatory behavior in order to test the hypothesis that sexual receptivity decreases after ovulation. Latencies of all behaviors (tail raising, squatting, urination and mounting) were examined within the peri-ovulatory context.

The peri-ovulatory data for tail raising was more bountiful than for the other behaviors. However, several mares were eliminated from all of the analyses because of missing data beyond day +1. Values for estimated missing data on days +4, +3, +2 and +1 were incorporated into the

analyses on latencies to squatting, urination and mounting (Table A11).

(a) Intramare Linear Regressions

Intramare linear regressions (ILRs) were performed on latencies to tail raising, urination, squatting and mounting for all days of estrus (Tables A1, A2, A3, A4 and A11). Only data from estrous periods that extend to at least day Ov were used.

Tail raising latencies in untreated horse mares decreased significantly (Figure 31, Table 8) from 48.7 seconds on day +7, at a rate of 4.1 seconds per day to 19.9 seconds on day Ov and 11.7 seconds on day -2. These results initially suggest that sexual receptivity continued to increase past ovulation because the linear slope that continued to decrease post-ovulation was significant (Table 8). However, the peri-ovulatory ILR slope on tail raising latencies (b_1 = 4.1) was smaller than the pre-ovulatory slope ($b_{1\ pre}$ = 7.5)[4]

[4]Recalculation of the pre-ovulatory slope on tail raising latencies without the data from mare number 10 reveals the slope of 9.1 which is significant ($P < 0.05$) and larger than the original pre-ovulatory slope and the peri-ovulatory slope, so the possibility of increased variation or curvature post-ovulation remains.

(Figure 31), indicating perhaps an increase in the variation after ovulation or possibly a curvature, both would suggest a decrease in sexual receptivity after ovulation. The possibility of a curvature is tested in the next section.

Mounting latency in untreated horse mares surprisingly decreased significantly (Figure 32, Table 8) from 90.5 seconds on day +7, with a slope of 5.5 seconds per day to 52.1 seconds on day Ov and 41.2 seconds on day -2. These results suggest that the readiness of the stallion to copulate continued to increase after ovulation. It was previously assumed that because it was physically awkward for the pony stallion to mount the horse mares, variation in the latency times were too great to show significance of the pre-ovulatory ILR slope. However, in comparing the two slopes, the pre-ovulatory slope ($b_{1\ pre} = 2.8$) was smaller than the peri-ovulatory slope ($b_1 = 5.5$) (Figure 32), suggesting that variation was reduced after ovulation. The

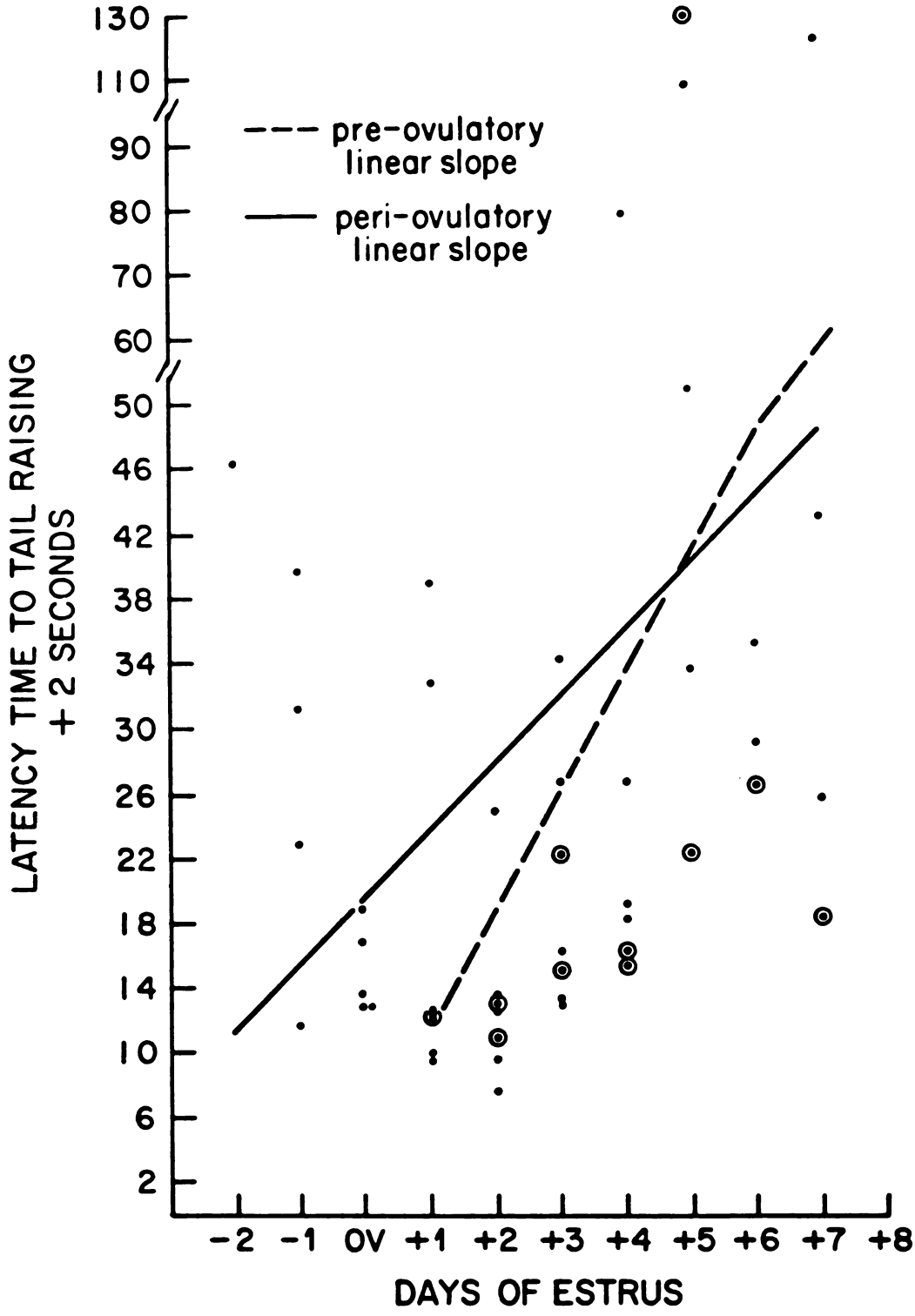

Figure 31. Data and slopes of pre-ovulatory and peri-ovulatory ILRs on tail raising latencies of untreated horse mares in Experiment 1.†

†Circled data points - used only for pre-ovulatory ILR.

significance of the peri-ovulatory slope could have been due to the elimination of the data of mare number 10.[5] With a small number of horse mares, it became apparent that data from one mare could influence the results markedly for this form of analysis.

Urination and squatting latencies of untreated horse mares decreased 3.5 seconds per day each but the two slopes were not significant (Table 8). The peri-ovulatory slope of urination latencies ($b_1 = 3.5$) was smaller than the pre-ovulatory slope ($b_{1\ pre} = 4.1$) (Table 7), which was also not significant from zero. The variation of both pre- and peri-ovulatory slopes on urination latencies was high, suggesting that this behavioral latency was not as meaningful for determining the change in sexual receptivity as were latencies to tail

[5]Recalculation of the pre-ovulatory slope on latency to mounting without data from mare number 10 reveals that the new pre-ovulatory slope ($b_{1\ pnew} = 7.3$) is even larger than the peri-ovulatory slope; but is not significant ($P > 0.1$) because the variation remained large without the data from mare number 10. So the suggestion that variation decreases after ovulation remains.

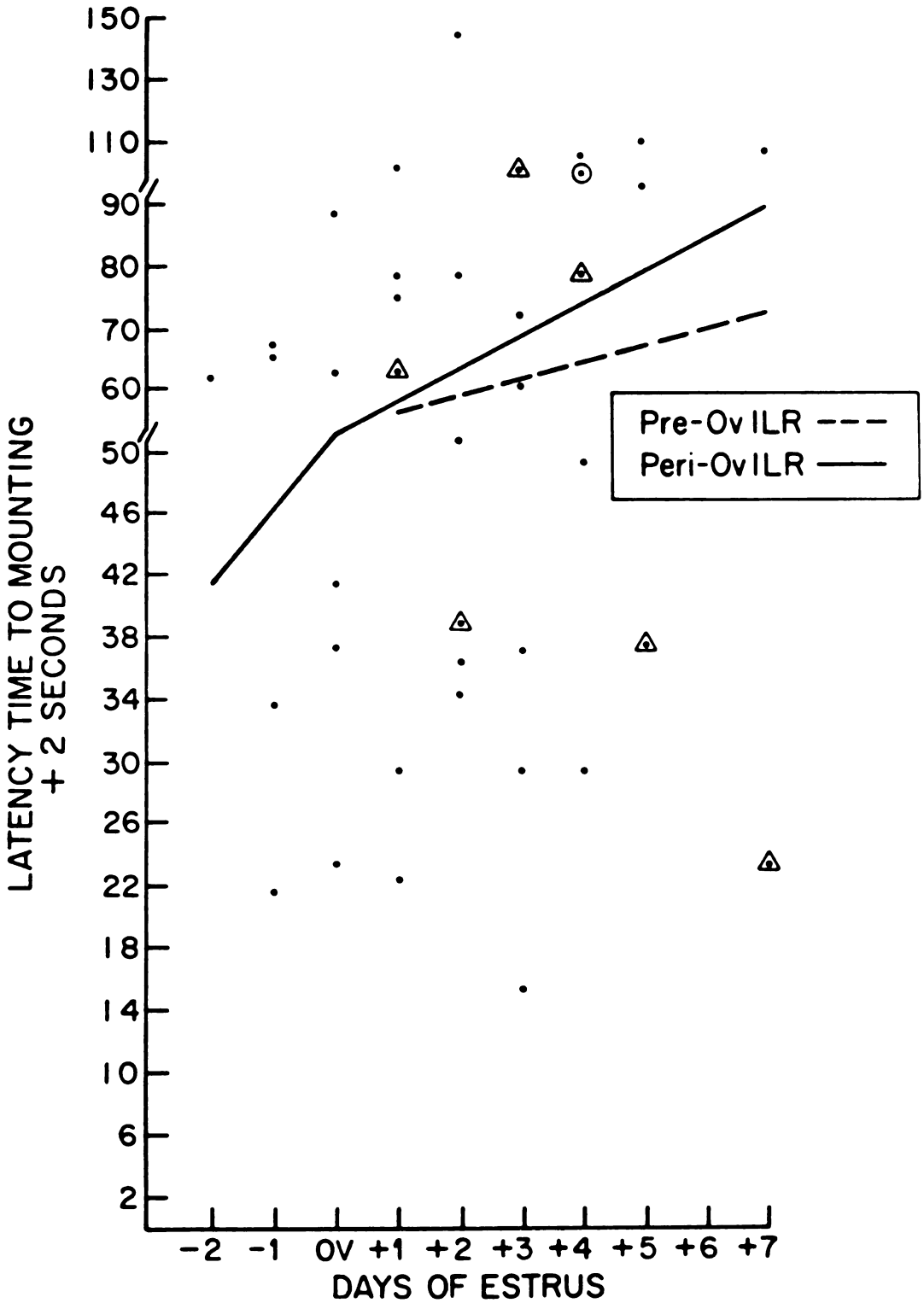

Figure 32. Data and slopes of pre-ovulatory and peri-ovulatory ILRs on mounting latencies of the pony stallion in Experiment 1.†

†Circled data points - estimated values of missing data. Data points within triangles - used only for pre-ovulatory ILR.

Table 8. Results of peri-ovulatory ILRs - Experiment 1.

	Latency to Tail Raising (+2 sec)	Latency to Urination (+2 sec)	Latency to Squatting (+2 sec)	Latency to Mounting (+2 sec)
Mares	1,5,7 and 11	1,5,7,9 and 11	5,7 and 9	1,5,7,9 and 11
d.f.	27	22	11	22
b_1(slope)	4.1	3.5	3.5	5.5
b_o(origin)	19.9	29.2	47.1	52.1
H:$\beta_1 = 0$				
H:$\beta_2 \neq 0$				
t =	2.4**	$1.7^{n.s.}$	$1.1^{n.s.}$	2.1**
95% C.I. estimate				
Upper	+7.61			+10.9
Lower	+0.62			+ 0.02

** = ($P < 0.05$)
n.s. = ($P > 0.05$)

raising and mounting. The non-significant peri-ovulatory ILR slope on squatting latencies (b_1 = 3.5) (Table 8)[6] was also smaller than the pre-ovulatory slope ($b_{1\ pre}$ = 6.0) (Table 7), the latter was slightly significant ($P < 0.07$) from zero.

Conclusions about the changes in sexual receptivity in relation to ovulation were not drawn until the hypothesis of curved slopes could be tested in the next section.

(b) Intramare Curvilinear Regressions

To test the possibility that the behavioral latencies curved intramare curvilinear regressions (ICRs) were performed on the data of untreated horse mares (Tables A1, A2, A3, A4 and A11) on all days of estrus. Computational formulae used in addition to those for an ILR are given in Appendix B (Section B.).

[6]The peri-ovulatory slope on latency to squatting was derived from data on only three mares which this experimenter considers an inadequate sample size to provide a valid statistical test. Gill (1978) suggests n < 15 creates a bias, the sample size in the peri-ovulatory ILR provided only n = 17.

The curve of tail raising latencies of untreated horse mares decreased from 66.1 seconds on day +7 to a minimum of 19.2 seconds on day +1, then increased to 32.4 seconds on day -2 (Figure 33, Table 9). Only the curvature (and not the slope) of the tail raising latencies approached significance ($P < 0.07$) (Table 9). The curvature was suggested as a possibility in the previous section where the peri-ovulatory ILR slope was significant but smaller than the pre-ovulatory ILR slope. These results, although only approaching statistical significance, tend to confirm the hypothesis that sexual receptivity of untreated horse mares decreases after ovulation (which occurred between days +1 and Ov).

The other slopes and curves were not significant (Table 9) indicating that for urination and squatting latencies, the peri-ovulatory data varied too greatly to fit either the linear or curvilinear models. However, the peri-ovulatory latency to mounting data did fit the linear model in the previous section with a significant slope, indicating that the readiness of the

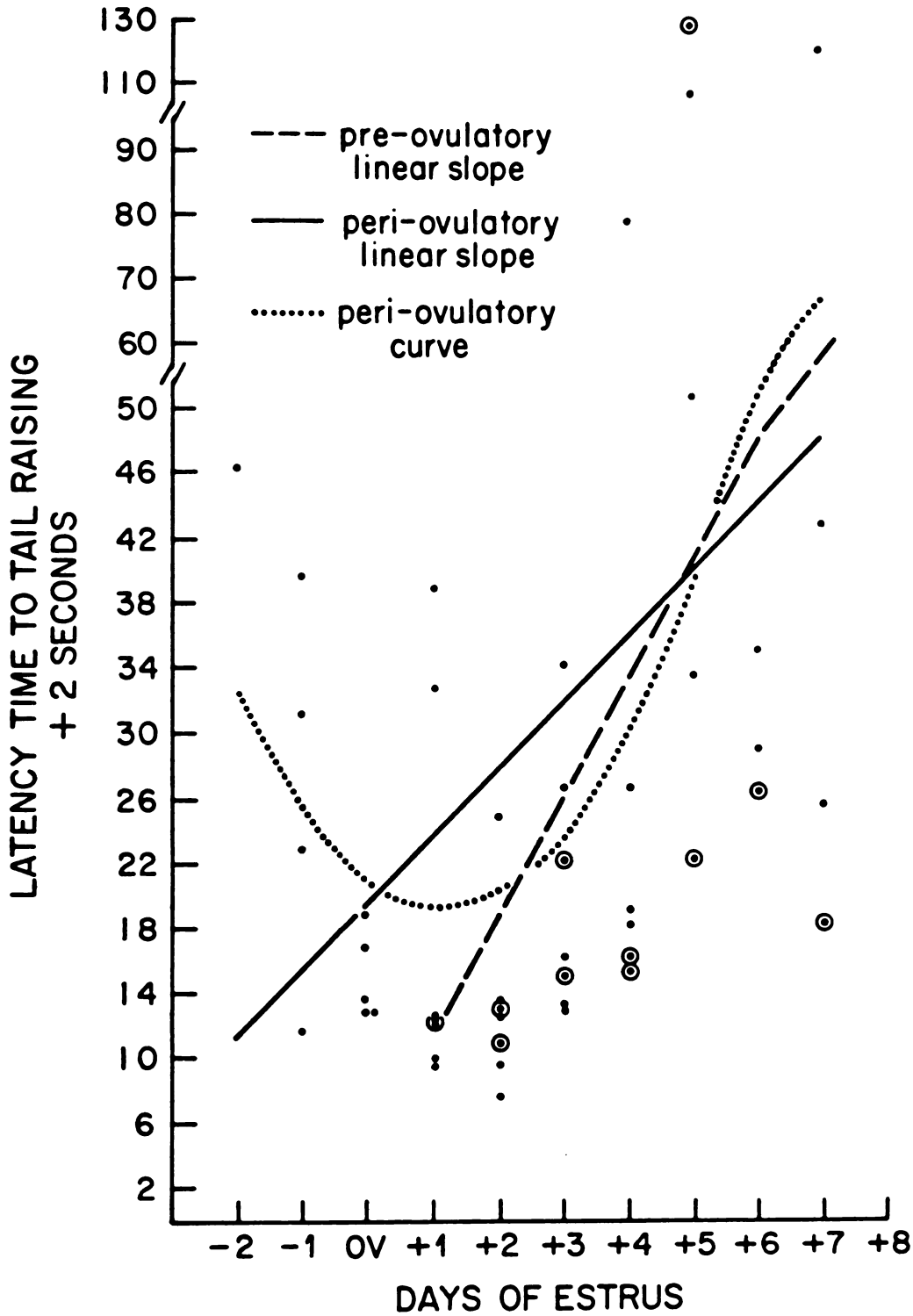

Figure 33. Pre-ovulatory ILR and peri-ovulatory ILR and ICR on tail raising latencies of untreated horse mares in Experiment 1.†

†Circled data points - used only for pre-ovulatory ILR.

Table 9. Results of peri-ovulatory ICRs - Experiment 1.

	Latency to Tail Raising (+2 sec)	Latency to Urination (+2 sec)	Latency to Squatting (+2 sec)	Latency to Mounting (+2 sec)
Mares	1,5,7,9 and 11	1,5,7,9 and 11	5,7 and 9	1,5,7,9 and 11
d.f.	22	17	8	17
b_1(slope)	-3.0	-2.0	-6.2	1.2
H:$\beta_1 = 0$				
t =	$-0.75^{n.s.}$	$-0.4^{n.s.}$	$-0.8^{n.s.}$	$0.2^{n.s.}$
b_2(curve)	1.4	1.0	2.1	1.0
H:$\beta_2 = 0$				
t =	1.9*	$1.1^{n.s.}$	$1.3^{n.s.}$	$0.9^{n.s.}$
b_o(origin)	20.9	34.2	51.3	52.0

* = ($P < 0.07$)
n.s. = ($P > 0.1$)

stallion to copulate continued to increase after ovulation and suggesting that the stallion was not perceptive of the ovulatory state of the mares.

b) Experiment 2

Although twenty-four (24) pony mares began in Experiment 2, complete data was gathered for only 15 mares[7] during one estrous period. Data for behavioral latencies (tail raising, urination, squatting and mounting) are in Tables A7, A8, A9 and A10 respectively. Table A14 gives the data for each mare on days +4 to +1 for all latencies with the estimated values for missing data where possible.

(1) Pre-Ovulatory Behavior

(a) Comparisons Between Naive and Experienced Mares

Since both experienced mares (mares which had been in estrus in previous years and possibly bred) and naive mares (in their

[7] Incomplete or no data for the remaining nine mares were due to: Number 19 developing a tear in the rectum, so palpation was discontinued before ovulation detection. Mare numbers 18 and 21 had anovulatory estrous cycles. An incompleted estrus was recorded for mare number 6 early in the experiment. Mare numbers 10, 14, 22 and 759 were pregnant from the beginning of the experiment and this was not detected until the experiment was almost over, none of these mares displayed estrous behavior. Mare number 790 never displayed estrous behavior and was not diagnosed as pregnant.

first estrous season) were observed in this experiment, it was necessary to see if they differed prior to pooling the data. The results of an orthogonal polynomial comparison (Gill, personal communication: Appendix B, Section C.) showed that only the mean squatting latencies in the linear comparison were slightly longer ($0.05 < P < 0.1$) for naive than for experienced pony mares on days +4, +3, +2 and +1 (Table 10). Thus the data from all the pony mares were used in the analyses that follow.

(b) Intramare Linear Regressions

Separate intramare linear regressions (ILRs) on tail raising, urination, squatting and mounting latencies on pre-ovulatory days of estrus were performed to determine if sexual receptivity increased prior to ovulation. The ILRs were calculated in the same way as those for Experiment 1 in section IV.D.2.a)(1).[8]

Tail raising latencies decreased significantly (Figure 34, Table 11) from 35.1

[8]Mare number 34 was excluded for all the ILRs because of too few data points. Mare number 24 was excluded from the ILR on mounting latencies for the same reason.

