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EFFECTS OF PROSTAGLANDIN $F_2\alpha$ AND GONADOTROPIN RELEASING HORMONE ON ESTRUS, OVULATION AND SERUM HORMONES IN MARES

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# EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$ AND GONADOTROPIN RELEASING HORMONE ON ESTRUS, OVULATION AND SERUM HORMONES IN MARES

By

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ABSTRACT

EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$ AND GONADOTROPIN RELEASING HORMONE ON ESTRUS, OVULATION AND SERUM HORMONES IN MARES

By

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This study was designed to determine if 3 injections of GnRH at 8-hour intervals with or without pretreatment with estradiol-17β would elevate serum LH sufficiently to shorten and/or synchronize the $PGF_{2\alpha}$ induced luteolysis to ovulation interval in mares.

Twelve horse mares received 1 of 3 treatments over 3 consecutive estrous cycles utilizing a balanced Latin square crossover design. Mares were injected with $PGF_{2\alpha}$ (treatment A), $PGF_{2\alpha}$ + GnRH (treatment B) or $PGF_{2\alpha}$ + $E_2$-17β + GnRH (treatment C). The mean interval in days from $PGF_{2\alpha}$ to 1st ovulation averaged 1 day less ($p<.10$) for treatments B ($6.7 \pm 0.6$) and C ($6.2 \pm 0.3$) than for treatment A ($7.6 \pm 0.7$). Mares treated with GnRH (treatments B and C) showed a significant ($p<.01$) 2- to 3-fold increase in LH levels when compared to controls. Pretreatment with estradiol-17β did not appear to enhance LH release following GnRH.

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# INTRODUCTION

Ovulation control in the mare would be desirable from the standpoint of developing a preplanned breeding program for an individual mare or a group of mares. Management of the stallion would also be more efficient by reducing the number of covers or matings required per mare and allowing more efficient use of semen collected artificially.

In the mare, duration of the estrous cycle is controlled by the life span of the corpus luteum (CL). Estrus occurs within 2 to 4 days after regression of the corpus luteum. This regression is termed luteolysis. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been demonstrated to be an effective luteolytic agent and may be the natural luteolytic agent. Administration of exogenous $PGF_{2\alpha}$ caused rapid regression of the mature CL (at least 5 days postovulation) and mares returned to estrus in 3 to 5 days. The ability to control the onset of estrus does not imply a control of ovulation time since there is a 3 to 7 day variation among mares in the interval between the onset of estrus and ovulation.

Researchers have tried to induce ovulation during estrus in mares with various hormone treatments. Luteinizing hormone (LH) or compounds with LH activity have been used to induce ovulation in several species including mares. One such compound, human chorionic gonadotropin (HCG), has been reported to induce ovulation in mares (Loy and Hughes, 1966). However, Sullivan et al. (1973) reported a delay in ovulation for mares treated during 3 successive estrous periods with HCG. It appeared that repetitive use of HCG may actually interfere with ovulation in mares similarly to other species. Reel et al. (1976) reported that following multiple treatment with HCG there was a progressive decline in the number of eggs shed and corpora lutea formed in rabbits, mice and cows. Experiments have suggested that the development of this ovulatory refractiveness was due to the formation of antibodies against the foreign protein (Greenwald, 1970).

A new synthetic peptide containing 10 amino acids identified as gonadotropin releasing hormone (GnRH) has been used to induce endogenous LH release and thus prevent the injection of foreign proteins to induce ovulation. Mares treated with GnRH had increased LH concentrations (Garcia and Ginther, 1975; Ginther and Wentworth, 1974; Irvine et al., 1975; Oxender et al., 1977b). Summarizing data from the above authors indicated that GnRH increased LH concentration significantly for approximately 8 hours following treatment and then returned to baseline within 24 hours. The transient LH surge following a single injection of GnRH did not reduce the interval from estrus to ovulation (Ginther and Wentworth, 1974; Garcia and Ginther, 1975; Oxender et al., 1977b). However, LH concentrations normally remain elevated 2 to 4 days prior to ovulation in mares (Whitmore et al., 1973; Noden et al., 1974; Pattison et al., 1974). Thus, it has been suggested that repeated injections of GnRH may be necessary to duplicate the normal LH response of the mare in stimulating follicular development and ovulation (Ginther and Wentworth, 1974).

Estradiol concentrations in peripheral blood reached maximal values 1 to 2 days prior to ovulation and appeared to influence pituitary LH release. Data reported by Garcia and Ginther (1975) indicated that pretreatment with estradiol-17β ($E_2$-17β) enhanced the LH response to a dose of GnRH. Therefore, it was decided to test the effects of estradiol and repeated GnRH treatments on the interval from luteolysis to ovulation.

The objective of this research was to determine if repeated GnRH treatments with or without estradiol pretreatment would maintain elevated LH concentrations, stimulate follicular development and synchronize ovulation following $PGF_{2\alpha}$ induced luteolysis.

# LITERATURE REVIEW

## Equine Estrous Cycle

### Seasonal Variation

An important feature of reproduction in the mare is that she is classified a seasonal breeder. Changes in the duration of daylight have influenced the mare's reproductive pattern while environmental temperature and nutrition have been shown to modify but not change the basic effect of light (Quinlan et al., 1951).

Mares appear to recognize and respond to the changing duration of daylight between 4 important days in the light year. One is the longest day of the year (June 21), the summer solstice, and another is the shortest day of the year (December 21), the winter solstice. The other 2 days are the spring (March 21) and fall (September 21) equinoxes, points halfway between the solstices when the duration of daylight and darkness is equal (Greenhoff and Kenney, 1975). The major population of mares reached its peak of fertility before, around and after the summer solstice and its low point of fertility around the winter solstice. The fertility was either decreasing or increasing the remainder of the year (Hutton and Meacham, 1968). The peak incidence of ovulation occurred around the summer solstice in both the northern and southern hemispheres (Kenney et al., 1975).

Individual mares may exhibit regular estrous cycles the year round, while others exhibit a variable period of anestrus during the winter (Day, 1940). The ovaries of mares in deep anestrus are described as being small, firm and containing follicles less than 5 mm in diameter but no corpora lutea were found (Van Niekerk et al., 1973).

During late winter and early spring when estrous periods begin, follicles tend to persist for prolonged periods with irregular estrous periods often lasting more than 10 days and not always accompanied by ovulation (Greenhoff and Kenney, 1975). Because follicles often become atretic rather than ovulate, and because the mares are in estrus, Greenhoff and Kenney (1975) term this the period of "anovulatory receptivity." By April, ovulations occur in over 50% of the estrous periods (Arthur, 1958; Osborn, 1966) and the duration of estrus shortens (Andrews and McKenzie, 1941; Trum, 1950; Van Niekerk, 1967), probably because ovulations occur sooner.

In summary, associated with increasing daylight, mares showed an increased incidence of psychic estrus, an increased incidence of ovulation and a decreased length of estrus.

## Estrus and Ovulation

During the period of ovulatory receptivity or natural breeding season, the estrous cycle ranged from 19 to 23 days, diestrus from 14 to 16 days, and estrus from 4 to 9 days (Andrews and McKenzie, 1941; Trum, 1950; Quinlan et al., 1951; Van Niekerk, 1967; Ginther et al., 1972; Stabenfeldt et al., 1972; Ginther, 1974).

Most researchers indicated that ovulation occurred 24 to 48 hours before the end of estrus (Day, 1940; Andrews and McKenzie, 1941; Trum, 1950; Ginther et al., 1972; Plotka et al., 1972; Stabenfeldt et al., 1972; Whitmore et al., 1973; Ginther et al., 1974). The size of the follicle just prior to ovulation has been reported to be 4.5 to 6.5 cm in Thoroughbreds and 2.5 to 3.5 cm in ponies (Day, 1940). These values are within the range reported by subsequent researchers (Andrews and McKenzie, 1941; Witherspoon and Talbot, 1970; Ginther et al., 1972). Most mares are reported to ovulate only 1 follicle during a given estrous period. The frequency of multiple ovulations in horse mares has been reported as low as 3.8% (Andrews and McKenzie, 1941), up to 25% (Stabenfeldt et al., 1972), to a maximum of 42% (Warszawsky et al., 1972). Stabenfeldt et al. (1972) reported that the interval between double ovulations was about 24 hours. Ovulations were also reported to occur after the end of estrus (Stabenfeldt et al., 1972; Ginther, 1974).

## Hormonal Changes During the Estrous Cycle

### Luteinizing Hormone (LH)

Serum LH has been quantified in the peripheral blood of mares by several investigators (Anand et al., 1973; Whitmore et al., 1973; Noden et al., 1975; Evans and Irvine, 1976; Garcia and Ginther, 1976a). The reported LH concentrations by the above authors differ greatly; however, several different assay procedures and LH standards were used. There was general agreement that LH concentrations are low during mid-diestrus and begin to increase a few days prior to the onset of estrus, reaching maximum concentrations 1 to 2 days postovulation. A gradual decrease in LH occurred during the next 4 to 6 days. The pattern of an extended period of increasing LH concentration during estrus and peaking beyond ovulation was in sharp contrast to the short duration of LH release prior to ovulation in cows, ewes and sows (Swanson et al., 1972; Hansel and Ecternkamp, 1972). The prolonged LH surge in the mare may be related to the long periods of follicular growth and estrus in mares compared with other domestic animals.

The total biological role of LH is not yet known. It has been suggested by Evans and Irvine (1975) that LH may play an important role in the maturation of the ovulatory follicle as well as have a luteotropic effect in the mare. The findings of Pineda et al. (1972) and Pineda et al. (1973) utilizing an antiserum against an equine pituitary fraction having follicle stimulating hormone (FSH) and LH activity supported these views. They showed that the largest follicle in antiserum treated mares was reduced in volume, weight and diameter versus controls, and the weights of corpora lutea were significantly less for antiserum treated mares.

The persistence of high LH concentrations into the postovulatory period has been proposed as the reason for double ovulations often reported (Geschwind et al., 1975). Garcia and Ginther (1976a) used ovariectomized mares to study seasonal variation in LH. The LH concentrations were low in these mares during the anovulatory season and high during the ovulatory season, indicating an environmental influence affecting basal LH levels in the absence of ovarian steroids.