Table 10. Results of the linear (ξ_1-), quadratic (ξ_2-) and cubic (ξ_3-) orthogonal polynomial comparisons between naive and experienced pony mare behavioral latencies on days +4, +3, +2 and +1.

Latency to Tail Raising +10 Seconds		
Linear (ξ_1-)	Quadratic (ξ_2-)	Cubic (ξ_3-)
$t = -0.7998^{n.s.}$	$t = 0.8683^{n.s.}$	$t = -0.5727^{n.s.}$
Latency to Urination +10 Seconds		
Linear (ξ_1-)	Quadratic (ξ_2-)	Cubic (ξ_3-)
$t = -1.3303^{n.s.}$	$t = 0.9601^{n.s.}$	$t = -1.0289^{n.s.}$
Latency to Mounting +10 Seconds		
Linear (ξ_1-)	Quadratic (ξ_2-)	Cubic (ξ_3-)
$t = 0.5649^{n.s.}$	$t = -0.1359^{n.s.}$	$t = -0.2437^{n.s.}$
Latency to Squatting +10 Seconds		
Linear (ξ_1-)	Quadratic (ξ_2-)	Cubic (ξ_3-)
$t = -1.9709^{*}$	$t = 1.7632^{n.s.}$	$t = -0.2801^{n.s.}$

n.s. = ($P > 0.1$)
* = ($0.05 < P < 0.1$)

seconds on day +10, at a rate of 2.9 seconds per day to day +1 and the origin on day Ov (b_o = 6.3 seconds). Squatting latencies decreased (Figure 35, Table 11) from 41.6 seconds on day +9 with a slope of 2.9 seconds per day to day +1 and the origin on day Ov (b_o = 15.2 seconds), however the slope only approached significance ($P < 0.07$) (Table 11). Urination latencies decreased 2.2 seconds per day, but the slope was not significantly different from zero (Table 11).

Mounting latencies by the four pony stallions decreased significantly (Figure 36, Table 11) from 80.8 seconds on day +10, at a rate of 4.3 seconds per day to day +1 and the origin on day Ov (b_o = 37.8 seconds).

As with the untreated horse mares in Experiment 1 [Section IV.D.2.a)(1)], only the significantly decreasing tail raising latencies support the hypothesis that sexual receptivity increases as ovulation approaches in untreated pony mares. Although the slope of squatting latencies approached significance, as it did in untreated horse mares, the results tend to support the hypothesis.

Significantly decreasing mounting latencies suggest that stallions may be

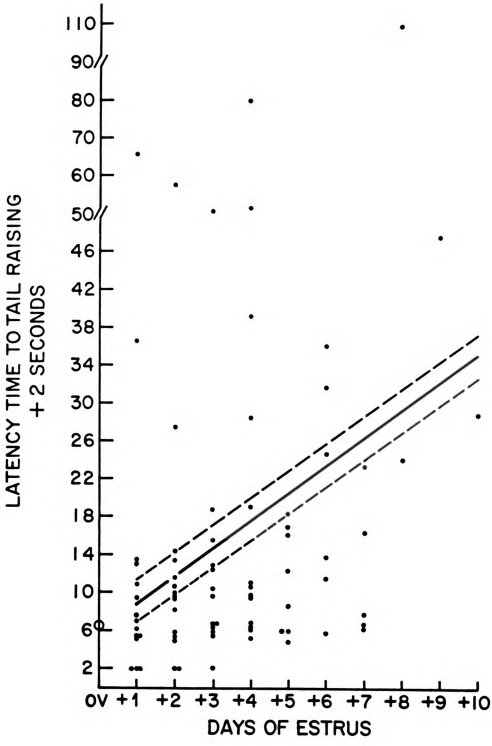

Figure 34. Data, slope and 95% C.I. estimate around the slope of the pre-ovulatory ILR on tail raising latencies of untreated pony mares in Experiment 2.

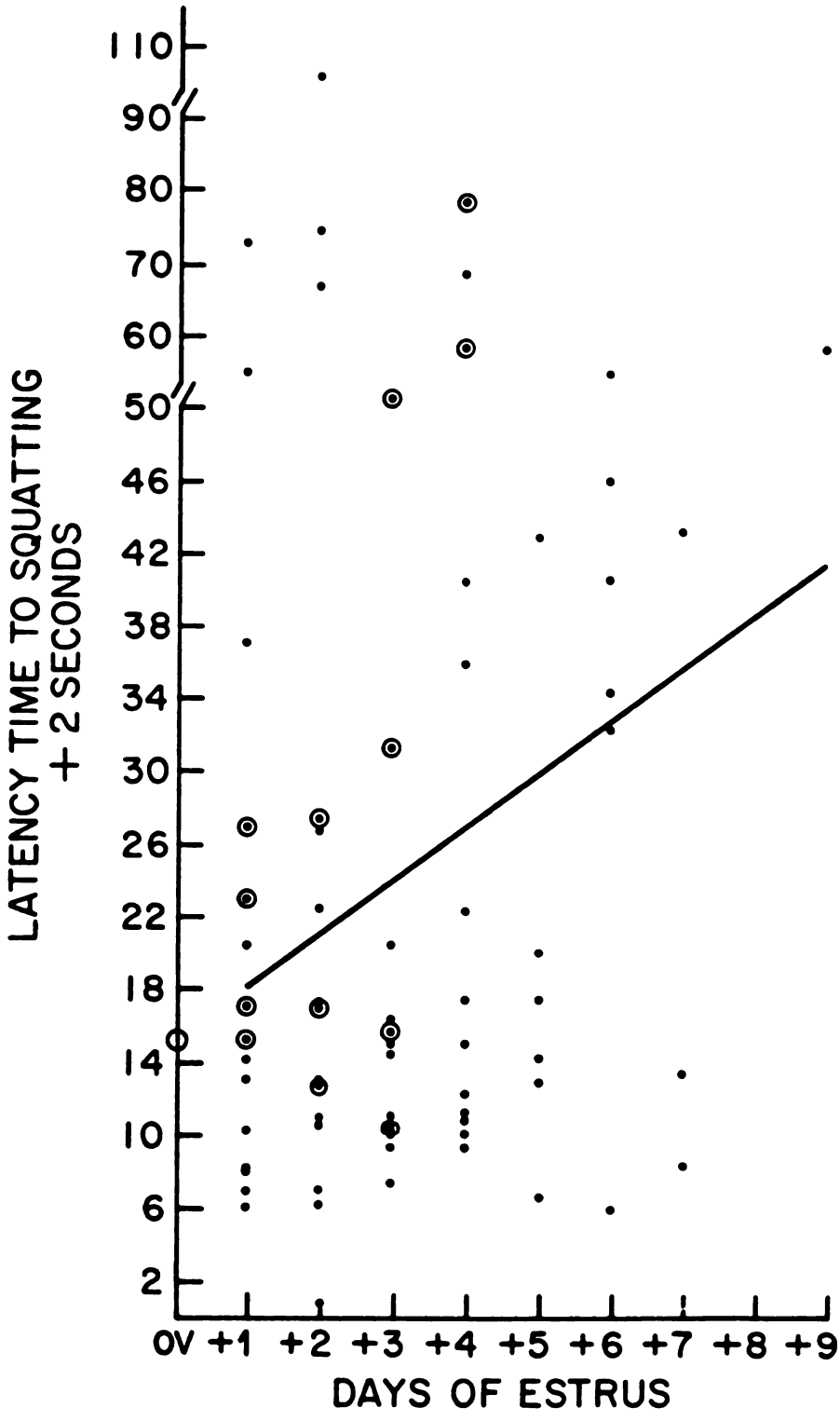

Figure 35. Data and slope of the pre-ovulatory ILR on squatting latencies of untreated pony mares in Experiment 2.†

†Circled data points - values of estimated missing data.

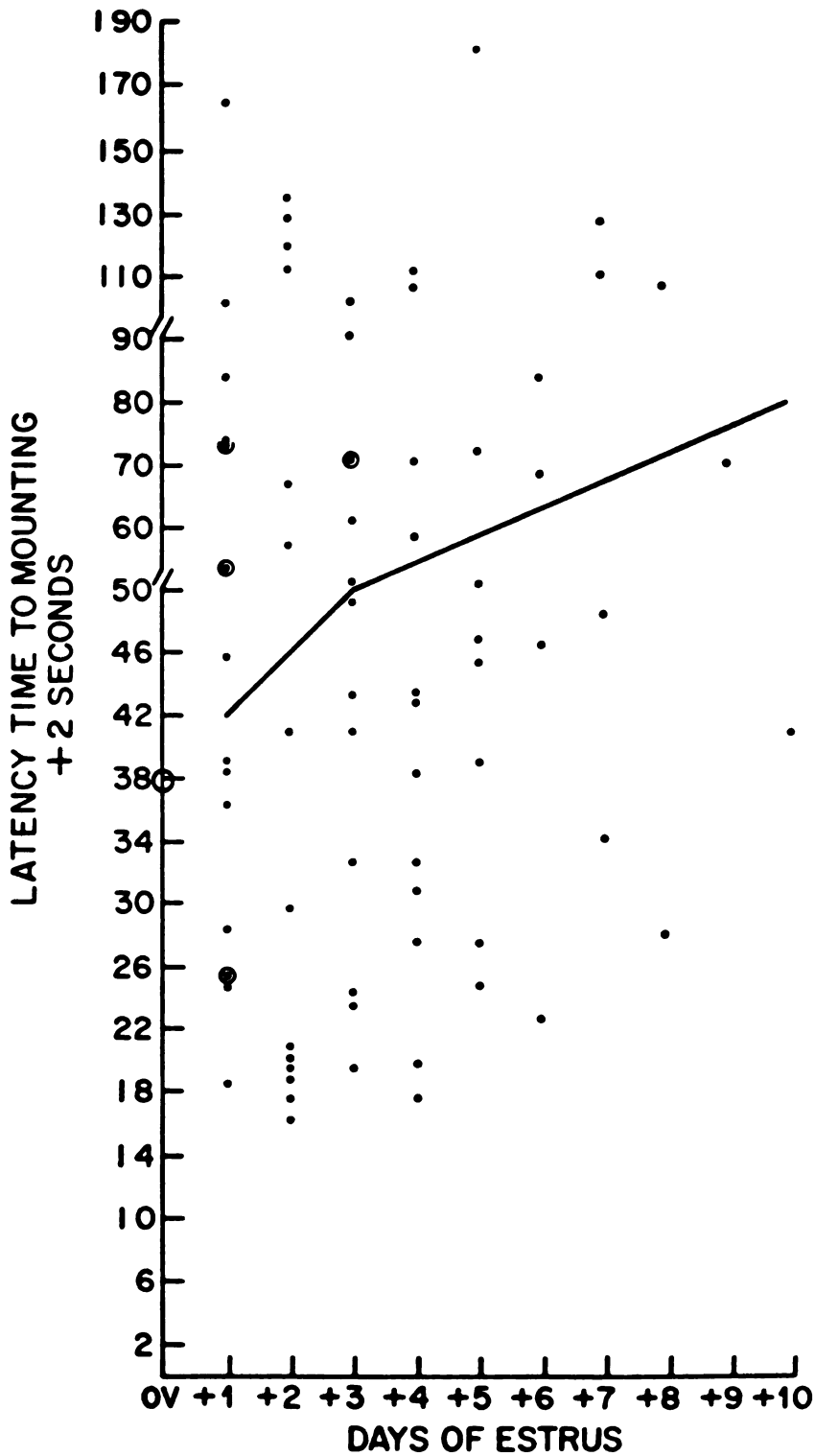

Figure 36. Data and slope of the pre-ovulatory ILR on mounting latencies of pony stallions in Experiment 2.†

†Circled data points - values of estimated missing data.

Table 11. Results of pre-ovulatory ILRs - Experiment 2.

	Latency to Tail Raising (+2 sec)	Latency to Urination (+2 sec)	Latency to Squatting (+2 sec)	Latency to Mounting (+2 sec)
Mares	12,15,16, 17,23,24, 25,26,35, 36,466, 725,757 and 770	12,15,16, 17,23,24, 25,26,35, 36,466, 725,757 and 770	12,15,16, 17,23,24, 25,26,35, 36,466, 725,757 and 770	12,16,17, 23,25,26, 35,36,466, 725,757, and 770
ν = d.f.	49	43	42	44
b_1(slope)	2.9	2.2	2.9	4.3
b_o(origin)	6.3	18.2	15.2	37.8
H:$\beta_1 = 0$				
$\bar{H}$:$\beta_1 \neq 0$				
t =	2.6**	$1.7^{n.s.}$	1.9*	2.1**
95% C.I. estimate				
Upper	+5.18		+5.99	+8.52
Lower	+0.61		-0.12	+0.09

n.s. = (P > 0.1)
* = (P < 0.07)
** = (P < 0.05)

perceptive of increasing sexual receptivity of the untreated pony mares.

(2) Peri-Ovulatory Behavior

(a) Intramare Linear Regressions

Intramare linear regressions (ILRs) were performed on the data of latencies to tail raising, squatting, urination and mounting on all peri-ovulatory days of estrus to test the hypothesis that sexual receptivity of untreated pony mares decreased after ovulation. Data came from Tables A7, A8, A9, A10 and A14. Only data from mares whose estrus extended to at least day Ov. were used. This excluded data from several mares in each ILR.

None of the peri-ovulatory ILR slopes of untreated pony mares were significantly different from zero (Table 12). These peri-ovulatory results indicate that either the variances increased after ovulation and contributed to the nonsignificance of the slopes or there was a curvature to the slopes near the day of ovulation. The possibility of slope curvature is tested in the next section.

Table 12. Results of peri-ovulatory ILRs - Experiment 2.

	Latency to Tail Raising (+2 sec)	Latency to Urination (+2 sec)	Latency to Squatting (+2 sec)	Latency to Mounting (+2 sec)
Mares	12,15,16, 17,23,24, 25,26,35, 36,466, 725,757 and 770	12,15,23, 25,26,35, 36,466, 725,757 and 770	15,23,25, 35,36,466, 725 and 757	12,16,17, 23,25,26, 35,36,466, 725,757, and 770
d.f.	70	52	38	62
b_1(slope)	0.6	0.5	1.2	2.5
b_o(origin)	17.0	23.8	18.1	46.2
$H:\beta_1 = 0$				
$\bar{H}:\beta_1 = 0$				
t =	$0.7^{n.s.}$	$0.3^{n.s.}$	$1.0^{n.s.}$	$1.5^{n.s.}$

n.s. = ($P > 0.1$)

The results indicate that sexual receptivity of the pony mares and the readiness of the stallions to copulate do not continue to increase after ovulation, rather a decrease or dissipation occurs.

(b) Intramare Curvilinear Regressions

To test the possibility that the behavioral latencies curved, intramare curvilinear regressions (ICRs) were performed on the data of latencies to tail raising, urination, squatting and mounting on all peri-ovulatory days of estrus.

Only the average slope and curvature of tail raising latencies of the untreated pony mares were significant (Figure 37, Table 13). The curve decreased from 55.6 seconds on day +10 to the minimum of 13.3 seconds on day +3 and then increased to 33.7 seconds on day -2. It was noted that the rate of decrease from day +4 to day +3 was about one second and the rate of increase from day +3 to day +2 was less than one second, indicating a shallowness to the curve between days +4 and +2, which could be considered the 'minimum days'. These results indicate that tail

raising latencies of untreated pony mares fit a curvilinear model.

Only the ICR results of tail raising latencies support the hypothesis that sexual receptivity increases rapidly to day +4, remains at a high level to about day +2, then decreases up to and after ovulation. It was noted that the beginning of the decrease came before ovulation.

The slopes and curvatures on latencies to urination, squatting and mounting were not significant (Table 13). These results in conjunction with the results of the peri-ovulatory ILRs (Table 12) suggest that the variances of these latencies increased beyond the day of ovulation sufficiently to produce non-significant linear and curvilinear results. The linear relationships shown for the pre-ovulatory latencies to squatting and mounting (Table 11) no longer exist beyond ovulation. This suggests that sexual receptivity and readiness to copulate, as reflected by the once significant slope of mounting and the near significant slope of squatting latencies now appear to dissipate after ovulation. Urination latencies do not appear to be an

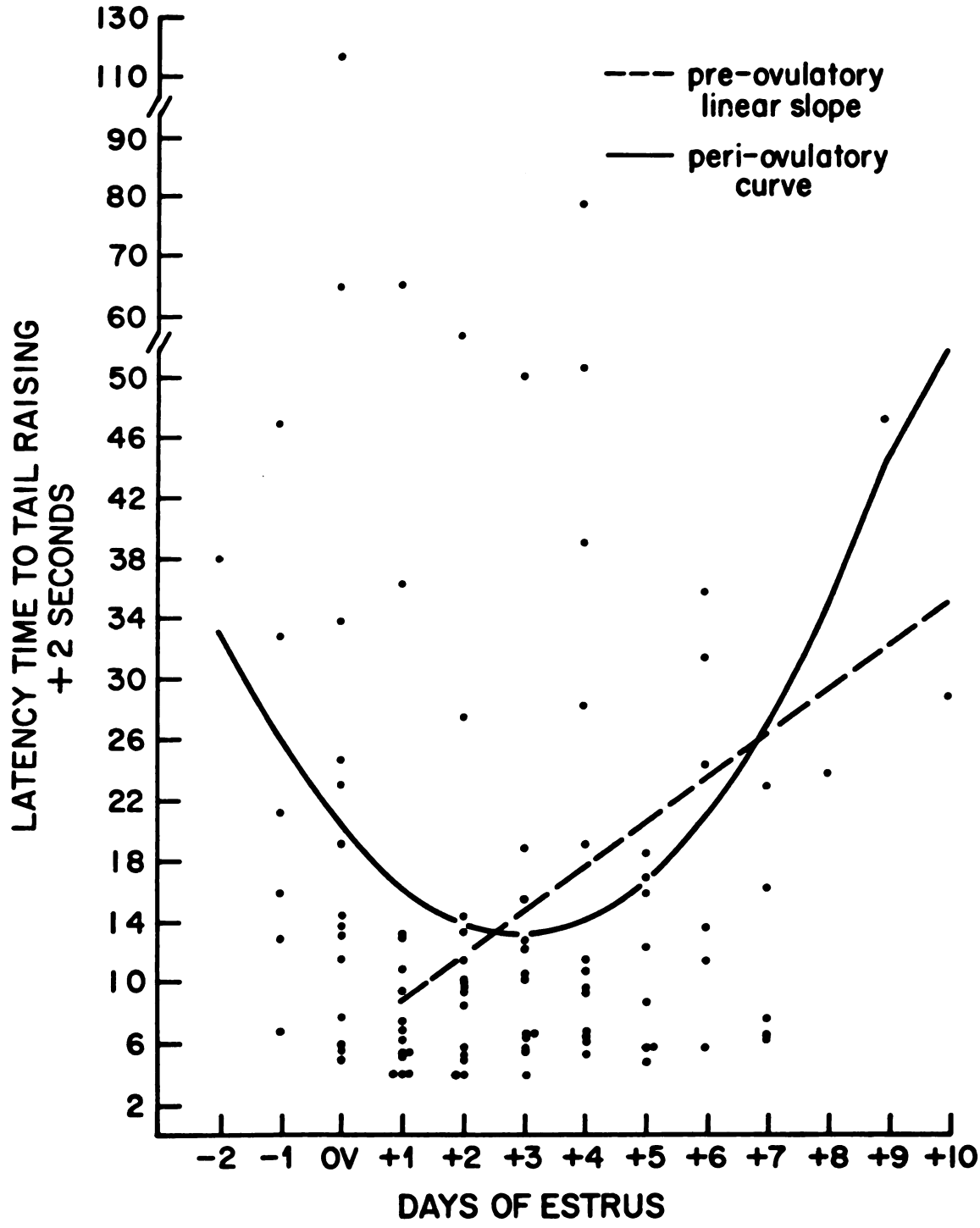

Figure 37. Data and slopes of the pre-ovulatory ILR and peri-ovulatory ICR on tail raising latencies of untreated pony mares in Experiment 2.

Table 13. Results of the peri-ovulatory ICRs - Experiment 2.

	Latency to Tail Raising (+2 sec)	Latency to Urination (+2 sec)	Latency to Squatting (+2 sec)	Latency to Mounting (+2 sec)
Mares	12,15,16 17,23,24, 25,26,35, 36,466, 725,757 and 770	12,15,23, 25,26,35, 36,466, 725,757, and 770	15,23,25, 35,466, 725 and 757	12,16,17, 23,25,26, 35,36,466, 725,757 and 770
d.f.	56	41	30	50
b_1(slope)	-4.9	-5.4	-2.2	-0.6
H:$\beta_1 = 0$				
t =	-2.3**	$-1.2^{n.s.}$	$-0.7^{n.s.}$	$-0.2^{n.s.}$
b_2(curve)	0.8	1.0	0.5	0.5
H:$\beta_2 = 0$				
t =	2.9**	$1.4^{n.s.}$	$1.1^{n.s.}$	$0.8^{n.s.}$
b_o(origin)	20.5	39.0	20.5	48.2

n.s. = ($P > 0.05$)
** = ($P < 0.01$)

appropriate measure of sexual receptivity, because all of the slopes (Tables 11, 12 and 13) were non-significant due to large variances in the data. This was also shown for untreated horse mares in Experiment 2 (Tables 7, 8 and 9).

c) Comparisons of Untreated Horse and Pony Mares

(1) Pre-Ovulatory Comparisons

Slopes and origins of relevant pre-ovulatory ILRs from Tables 7 and 9 were compared between horse and pony mares. Formulae for the t-statistics and Welch's approximation of the degrees of freedom (Gill, personal communication) are given in Appendix B (Sections A.3. and A.4.).