Follicle Stimulating Hormone (FSH)

It was only recently that a method of measuring serum concentrations of equine FSH was developed (Evans and Irvine, 1975, 1976). The authors quantified circulating concentrations of FSH during the estrous cycle and early pregnancy in the mare. They showed that serum FSH levels were elevated during late estrus and early diestrous period and again at mid-diestrus, 10 to 13 days before the next ovulation. These reported surges of FSH seemed to coincide with periods of follicular development as demonstrated by Pineda and Ginther (1972) and Pineda et al. (1973). Evans and Irvine (1975) suggested that the mid-diestrus surge of FSH was important in triggering the maturation of the next ovulatory follicle. Work in pony mares (Miller et al., 1977) also showed a definite surge of FSH 9 to 11 days prior to ovulation which would agree with the work in horse mares. However, the data from the pony mares did not show a rise in FSH serum levels prior to ovulation as was found in horse mares. No explanation can be given for these differences at this time.

Initiation of corpus luteum development and progesterone production occurred rapidly following ovulation. Follicle stimulating hormone levels have been shown to be increased during this time and LH levels were still quite high, gradually decreasing to baseline levels by 5 days postovulation. The presence of high levels of gonadotropins at this stage has been postulated as being important for early luteal development.

The dependency of corpora lutea on pituitary hormones has been tested utilizing antibodies to equine pituitary fractions with FSH and LH activity (Pineda et al., 1972, 1973). The mean weight of the corpus luteum was significantly less for antiserum-treated mares than for control mares, indicating that gonadotropins were necessary to maintain the corpus luteum. Garcia and Ginther (1974) administered pituitary hormones to determine if they would extend luteal life span or prevent the luteolytic effect of other treatments. In one trial, pony mares were treated with 2,000 units of HCG (shown to have LH and FSH activity) each day from days 11 to 16 of diestrus and this treatment failed to prevent luteolysis. In a second experiment, HCG or an equine pituitary extract was given daily on days 10 to 17. There was a significant reduction in progesterone concentrations by day 13 in control mares and day 17 in HCG-treated mares. However, progesterone did not decrease in the pituitary extract-treated mares, suggesting maintenance of the corpus luteum. Although research to date has failed to identify the active luteotropic substance in mares, the studies certainly indicated that maintenance of the equine corpus luteum was dependent upon circulating gonadotropins.

## Progesterone

Progesterone is a steroid produced by the corpus luteum. Recently, numerous studies have reported progesterone concentrations during the estrous cycle in mares. The earlier work was done with competitive protein binding assay techniques (Smith et al., 1970; Stabenfeldt et al., 1972; Plotka et al., 1972; Sharp and Black, 1973; Allen and Hadley, 1974; Van Niekerk et al., 1975), while later studies utilized radioimmunoassay (RIA) techniques (Squires et al., 1974; Noden et al., 1975; Palmer and Jousset, 1975a). Although there was variation among the reports, there was general agreement that serum progesterone concentrations were low during estrus. Most reports indicated less than 1 ng/ml serum progesterone during estrus. Progesterone concentrations increased within 24 to 36 hours following ovulation and proceeded to maximal values by 5 to 7 days after ovulation. Although there were fluctuations in progesterone concentrations during diestrus, they generally remained elevated until luteolysis on day 13 or 14. Following luteolysis, concentrations decreased rapidly until the low estral levels were reached 2 to 3 days later. Of interest, Stabenfeldt et al. (1972) noted no significant difference in the progesterone levels between mares with 1 or 2 corpora lutea. When 2 ovulations occurred, the values did not increase until 24 hours after the 2nd ovulation. It was proposed that the presence of a large preovulatory follicle was probably inhibiting the steroidogenic capabilities of the corpus hemorrhagicum formed by the 1st ovulation.

## Estrogens

The determination of the circulating concentrations of estrogens has been more difficult than progesterone due to much lower circulating concentrations and the presence of several estrogen compounds. Thus, these estrogens cross react with estrogen antisera and complicate quantification procedures and interpretation of the results.

Reported levels in plasma or serum vary greatly (Pattison et al., 1974; Noden et al., 1975; Plotka et al., 1975). However, the relative changes reported between estrus and diestrus were similar. Serum estradiol concentrations reported by Pattison et al. (1974) for estrous and diestrous mares were 141 pg/ml and 20 pg/ml, respectively, while values reported by Noden et al. (1975) were 11.5 pg/ml and 2.2 pg/ml. Plasma estrone as reported by Noden et al. (1975) ranged from 9.2 to 12.5 pg/ml throughout the estrous cycle, showing no significant difference among the days. The estrogen values obtained by Plotka et al. (1975) combined estradiol and estrone; as a result, they failed to show the changes in estrogen levels around the time of ovulation as reported by the others.

In general, near the beginning of estrus the blood concentrations of estradiol-17β began increasing, peaking approximately 1 to 2 days before ovulation. This increase in estradiol occurred parallel with an increase in follicular size. Estradiol concentrations appeared to decrease by the time ovulation had occurred and reached diestrus levels within a day or two after ovulation.