The results of the t-tests show that the slopes and origins of the latencies to tail raising, squatting and mounting are not significantly different ($P > 0.05$) between the groups of horse and pony mares in Experiments 1 and 2 (Table 14). This suggests that sexual receptivity of untreated horse and pony mares increases at the same rate prior to ovulation.

The one pony stallion in Experiment 1 and the four pony stallions in Experiment 2 apparently responded to the increasing sexual receptivity of the horse and pony mares in a

like fashion, although the single pony stallion in Experiment 1 failed to show a significant decrease in pre-ovulatory mounting latencies (Table 7).

Table 14. Comparisons of the slopes and origins of the pre-ovulatory ILRs on tail raising, squatting and mounting latencies between horse and pony mares.

Latencies	Slope Comparison H: $\beta_1^1 = \beta_1^2$ $\bar{H}$: $\beta_1^1 \neq \beta_1^2$	Origin Comparison H: $\beta_o^1 = \beta_o^2$ $\bar{H}$: $\beta_o^1 \quad \beta_o^2$
Tail Raising	$t = 1.6146^{n.s.}$	$t = -0.2032^{n.s.}$
Squatting	$t = 0.8699^{n.s.}$	$t = 0.8950^{n.s.}$
Mounting	$t = -0.0663^{n.s.}$	$t = 0.9888^{n.s.}$

n.s. = not significant = ($P > 0.1$)

Since the slope and origin comparisons were not significant I compared the horse and pony mare latencies (tail raising, urination and squatting),[9] prior to ovulation more precisely. A multivariate T^2-test and a univariate Approximate F-test described by Gill and Hafs (1971) were recommended by Gill (personal communication). Because these tests require that the sample size

[9] Mounting latencies were not compared because of the differences in stallion numbers and design between the experiments which did not fit the model of these tests.

for each mare (i.e. the number of days of estrus) have to be equivalent, the tests were limited to the behavioral latencies on days +4, +3, +2 and +1 of estrus.[10] Coincidentally, these were the days that Hammond (1938) recognized as being the most critical for possible conception, so they were biologically relevant.

The comparisons described by Gill and Hafs (1971) were used because the means and standard deviations of the mare groups for each behavioral latency (Figures 38, 39 and 40), suggested that some of the variances were heterogeneous. This heterogeneity of variance would invalidate a typical univariate F-test. In addition, the multivariate procedure makes use of correlation among the data (by incorporating the covariances between days), whereas a univariate test does not. The approximate F-test used, although univariate, is appropriate for cases of variance-covariance heterogeneity, as is the multivariate test.

The main null hypothesis tested for each comparison was: the profiles of response over time were identical for the two mare groups.

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[10]Adjustments for unequal group sample size were made by formulae supplied by Gill (personal communication).

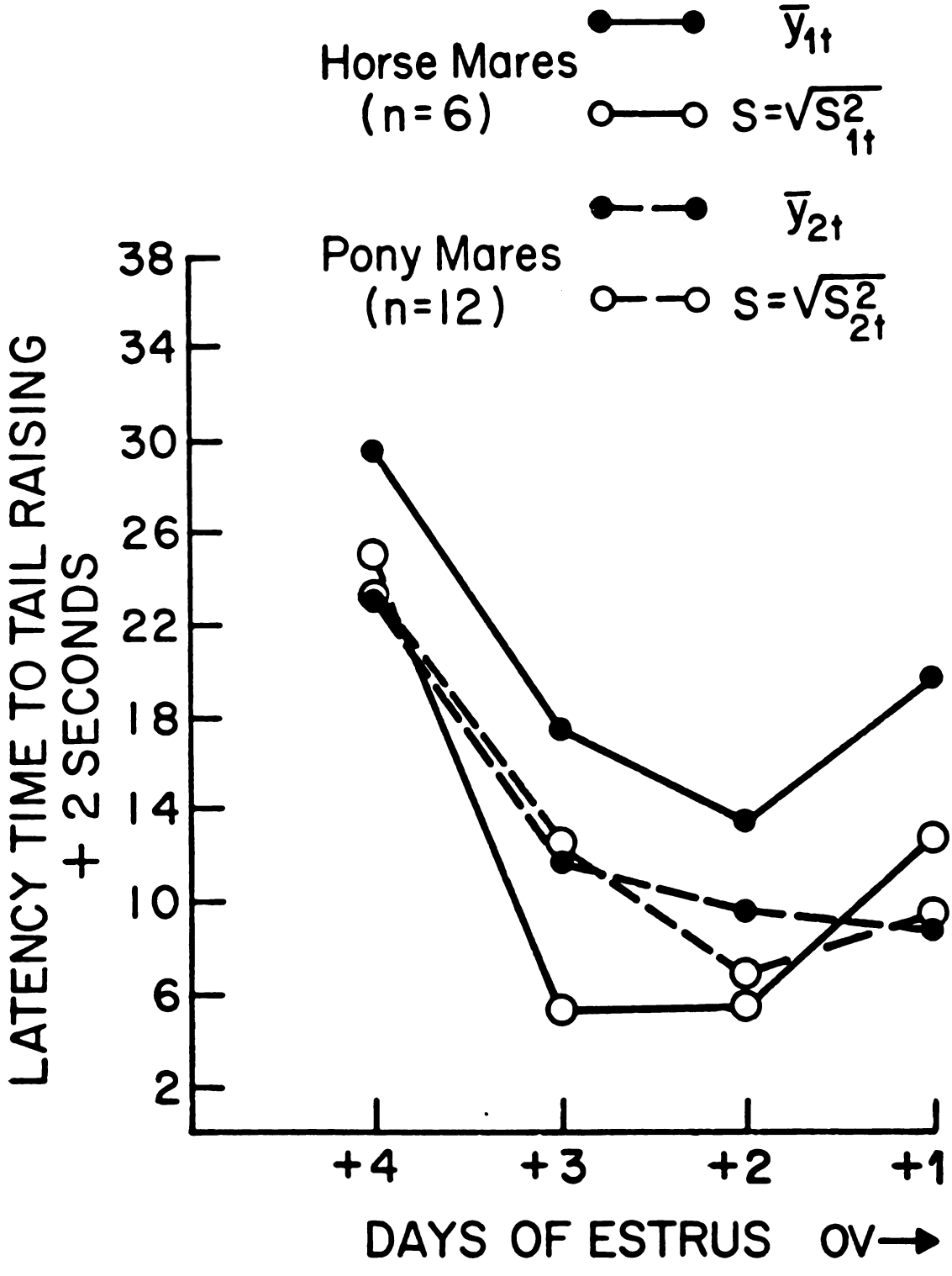

Figure 38. Means and standard deviations of tail raising latencies of horse and pony mares for comparisons.

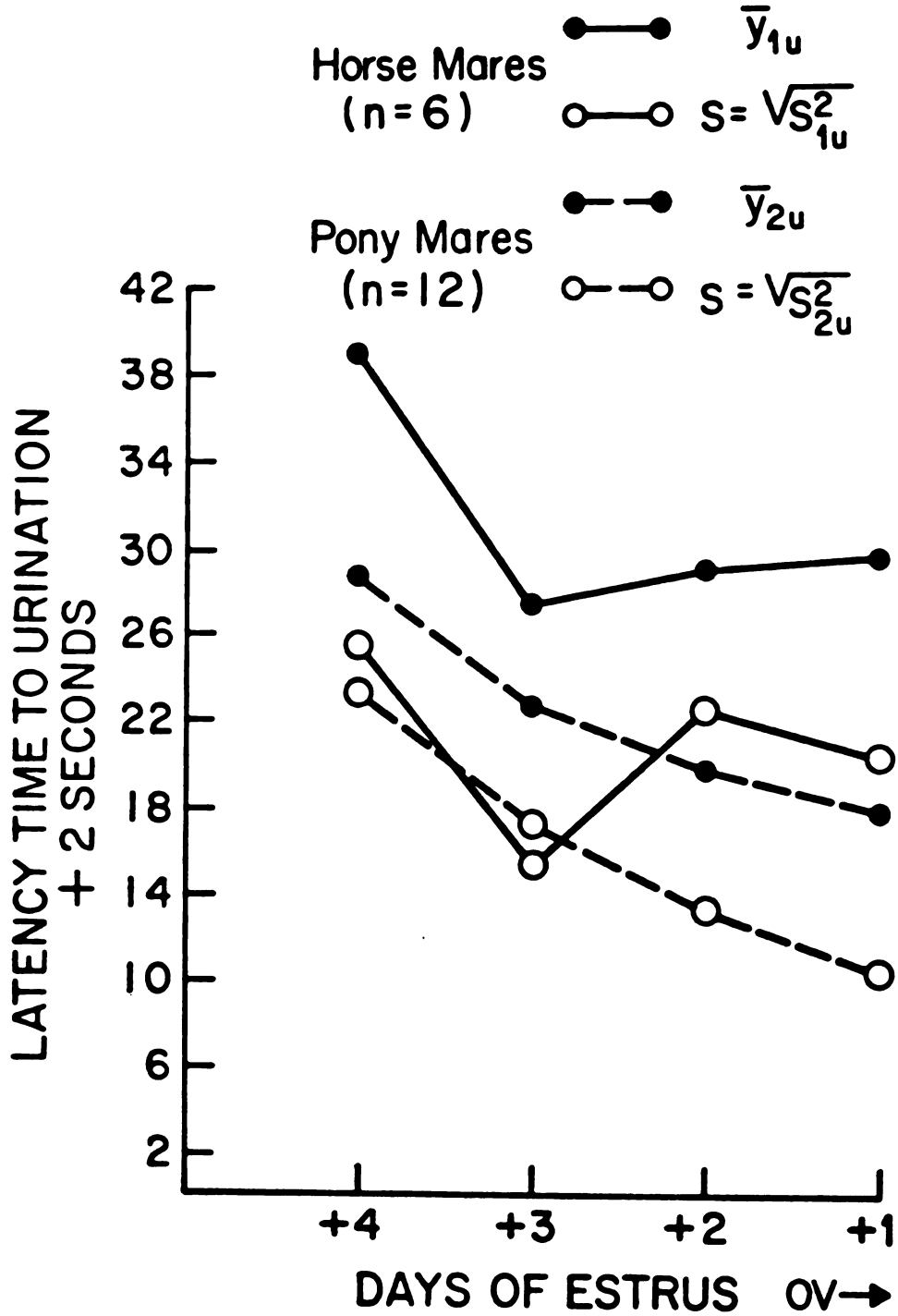

Figure 39. Means and standard deviations of urination latencies of horse and pony mares for comparisons.

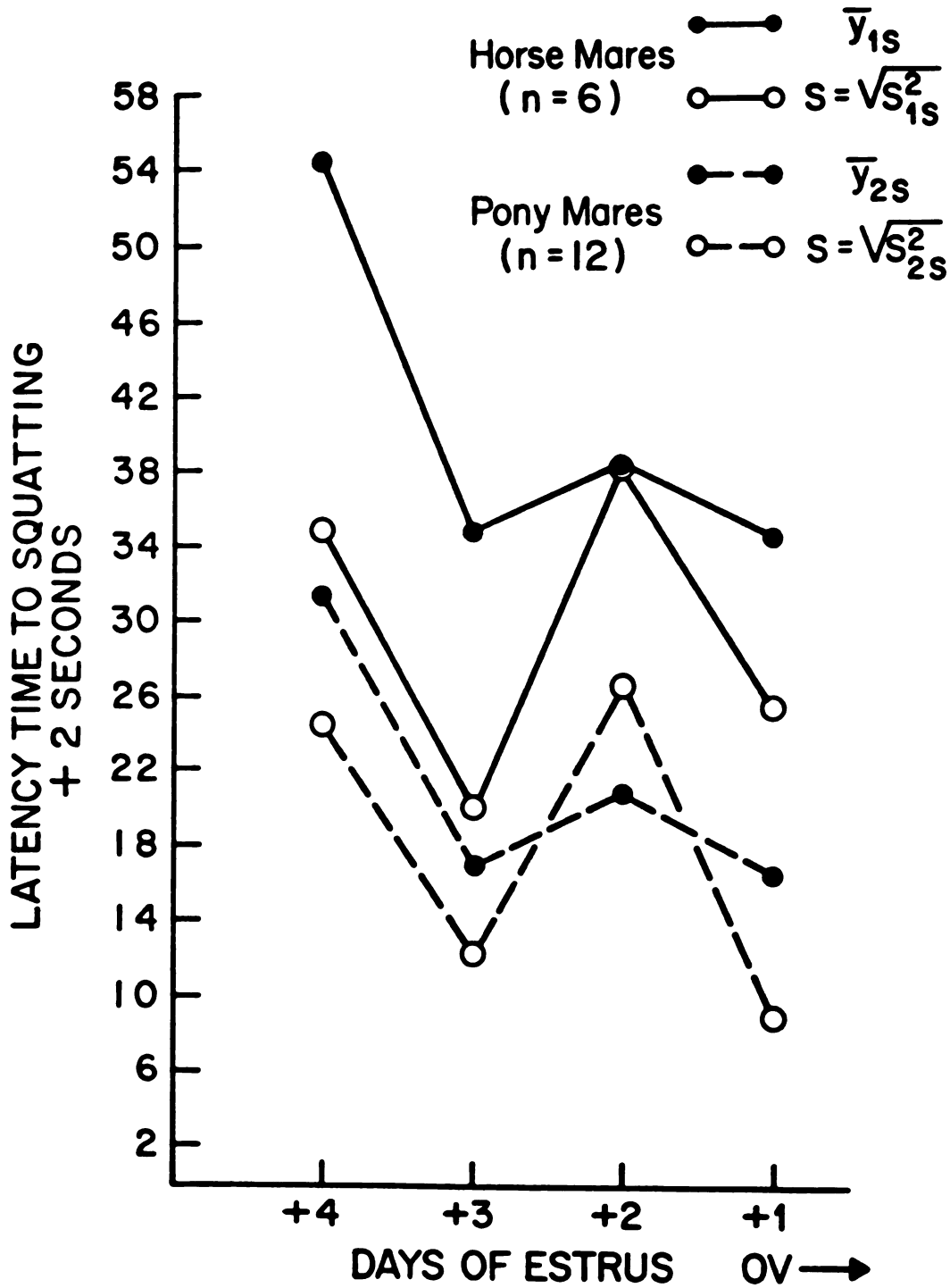

Figure 40. Means and standard deviations of squatting latencies of horse and pony mares for comparisons.

The alternative hypothesis was: the two profiles were non-coincident (whereas, a univariate alternative hypothesis of no interaction is merely that the two profiles are not parallel). The main null hypothesis was confirmed, which indicates that the profiles of response over time of the mare groups were similar for latencies to tail raising, urination and squatting (Table 15).

The approximate F-test tested the interaction of mare groups with days and the null hypothesis was: there were no differences between horse and pony mare groups within each day. The results of the approximate F-tests show that on day +1 the latency to tail raising was longer for horse mares than for pony mares with only 90% confidence (Table 15). Horse and pony mares, despite the difference in their size and morphology, showed little difference in the latencies to tail raising, urination and squatting over the four days of estrus that were tested. This indicates that increasing sexual receptivity in horse and pony mares is temporally alike.

The above results are concomitant with the results of the pre-ovulatory ILRs and the slope and origin comparisons between mare groups, in that horse and pony mares similarly increase

Table 15. Results of the pre-ovulatory comparisons of horse and pony mares in Experiments 1 and 2.

Multivariate T^2-Test H: Identical Profiles		Approximate F-Tests H: No differences between mare groups within days
Latency to Tail Raising +2 Seconds	Ho can be accepted$^{n.s.}$	Day +4 accept Ho$^{n.s.}$ Day +3 accept Ho$^{n.s.}$ Day +2 accept Ho$^{n.s.}$ Day +1 accept Ho*
Latency to Urination +2 Seconds	Ho can be accepted$^{n.s.}$	Day +4 accept Ho$^{n.s.}$ Day +3 accept Ho$^{n.s.}$ Day +2 accept Ho$^{n.s.}$ Day +1 accept Ho$^{n.s.}$
Latency to Squatting +2 Seconds	Ho can be accepted$^{n.s.}$	Day +4 accept Ho$^{n.s.}$ Day +3 accept Ho* Day +2 accept Ho$^{n.s.}$ Day +1 accept Ho$^{n.s.}$

n.s. = ($P > 0.1$)
* = ($0.05 < P < 0.1$)

sexual receptivity in close temporal relation to ovulation.

(2) Peri-Ovulatory Comparisons

Slope and origin comparisons were not performed on the peri-ovulatory behavioral latency data between Experiments 1 and 2 because of the results of the peri-ovulatory ILRs and ICRs (Tables 8, 9, 12 and 13). Only the peri-ovulatory data on tail raising latencies appeared to have biologically relevant results for both horse and pony mares [Sections IV.D.2.a)(2) and IV.D.2.b)(2), Tables 8, 9 and 13]. Thus I decided to compare sexual receptivity as reflected by latency to tail raising between horse and pony mares on the days surrounding ovulation (days +4, +3, +2, +1 and Ov). Figure 41 gives the means and standard deviations of latency to tail raising for horse and pony mares on the days examined.

A multivariate T^2-test and a univariate approximate F-test described by Gill and Hafs (1971) were performed on the data. Further discussion about these tests appear in the previous section. Only data that were complete

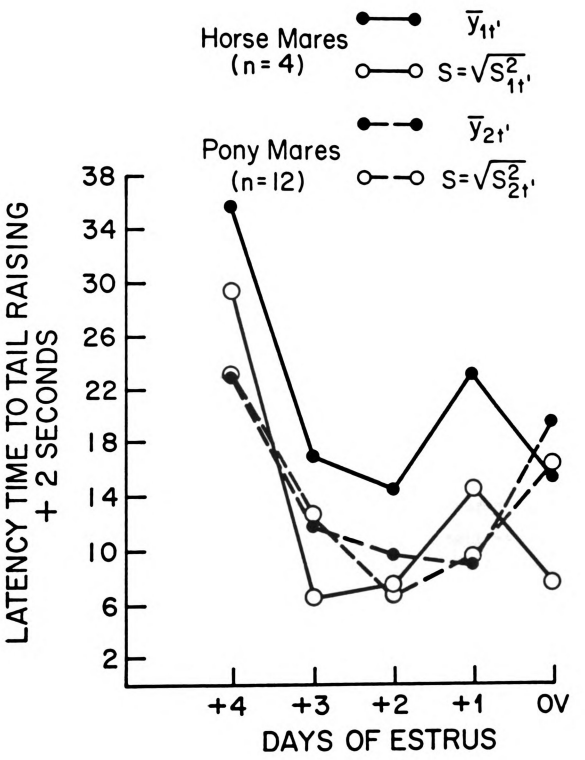

Figure 41. Means and standard deviations of tail raising latencies of horse and pony mares on days +4, +3, +2, +1 and Ov, for comparisons.

for each mare were used,[11] thus data from n = 4 horse mares and n = 12 pony mares were compared.[12]

The main null hypothesis of identical profiles was not rejected with at least 90% confidence (Table 16), concluding that the profiles of response over time of the mare groups were identical for latency to tail raising. This suggests that the changes in sexual receptivity of the mare groups around the time of ovulation were also similar.

The approximate F-tests were performed to test the interaction of mare groups with days by the null hypothesis of no differences between horse and pony mare groups within each day. The results (Table 16) show that on day +1, the mare groups differ with 90% but not 95% confidence. This indicates that the latency to tail raising is slightly longer for horse mares than for pony mares on day +1. There was no significant difference in latency to tail raising between the horse and pony mare groups on the other days of estrus tested (+4, +3, +2 and Ov). These results suggest that horse and pony mares despite

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[11]This eliminated data from horse mares number 2, 10 and 11 and pony mares number 15, 24 and 34.

[12]Adjustments for unequal groups sample size were made from formulae supplied by Gill (personal communication).

Table 16. Results of peri-ovulatory tests comparing tail raising latencies of horse and pony mares in Experiments 1 and 2.

Multivariate T^2-Test H: Identical Profiles		Approximate F-Tests H: No differences between mare groups within periods (days).
Latency to Tail Raising +2 Seconds	Ho can be accepted$^{n.s.}$	Day +4 accept Ho$^{n.s.}$ Day +3 accept Ho$^{n.s.}$ Day +2 accept Ho$^{n.s.}$ Day +1 accept Ho* Day Ov accept Ho$^{n.s.}$

n.s. = ($P > 0.1$)
* = ($0.05 < P < 0.1$)

the difference in their size and morphology showed little difference in sexual receptivity as reflected by latency to tail raising over the days of estrus that were tested.

d) Day by Day Comparisons of Data from Experiments 1 and 2 Combined.