## Androgens

The physiological role of the androgens as they relate to the mare's estrous cycle is not known. Recent studies in rats (Schreiber and Ross, 1976) suggest the possibility that androgens may be linked with control of atresia and preantral follicular maturation. Androstenedione, an estrogen precursor, has been shown to increase prior to ovulation (Noden et al., 1975; Ganjam and Kenney, 1976) at a time when the large nonovulatory follicles are regressing. Recently, a preovulatory rise in dehydroepiandrosterone levels has been reported in mares (Rance et al., 1976). The significance or source is not yet known.

## Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)

Although $PGF_{2\alpha}$ is usually not thought of as being a reproductive hormone, it does fit the classical definition of a hormone. It is secreted by an organ, the uterus, and travels through the blood to exert its effect on a distant target organ, the ovary. The concentration of the F-series of prostaglandins has been measured in the uterine veins during the estrous cycle of the mare by Douglas and Ginther (1976). The PGF concentration increased significantly between days 10-14 of diestrus near the time luteolysis occurs. It was assumed by the authors that the primary prostaglandin measured was $PGF_{2\alpha}$. The implications of the findings add an important piece of evidence to the growing body of knowledge indicating that prostaglandin may be the natural uterine luteolysin in the mare.

During the 1970s, investigators had demonstrated that prostaglandins were potent luteolysins in many species. Work in the mare (Douglas and Ginther, 1972) showed that a single subcutaneous injection of $PGF_{2\alpha}$ caused mares to return to estrus in 3 days. Subsequent work confirmed the luteolytic effect of exogenous $PGF_{2\alpha}$ in the cycling mare (Noden et al., 1974), pseudopregnant and pregnant mares (Kooistra and Ginther, 1976) and hysterectomized mares (Douglas et al., 1975). The post-treatment effects on the behavioral, hormonal and ovulatory events of the ensuing estrus were similar to controls (Noden et al., 1974). It is of interest that the newly developing corpus luteum (1st 4 days after ovulation) appeared resistant to the luteolytic effects of $PGF_{2\alpha}$ (Douglas and Ginther, 1975a; Oxender et al., 1975a). The reason has not been determined.

The minimal effect dose of exogenous $PGF_{2\alpha}$ in horse and pony mares averaged approximately 8 μg/kg of body weight (Douglas and Ginther, 1972; Oxender et al., 1975a). This is in marked contrast to the sheep, in which the minimal effective dose is 144 μg/kg (Douglas and Ginther, 1973b). The unusual sensitivity of mares to $PGF_{2\alpha}$ may relate to the greater affinity of the membrane of the corpus luteum from mares for binding $PGF_{2\alpha}$ when compared with the cow (Kimball, 1976).

## Gonadotropin Releasing Hormone (GnRH)

Gonadotropin releasing hormone is a peptide containing 10 amino acids which is produced by the hypothalamus and is transported to the adenohypophysis through a system of portal vessels. Natural and synthetic GnRH has been shown to cause pituitary release of both LH and FSH in several species (Schally et al., 1976). Ginther and Wentworth (1974) demonstrated that single intravenous injections of GnRH in anestrous pony mares caused an immediate increase in plasma LH concentration that gradually decreased within 2.5 hours to pre-treatment levels. Administration of GnRH subcutaneously on day 2 of estrus caused a more prolonged rise in LH values which reached maximum 1 hour post-treatment and then decreased to pretreatment levels by 8 hours. In these experiments there was no effect on the GnRH treatment on follicular development, time of ovulation or length of estrus. Evans and Irvine (1976) further characterized the effects of GnRH in the mare by measuring both FSH and LH following injection of GnRH into 7 anestrous mares. They reported significant increases in both FSH and LH following GnRH treatment. In fact, FSH release was significantly greater than that for LH. This is of interest because it had been suggested that GnRH was primarily LH releasing in the human, rat, sheep and cow when the FSH and LH changes were compared by expressing the increases as a fraction of the baseline concentration (Evans and Irvine, 1976). The authors suggested that the difference in FSH and LH response of the mare to GnRH in comparison to other species was due to a difference in the pituitary response.

Pursuing this difference in pituitary response between species still further, experiments on women seem to indicate that GnRH caused the release of stored gonadotropins rather than active synthesis and that the pituitary could be depleted of gonadotropin (Newton et al., 1973).

A refractory period in LH release following repetitive injections of GnRH has been demonstrated in the ewe and the steer (Rippel et al., 1974; Tannen and Convey, 1977). Rippel et al. (1974) concluded from their data that both the anestrous and ovariectomized ewe had a refractory period in the mechanism controlling GnRH induced LH release. Successive injections of GnRH at 96-hour intervals resulted in LH release similar to the initial dose. However, a decline in LH release occurred at 72-hour injection intervals. Pituitary concentrations of LH and FSH were not depleted, which suggested that pituitary secretion was not necessarily related to hormone content of the gland. In recent work by Bailey and Douglas (1977) where anestrous mares were injected with GnRH every 8 hours for 14 days, a period of refractivity was not apparent.

## Artificial Control of Estrus and Ovulation in Mares

Understanding how the previously mentioned reproductive hormones interact with one another to control the mare's estrous cycle is, of necessity, very important if we are to try to artificially manipulate this dynamic process. Studies on cause and effect relationships are difficult to devise. So far, studies involving only 2 or 3 hormones have been done, and conclusions drawn from the information pieced together may not be valid. Studies thus far have been on the effects of one hormone upon the circulating concentrations of another or simply comparing the interrelationships in concentrations over time; for example, one hormone increasing in concentration when another is decreasing. Thus, one should proceed with caution to make generalizations about what is known at this time.

The estradiol and LH curves are roughly synchronized, except that the estradiol peak occurred approximately 2 days before the LH peak and the estradiol concentration decreased sooner following ovulation than LH (Pattison et al., 1974; Noden et al., 1975).

In contrast, estradiol and progesterone fluctuated reciprocally, as would be expected; progesterone characterized the diestrous phase and estrogen characterized the estrous phase. The progesterone decrease at the end of diestrus preceded the increase in LH (Noden et al., 1974), while the progesterone increase after ovulation preceded the reduction in LH concentrations (Anand et al., 1973).

The above mentioned relationships between estradiol, progesterone and LH suggested that progesterone had a negative effect on LH output while estrogen had a positive effect on pituitary LH release. As was noted earlier, LH concentrations followed a seasonal pattern in ovariectomized mares. In a subsequent study, Garcia and Ginther (1976b) examined the effects of exogenous estradiol and progesterone on LH in ovariectomized mares during the ovulatory season. Their conclusions were that estradiol-17β treatment caused a transient increase in plasma LH levels while progesterone treatment decreased plasma LH. However, the concurrent administration of estradiol-17β and progesterone caused a greater decrease in plasma LH levels than progesterone alone. In summary, it appeared that the ovarian steroids were exerting their influence upon the hypothalamus, altering the release of GnRH and the subsequent release of LH by the anterior pituitary, or that they had a direct effect on the pituitary or perhaps the ovarian steroids were controlling both the hypothalamus and pituitary.

Reeves et al. (1971a) showed that LH release in response to GnRH in ewes was greatest during an 8-hour period on day 1 of the estrous cycle, providing evidence to indicate elevated serum concentrations of estradiol increased LH release after GnRH treatment. In addition, pretreatment of ewes with 250 to 500 mg of estradiol benzoate 20 hours before GnRH treatment increased LH release compared to control ewes (Reeves et al., 1971b). Zolman et al. (1973), however, failed to detect a significant variation in the magnitude of LH release in heifers treated with GnRH on different days of the estrous cycle.

Estradiol-17β pretreatment enhanced the LH response in mares to a dose of GnRH, which was ineffective in eliciting a response when given without estradiol (Garcia and Ginther, 1975). The data indicated that estradiol treatment alone might also induce an LH response.

While it is evident that the ovarian steroids modify the hypothalamic pituitary response, the mechanisms involved are not known. It appeared that the role of estradiol and progesterone on LH release was complex and may involve modulation at both the hypothalamic and pituitary level. While the control mechanisms of estrus and ovulation appear complex, several researchers have successfully changed the functional life of the corpus luteum. In addition, there has been limited success with induction of ovulation in mares. Following is a brief review of the methods used to control estrus and ovulation in mares.

## Prolongation of the Luteal Phase

Progestins, a term used to identify several compounds with progesterone activity, are the synchronizing agents used most extensively in many species. The theory is to maintain progestin blood concentrations and keep the animals in a diestrous state artificially until the corpora lutea in all animals of the group have regressed. When the progestin is withdrawn, estrus and ovulation occur in a synchronized fashion. The original studies utilizing exogenous progesterone in the mare were conducted by Loy and Swan (1966). Injections of 100 mg of progesterone per day and higher amounts beginning in mid-diestrus blocked estrus and ovulation in all mares. Follicular development occurred prior to the end of treatment when the treatment period extended beyond 20 days after ovulation. Fifty milligrams per day prevented estrus but not ovulation. In contrast, treatment with 100 mg of progesterone per day starting on the 1st day of estrus failed to prevent estrus and ovulation.

Synthetic progestins were also studied by Loy and Swan (1966). One of these, 6α-methyl-17α-acetoxyprogesterone (MAP), administered orally to mares in midcycle or to mares showing erratic estrous behavior, failed to inhibit estrus or ovulation at levels of from 400 to 1,782 mg per day. Melengestrol acetate (MGA) also was ineffective in cycling mares when given orally at 10 to 20 mg per day. Another orally administered synthetic progestin (17-α-allyl-estratriene-4-9-11, 17-β-ol-3-one) was reported to be effective in cycling mares (Webel, 1975). This new progestin in a dose of 15 or 20 mg daily for 18 days resulted in a withdrawal-to-estrus interval of $5.4 \pm 3.1$ and $5.8 \pm 2.7$ days, respectively.