(1) Pre-Ovulatory

The Multivariate T^2-tests and Approximate F-tests in the Section IV.D.2.c)(1) showed that behavioral latencies and sexual receptivity on days +4 to +1 were alike between horse and pony mare groups. Latency data from both mare groups were therefore statistically combined. A multivariate T^2-test (Gill and Hafs, 1971) revealed that an average time trend existed from day +4 to day +1 for each behavioral latency (Table 17). Time trends were also seen for the pre-ovulatory ILR slopes on tail raising and squatting latencies (Tables 7 and 11), but here the time trends were demonstrated for the short period of time just before ovulation. Urination latencies also showed a significant time trend from day +4 to +1, although the pre-ovulatory ILR slopes were not significant (Tables 7 and 11).

Specific day comparisons were made using Scheffé's comparison of means (a posteriori).

The specific day comparisons showed that the mean latencies on day +4 were significantly longer ($P < 0.05$) than the mean latencies on day +2 and +1 for tail raising and urination. Also the mean squatting latency on day +4 was significantly longer ($P < 0.05$) than the mean latencies on days +3 and +1 (Table 17). The decrease in latency between day +4 and +1 for all the behaviors could be a meaningful indicator of increased sexual receptivity in relation to approaching ovulation.

(2) Peri-Ovulatory

The multivariate T^2-test and the approximate F-tests in the Section IV.D.2.c)(2) showed that sexual receptivity as reflected by tail raising latencies on days +4 to Ov were alike for horse and pony mare groups. Latency data for both mare groups were statistically combined. A multivariate T^2-test (Gill and Hafs, 1971) revealed that an average time trend existed from day +4 to day Ov, when the null hypothesis of no time effects was rejected (Table 18). Significant time trends were also seen in the peri-ovulatory ILR and ICR slopes (Tables 8, 9 and 13), but here the time trend was demonstrated for a shorter period of time surrounding ovulation.

Table 17. Results of pre-ovulatory day by day comparisons of Experiments 1 and 2 combined.

	Multivariate T^2-Test H: No Time Effects	Significant Scheffé Comparisons of Day Means
Latency to Tail Raising	Ho can be rejected***	day +4 - day +2** (26.0 sec - 11.9 sec) day +4 - day +1** (26.0 sec - 14.3 sec)
Latency to Urination	Ho can be rejected***	day +4 - day +2** (26.0 sec - 11.8 sec) day +4 - day +1** (26.0 sec - 14.3 sec)
Latency to Squatting	Ho can be rejected****	day +4 - day +3** (42.9 sec - 25.8 sec) day +4 - day +1** (42.9 sec - 25.6 sec)

** = ($P < 0.05$)
*** = ($0.01 < P < 0.025$)
**** = ($P < 0.001$)

Specific day comparisons using Scheffé's comparison of means test showed that the mean tail raising latency on day +4 was significantly longer than the mean latencies on days +3, +2 and +1 for horse and pony mares combined (Table 18). There were no other significant comparisons. The mean tail raising latency on day +4 (29.4 seconds) was not significantly longer than that on day Ov (17.8 seconds) suggesting a curve to the combined data with a minimum on day +2. This proposed minimum fell on a day intermediate to those for the ICRs on tail raising latencies of horse mares (Figure 33) and of pony mares (Figure 37). This result suggests that prior to ovulation sexual receptivity begins to decrease rather than at ovulation, but after ovulation sexual receptivity is indeed diminished.

3. Behavior of Treated Horse Mares

In order to test the hypothesis that sexual receptivity could be temporally altered by the hormonal treatments in the studies by Oxender et al. (1977), I examined the pre-ovulatory tail raising latencies from the studies in several ways. First split-plot analyses of variance were performed on the tail raising latencies of the days closest to ovulation (days +4, +3, +2 and +1)

Table 18. Results of peri-ovulatory day by day comparisons of Experiments 1 and 2 combined.

	Multivariate T^2-Test H: No Time Effects	Significant Scheffé Comparisons of Day Means
Latency to Tail Raising	Ho can be rejected**	Day +4 - Day +3** (29.4 sec - 14.5 sec) Day +4 - Day +2** (29.4 sec - 12.3 sec) Day +4 - Day +1** (29.4 sec - 16.3 sec)

** = ($P < 0.05$)

for each experiment (Experiment 3 and Experiment 4) and for the two treatments (Saline and 5 mg GnRH once) that both experiments had in common. Secondly pre-ovulatory intramare linear regressions (ILRs) were performed on tail raising latencies on (1) saline treatment from both studies, (2) 5 mg GnRH once treatment from both studies and (3) all treatments from both studies. The slopes and origins of the pre-ovulatory ILRs on treated horse mares were then compared to the slope and origin of the pre-ovulatory ILR on tail raising latencies of untreated horse mares in Experiment 1 [Section IV.D.2.a.)(1)], in order to test the hypothesis that treatments altered the change in sexual receptivity prior to ovulation, although it was recognized that the data sets were only partially independent.

a) Pre-Ovulatory Split-Plot Analyses

In order to test the null hypotheses that there were no differences in tail raising latencies among treatments and among mares split-plot analyses of variance (Gill, 1978) were performed on the tail raising latencies on days +4, +3, +2 and +1 of all the treatments in the experiments. Secondarily the split-plot analysis of variance tested the null hypotheses that there were no differences among days, no interaction of treatments with days and no interaction of mares with days. Observed data came

from Tables A5 and A6 with estimated values for missing data from Tables A12 and A13.

(1) Experiment 3

There were no significant differences in tail raising latencies among treatments (saline, 1, 2, and 5 mg GnRH), or among mares (numbers 1, 5 and 9)[13] and no interaction of treatments with days (Table 19). Significant differences were found among days and for the interaction of mares with days (Table 19). Post data comparisons of means using Scheffé's procedure (Kirk, 1968) were performed on the mean latencies on each day and on the mean latencies of the mares on each day. The mean tail raising latency on day +4 was significantly ($P < 0.05$) longer than the mean latencies on days +3, +2 and +1 for all treatments combined. On day +4, mare number 9 had significantly longer tail raising latencies than did mare numbers 1 and 5 ($P < 0.05$). No other day comparisons were significant. It was noted that the differences between mares on day +4 and possibly the differences between day +4 means and those of the other days, were due to

[13]Data from mare number 11 could not be used in this analysis because she did not receive the 5 mg GnRH treatment causing missing data and estimation problems (Table A12).

Table 19. Split-plot analysis of variance for Experiment 3.

Source of Var.	d.f.	SS	MS	F-Ratios
A = Treatments	3	443.6921	147.8974	$F=MS_A/MS_{AB}=0.15^{n.s.}$
B = Mares	2	3882.3254	1941.1627	$F=MS_B/MS_{AB}=1.94^{n.s.}$
A × B = Trts. × Mares (Error a)	6	5993.4340	998.9057	
C = Days	3	3570.5023	1190.1674	$F=MS_C/MS_{ABC}=3.54^{*}$
A × C = Trts. × Days	9	2443.8677	271.5409	$F=MS_{AC}/MS_{ABC}=0.81^{n.s.}$
B × C = Mares × Days	6	7791.9237	1298.6595	$F=MS_{BC}/MS_{ABC}=3.86^{**}$
ABC = Trts. × Mares × Days (Error b)	14#	4710.7823	336.4845	

\# = -4 for missing cells
n.s. = ($P > 0.1$)
* = ($P < 0.05$)
** = ($P < 0.025$)

the long tail raising latencies of one mare (number 9) on day +4. Also one-half of mare number 9's values on day +4 (1 and 5 mg GnRH treatments) were the result of estimation of missing data (Table A12). Thus the differences may be erroneous.

Oxender et al. (1977) found that GnRH treatments did not shorten the time interval to ovulation, thus follicular maturation and ovulation were not temporally altered. The split-plot analysis of variance and the post data tests indicated that the dose levels of GnRH did not affect the tail raising latencies or sexual receptivity on days +4, +3, +2 and +1. Therefore the hypotheses that follicular maturation, ovulation and sexual receptivity could be temporally altered by hormonal treatments was rejected for the days tested.

(2) Experiment 4

There were no significant differences in tail raising latencies among the treatments (Saline, 5 mg GnRH once and 5 mg GnRH daily), among mares (numbers 2, 3, 4, 6, 8 and 10) or among days (+4, +3, +2 and +1) (Table 20). There were highly significant interactions of treatments with days and mares with days (Table 20).

Table 20. Split-plot analysis of variance for Experiment 4.

Source of Var.	d.f.	SS	MS	F-Ratios
A = Treatments	2	7521.8979	3760.9489	$F=MS_A/MS_{AB}=1.00^{n.s.}$
B = Mares	5	12322.0222	2464.4044	$F=MS_B/MS_{AB}=0.66^{n.s.}$
A × B = Trts. × Mares (Error a)	10	37413.4389	3741.3439	
C = Days	3	757.8399	252.6133	$F=MS_C/MS_{ABC}=2.33^{n.s.}$
A × C = Trts. × Days	6	8314.6079	1385.7680	$F=MS_{AC}/MS_{ABC}=12.80^{**}$
B × C = Mares × Days	15	12650.6057	843.3737	$F=MS_{BC}/MS_{ABC}=7.79^{**}$
A × B × C = Trts. × Mares × Days (Error b)	19#	2057.1827	108.2728	

# = -11 for missing cells.
n.s. = (P > 0.1)
** = (P < 0.01)

Post data comparisons of mean latencies using Scheffé's procedure (Kirk, 1968) compared the mean latencies of treatments within days and the mean latencies of mares within days. To simplify the results of the Scheffé comparisons, means not significantly different were underlined. In the comparisons of the mean latencies of the treatments within days the significant differences ($P < 0.05$) on all days are the same and can be symbolized by: GnRH daily <u>Saline, GnRH once</u>, showing that the mean tail raising latencies during GnRH daily treatment were significantly longer than during both Saline and GnRH once treatments. For the comparisons of means of mares within days this symbolism can be used to show all the significant differences ($P < 0.05$) within 1 day. For example on day +4:

<u>#10 #3</u> <u>#8 #6 #2 #4</u> shows the mare mean latencies in descending order, and that mares no. 10 and 3 have significantly longer mean tail raising latencies than mares no. 8, 6, 2 and 4. Below the symbolism is used for days +3, +2 and +1.

Day +3: #10 <u>#3 #4 #6 #8 #2</u>

Note: in addition to mare #10 having significantly longer mean tail raising latencies than the other mares, mare #3 was also significantly different from mare #2.

Day +2: #10 <u>#3 #4 #8 #2 #6</u>

Day +1: #10 <u>#3 #4 #8 #6 #2</u>

It was apparent that mare no. 10 had significantly longer mean tail raising latencies than all other mares on all days. This was due to very long tail raising latencies during the 5 mg GnRH daily treatment (Table A13), three-fourths of which were the result of estimated missing values. Thus, the differences in the Scheffé comparisons could have been erroneous. Mare no. 3 also had long tail raising latencies on days +4 and +3, but they were not as high as those of mare no. 10; nor were they due to estimation of missing data. The differences between treatments within days are partially due to the long tail raising latencies of mare no. 10 during the 5 mg GnRH daily treatment, but not totally as can be seen from the data in Table A13.

Oxender et al. (1977) found that the GnRH treatments did not shorten the time interval to ovulation, thus follicular maturation and ovulation were not temporally altered by the hormonal treatment. The split-plot analysis of variance and the post data tests indicated that the GnRH treatment regimes did not affect the latency to tail raising or sexual receptivity on

days +4, +3, +2 and +1 of estrus. Therefore the hypotheses that follicular maturation, ovulation and sexual receptivity could be temporally altered by hormonal treatments was rejected for the days tested.

(3) Partial Treatment Comparison of Experiments 3 and 4 Combined

A split-plot analysis of variance was performed on tail raising latiencies on days +4, +3, +2 and +1 of estrus for the two treatments that Experiments 3 and 4 had in common (Saline and 5 mg GnRH once). Although the degrees of freedom (d.f.) for treatments were drastically reduced in this analysis, the total d.f. for mares were increased. The null hypotheses of no differences in tail raising latencies among treatments and among mares were perfunctory, since there were no differences found when each experiment was tested separately (the previous two sections). The tests of primary interest were those testing the null hypotheses of no differences among days and no interactions of treatments with days or interactions of mares with days.

There were no significant differences in tail raising latencies among treatments or among mares, which was expected (Table 21).

Significant differences in tail raising latencies were found among days and for the interactions of treatments with days and mares with days (Table 21).

Post data comparisons of mean tail raising latencies using Scheffé's procedure (Kirk, 1968) were performed for all possible differences of means for days, treatments within days and mares within days. Simplification of the results used the symbolism found in the previous section. For comparisons of mean latencies among days the significant difference ($P < 0.05$) was:
day +4 <u>day +3 day +2 day +1</u>; i.e. the mean latencies for all treatments and mares on day +4 were significantly longer than on all other days. For the comparison of mean tail raising latencies of treatments within days, only the mean latencies on day +4 were significantly ($P < 0.05$) longer for Saline treatment than for 5 mg GnRH once treatment. For the comparisons of mean latencies of mares within days the mean latencies were written in descending order for the mares and the mares not significantly different ($P < 0.05$) were underlined on the successive descending lines:

Day +4: #9 #3 #4 #1 #10 #5 #8 #6 #2

Day +3: #1 #9 #10 #4 #8 #3 #6 #5 #2

Day +2: #9 #10 #1 #4 #5 #3 #8 #2 #6

Day +1: #1 #4 #3 #5 #9 #10 #6 #2 #8

It can be seen that the number of significant differences between mares diminishes as ovulation approaches which suggests that the data for these treatments may be linearly related to the days of estrus prior to ovulation. In contrast to the analyses for Experiments 3 and 4 estimated values of missing cells were not responsible for the mares with the longest mean tail raising latencies (Tables A12 and A13, Saline and 5 mg GnRH treatments only).

The hypothesis that sexual receptivity could be temporally altered by hormone treatments was rejected in the two previous sections, for the days tested.

Table 21. Split-plot analysis of variance for Experiments 3 and 4 combined.[†]

Source of Var.	d.f.	SS	MS	F-Ratios
A = Treatments	1	102.8895	102.8895	$F=MS_A/MS_{AB}=0.22^{n.s.}$
B = Mares	8	2581.3771	322.6721	$F = MS_B/MS_{AB}=0.68^{n.s.}$
A × B = Trts. × Mares (Error a)	8	3790.3619	473.7952	
C = Days	3	1352.7769	450.9256	$F=MS_C/MS_{ABC}=6.86^{**}$
A × C = Trts. × Days	3	1674.9648	558.3216	$F=MS_{AC}/MS_{ABC}=8.49^{**}$
B × C = Mares × Days	24	5726.7223	238.6134	$F=MS_{BC}/MS_{ABC}=3.63^{**}$
A × B × C = Trts. × Mares × Days (Error b)	18#	1183.2294	65.7350	

\# = -6 for missing cells
n.s. = (P > 0.1)
** = (P < 0.005)

[†]Saline and 5 mg. GnRH once treatments only.

b) Pre-Ovulatory Intramare Linear Regressions

Intramare linear regressions (ILRs) were performed on tail raising latencies from all pre-ovulatory days of estrus (Tables A5, A6, A12 and A13) for Saline treatment, 5 mg GnRH once treatment and all treatments from Experiments 3 and 4.[14]

(1) Saline Treatment - Experiments 3 and 4

Tail raising latencies during saline treatment (Experiments 3 and 4) decreased significantly (Table 22, Figure 42) from 35.5 seconds on day +8 with a slope of 4.2 seconds per day to day +1 and the origin on day Ov (b_o = 2.0 seconds). The results suggest that sexual receptivity increases prior to ovulation.

(2) 5 mg GnRH Once Treatment - Experiments 3 and 4

Tail raising latencies during 5 mg GnRH once treatment (Experiments 3 and 4) decreased significantly (Table 22, Figure 43) from 29.7 seconds on day +7 with a slope on 4.1 seconds per day to day +1 and the origin on day Ov

[14]Peri-ovulatory ILRs and ICRs were also performed on the same data. The results of these confirm the results of the peri-ovulatory ILR and ICR on tail raising latencies of untreated horse mares in Experiment 1, Section IV.D.2.a)(2), but were not relevant here. Peri-ovulatory slopes and curves can be seen in the figures of this section.

(b_o = 1.0 seconds). The results suggest that sexual receptivity increases prior to ovulation.

(3) All Treatments - Experiments 3 and 4

Tail raising latencies during all treatments (Experiments 3 and 4) decreased significantly (Table 22, Figure 44) from 38.6 seconds on day +8 with a slope of 4.3 seconds per day to day +1 and the origin on day Ov (b_o = 4.1 seconds). The results suggest that sexual receptivity during all treatments increased prior to ovulation.

It was possible to test for non-linearity because there were several Y-values for each X-value for each mare. The formulae for this test are given in Appendix B (Section A.2.). The residual error sum of squares SS_E = 22440.34 was partitioned into "pure" error ($SS_{y|x}$) = 16184.33 and that due to non-linearity $SS_{NL} = SS_E - (SS_{y|x})$ = 6256.01. The hypothesis that error due to non-linearity equals zero (H: $\sigma^2_{NL} = 0$) was tested and the result was $f = 0.918$ which was not significant ($P > 0.05$). Therefore, the hypothesis was accepted and the conclusion was that the ILR was linear.

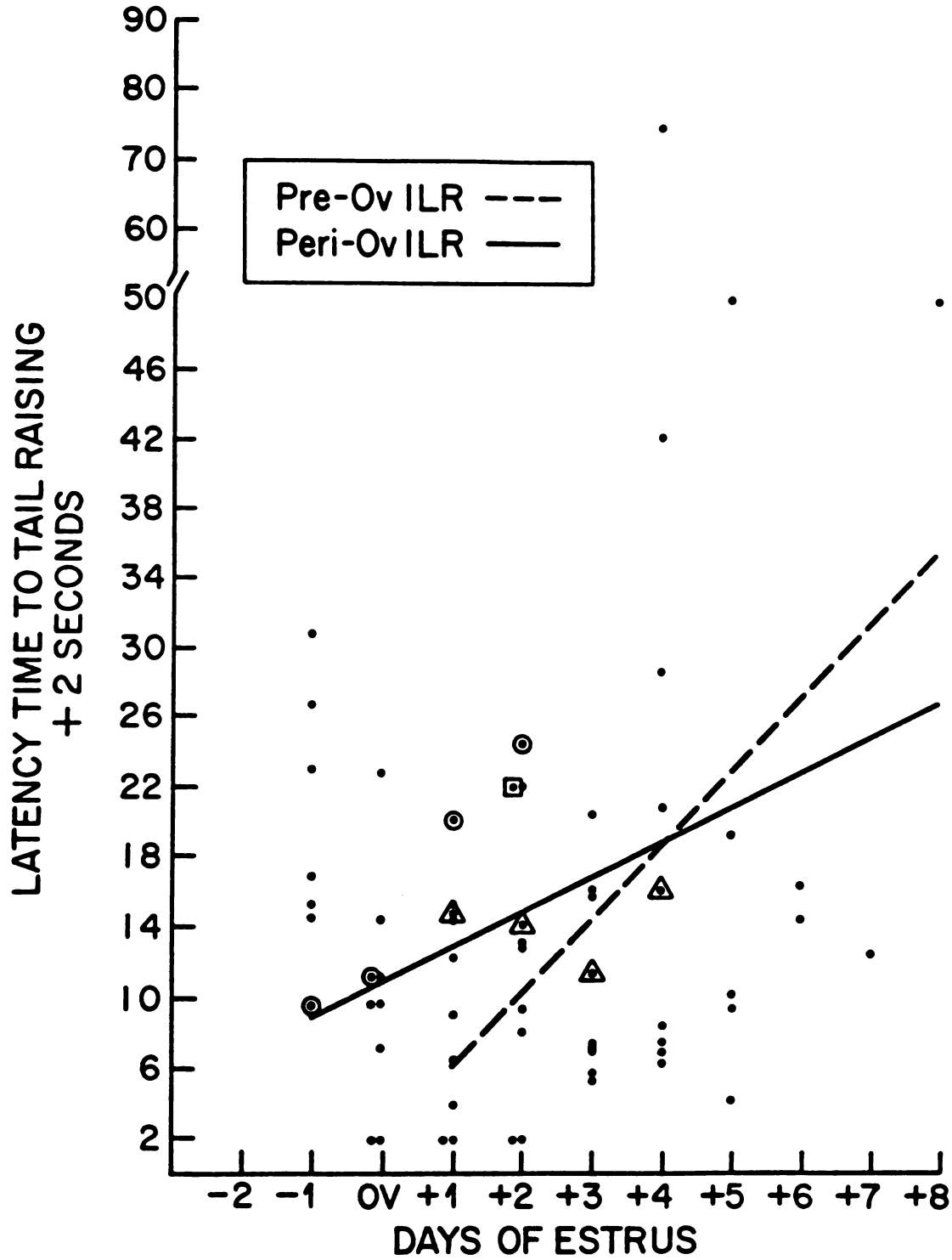

Figure 42. Pre- and peri-ovulatory ILRs on tail raising latencies of saline treated horse mares in Experiments 3 and 4.†

†Circled data points - used only for peri-ovulatory ILR. Data points in triangles - used only for pre-ovulatory ILR. Data point in square - estimated missing value.