Another method of prolonging the luteal phase or delaying follicular development has been to block pituitary gonadotropin release. Methallibure, a potent inhibitor of gonadotropin secretion, was reasonably successful in synchronizing estrus in mares, but induced inappetence and other side effects made it unsuitable (First, 1973). Thus, exogenous progestins appeared to be more satisfactory than methalibure in delaying estrus and ovulation in mares.

Termination of the Luteal Phase

One of the analogues of prostaglandin $F_{2\alpha}$ or $PGF_{2\alpha}$ itself caused rapid regression of the mature corpus luteum. Several reviews on the applied uses of $PGF_{2\alpha}$ are available (Oxender et al., 1974; Allen and Cooper, 1975; Lauderdale and Miller, 1975; Nelson, 1976). Prostaglandin $F_{2\alpha}$ administration caused a predictable return to estrus in 2 to 4 days if the mare had a mature corpus luteum. As had been mentioned previously, the corpus luteum was refractory to $PGF_{2\alpha}$ treatment for 4 to 5 days following ovulation (Allen and Rowson, 1973; Douglas and Ginther, 1973a; Oxender et al., 1975a). In addition to the 1st 4 days postovulation, mares already in estrus could not be synchronized with a group treated with $PGF_{2\alpha}$. Therefore, only about 60% of a group of mares treated at any one time with $PGF_{2\alpha}$ would be in estrus 2 to 4 days later. Two $PGF_{2\alpha}$ treatments 12 to 14 days apart have been used to synchronize estrus in group treated mares (Palmer and Jousset, 1975b). Although $PGF_{2\alpha}$ treatment induced luteolysis in most mares on day 5 to 14 postovulation, and the interval from $PGF_{2\alpha}$ treatment to ovulation averaged 8.5 to 9.0 days, the variation of response for individual mares ranged from 3 to 12 days. This lack of precision for predicting the time of ovulation in advance has caused researchers to pursue additional methods to control the time of ovulation in mares.

## Induction of Ovulation

Several studies have been done on the effects of HCG on estrus and ovulation (Loy and Hughes, 1966; Ginther, 1971; Sullivan et al., 1973; Voss et al., 1975). Data from the above work consistently showed that the duration of estrus was reduced in mares treated with HCG. The variation in length of estrus was also reduced (Loy and Hughes, 1966). A large percentage, 88.9% (Loy and Hughes, 1966) and 82.4% (Sullivan et al., 1973), ovulated within 48 hours post-treatment. The work by Sullivan and his co-workers showed that, although the treatment was effective during the 1st cycle, treatment during the 2nd and 3rd cycles was ineffective in reducing the estrus to ovulation interval or the length of estrus. In fact, the 3rd consecutive treatment resulted in a significant lengthening of estrus. Although there appears to be some controversy over the effects of repeated HCG treatment in mares, HCG administration has not been shown to have adverse effect on conception rates (Loy and Hughes, 1966; Voss et al., 1975).

Gonadotropin releasing hormone has also been used to induce ovulation in mares (Ginther and Wentworth, 1974; Irvine et al., 1975; Heinze and Klug, 1975). It has been demonstrated to cause a significant increase in serum LH levels after a single administration. Irvine et al. (1975) showed that 2 mg GnRH given on day 2 of estrus significantly shortened estrus, but the time of ovulation was not changed. However, when 2 mg GnRH was administered daily, the duration of estrus and the time of ovulation were significantly shorter than for the controls.

In clinical tests demonstrating the efficacy of GnRH, Heinze and Klug (1975) showed that all but 1 of 39 mares having a mature follicle ovulated within 48 hours of an injection of 4 mg GnRH when the estrus lasted greater than 9 days. Only 11 of 16 control mares ovulated within the same period. This indicates that at least in some mares GnRH treatment may be successful in inducing ovulation of a mature follicle.

Synchronization of Estrus and Ovulation

The research results reported above led some researchers to combine progestin and luteolytic treatments with gonadotropin treatment in an attempt to increase the synch rony of ovulation in mares. Holtan et al. (1974, 1977) administered 50 mg progesterone per day for 19 days followed by HCG 6 days after withdrawal to 21 pony mares. Fifteen of the 21 mares ovulated between days 6 to 9 after withdrawal. A comparative study by the same authors utilized $PGF_{2\alpha}$ and HCG. The $PGF_{2\alpha}$ was given on days 0 and 18 and the HCG was given 6 days following the 2nd injection of prostaglandin. The use of 2 injections of $PGF_{2\alpha}$ 18 days apart was designed to induce luteolysis if a mature corpus luteum was present at the 1st injection and hopefully all corpora lutea in all mares would be approximately 10 to 19 days old by the time of the 2nd injection. Results indicated that better synchronization of the day of ovulation occurred following the progesterone-HCG treatment than with the $PGF_{2\alpha}$-HCG treatment.

However, a French research group used the $PGF_{2\alpha}$-HCG combination on a different schedule and obtained favorable results. Palmer and Jousset (1975b) tested a regime as follows: day 0, prostaglandin; day 6, HCG; day 14, prostaglandin; day 20, HCG. Treatment was started without regard to the stage of the estrous cycle. Because the onset of estrus is more synchronized than the day of ovulation using $PGF_{2\alpha}$, an ovulating hormone such as HCG is useful to reduce this variability. About 50% of the 48 mares treated ovulated within 48 hours of the 2nd HCG injection.