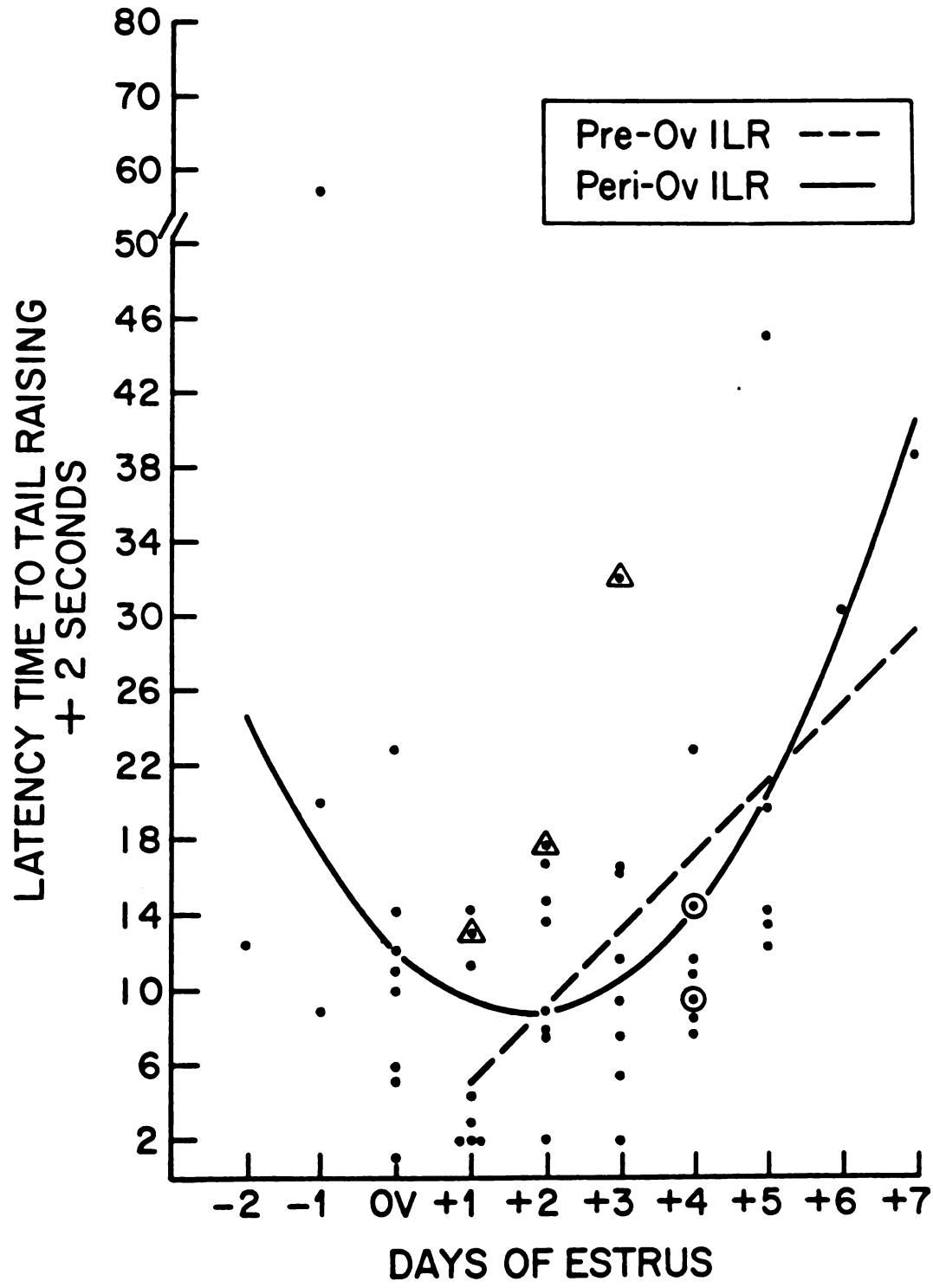

Figure 43. Pre-ovulatory ILR and peri-ovulatory ICR on tail raising latencies of horse mares treated with 5 mg GnRH once in Experiments 3 and 4.†

†Circled data points - estimated missing values.
Data points in triangles - used only for pre-ovulatory ILR.

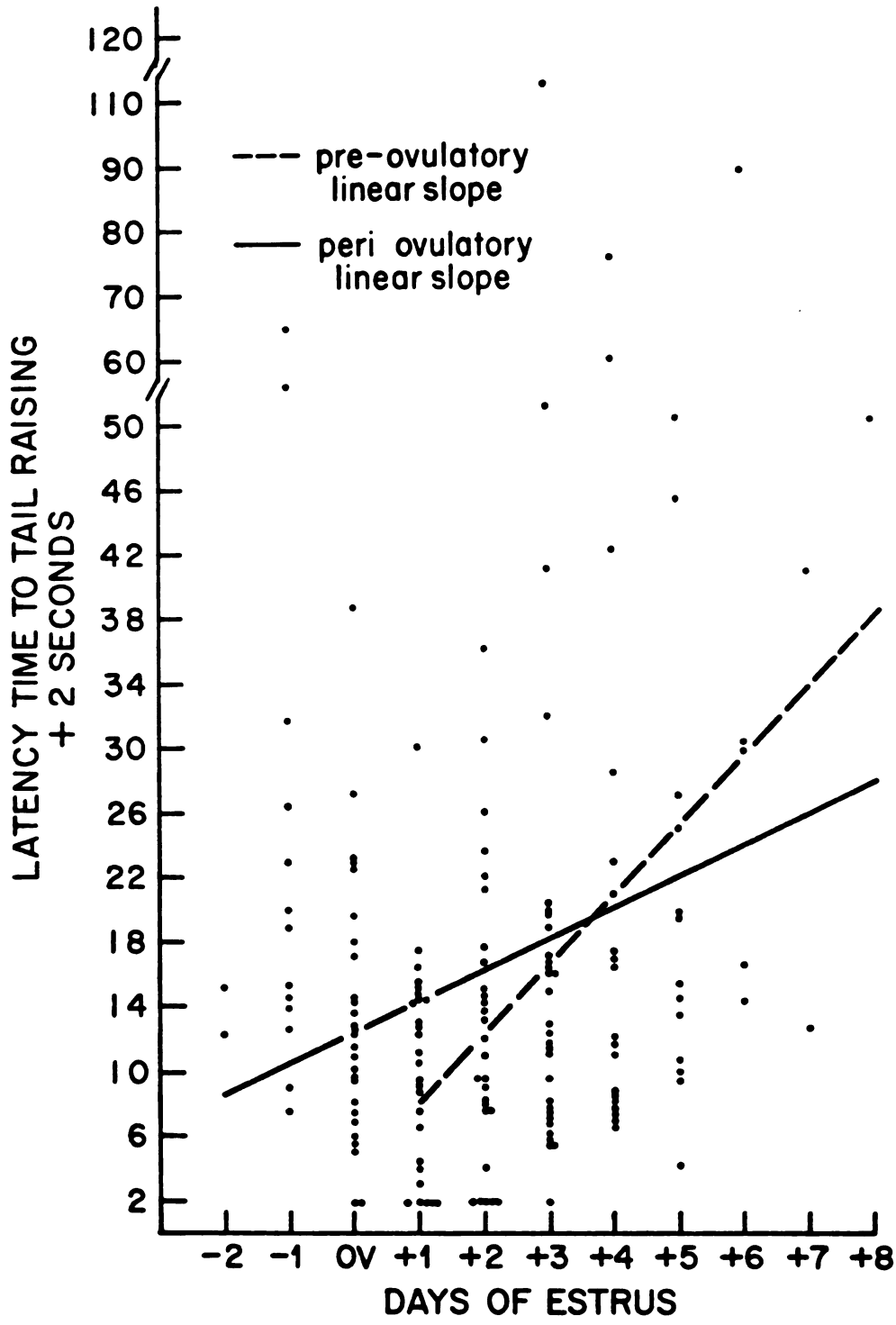

Figure 44. Pre- and peri-ovulatory ILRs on tail raising latencies of horse mares given all treatments in Experiments 3 and 4.†

†Estimated missing values not circled.

Table 22. Results of pre-ovulatory ILRs on tail raising latencies of treated horse mares in Experiments 3 and 4.

Treatments	Mares	d.f.	b_1(slope)	b_o(origin)
Saline	1,5,9,2,3,4,6, 8 and 10.	27	4.2***	2.0
5 mg GnRH Once	1,5,9,2,4,6,8 and 10.	21	4.1***	1.0
All Treatments	1,5,9,2,3,4,6 8 and 10.	108	4.3***	4.1

*** = ($P < 0.01$)

(4) Comparison of Treated Horse Mares with Untreated Horse Mares

Slopes and origins of the pre-ovulatory ILRs on tail raising latencies of treated horse mares (Table 22) were compared with the pre-ovulatory slope and origin of the ILR on tail raising latencies of untreated horse mares in Experiment 1 (Table 7). Formulae for the t-statistics are given in Appendix B (Sections A.3. and A.4.). The results of the t-tests show that none of the slopes and origins of the treated horse mares (Saline, 5 mg GnRH once and all treatments) were different ($P > 0.1$) from the slope and origin of the untreated horse mares in Experiment 1 (Table 23). These results suggest that the 5 mg GnRH once treatment and the saline treatment do

not change the pre-ovulatory rate of increase of sexual receptivity in horse mares. This confirms the findings of Oxender et al. (1977) and contributes to fully rejecting the hyoptheses that follicular maturation, ovulation and sexual receptivity could be temporally altered by hormonal treatments with GnRH.

Table 23. Comparisons of the pre-ovulatory ILR slopes and origins on tail raising latencies between treated horse mares in Experiments 3 and 4 and untreated horse mares in Experiment 1.

Experiment 1 vs.	Slope Comparison	Origin Comparison
Saline (Expts. 3 and 4)	$t = 0.3884^{n.s.}$	$t = 0.1864^{n.s.}$
5 mg GnRH once (Expts. 3 and 4)	$t = 0.4417^{n.s.}$	$t - 0.2796^{n.s.}$
All treatments (Expts. 3 and 4)	$t = 1.1600^{n.s.}$	$t = -0.0047^{n.s.}$

n.s. = ($P > 0.1$)

V. DISCUSSION

A. Introduction

The purpose of this work was to quantitatively analyze the changes in sexual behavior and receptivity of domestic mares during estrus in relation to ovulation. The variable length of estrus and variable time intervals from estrus onset to ovulation make the time of ovulation difficult to predict. This results in a low conception rate in domestic mares under highly controlled breeding programs.

Researchers have investigated the problem of low conception rate by intervening physiologically to: (1) shorten diestrus, thus increasing the number of estrous cycles per year and (2) synchronize ovulation (as yet only attempted). Many researchers have recognized the behavioral component to this physiological problem and have attempted to analyze sexual behavior of the mare in order to predict ovulation through behavior. Unfortunately most of their measurements and analyses were not appropriate. As a result their studies could not diagnostically predict ovulation.

In the review by Andrews and McKenzie (1941) and the data provided by Hammond (1938), it was suggested that mares bred once during the four days prior to ovulation had a greater probability of conception than those bred on any

other day during estrus (65% vs. 18%). Although these data were old and questionable, they may validly indicate an optimum time for breeding relative to ovulation. Thus the problem was redefined: to use behavior to predict the optimum time for breeding prior to ovulation. With the information about the physiological changes during estrus, I hypothesized that sexual receptivity of normal mares would increase prior to ovulation and decrease after ovulation. If the change of behavioral measures support the hypothesis, then the behavioral measurements could be used for predictions. I also hypothesized that sexual receptivity could be temporally altered by hormonal treatments if the treatments also altered the rate of follicular maturation and shortened the interval to ovulation. Both hypotheses indicate reproductive success in domestic equids.

B. Results

1. Studies on Untreated Mares

I hypothesized that sexual receptivity of normally cycling mares would increase before ovulation and decrease after ovulation. For purposes of analysis the hypothesis was broken down into two parts: (1) analysis of pre-ovulatory behavior to determine if sexual receptivity increases prior to ovulation and (2) analysis of peri-ovulatory behavior to determine if sexual receptivity decreased after ovulation.

The pre-ovulatory results showed that only the significantly decreasing tail raising latencies of untreated horse and pony mares fully supported the hypothesis that sexual receptivity increased prior to ovulation. Squatting latencies of horse and pony mares were consistant with the hypothesis, but only approached significance.

Mounting latencies of the pony stallions were used to determine if stallions perceived the ovulatory state of the mares. Only significantly decreasing mounting latencies of the pony stallions used to tease the pony mares reflected a sensitivity of the stallions to the pre-ovulatory state of the pony mares.

The results of the peri-ovulatory behavior analyses were not as unequivocal as the results of the pre-ovulatory analyses. Tail raising latencies most strongly supported the hypothesis that sexual receptivity decreased after ovulation with some qualification. Tail raising latencies of horse mares continued to decrease after ovulation, but a curvilinear trend that approached significance ($P < 0.07$) showed a decrease to the day before ovulation then an increase between days +1 and Ov. This suggested that sexual receptivity decreases very close to ovulation. In pony mares, tail raising latencies decreased significantly to day +3 then slowly began to increase until day +2 and continued to increase up to and after ovulation, suggesting that the decrease

in sexual receptivity began before ovulation. This reduction of receptivity before ovulation probably can be accounted for by the equation used for the ICR curvilinear line, which describes a symmetrical parabola and therefore the minimum may be inaccurately placed. A more precise calculation of the curve (a least squares computation), would have eliminated the intrasubject format of the data. Biologically, if the point of maximal receptivity (i.e. minimum of the curve) were correctly placed, at day +3, the optimum time for pony mares to breed is a few days prior to ovulation. Such early breeding may indicate the necessity for capacitance of the sperm within the mare before fertilization could occur. Studies on sperm capacitance have not been performed on equids, but in bovids it explains why ovulation occurs about 11 hours after estrus concludes (Section II.D.2.).

The peri-ovulatory mounting latencies of the single pony stallion used to tease the horse mares changed significantly throughout estrus. His mounting latencies continued to decrease significantly after ovulation, which suggests that this stallion did not perceive the ovulatory state of the horse mares. The variation of mounting latencies of the four pony stallions used to tease the pony mares increased after ovulation, which indicates a possible dissipation of copulatory readiness after ovulation of the pony mares. This suggests

that the four stallions were sensitive to the ovulatory state of the pony mares. Future experiments are needed to test the hypothesis that stallions can perceive the ovulatory state of the mares via behavioral and other changes.

The hypothesis that sexual receptivity increases prior to ovulation and decreases after ovulation may not apply to non-domesticated equids. The little evidence in the literature on various non-domestic equine species, including horses, indicates that mares in estrus copulate repeatedly for only 1 to 4 days (means and variances were not given) (Dubroruka, 1961; Joubert, 1972; Welsh, 1975). Ovulation cannot be detected in non-domestic equids without peril to the palpator, so we do not know when ovulation occurs in relation to the period of copulation.

The similarities in slopes, origins, profiles and latency times on a daily basis of pony and horse mares were expected because horses and ponies are only morphological variants or races of the same species. Although the behavior patterns of pony mares (i.e. tail raising and squatting) appear more subtle than the same behavior patterns in horse mares, it is only because the pony mares are smaller and their tail placement in relation to the croup differs from horses'.

2. Studies on Treated Horse Mares

The hypothesis that sexual receptivity could be temporally altered by hormonal treatments with GnRH was rejected in two ways: (1) Tail raising latencies of GnRH treatments did not differ significantly from Saline treatment during the four days prior to ovulation. (2) The slopes and origins of 5 mg GnRH once treatment and Saline treatment during the pre-ovulatory part of estrus did not differ from the pre-ovulatory slope and origin of tail raising latencies of untreated horse mares in Experiment 1. These results agreed with Oxender et al.'s (1977) conclusion that follicular maturation and ovulation were not temporally altered by hormonal treatments with GnRH.

If the treatments with GnRH had affected earlier follicular maturation and ovulation, I would have expected to find tail raising latencies of GnRH treated mares to decrease more rapidly prior to ovulation than did the mares during Saline treatments or those of untreated horse mares.

Although Experiments 3 and 4 were set up as Latin Square designs, this design was not carried out due to uncontrollable events. However, the Latin Square design would have complicated the behavioral analyses for the four days prior to ovulation. I would have preferred an experimental design for a simpler form of analysis of variance (e.g. complete block design), but

this would have involved five or six times the number of mares than were used in the experiments.

C. Problems with the Present Work

1. Observation and Data Collection

In behavioral research problems arise which the researchers must recognize and analyze for the benefit of future research in that area. In these particular studies, problems in observation and data collection could have been reduced with a larger sample size of animals, another observer and more technical equipment.

a) Sample Size

In all published experiments with animals that are expensive to procure and/or maintain, the studies have been restricted to small sample sizes of from three females (rhesus monkeys; Michael and Welegalla, 1968; Michael et al., 1966) to a maximum of twenty-three females (thick-tailed bushbabies; Eaton et al., 1973). The problem of cost in the study of equids is extreme. The horses used in Experiments 1, 3 and 4 were donated to the university, but they presently cost from $1,004.00 (on pasture) to $1,460.00 (in barn) per subject per year to maintain (Oxender, personal communication), without the added cost of experimentation. Ponies are somewhat less expensive to maintain, but Dr. Douglas rarely obtained donated animals and had to pay from $20.00 to $50.00 per

subject in addition to yearly maintenance (Douglas, personal communication). Another concern with large animals is adequate space for proper maintenance. In this respect the facilities of Michigan State University were adequate.

Personnel are expensive and at least three people were needed in addition to the observer to conduct the teasing sessions each day. The number of available personnel had to be at least twice what was needed each day. Thus, the problems of large animal research are greater than those in small laboratory species for the same sort of experimentation.

b) Data Collection, Equipment and Observers

During a teasing session equids displayed behavior patterns in rapid progression so that I had to talk very rapidly to record the behavior sequence as accurately as possible. Precision in observational recording could have been enhanced by having two observers (for interobserver reliability testing) and electromagnetic recording devices that do not rely on voice and can quickly record behavioral events as they occur.

The most accurate form of recording behavioral data is of course film or video tape because it can be viewed repeatedly after the behavioral interaction

and ultimate accuracy for frequencies, latency and duration can be obtained. Such high cost personnel and equipment were not available for this work.

Future experiments on estrous behavior should also collect data two or three times a day to pinpoint more accurately the changes in behavior around the time of ovulation.

2. Behavioral Measurements Used for Analysis

a) Frequency Data

Another problem with these studies and their experimental design was the collection and use of frequency data. The teasing sessions were of varying lengths in contrast to set times of testing periods in other experiments on rodents, primates and canids mentioned in the literature review. Also copulation was not allowed and even actively prevented by the handlers. The mares in the studies were used for successive estrous cycles thus interruption of cycling due to pregnancy would have terminated their use. Teasing sessions of equal duration with copulation as the culminating event were impossible in the experimental design. Thus the frequency of a behavior per teasing session was the measure obtained. If the data were converted to frequency per minute of a teasing session, one assumes that the behavioral

patterns were evenly distributed and of equal duration during each teasing session, which they were not. So frequency of behavior could not be statistically compared among subjects or analyzed throughout estrus.

Other research on sexual receptivity has shown that the frequency of behavior patterns of females is not valuable for discerning change of behavior over time (Eaton and Resko, 1974; Eaton et al., 1973; Scrunton and Herbert, 1970). Frequency of male behavior patterns alone or in ratio to female behaviors are reasonably valuable measures for discerning temporal change in behavior during the estrous or menstural cycle (Bullock et al., 1972; Hardy, 1972; Kuehn and Beach, 1963; Michael and Welegalla, 1968; Michael and Zumpe, 1970; Scrunton and Herbert, 1970).

b) Latency Data

The latency to a behavior response has been used primarily for male behavior patterns during sexual testing (e.g. on stallions, Pickett and Voss, 1972; Pickett et al., 1970; Wiersbowski, 1959). Male behavior latencies have shown temporal changes in response to the changes in the ovulatory state of the females during the phases of the menstrual cycle (Bullock et al., 1972) or during the estrous

period (Eaton et al., 1973). Beach (1976) refers to measures of male response as measurement of female attractivity. But to my knowledge, latency to behavioral response of females has not been used as a measurement of female sexual receptivity.

I feel that latency to behavioral response by females is a logical measurement of sexual receptivity. The readiness of a female to allow copulation during a teasing session is intrinsically a time measurement.

c) Duration of Behavior

Duration of behavior patterns, which was not measured in these studies, has proved valuable in other studies not related to sexual behavior. Kuehn and Beach (1963) did mention measuring duration of lordosis of female rats, but their measurements appeared to be correlated more with the quality of the sexual interaction (mounting only, mounting with intromission, or mounting with intromission and ejaculation) than the time during estrus. Further examination is needed to determine the value of duration measures for studies on sexual receptivity.

D. Application of Results in Comparison with Commonly Used Breeding Schemes

According to Willis (1973) most breeding farms use one of two schemes of breeding schedules for hand breeding (when stallions are bred to mares under highly controlled situations). Either mares are first bred on the third day the mare is in estrus (counted from the beginning of estrus and not to be confused with day +3 of my data arrangement) and bred the second time on the sixth day, if the mare is still in estrus (Scheme 1, Tables 24 and 25); or mares are bred on the second and fourth days of estrus and again on the seventh day, if the mare is still in estrus (Scheme 2, Tables 24 and 25).

I devised three schemes using tail raising latencies (+2 seconds) of horse and pony mares combined for days +4, +3 and +2 from Section IV.D.2.d)(2) (29.4, 14.5 and 12.3 seconds, respectively). Scheme A resembles Scheme 1 in the number of breedings per mare (i.e. two): mares are first palpated and bred on the first day that tail raising latency is at 29.4[1] seconds or less, they are bred a second time when tail raising latencies decrease to 14.5 seconds or less. Scheme B is like Scheme A: first breeding ≤29.5 seconds, second breeding ≤14.5 seconds with a third breeding on the day when tail raising latency is 12.3 seconds or less.

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[1]As was previously noted these are adjusted tail raising latencies so that the observed time would be two seconds less.

Scheme B resembles Scheme 2 in the number of breedings that are possible for each mare (i.e. three). Scheme C is like Scheme B: first breeding ≤29.5 seconds, second breeding ≤ 14.5 seconds, third breeding ≤12.3 seconds with a fourth breeding two days after the third breeding if the mare is still in estrus with a tail raising latency of 12.3 seconds or less.

Information on the onset and termination of estrus for mares in Experiments 1 and 2 along with the tail raising latencies from Tables A1 and A7, were compiled with all of the breeding schemes (Tables 24 and 25). I then calculated the number and percentage of horse and pony mares that would have been bred once or twice during days +4, +3, +2 and +1 of my data arrangement [the days that Hammond (1938) considered optimal for conception] for all the breeding schemes (Table 26).

Table 24. Breeding Schemes 1, 2, A, B and C for untreated horse mares in Experiment 1.†

Mare No.	Day of Estrus +7	+6	+5	+4	+3	+2	+1	Ov	-1	-2
1		2	1	ABC 2	ABC	1	2			--
2		--		ABC 2	1	ABC 2	BC	--	--	--
5	--	--	--	ABC	ABC 2	BC 1	2		1	--
7		2	1	ABC 2		1	2		ABC	
9	ABC	2	1	2		ABC 1	2			--
10	ABC	2	1	2		ABC 1	BC 2	--	--	--
11	--	--	--	--		ABC 2	ABC 1	2	--	--

†Mare not in estrus on day -- appears in table.

Table 25. Breeding Schemes 1, 2, A, B and C for untreated pony mares in Experiment 2.†

Mare No.	Day of Estrus +10	+9	+8	+7	+6	+5	+4	+3	+2	+1	Ov	-1	-2
12	--	--	--	--	--	--	ABC	ABC 2	BC 1	2		1	--
15	--	--	--	--	--	--	ABC	2	ABC 1	BC 2		--	--
16	--	--	--	--	--	--		ABC 2	ABC 1	2		--	--
17	--	--	--	--	--	ABC	ABC 2	1	2	BC	1	--	--
23	--	--	--	ABC	ABC 2	BC 1	2	C	1	2			--
24	--	--	--	--	--	--	--	ABC	2	1	2	--	--
25	--	--	--	--	--	ABC	ABC 2	BC 1	2		1	--	--
26	--	--	--	ABC	2	ABC 1	BC 2		C 1	2		--	--
34	--	--	--	--	--	--	--	--	ABC	ABC 2	--	--	--
35	--	--	--	--	--	--	ABC	ABC 2	BC 1	2		1	2
36	ABC	2	1	ABC 2	BC	1	C 2						--
466	--	--	--	ABC	ABC 2	BC 1	2	C	1	2			--
725	--	--	--	--	ABC	2	ABC 1	BC 2		C 1	2		--
757	--	--	--	--	--	ABC	2	1	ABC 2	BC	1	--	--
770	--	--	ABC	2	1	2		1	ABC 2	BC		--	--

†Mare not in estrus on day -- appears in table

Table 26. Number and percent of mares bred once or twice during days +4, +3, +2 and +1 using breeding schemes 1, 2, A, B and C.

Scheme	Bred Once		Bred Twice	
	Horse	Pony	Horse	Pony
1	7 (100%)	13 (86.6%)	0 (0%)	1 (6.6%)
2	7 (100%)	15 (100%)	6 (85.7%)	10 (66.6%)
A	7 (100%)	11 (73%)	4 (56%)	5 (33%)
B	7 (100%)	12 (80%)	5 (71%)	10 (66.6%)
C	7 (100%)	15 (100%)	5 (71%)	11 (73%)

Scheme 2 appears more efficient than Scheme 1, if more than one breeding is needed on the optimal days for conception (Table 26). Scheme A appears to be an improvement over Scheme 1 in the number and percentage of mares bred twice during the optimal days (Table 26). Scheme B appears to be not as efficient as Scheme 2 in the number and percentage of pony mares bred once and horse and pony mares bred twice during the optimal days (Table 26). Scheme C appears comparable to Scheme 2 for pony mares, but less efficient for the number and percentage of horse mares bred twice on the optimal days (Table 26). Scheme C offers the advantage that 5 of the horse mares and 9 of the pony mares would have been bred on two successive days during days +4 to +1, whereas Scheme 2 does not allow for mares to be bred on successive days. However, the total number of breedings for horse and pony mares using Scheme C is 17 and 46, respectively;

whereas Scheme 2 breeds a total of 18 times for horse mares and 36 times for pony mares, a decrease of 9 breedings for the stallion(s) which may be a more economical use of the stallion(s). If breedings outside the optimal period (days +4 to +1) are considered wasted, then 16 or 29.6% of all the breedings for Scheme 2 and 19 or 30% of all the breedings for Scheme C would be wasted, amounts that are about statistically equivalent.

All of the schemes require that a daily log be kept on the mares, which is normally done on breeding farms. The schemes that I devised (A, B and C) also require a teasing set up that would permit tail raising latencies to be timed and recorded. I feel that more work is needed on a larger sample size of mares of uniform breeds which are bred, in order to evaluate the efficacy of Scheme C versus Scheme 2 and vice versa. I do feel that tail raising latencies might be employed by breeders as a guide for palpation. Instead of palpating mares on the first day that estrous behavior is displayed to the teasing stallion, palpation could begin on the day that tail raising latency decreases to day +4 (e.g. 29.5 seconds) or day +3 (e.g. 14.5 seconds) levels. This would reduce the time the veterinarian or technician spent in palpating many estrous mares each day.

E. Future Research

Correlations between ovulation, plasma hormone concentrations and estrous behavior have not been investigated in

any species, to my knowledge, thus far. Although Eaton et al. (1973) did not correlate the data on estradiol concentrations in female thick-tailed bushbabies with the latencies to intromission by the males, the graphic presentation of the data suggest that they may be associated temporally. The equine presents an ideal model for investigation because ovulation is easily detected via palpation, blood samples in 10 cc or greater volumes can be collected frequently and this dissertation presented a framework for temporal analysis of estrous behavior.

An extension of the present studies on normally cycling domestic mares could precisely establish the relationships of: (1) plasma hormone concentrations (estrogens, LH and androgens) with ovulation and (2) behavioral measures with plasma hormone concentrations throughout estrus in relation to ovulation. A sample size of 50 mares and 6 or more sexually vigorous stallions would be needed. Mares would be teased, palpated and blood collected at least twice, optimally three times daily to determine as precisely as possible when ovulation occurs. More than one estrous cycle should be examined. In addition to the information gathered and relationships tested, the results of such a study may find that individual variation, e.g. in tail raising latencies, correlate inversely with plasma levels of testosterone and other androgens. This is speculation, but it may explain the consistent low tail raising latencies of pony mare #466 (a behaviorally dominant mare) throughout

estrus, compared with the tail raising latencies of other pony mares (Table A7).

In another study, feral conditions could be simulated by establishing herds of domestic mares and stallions on pasturage. The mares could be brought in from pasture daily for palpation and blood collection. This design would be an attempt to mimic feral, free-ranging or pasture breeding social conditions with the advantage of monitoring estrus, ovulation and plasma hormone concentrations in the mares. Behavioral observation of the pastured subjects would provide information on the periods of estrous behavior and copulation which could then be correlated with ovulation time. If the herds were maintained for several years under the experimental conditions, the social organization and behavior of the subjects may approach that observed under feral conditions (e.g. Sable Island, Prior Mountain Horse Range, Assateague Island and the New Forest) with the added benefit of being able to monitor ovulation and plasma hormone levels. If an experiment on domestic mares (i.e. sexually segregated, yet bred when teased) were conducted along with the experiment on the pastured subjects (after the herds were well established), the hypothesis that mares under highly controlled breeding conditions have a lower threshold of behavioral response to estrogen plasma levels and thus exhibit a longer duration of estrus could be tested.

Each one of these proposed studies would further expand our knowledge about reproduction, with the future goal of

increasing conception rate hence, reproductive success, in domestic equids.

VI. SUMMARY

The hypothesis that sexual receptivity in mares increases prior to ovulation and decreases after ovulation was tested in two parts for untreated horse and pony mares in Experiments 1 and 2. Pre-ovulatory tail raising latencies decreased significantly for both horse and pony mares before ovulation supporting the hypothesis that sexual receptivity increased prior to ovulation. Pre-ovulatory squatting latencies decreased prior to ovulation for both mare groups, approached significance and further supported the same hypothesis.

Peri-ovulatory tail raising latencies of horse mares decreased linearly, but also showed a curve that began to increase around the time of ovulation (between days +1 and Ov). The curve approached significance and generally supported the hypothesis that sexual receptivity in horse mares decreased after ovulation. Peri-ovulatory tail raising latencies of pony mares began to increase before ovulation (between days +3 and +2) supporting the hypothesis that sexual receptivity decreased after ovulation with the qualification that the decrease began before ovulation. The possible necessity for sperm capacitance in equids is discussed as the factor that could be responsible for the

decrease in sexual receptivity before ovulation. The mean tail raising latencies on the four days prior to and on the day of ovulation were similar in horse and pony mares.

The pony stallions used to tease the pony mares showed significant decreasing mounting latencies prior to ovulation, but not throughout estrus. These results suggested that stallions perceived the ovulatory state of mares and responded accordingly in their readiness to copulate. The pony stallion used to tease the horse mares did not show sensitivity to the ovulatory state throughout estrus.

The hypothesis that sexual receptivity of horse mares could be temporally altered by hormonal treatments with GnRH was rejected for the four days prior to ovulation and for the pre-ovulatory part of estrus. These results agree with the conclusion of Oxender et al. (1977) that follicular maturation and ovulation could not be temporally altered by hormonal treatments with GnRH.

Despite the limitations of sample size and experimental procedures in large, expensive animals, this research demonstrated changes in sexual receptivity relative to ovulation. Future work could correlate plasma hormone concentrations and measurements of behavior with ovulation. This would further expand our knowledge about reproduction, and is consistent with the goal of increasing conception hence, reproductive success, in domestic equids.

APPENDIX A

ADJUSTED LATENCY DATA WITH ESTIMATED VALUES FOR MISSING DATA AND FREQUENCY DATA

Table A1. Tail raising latencies +2 seconds on all estrus days and date of ovulation for untreated horse mares in Experiment 1.

Mare No.	Date Ovulated	Days of Estrus in Relation to Ovulation (Ov)										
		+8	+7	+6	+5	+4	+3	+2	+1	Ov	-1	-2
1	5/22/75		120.2	31.3	33.8	18.3	12.7	13.6	12.4	18.7	23.2	
2	5/15/75				129.5	16.3	22.3	11.0	12.0			
5	5/12/75					26.5	12.8	7.7	10.0	12.7	39.3	
7	5/20/75		45.1	35.3	52.7	19.0	16.2	25.0	32.7	17.0	11.8	46.2
9	5/21/75		25.9	. .	108.2	79.8	26.7	12.4	39.1	13.7	31.3	
10	5/20/75		18.2	26.5	22.4	15.6	15.2	13.1	12.2			
11	6/15/75						34.4	9.7	9.5	12.7		

Table A2. Urination latencies +2 seconds on all estrus days and date of ovulation for untreated horse mares in Experiment 1.

Mare No.	Date Ovulated	Days of Estrus in Relation to Ovulation (Ov)									
		+7	+6	+5	+4	+3	+2	+1	Ov	-1	-2
1	5/22/75	125.9	. .	46.2	27.8	14.4	15.7	17.0	20.9	34.7	
2	5/15/75			. .	18.6	24.4	13.0	13.8	. .		
5	5/12/75				. .	17.9	14.1	11.5	14.3	. .	
7	5/20/75	. .	48.1	62.3	. .	57.6	71.5	48.2	75.7	13.5	. .
9	5/21/75	28.8	. .	. .	84.7	. .	. .	60.4	15.7	72.9	
10	5/20/75	31.0	29.9	28.4	21.3	18.1	30.7	25.6	. .		
11	6/15/75					. .	10.8	40.0	27.6	24.8	

Table A3. Squatting latencies +2 seconds on all estrus days and date of ovulation for untreated horse mares in Experiment 1.

Mare No.	Date Ovulated	Days of Estrus in Relation to Ovulation (Ov)									
		+7	+6	+5	+4	+3	+2	+1	Ov	-1	-2
1	5/22/75	124.7	37.8	. .	. .	13.6	14.9	26.2	. .	. .	
2	5/15/75			. .	19.5	23.3	12.6	12.1	. .		
5	5/12/75				. .	17.5	9.8	10.5	15.1	. .	
7	5/20/75	. .	114.0	. .	. .	57.1	110.5	. .	73.6	. .	
9	5/21/75	. .	. .	. .	. .	. .	. .	63.9	23.8	76.9	
10	5/20/75	30.1	29.0	. .	83.1	59.3	. .	24.5	. .		
11	6/15/75					. .	10.3	39.1	14.7	. .	

Table A4. Mounting latencies +2 seconds on all estrus days and date of ovulation for untreated horse mares in Experiment 1.

Mare No.	Date Ovulated	Days of Estrus in Relation to Ovulation (Ov)									
		+7	+6	+5	+4	+3	+2	+1	Ov	-1	-2
1	5/22/75	109.0	. .	. .	29.3	29.7	34.3	29.5	63.9	67.5	
2	5/15/75			. .	. .	. .	. .	. .	. .		
5	5/12/75				49.5	61.0	36.3	22.6	37.5	65.9	
7	5/20/75	. .	. .	96.9	. .	72.7	145.8	101.7	88.6	33.9	62.2
9	5/21/75	. .	. .	111.3	106.7	37.4	52.1	75.0	41.7		
10	5/20/75	23.3	. .	37.9	79.4	101.0	39.0	63.4	. .		
11	6/15/75					15.4	78.0	78.7	23.7	21.5	

Table A5. Tail raising latencies +2 seconds on all estrus days, date of ovulation and treatments for treated horse mares which completed Experiment 3.†

Mare No.	Date Ovulated	Treatment	Days of Estrus in Relation to Ovulation (Ov)										
			+8	+7	+6	+5	+4	+3	+2	+1	Ov	-1	-2
1	6/5/75	Saline					21.2	16.2	13.3	15.6	14.4	26.7	
1	6/21/75	1mg GnRH			29.7	25.05	16.9	13.15	9.45	17.55	22.25		
1	7/6/75	2mg GnRH					17.4	16.65	21.15	13.0	13.0		
1	7/18/75	5mg GnRH						32.0	17.9	12.95			
5	6/12/75	5mg GnRH				13.55	11.8	5.4	9.0	11.2	6.0		
5	6/27/75	2mg GnRH			88.9	. .	t.b.	15.05	2.0	9.0	12.15		
5	7/14/75	1mg GnRH				15.4	6.8	8.1	11.2	14.0	18.0		
5	8/2/75	Saline	50.3	12.65	14.4	9.5	6.3	5.9	13.0	6.5	7.35		
9	6/1/75	1mg GnRH						19.8	36.1	10.7	5.25		
9	6/17/75	5mg GnRH				12.4	22.8	16.7	14.8	2.0	14.3	55.9	
9	7/3/75	Saline				50.5	75.15	20.5	22.05	14.6	22.8	31.8	
9	7/17/75	2mg GnRH						53.4	26.0	31.1	27.3	64.1	
11	6/29/75	2mg GnRH							31.3	. .	20.85		
11	7/13/75	Saline							24.45	20.05	11.25	9.50	
11	7/30/75	1mg GnRH						82.0	. .	31.0	. .		

†t.b. = tape blank--data not recorded.

Table A6. Tail raising latencies +2 seconds on all estrus days, date of ovulation and treatments for horse mares in experiment 4.†

Mare No.	Date Ovulated	Treatment	Days of Estrus in Relation to Ovulation (Ov)									
			+7	+6	+5	+4	+3	+2	+1	Ov	-1	-2
2	5/28/75	5mg GnRH daily				8.3	6.1	4.0	2.0	7.0		
2	7/13/75	Saline			19.25	8.6	7.1	8.2	2.0	2.0	23.0	
2	7/20/75	5mg GnRH 1x			14.20	7.9	2.0	2.0	4.5	5.6		
3	6/09/75	5mg GnRH 1x						7.85	9.25	19.75		
3	6/24/75	5mg GnRH daily				60.2	41.15	30.65	t.b.	17.25	12.75	
3	7/09/75	Saline				28.7	5.6	9.4	9.1	9.8	15.4	
4	6/25/75	Saline				42.3	16.0	t.b.	12.35	11.25		
4	7/13/75	5mg GnRH daily			27.0	12.0	10.95	12.0	14.05	38.6		
4	7/30/75	5mg GnRH 1x	40.8	30.4	19.75	8.5	9.25	8.0	14.25	12.1		
6	5/18/75	5mg GnRH 1x					7.8	7.8	3.0	11.2	9.0	
6	6/02/75	5mg GnRH daily					19.1	2.0	7.4	8.1	7.8	15.3
6	6/20/75	Saline		16.65	10.2	7.6	7.6	2.0	4.0	9.8	14.75	
8	5/29/75	Saline			4.2	7.0	7.4	2.0	2.0	2.0	17.1	
8	6/16/75	5mg GnRH 1x			45.4	11.0	11.8	13.8	2.0	10.0		
8	6/30/75	5mg GnRH daily					12.35	23.9	14.4	13.4	14.0	
10	6/04/75	5mg GnRH 1x					16.4	16.9	2.0	22.9	20.0	12.3
10	6/23/75	Saline				16.4	11.45	14.4	14.8	t.b.		
10	7/08/75	5mg GnRH daily					105.0	. .	. .	. .		

†t.b. = tape blank--data not recorded.

Table A7. Tail raising latencies +2 seconds on all estrus days and date of ovulation for untreated pony mares in Experiment 2.

Mare No.	Date Ovulated	Days of Estrus in Relation to Ovulation (Ov)												
		+10	+9	+8	+7	+6	+5	+4	+3	+2	+1	Ov	-1	-2
12	6/13/76							28.5	10.4	11.5	13.0	13.9	46.9	
15	6/29/76							9.3	. .	5.9	11.1	5.7		
16	6/02/76							52.0	12.9	14.5	13.3	19.2		
17	6/28/76						17.0	11.5	12.5	13.4	2.0	65.1		
23	5/30/76				23.2	13.9	8.7	78.4	9.8	27.8	36.5	23.3	21.5	
24	5/18/76								18.9	57.5	65.2	116.0		
25	6/06/76						18.5	6.3	2.0	2.0	2.0	11.8		
26	5/28/76				6.7	36.0	12.3	6.8	5.5	9.4	7.1	13.2		
34	6/26/76									10.3	7.5			
35	5/14/76							9.6	6.4	8.5	5.4	7.9	16.0	38.0
36	5/26/76	29.0	47.7	109.6	7.0	11.4	5.9	6.1	6.3	2.0	2.0	24.7	33.0	
466	5/15/76				6.3	5.9	5.0	5.3	5.8	5.6	5.2	5.4	6.9	
725	5/16/76					24.6	16.0	11.0	6.4	5.2	5.4	5.8	12.8	
757	5/14/76						5.9	19.2	50.2	9.6	9.3	15.5		
770	5/16/76			24.0	16.3	31.8	. .	39.2	15.5	9.9	6.2	34.0		

Table A8. Urination latencies +2 seconds on all estrus days and date of ovulation for untreated pony mares in Experiment 2.