Although these treatments can be used to obtain synchrony of ovulation, there remains the question about the fertility of controlled ovulations. In an experiment Holtan, Douglas and Ginther (1977) used a combination of progesterone, $PGF_{2\alpha}$ and HCG together followed by single or multiple breedings to determine pregnancy rate. The treated pony mares received 75 mg progesterone for 10 days in addition to $PGF_{2\alpha}$ (1.25 mg) on day 7 during progesterone treatment. The prostaglandin was used to prevent survival of any corpora lutea beyond the progesterone withdrawal period. Human chorionic gonadotropin (2,000 IU) was given 5 days following progesterone withdrawal. There was no significant difference in the pregnancy rate between the control mares or the treated mares that received either single or multiple breedings.

Recent work by Oxender et al. (1977b) was done to determine the dose of GnRH required to cause increased serum LH in mares and to see if this increase would synchronize ovulation following induction of luteolysis with $PGF_{2\alpha}$. Results showed a significant rise in LH levels using GnRH (1 to 5 mg sc). However, the interval between $PGF_{2\alpha}$ and ovulation was not significantly reduced, nor was the time of ovulation synchronized.

Although much has been learned about the controls of estrus and ovulation in mares, it appears much more research will be necessary before we can accurately predict in advance the day of ovulation for mares.

## METHODS AND MATERIALS

### Animals

Twelve mares of mixed breeding, 4 to 21 years of age and weighing 300 to 500 kg, were used in this experiment, which was conducted during June and July of 1976. The mares were kept on pasture throughout the experiment.

### Experimental Design

Twelve mares were teased daily to determine estrous behavior and time of ovulation in late May 1976. On the day of ovulation (day 0), which occurred during estrus of a control cycle, each mare was assigned to 1 of 4 squares in a balanced Latin square crossover design (Table 1). Each of the 12 mares received 1 of 3 treatments in each of 3 consecutive estrous cycles such that each mare received all 3 treatments (A, B and C) during the experimental period. The order of treatments for each mare was fixed. The design insured that each treatment preceded and followed the other 2 treatments an equal number of times. This design allowed for detection of period effects as well as residual effects of one treatment on subsequent treatments.

The 3 experimental treatments were: 1) A, Controls, Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$); 2) B, $PGF_{2\alpha}$ and Gonadotropin releasing hormone (GnRH); and 3) C, $PGF_{2\alpha}$, GnRH and Estradiol-17β ($E_2$-17β). The $PGF_{2\alpha}$ (10 mg sc) was injected on day 7 postovulation; peanut oil (PO) or

Table 1. Balanced Latin square crossover design utilizing 12 mares

| Mares | Estrous Periods 1 | 2 | 3 |
|---|---|---|---|
| 1 | A | B | C |
| 2 | B | C | A |
| 3 | C | A | B |

| Mares | Estrous Periods 1 | 2 | 3 |
|---|---|---|---|
| 4 | B | A | C |
| 5 | A | C | B |
| 6 | C | B | A |

| Mares | Estrous Periods 1 | 2 | 3 |
|---|---|---|---|
| 7 | A | B | C |
| 8 | B | C | A |
| 9 | C | A | B |

| Mares | Estrous Periods 1 | 2 | 3 |
|---|---|---|---|
| 10 | B | A | C |
| 11 | A | C | B |
| 12 | C | B | A |

$E_2$-17β (3 mg sc) was injected on days 8 and 9; and saline or GnRH (0.2 mg sc) was injected at 1630 and 2400 hours on day 10 and at 0800 hours on day 11 (Table 2). In the case of mares having 2 or more ovulations during estrus, $PGF_{2\alpha}$ was administered 7 days following the last ovulation. Diestrus ovulations were not considered when calculating the day for $PGF_{2\alpha}$ administration.

Blood sampling, estrus detection and rectal palpation were performed according to plan throughout the 3 consecutive estrous periods stopping 15 days postovulation following the final treatment regime.

## Estrus Detection

Mares were teased daily each afternoon at 1630 hours with a mature stallion. A teasing criterion was established and behavioral signs were noted as follows:

T = Raising tail prior to being mounted or after being dismounted

U = Urinating anytime during teasing

W = Winking vulva anytime during teasing

Sq = Squatting

R = Raising tail while being mounted

S = Switching of tail anytime during teasing

K = Kicking (includes strikes) during teasing

B = Attempts to bite the teaser

M = Movement to prevent mounting by the teaser

N = Failure to stand firmly while being mounted

D = Standing firmly while being mounted but failing to elevate tail

X = Failure of teaser to mount

Table 2. Schedule of treatment events, frequency of teasing (T), bleeding (B) and rectal palpation (P) following ovulation