Mare No.	Date Ovulation	Days of Estrus in Relation to Ovulation (Ov)								
		+7	+6	+5	+4	+3	+2	+1	Ov	-1
12	6/13/76				45.0	11.6	19.8	. .	16.5	
15	6/29/76				10.9	. .	23.1	67.4	11.7	
16	6/02/76				55.3	14.1	15.5	20.6	. .	. .
17	6/28/76			22.6	14.0	19.2	16.4	. .	. .	
23	5/30/76	43.8	41.9	16.6	80.2	16.2	28.2	38.8	26.4	23.8
24	5/18/76					20.0	61.6	68.0	. .	
25	6/06/76			. .	15.3	34.5	45.4	24.2	13.5	
26	5/28/76	89.7	41.7	13.6	8.1	7.5	13.3	13.6	22.0	
34	6/26/76						11.8	9.0		
35	5/14/76				10.9	9.1	12.3	9.5	10.0	23.8
36	5/26/76	29.3	49.6	. .	22.3	58.4	. .	. .	35.0	
466	5/15/76	7.1	7.1	11.5	6.2	10.2	7.8	7.3	7.4	11.5
725	5/16/76		37.6	18.5	12.6	11.5	6.8	7.9	7.7	14.3
757	5/14/76			8.3	20.8	54.8	30.3	11.8	186.5	
770	5/16/76	29.5	33.9	. .	. .	23.6	. .	29.4	36.6	

Table A9. Squatting latencies +2 seconds on all estrus days and date of ovulation for untreated pony mares in Experiment 2.

Mare No.	Date Ovulated	Days of Estrus in Relation to Ovulation (Ov)												
		+10	+9	+8	+7	+6	+5	+4	+3	+2	+1	Ov	-1	-2
12	6/13/76							68.7	11.0	13.0	. .	. .	. .	
15	6/29/76							9.3	. .	22.5	55.0	7.0		
16	6/02/76							. .	. .	. .	20.6	. .	. .	
17	6/28/76						43.0	36.0	14.5	26.8	. .	. .		
23	5/30/76				43.3	40.4	20.1	. .	16.4	. .	37.3	. .	39.3	
24	5/18/76								20.7	67.0	73.5	. .		
25	6/06/76						. .	15.1	. .	101.8	. .	12.6		
26	5/28/76				. .	55.0	. .	17.6	10.1	. .	13.3	. .		
34	6/26/76									74.9	8.0			
35	5/14/76							10.1	7.4	10.6	8.1	11.7	24.3	
36	5/26/76	. .	58.1	. .	13.3	46.1	14.2	11.3	14.9	17.5	14.2	34.5	. .	
466	5/15/76				8.3	6.3	13.0	10.9	9.5	7.1	6.1	8.6	. .	
725	5/16/76					34.5	17.5	12.3	10.9	6.3	7.0	7.2	14.3	
757	5/14/76						6.7	22.2	. .	11.1	10.2	16.7		
770	5/16/76			. .	. .	32.5	. .	40.5	. .	. .	. .	. .		

Table A10. Mounting latencies +2 seconds on all estrus days and date of ovulation for untreated pony mares in Experiment 2.

Mare No.	Date Ovulated	Days of Estrus in Relation to Ovulation (Ov)												
		+10	+9	+8	+7	+6	+5	+4	+3	+2	+1	Ov	-1	-2
12	5/13/76							38.3	61.4	136.2	. .	32.5	59.9	
15	6/29/76							. .	. .	113.1	84.3	72.5		
16	6/02/76							113.5	51.9	19.4	74.6	. .	128.8	
17	6/28/76						72.8	42.9	90.7	57.6	. .	67.3		
23	5/30/76				129.3	. .	183.7	108.1	24.2	29.7	101.9	32.7	96.3	
24	5/18/76								. .	129.9	166.1	112.4		
25	6/06/76						24.7	71.5	41.0	120.0	24.7	29.0		
26	5/28/76				112.2	68.2	27.5	32.6	49.3	67.7	18.7	22.2		
34	6/26/76									81.4	63.5			
35	5/14/76							17.6	32.7	18.8	45.9	39.8	43.5	36.8
36	5/26/76	41.0	71.7	108.5	. .	84.2	45.4	58.5	103.4	21.0	38.4	36.7	28.3	
466	5/15/76				48.6	22.6	51.5	27.6	23.6	16.2	39.0	17.6	. .	
725	5/16/76					. .	46.9	19.9	43.3	20.2	. .	13.0	25.6	
757	5/14/76						39.0	30.7	. .	41.2	36.1	193.7		
770	5/16/76			28.1	34.1	46.7	. .	43.5	19.4	17.5	28.3	37.5		

Table A11. Tail raising (t↑), urination (ur.), squatting (sqt.) and mounting (mt.) latencies (±? seconds) with estimated values of missing data (*) for days +4, +3, +2 and +1 for untreated horse mares in Experiment 1.†

Mare No. 1 Day	t↑	ur.	sqt.	mt.
+4	18.3	27.8	23.13*	29.3
+3	12.7	16.4	13.6	29.7
+2	13.6	15.7	14.9	34.3
+1	12.4	17.8	26.2	29.5

Mare No. 2 Day	t↑	ur.	sqt.	mt.
+4	16.3	18.6	19.5	. .
+3	22.3	24.4	13.3	. .
+2	11.0	13.0	12.6	. .
+1	12.0	13.8	12.1	. .

Mare No. 5 Day	t↑	ur.	sqt.	mt.
+4	26.5	27.43*	25.53*	49.5
+3	12.8	17.9	17.5	61.0
+2	7.7	14.1	9.8	36.3
+1	10.0	11.5	10.5	22.6

Mare No. 7 Day	t↑	ur.	sqt.	mt.
+4	19.0	53.47*	76.89*	100.9*
+3	16.2	57.6	57.1	72.1
+2	25.0	71.5	110.5	145.8
+1	32.7	48.2	79.97*	101.7

Mare No. 9 Day	t↑	ur.	sqt.	mt.
+4	79.8	84.7	96.13*	106.7
+3	26.7	29.45*	36.92*	37.4
+2	12.4	29.65*	37.12*	52.1
+1	39.1	60.4	63.9	75.0

Mare No. 10 Day	t↑	ur.	sqt.	mt.
+4	15.6	21.3	83.1	79.4
+3	15.2	18.1	59.8	101.0
+2	13.1	30.7	44.31*	39.0
+1	12.2	25.6	24.5	63.4

Mare No. 11 Day	t↑	ur.	sqt.	mt.
+4	. .	. .	. .	. .
+3	34.4	. .	. .	15.4
+2	9.7	10.8	10.3	78.0
+1	9.5	40.0	39.1	78.7

†Mare nos. 2 and 11 had too many missing cells causing the simultaneous equations to be algorithmically singular i.e. without missing solutions.

Table A12. Tail raising latencies +2 seconds of treated horse mares on days +4, +3, +2 and +1 in Experiment 3 and estimated values of missing data (*).

Mare No. 1 trt.	Days +4	+3	+2	+1	Mare No. 2 trt.	Days +4	+3	+2	+1
saline	21.20	16.20	13.30	15.60	saline	6.30	5.90	13.00	6.50
1 mg GnRH	16.90	13.15	9.45	17.55	1 mg GnRH	6.80	8.10	11.20	14.00
2 mg GnRH	17.40	16.65	21.15	13.00	2 mg GnRH	7.62*	15.05	2.00	9.00
5 mg GnRH	24.33*	32.00	17.90	12.95	5 mg GnRH	11.80	5.40	9.00	11.20
Mare No. 9	**Days**				**Mare No. 11**	**Days**			
trt.	**+4**	**+3**	**+2**	**+1**	**trt.**	**+4**	**+3**	**+2**	**+1**
saline	75.15	20.50	22.05	14.60	saline	. .	. .	24.45	20.05
1 mg GnRH	56.06*	19.80	36.10	10.70	1 mg GnRH	. .	82.00	. .	31.00
2 mg GnRH	70.70*	53.40	26.00	31.10	2 mg GnRH	. .	. .	31.30	. .
5 mg GnRH	22.80	16.70	14.80	2.00	5 mg GnRH	. .	. .	. .	. .

Table A13. Tail raising latencies +2 seconds of treated horse mares on days +4, +3, +2 and +1 in Experiment 4 and estimated values of missing data (*).

Mare No. 6 trt.	Days +4	+3	+2	+1
saline	7.60	7.60	2.00	4.00
5 mg GnRH 1x	9.26*	7.80	7.80	3.00
5 mg GnRH daily	12.56*	19.10	2.00	7.40

Mare No. 8 trt.	Days +4	+3	+2	+1
saline	7.00	7.40	2.00	2.00
5 mg GnRH 1x	11.00	11.80	13.80	2.00
5 mg GnRH daily	19.38*	12.35	23.90	14.40

Mare No. 10 trt.	Days +4	+3	+2	+1
saline	16.40	11.45	14.40	14.80
5 mg GnRH 1x	14.62*	16.40	16.90	2.00
5 mg GnRH daily	106.58*	105.00	106.725*	99.425*

Mare No. 2 trt.	Days +4	+3	+2	+1
saline	8.60	7.10	8.20	2.00
5 mg GnRH 1x	7.90	2.00	2.00	4.50
5 mg GnRH daily	8.30	6.10	4.00	2.00

Mare No. 3 trt.	Days +4	+3	+2	+1
saline	28.70	5.60	9.40	9.10
5 mg GnRH 1x	31.30*	10.22*	7.85	9.25
5 mg GnRH daily	60.20	41.15	30.65	37.66*

Mare No. 4 trt.	Days +4	+3	+2	+1
saline	42.30	16.00	22.05*	12.35
5 mg GnRH 1x	8.50	9.25	8.00	14.25
5 mg GnRH daily	12.00	10.95	12.00	14.05

APPENDIX B

STATISTICAL ANALYSES

Table A14. Tail raising (t↑), urination (ur.), squatting (sqt.) and mounting (mt.) latencies (+2 seconds) with estimated values of missing data (#) on days +4, +3, +2 and +1 for untreated pony mares in Experiment 2.†

Mare No. 12				
Day	t↑	ur.	sqt.	mt.
+4	28.5	45.0	68.7	38.3
+3	10.4	11.6	11.0	61.4
+2	11.5	19.8	13.0	136.2
+1	13.0	21.66#	27.1#	74.83#

Mare No. 16				
Day	t↑	ur.	sqt.	mt.
+4	52.0	55.3	58.1#	113.5
+3	12.9	14.1	10.73#	51.9
+2	14.5	15.5	0.9#	19.4
+1	13.3	20.6	20.6	74.6

Mare No. 17				
Day	t↑	ur.	sqt.	mt.
+4	11.5	14.0	36.0	42.9
+3	12.5	19.2	14.5	90.7
+2	13.4	16.4	26.8	57.6
+1	2.0	6.06#	15.3#	53.26#

Mare No. 23				
Day	t↑	ur.	sqt.	mt.
+4	78.4	80.2	77.84#	108.1
+3	9.8	16.2	16.4	24.2
+2	27.8	28.2	17.52#	29.7
+1	36.5	38.8	37.3	101.9

Mare No. 25				
Day	t↑	ur.	sqt.	mt.
+4	6.3	15.3	15.1	71.5
+3	2.0	34.5	31.86#	41.0
+2	2.0	45.4	101.8	120.0
+1	2.0	24.2	23.0#	24.7

Mare No. 26				
Day	t↑	ur.	sqt.	mt.
+4	6.8	8.1	17.6	32.6
+3	5.5	7.5	10.1	49.3
+2	9.4	13.3	27.22#	67.7
+1	7.1	13.6	13.3	18.7

Mare No. 35 (No Missing Cells)				
Day	t↑	ur.	sqt.	mt.
+4	9.6	10.9	10.1	17.6
+3	6.4	9.1	7.4	32.7
+2	8.5	12.3	10.6	18.8
+1	5.4	9.5	8.1	45.9

Mare No. 36				
Day	t↑	ur.	sqt.	mt.
+4	6.1	22.3	11.3	58.5
+3	6.3	58.4	14.9	103.4
+2	2.0	20.43#	17.5	21.0
+1	2.0	25.13#	14.2	38.4

Mare No. 466 (No Missing Cells)				
Day	t↑	ur.	sqt.	mt.
+4	5.3	6.2	10.9	27.6
+3	5.8	10.2	9.5	23.6
+2	5.6	7.8	7.1	16.2
+1	5.2	7.3	6.1	39.0

Mare No. 725				
Day	t↑	ur.	sqt.	mt.
+4	11.0	12.6	12.3	19.9
+3	6.4	11.5	10.9	43.3
+2	5.2	6.8	6.3	20.2
+1	5.4	7.9	7.0	25.34#

Mare No. 757				
Day	t↑	ur.	sqt.	mt.
+4	19.2	20.8	22.2	30.7
+3	50.2	54.8	50.17#	71.66#
+2	9.6	30.3	11.1	41.2
+1	9.3	11.8	10.2	36.1

Mare No. 770				
Day	t↑	ur.	sqt.	mt.
+4	39.2	50.5#	40.5	43.5
+3	15.5	23.6	15.6#	19.4
+2	9.9	22.85#	12.85#	17.5
+1	6.2	29.4	17.4#	28.3

Mare No. 15				
Day	t↑	ur.	sqt.	mt.
+4	9.3	10.9	9.3	. .
+3	. .	. .	. .	. .
+2	5.9	23.1	22.5	113.1
+1	11.1	67.4	55.0	84.3

Mare No. 24				
Day	t↑	ur.	sqt.	mt.
+4	. .	. .	. .	. .
+3	18.9	20.0	20.7	. .
+2	57.5	61.6	67.0	129.9
+1	65.2	68.0	73.5	166.1

Mare No. 35				
Day	t↑	ur.	sqt.	mt.
+4	. .	. .	. .	. .
+3	. .	. .	. .	. .
+2	10.3	11.8	74.9	81.4
+1	7.5	9.0	8.0	63.5

†Mare nos. 15, 24, and 34 had too many missing cells causing simultaneous equations to be algorithmically singular i.e. without unique solutions.

Table A15. Means and standard deviations of the frequencies of behavior patterns displayed by mares (per mare per day of estrus) - Experiment 1

Behavior Pattern	Days of Estrus +7		+6		+5		+4		+3		+2		+1		Ov		-1		-2	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Social Behaviors																				
Naso-Nasal	1.0	±0.82	0.2	±0.50	0.8	±0.45	0.3	±0.52	0.7	±0.49	0.3	±0.49	0.4	±0.53	0.2	±0.45	0.0	±0.00	0.0	±0.00
Whinney	0.2	±0.50	0.5	±0.58	0.6	±0.55	0.8	±0.41	0.3	±0.49	0.3	±0.75	0.1	±0.34	0.0	±0.00	0.0	±0.00	1.0	±0.00
Investigatory Behaviors																				
Smells Stallion	0.0	±0.00	0.5	±0.00	0.6	±0.55	0.6	±0.85	0.0	±0.00	0.1	±0.39	0.3	±0.49	0.2	±0.45	0.6	±0.89	1.0	±0.00
Gender Behaviors																				
Tail Raising	1.0	±0.00	0.7	±0.50	1.0	±0.00	1.3	±0.52	1.0	±0.00	1.3	±0.49	1.3	±0.49	1.2	±0.45	1.0	±0.71	1.0	±0.00
Squatting	0.5	±0.58	0.7	±0.96	0.0	±0.00	0.5	±0.84	1.1	±0.90	1.3	±1.11	1.3	±0.95	0.8	±0.45	0.2	±0.45	0.0	±0.00
Urination	0.7	±0.50	1.0	±1.15	0.8	±0.84	1.2	±0.98	1.0	±0.82	1.6	±1.13	1.3	±0.49	1.6	±0.89	1.0	±0.71	0.0	±0.00
Winking	1.7	±0.96	1.2	±0.96	1.8	±2.05	0.8	±0.41	1.4	±0.79	1.4	±1.27	1.4	±1.27	1.0	±1.22	0.8	±0.45	1.0	±0.00
Stand for Mount	0.0	±0.00	0.0	±0.00	0.8	±0.84	0.5	±0.55	0.8	±0.38	0.8	±0.38	0.8	±0.38	1.0	±0.00	0.6	±0.55	1.0	±0.00
Not Stand for Mount	0.2	±0.50	0.0	±0.00	0.2	±0.45	0.0	±0.00	0.0	±0.00	0.0	±0.00	0.0	±0.00	0.0	±0.00	0.2	±0.45	0.0	±0.00
Withdrawl																				
Withdrawl	0.0	±0.00	0.0	±0.00	0.2	±0.45	0.2	±0.45	0.1	±0.38	0.1	±0.38	0.1	±0.38	0.4	±0.89	0.0	±0.00	0.0	±0.00
Aggression																				
Kick	1.0	±0.82	0.5	±0.58	0.4	±0.55	0.3	±0.52	0.1	±0.38	0.0	±0.00	0.0	±0.00	0.0	±0.00	0.2	±0.45	0.0	±0.00
Squeal	1.2	±0.96	1.2	±0.96	2.0	±2.55	0.5	±0.84	0.4	±0.79	0.8	±1.21	1.0	±1.15	0.0	±0.00	0.6	±0.55	0.0	±0.00
Ears Back	2.0	±2.16	3.5	±1.73	3.2	±1.64	2.3	±2.50	1.8	±2.27	2.0	±1.41	2.1	±1.77	0.8	±0.84	1.0	±1.22	1.0	±0.00
Number of Mares/Day	4		4		5		6		7		7		7		5		5		1	

Table A16. Means and standard deviations of the frequencies of behavior patterns displayed by the stallion (per mare per day of estrus) - Experiment 1.

Behavior Pattern	Day of Estrus +7	+6	+5	+4	+3	+2	+1	Ov	-1	-2
	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD
Social Behaviors										
Naso-Nasal	0.7±0.50	0.2±0.50	0.8±0.45	0.3±0.52	0.7±0.49	0.4±0.53	0.6±0.53	0.2±0.45	0.0±0.00	0.0±0.00
Whinney	1.2±0.96	0.2±0.50	0.2±0.45	1.0±0.63	1.4±0.53	0.3±0.76	1.1±0.69	1.2±0.45	1.0±0.71	1.0±0.00
Investigation										
Smell Fore Body	1.0±1.50	1.0±0.08	1.0±1.00	1.0±0.89	0.8±1.21	0.8±0.90	1.8±1.21	1.0±0.71	0.8±0.84	1.0±0.00
Smell Rear Body	2.7±1.89	2.0±1.41	2.2±1.48	2.5±1.38	2.4±1.72	2.0±2.24	1.8±1.34	0.6±0.55	1.4±1.67	1.0±0.00
Total Smelling	3.7±2.63	3.0±0.82	2.8±2.17	3.5±2.07	3.3±1.25	2.8±1.57	3.7±1.89	1.6±1.14	2.2±1.90	2.0±0.00
Lick Fore Body	0.3±0.50	0.5±0.58	1.4±1.14	1.0±1.26	0.4±0.53	0.1±0.38	0.4±0.79	1.2±1.78	0.2±0.45	1.0±0.00
Lick Rear Body	2.0±1.83	2.3±2.06	3.6±2.88	2.8±3.82	1.4±2.15	3.1±3.84	1.6±2.70	1.6±1.52	3.8±3.27	2.0±0.00
Total Licking	2.3±2.22	2.7±1.71	5.0±3.08	3.8±3.97	1.7±2.21	3.2±3.73	2.0±2.52	2.8±3.11	4.0±3.24	3.0±0.00
Gender Behaviors										
Snort	1.0±0.82	0.7±0.50	0.8±0.84	1.0±1.09	1.3±1.11	1.4±1.51	1.6±1.40	1.3±1.14	0.8±0.84	0.0±0.00
Erection (Almost to full)	0.7±0.96	0.2±0.50	0.6±0.55	0.7±0.52	0.7±0.49	1.0±0.58	0.8±0.38	1.0±0.00	1.0±0.00	0.0±0.00
Mount with Erection (Half to full)	0.0±0.00	0.0±0.00	0.6±0.55	0.5±0.55	0.7±0.49	0.9±0.38	0.9±0.38	0.0±0.00	0.6±0.55	0.0±0.00
Flehmen	0.7±0.50	1.7±1.50	1.2±1.30	1.5±1.05	1.3±1.60	1.1±1.46	1.7±1.25	1.0±1.00	0.6±0.89	2.0±0.00
Aggression										
Squeal	0.5±0.58	0.7±0.96	0.2±0.48	0.0±0.00	0.3±0.49	0.1±0.38	0.3±0.49	0.0±0.00	0.2±0.45	1.0±0.00
Number of Mares/Day	4	4	5	6	7	7	7	5	5	1

Table A17. Means and standard deviations of the frequencies of behavior patterns displayed by mares (per mare per day of estrus) - Experiment 2.

Behavior Pattern	Days of Estrus +10	+9	+9	+7	+6	+5	+4	+3	+2	+1	Ov	-1	-2
	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD
Social Behaviors													
Naso-Nasal	1.0±0.00	3.0±0.00	1.5±0.71	1.2±0.45	0.8±0.75	0.9±0.33	0.2±0.44	0.6±0.75	0.9±0.64	0.5±0.83	0.3±0.50	0.7±0.49	1.0±0.00
Whinney	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.1±0.28	0.0±0.00	0.0±0.00	0.1±0.26	0.1±0.28	0.0±0.00	0.0±0.00
Investigation													
Smells Stallion	0.0±0.00	1.0±0.00	1.5±0.71	0.4±0.89	0.3±0.52	0.0±0.00	0.2±0.44	0.0±0.00	0.0±0.00	0.3±0.72	0.3±0.23	0.1±0.38	0.0±0.00
Gender Behaviors													
Tail Raising	1.0±0.00	1.0±0.00	1.0±0.00	1.0±0.00	1.3±0.89	0.9±0.33	1.0±0.00	0.9±0.27	1.1±0.35	1.1±0.46	1.0±0.00	1.3±0.75	1.0±0.00
Squatting	0.0±0.00	1.0±0.00	0.0±0.00	1.5±1.73	1.3±0.56	0.9±0.78	0.8±0.68	1.0±0.88	1.1±1.06	0.8±0.64	0.7±0.91	0.4±0.50	0.0±0.00
Urination	0.0±0.00	0.0±0.00	0.0±0.00	2.0±1.41	1.8±0.75	1.2±0.97	1.4±0.65	1.4±1.22	1.4±0.83	1.3±0.80	1.0±0.96	1.0±1.15	0.0±0.00
Winking	0.0±0.00	1.0±0.00	0.0±0.00	1.6±0.55	1.6±0.41	1.2±0.60	0.8±0.55	1.1±0.73	1.0±0.65	0.7±0.70	0.8±0.89	0.7±0.95	1.0±0.00
Stand for Mount	1.0±0.00	1.0±0.00	1.0±0.00	0.6±0.55	0.6±0.52	0.7±0.44	0.8±0.37	0.6±0.50	0.8±0.41	0.8±0.41	0.6±0.51	0.4±0.53	1.0±0.00
Not Stand for Mount	0.0±0.00	0.0±0.00	0.0±0.00	0.2±0.45	0.0±0.00	0.1±0.33	0.7±0.28	0.1±0.36	0.2±0.41	0.1±0.26	0.3±0.50	0.4±0.53	0.0±0.00
Withdrawal													
Withdrawal	0.0±0.00	0.0±0.00	0.5±0.71	0.2±0.48	0.6±0.54	1.0±1.50	0.4±0.88	0.4±0.64	0.5±0.83	0.4±0.73	0.3±0.63	0.4±0.53	1.0±0.00
Aggression													
Kick	0.0±0.00	0.0±0.00	0.0±0.00	0.2±0.48	0.2±0.41	0.0±0.00	0.0±0.00	0.3±0.61	0.3±0.72	0.0±0.00	0.3±0.47	0.3±0.75	0.0±0.00
Squeal	1.0±0.00	1.0±0.00	1.0±0.00	0.6±0.89	0.2±0.41	0.0±0.00	0.8±1.09	0.8±1.25	0.3±0.49	0.3±0.62	0.4±1.34	1.1±1.21	2.0±0.00
Ears Back	1.0±0.00	2.0±0.00	1.0±1.41	0.6±0.89	0.3±0.52	0.3±0.50	0.4±0.96	0.8±0.80	0.6±0.74	0.5±0.83	0.6±0.85	0.8±1.07	1.0±0.00
Number of Mares/Day	1	1	2	5	6	9	13	14	15	15	14	7	1

Table A18. Means and standard deviations of the frequencies of behavior patterns displayed by the stallions (per mare per day of estrus) - Experiment 2.

Behavior Pattern	Day of Estrus +10	+9	+8	+7	+6	+5	+4	+3	+2	+1	Ov	-1	-2
	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD
Social Behaviors													
Naso-Nasal	2.0±0.00	3.0±0.00	1.5±0.71	1.0±0.00	0.6±0.52	1.0±0.50	0.3±0.48	0.6±0.74	0.7±0.59	0.5±0.83	0.3±0.49	0.7±0.49	1.0±0.00
Whinney	1.0±0.00	1.0±0.00	1.0±1.41	2.2±1.09	1.1±0.75	1.7±0.83	1.3±1.26	1.7±1.26	1.3±1.23	1.3±1.04	1.2±1.05	1.4±0.97	2.0±0.00
Investigation													
Smell Fore Body	2.0±0.00	4.0±0.00	2.5±0.71	2.2±1.64	1.8±1.33	1.8±1.56	1.5±1.05	1.0±1.36	0.9±0.80	1.6±2.35	1.2±1.47	0.8±0.69	0.0±0.00
Smell Rear Body	1.0±0.00	1.0±0.00	0.5±0.71	3.6±3.78	4.1±3.60	2.6±3.08	3.1±3.70	3.0±2.48	2.2±2.54	2.4±2.16	2.6±2.40	3.6±3.46	0.0±0.00
Total Smelling	3.0±0.00	5.0±0.00	3.0±1.41	5.8±3.35	6.0±2.83	4.4±3.84	4.7±3.45	4.0±3.01	3.1±2.82	4.1±3.92	3.8±2.18	4.4±3.91	0.0±0.00
Lick Fore Body	0.0±0.00	1.0±0.00	0.0±0.00	0.2±0.45	0.0±0.00	0.0±0.00	0.1±0.27	0.1±0.36	0.4±0.74	0.4±1.05	0.0±0.00	0.0±0.00	0.0±0.00
Lick Rear Body	0.0±0.00	0.0±0.00	0.0±0.00	0.4±0.55	3.0±2.96	0.1±0.33	0.1±0.37	0.4±1.15	0.8±1.82	0.2±0.59	0.7±2.40	0.8±1.07	0.0±0.00
Total Licking	0.0±0.00	1.0±0.00	0.0±0.00	0.6±0.55	3.0±2.96	0.1±0.33	0.2±0.44	0.5±1.28	1.2±2.30	0.6±1.29	0.7±2.40	0.8±1.07	0.0±0.00
Total Head Resting	0.0±0.00	0.0±0.00	0.5±0.71	0.2±0.44	0.3±0.52	0.9±1.27	0.4±0.52	0.3±0.50	0.6±1.05	0.7±1.44	0.3±0.50	0.1±0.38	0.0±0.00
Gender Behaviors													
Snort	1.0±0.00	1.0±0.00	0.5±0.71	0.6±0.55	0.1±0.41	0.7±0.97	1.1±1.04	0.9±0.83	1.2±1.37	1.2±0.96	0.8±0.80	1.2±1.60	1.0±0.00
Erection (Almost to full)	1.0±0.00	1.0±0.00	1.0±0.00	1.0±0.00	0.5±0.55	0.7±0.66	0.7±0.44	0.6±0.51	0.6±0.49	0.6±0.51	0.6±0.51	0.6±0.53	0.0±0.00
Mount with Erection (Half to full)	1.0±0.00	1.0±0.00	1.0±0.00	0.8±0.45	0.6±0.52	0.6±0.50	0.7±0.48	0.6±0.50	0.7±0.46	0.7±0.46	0.6±0.51	0.6±0.53	1.0±0.00
Flehmen	0.0±0.00	0.0±0.00	0.0±0.00	2.8±1.92	2.5±3.78	0.7±1.7.	0.8±0.90	1.3±1.60	1.2±2.09	1.2±1.21	1.1±1.29	1.1±1.86	0.0±0.00
Aggression													
Squeal	0.0±0.00	2.0±0.00	0.5±0.71	0.8±0.84	0.3±0.52	0.5±1.01	0.4±0.66	0.2±0.58	0.1±0.26	0.3±0.82	0.3±0.63	1.0±1.15	0.0±0.00
Number of Mares/Day	1	1	2	5	6	9	13	14	15	15	14	7	1

APPENDIX B

Statistical Analyses

A. Intramare Linear Regression (ILR)

1. Application and Computation

Intramare (i.e. intra-subject) linear regressions (ILRs) were performed on all of the data on latency to behavior (tail raising, etc.) (Y = latency to behavior + 2 seconds)[1] on days relative to ovulation (X = day) for each experiment. An ILR compiles the regression of lines of all mares (i.e. subjects) and from this an average slope (b_1) and average origin (b_o) can be estimated and tests of hypotheses about these corresponding parameters can be made. Since weighted means ($\overline{X}_w$ and $\overline{Y}_w$) can be calculated, it is not necessary to have an equal number of data points for each subject, as is the case with this data, where the duration of estrus varies from mare to mare.

When one is interested in the relation of a behavioral latency to the time of ovulation it is particularly important to use an intrasubject regression if the subjects differ substantially in the average latency to the

[1]The data adjustment did not affect the slope, but it did increase the origin by 2 seconds.

behavior. In such cases, if all data are pooled together (subject or mare designation ignored except to pair X's and Y's), many of the deviations of individual latencies from the overall mean latency are very large, causing the estimate of the slope to be unnaturally exaggerated. Intramare or intrasubject regression avoids that problem by deviating individual latencies only from the mean latency of the subject or mare in question. Formulae for calculating the slope in both types of regression is given below:

$$\text{Slope ignoring subjects: } b = \frac{\sum(X-\overline{X})(Y-\overline{Y})}{\sum(X-\overline{X})^2}$$

$$\text{Intrasubject (mare) slope: } b_i = \frac{\sum(X-\overline{X}_i)(Y-\overline{Y}_i)}{\sum(X-\overline{X}_i)},$$

where $\overline{X}_i$ and $\overline{Y}_i$ = means for mare i. Table B1 shows computational formulae for an ILR that differ from those used in a pooled linear regression.

A test of the null hypothesis about the slope ($H:\beta_1=0$) and the computation of the 95% confidence interval estimate around the slope was performed for every ILR. Table B2 gives the computational formulae for these.

Table B1. Computational formulae for an ILR.

Sum of Sum of Squares of X	$\Sigma SS_x = \sum[\Sigma x^2-(\Sigma x)^2/n_i]$
Sum of Sum of Squares of Y	$\Sigma SS_y = \sum[\Sigma y^2-(\Sigma y)^2/n_i]$
Sum of Sum of Products of XY	$\Sigma SP_{xy} = \sum[\Sigma xy-(\Sigma x)(\Sigma y)/n_i]$
Weighted Mean of X	$\bar{X}_w = \sum \left[(\frac{n_i}{\Sigma n_i})\bar{X}_i\right]$
Weighted Mean of Y	$\bar{Y}_w = \sum \left[(\frac{n_i}{\Sigma n_i})\bar{Y}_i\right]$
Average Slope	$b_1 = (\Sigma SP_{xy})/(\Sigma SS_x)$
Average Origin	$b_o = (\bar{Y}_w - b_1\bar{X}_w)$
Regression Sums of Squares	$SS_R = b_1(\Sigma SP_{xy})$
Residual Error Sums of Squares	$SS_E = \Sigma SS_y - SS_R$
Degrees of Freedom	$\nu = \sum(n_i-2)$
Mean Square Error	$(S_y^2\|x) = SS_E/\nu$
Standard Error of the Slope	$\sqrt{(S_y^2\|x)/SS_x}$

Table B2. Computational formulae for H:β_1=0 and the 95% confidence interval estimate around the slope for an ILR.

$H: \beta_1 = 0$

$\bar{H}: \beta_1 \neq 0$

$$t = b_1/[\sqrt{(S_y^2|x)/\Sigma SS_x}]$$

t is compared with $t_{\alpha/2,\nu}$

95% confidence interval estimate around the slope

$$b_1 \pm t_{\alpha/2,\nu}[\sqrt{(S_y^2|x)/\Sigma SS_x}]$$

2. Test for Non-Linearity

For experiments 1 and 2 there was only one data point (Y) for each day (X), therefore it was not possible to test for non-linearity (H:σ^2_{NL}=0). However, for the ILR on all treatments of experiments 3 and 4 combined, for each mare there were several data points (Ys) for each day (X) due to treatments on successive estrous cycles. Thus for this ILR, it was possible to test for non-linearity. Table B3 gives the computational formulae for the test of non-linearity (H:σ^2_{NL}=0).

Table B3. Computational formulae for the test of non-linearity ($H:\sigma^2_{NL}=0$) for an ILR.

$H:\sigma^2_{NL} = 0$

$\overline{H}:\sigma^2_{NL} \neq 0$

SS_E (residual sum of squares of error) is partitioned into "pure" error ($SS_y|x$) and that due to non-linearity (SS_{NL})

$SS_{NL} = SS_E - (SS_y|x)$, where $(SS_y|x) = \sum \Sigma y_{ij}^2 - (\Sigma y_{ij})^2/r_i$

$f = \{SS_{NL}/[\nu-\Sigma(r_i-1)]\}/[(SS_y|x)/\Sigma(r_i-1)]$

f is compared with $f_{\alpha,\ \nu-\Sigma(r_i-1),\ \Sigma(r_i-1)}$.

3. Slope Comparisons

Slopes from two ILRs were compared using a t-test for a comparison of regression lines with unequal variance ($H:\ \beta_1^A = \beta_1^B$). For this test, a Welch's approximation for the degrees of freedom ($\hat{\nu}$) was used (Gill, personal communication). Table B4 gives the computational formulae for the t-test and the formulae necessary for the approximation of the degrees of freedom.

4. Origin Comparisons

Origins from two ILRs were compared using a t-test for a comparison of regression lines with unequal variance ($H:\beta_o^A = \beta_o^B$). For this test, a Welch's approximation for the degrees of freedom ($\hat{\nu}$) was used (Gill, personal

Table B4. t-test for comparison of ILR slopes with unequal variances and Welch's approximation for the degrees of freedom.

$$t = \frac{b_1^A - b_1^B}{\sqrt{\frac{(S_y^2|x)_A}{SS_x^A} - \frac{(S_y^2|x)_B}{SS_x^B}}} \quad ; \; t \text{ is compared with } {}^{+}t_{\alpha/2,\hat{\nu}}$$

Welch's Approximation for the degrees of freedom

$$g = \frac{(S_y^2|x)_A/(SS_x^A)}{(S_y^2|x)_B/(SS_x^B)}$$

$$\hat{\nu} = \frac{(1 + g)^2}{(\frac{g^2}{d.f.\ A} + \frac{1}{d.f.\ B})}$$

communication). Table B5 gives the computational formulae necessary for the approximation of the degrees of freedom.

Table B5. t-test for comparison of ILR origins with unequal variences and Welch's approximation for the degrees of freedom.

$$t = \frac{b_o^A - b_o^B}{\sqrt{\frac{(S_y^2|x)_A}{n^A} + \frac{(S_y^2|x)_B}{n^B} + (S_y^2|x)_A(\bar{X}_A^2/SS_x^A) + (S_y^2|x)_B(\bar{X}_B^2/SS_x^B)}}$$

$$g = \frac{\frac{(S_y^2|x)_A}{n^A} + (S_y^2|x)_A(\bar{X}_A^2/SS_x^A)}{\frac{(S_y^2|x)_B}{n^B} + (S_y^2|x)_B(\bar{X}_B^2/SS_x^B)}$$

Table B5 continued

$$\hat{\nu} = \frac{(1 + g)^2}{\frac{g^2}{d.f.A} + \frac{1}{d.f.B}}$$

t is compared with $\pm t_{\alpha/2,\hat{\nu}}$

B. Intramare Curvilinear Regression (ICR)

1. Application and Computation

Intramare Curvilinear Regressions (ICRs) are similar in concept to ILRs with an average curvature computed in addition to an average slope and origin. Computational formulae that were used for the ICR, and differing from those in Table B1 are given in Table B6.

Tests of null hypotheses about the slope ($H:\beta_1=0$) and the curvature ($H:\beta_2=0$) were performed for each ICR. Table B7 gives the computational formulae for these tests.

Table B6. Computational formulae for an ICR (in addition to those from Table B1).

Sum of Sum of Squares of Z	$\Sigma SS_z = \sum[\Sigma z^2 - (\Sigma z)^2/n_i]$, where $z=x^2$
Sum of Sum of Products of XZ	$\Sigma SP_{xz} = \sum[\Sigma xz - (\Sigma x)(\Sigma z)/n_i]$
Sum of Sum of Products of YZ	$\Sigma SP_{yz} = \sum[\Sigma yz - (\Sigma y)(\Sigma z)/n_i]$
Weighted Mean of Z	$\overline{Z}_w = \sum[(\frac{n_i}{\Sigma n_i})\overline{Z}_i]$
Average Slope	$b_1 = \frac{\Sigma SS_z \Sigma SP_{xy} - \Sigma SP_{xz}\Sigma SP_{yz}}{\Sigma SS_x \Sigma SS_z - (\Sigma SP_{xz})^2}$
Average Curvature	$b_2 = \frac{\Sigma SS_x \Sigma SP_{yz} - \Sigma SP_{xz}\Sigma SP_{xy}}{\Sigma SS_x \Sigma SS_z - (\Sigma SP_{xz})^2}$
Average Origin	$b_o = (\overline{Y}_w - b_1\overline{X}_w - b_2\overline{Z}_w)$
Degrees of Freedom	$\nu_E = \sum(n_i - 3)$
Residual Error Sum of Squares	$SS_E = \Sigma SS_y - b_1\Sigma SP_{xy} - b_2\Sigma SP_{yz}$
Mean Error Sum of Squares	$MS_E = SS_E/\nu_E$

Table B7. Computational formulae for H: $\beta_1=0$ (slope) and H: $\beta_2=0$ (curvature) for ICRs.

H: $\beta_1=0$

$\overline{H}$: $\beta_1\neq 0$

$$t = b_1/\text{s.e. } b_1$$

where s.e. $b_1 = \sqrt{MS_E(\Sigma SS_z)/(\Sigma SS_x \Sigma SS_z - \{\Sigma SP_{xz}\}^2)}$

t is compared with $t_{\alpha/2,\nu_E}$.

H: $\beta_2=0$

$\overline{H}$: $\beta_2\neq 0$

$$t = b_2/\text{s.e. } b_2$$

where s.e. $b_2 = \sqrt{MS_E(\Sigma SS_x)/(\Sigma SS_x \Sigma SS_z - \{\Sigma SP_{xz}\}^2)}$

t is compared with $t_{\alpha/2,\nu_E}$.

C. Orthogonal Polynomial Comparisons

In Experiment 2 both experienced mares (mares who had been in estrus during previous years and possibly bred) and naive mares (two year old mares in their first "estrous season") were observed. It appeared to this author and the handlers that during the teasing sessions the naive mares (Nos. 12, 15, 16, 17 and 24) were considerably more nervous during diestrus and sometimes during estrus than were the majority of experienced mares (Nos. 23, 25, 26, 34, 35, 36, 466, 725, 757 and 770). It was important to determine if

there was a difference between naive and experienced mares in the latencies (+10 seconds)[2] to tail raising, squatting, urinating and mounting before the data from all mares in Experiment 2 were pooled and compared with those from the untreated Horse mares in Experiment 1. Dr. John Gill of the Department of Dairy Science was consulted for an appropriate test that would discern differences between naive and experienced mares. He commented that: "There is a problem with the analysis of repeated measurement data because the correlation structure is often non-uniform." He decided that a form of comparison using orthogonal polynomials in time could test the relationships in linear, quadratic and cubic comparisons by "condensing the repeated measurements" and circumventing the problem, thus "permitting comparisons using single values for each animal" (Gill, personal communication). The null hypothesis of H: $\bar{q}_N = \bar{q}_E$ was tested for the latency (+10 seconds) to each behavior of estrus days +4, +3, +2 and +1. For each mare on these days $q = \Sigma c_i y_{ij}$ was calculated, where c_i = the polynomial coefficients and y_{ij} = the latency times. A mean q ($\bar{q} = \Sigma q/r_i$, where r_i = the number of mares per comparison) and Sums of Squares of q [$SS_q = \Sigma q^2-(\Sigma q)^2/r_i$], were used for the t-test. The formula used for the t-test of the Linear (ξ_1,) and comparison for latency to tail raising between naive (N) and experienced (E) mares is shown in

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[2]This is the only analysis that used +10 seconds to each behavior. Other analyses used the data adjustment discussed in Section II.E.3. of Methods and Materials.

Table B8. The same formula was used for the tests of the latencies to the other behaviors and comparative formulae were used for the quadratic (ξ_2-) and cubic (ξ_3-) comparisons with the appropriate $\bar{q}$'s and SS_q's.

Table B8. Linear (ξ_1-) comparison of latency to tail raising (+10 seconds) for days +4, +3, +2 and +1 between naive (N) and experienced (E) pony mares in Experiment 2.

$H: \bar{q}_N = \bar{q}_E$

$\bar{H}: \bar{q}_N \neq \bar{q}_E$

$$t = \frac{\bar{q}_{\xi_1\text{-}(N)} - \bar{q}_{\xi_1\text{-}(E)}}{\sqrt{\frac{SS_{q_{\xi_1\text{-}(N)}} + SS_{q_{\xi_1\text{-}(E)}}}{r_N + r_E - 2}} \sqrt{\frac{1}{r_N} + \frac{1}{r_E}}}$$

t is compared with $\pm t_{\alpha/2, r_N + r_E - 2}$.

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