| Days post-ovulation | Treatment[a] | | |
|---|---|---|---|
| | A | B | C |
| 7, 1630 hours | T,B,P-$PGF_{2\alpha}$ | T,B,P-$PGF_{2\alpha}$ | T,B,P-$PGF_{2\alpha}$ |
| 8, 1630 | T,B-PO | T,B-PO | T,B-$E_2$ |
| 9, 1630 | T,B-PO | T,B-PO | T,B-$E_2$ |
| 10, 1630 | T,B,P-S | T,B,P-GnRH | T,B,P-GnRH |
| 2400 | B-S | B-GnRH | B-GnRH |
| 11, 0800 | B-S | B-GnRH | B-GnRH |
| 1630 | T,B | T,B | T,B |
| 12, 1630 | T,B | T,B | T,B |
| 13, 1630 | T,B,P | T,B,P | T,B,P |
| 14, 1630 | T,B | T,B | T,B |
| 15, 1630 | T,B | T,B | T,B |

[a]$PGF_{2\alpha}$ = 10 mg sc

Estradiol-17β ($E_2$) = 3 mg in peanut oil sc

GnRH = 0.2 mg in saline sc

Peanut oil (PO) = 3 ml sc

Saline (S) = 1 ml sc

For purposes of determining onset of estrus and estrus duration, it was decided that a mare would not be considered in estrus until she stood firmly to be mounted with her tail raised along with a majority of positive signs, including winking, squatting and urinating. Cessation of estrus was noted when the mare no longer stood to be mounted.

## Ovulation Detection

Mares were palpated rectally to determine ovarian follicular changes at least every 3rd day unless a mare was in estrus or an ovarian follicle greater than 35 mm in diameter was detected. Mares in the latter category were palpated daily until ovulation or follicular atresia occurred.

## Blood Collection and Sample Storage

Each day at 1630 hours, at 1630 and 2400 on day 10 and 0800 on day 11, blood (20 ml) was collected by jugular venipuncture utilizing a 20 ml vacutainer tube with a 20 gauge 1 1/2 inch needle. The samples were placed on ice until transferred to the laboratory, where they were allowed to stand under refrigeration for 48 to 72 hours to allow sufficient clot retraction. After centrifugation, the decanted serum was placed in plastic vials and stored at -20 C for quantification of hormones.

## Hormone Quantification

Serum concentrations of luteinizing hormone (LH), progesterone ($P_4$) and estradiol-17β ($E_2$-17β) were determined using radioimmunoassay procedures.

Luteinizing Hormone (LH)

Plasma LH concentrations were assayed using a double antibody technique developed by Niswender et al. (1968) for rat LH and modified for equine LH by Noden et al. (1974). Dilution duplicates of equine serum were used, 200 µl for sample unknowns thought to be low in LH and 50 µl and 100 µl for sample unknowns thought to be high in LH concentration. All samples were diluted to 500 µl in phosphate buffered saline (PBS) with 0.1% Knox gelatin. Four sets of standard equine LH sera were placed at equal intervals throughout the assay. The standard equine LH (LER 1138-1, 0.27 U NIH LH-S1/mg) was supplied by Dr. L. E. Reichert of Emory University, Atlanta, Georgia. Each standard set consisted of 12 tubes containing a range from 2 to 100 ng of equine LH in 500 µl of PBS-0.1% Knox. Two hundred microliters of 1st antibody (rabbit antiovine LH #15 from Gordon Niswender, Colorado State University, Fort Collins, Colorado) was added to each culture tube at a dilution of 1:32,000 in 1:400 NRS (normal rabbit serum) in PBS:EDTA. Incubation of the tubes at 4 C for 24 hours allowed equilibration between the free antigen (LH), free antibody and bound antigen-antibody complex.

Purified ovine LH (LER-1056C2) was then radioiodinated using $^{125}I$ in a method similar to that described by Niswender et al. (1968). The stock solution of $^{125}I$ was diluted with PBS-0.1% Knox so that 100 µl contained approximately 20,000 counts per minute (CPM). One hundred microliters of $^{125}I$-LH solution was then added to each culture tube and the tubes were again incubated at 4 C for 24 hours. The labeled $^{125}I$-LH competes with the unlabeled antigen (LH) for the available antibody binding sites during the incubation period.

A 2nd antibody (sheep anti-rabbit gamma globulin) diluted 1:10 in PBS:EDTA was then added to form an antigen-antibody-antibody complex large enough to be precipitated by centrifugation. Two hundred microliters of the 2nd antibody was added to teach tube, followed by incubation for 72 hours. After incubation, 3 ml of cold PBS was added to each tube to dilute the unbound $^{125}I$-LH. Centrifugation at 3,000 rpm for 30 minutes in a refrigerated centrifuge precipitated the bound $^{125}I$-LH and the supernatant fluid was discarded. The bound $^{125}I$-LH in the precipitate was then quantified in an automatic gamma counter.

A standard curve was calculated from counts derived from the sets of standard sera using a computer program and multiple regression analysis procedures. The LH concentrations in the unknowns were computed by multiple regression analysis comparison with the standard curve.

Progesterone ($P_4$)

The assay technique used for $P_4$ has been described (Convey et al., 1977). Briefly, the assay utilized mare's serum in duplicate. Progesterone was extracted from 100 µl serum diluted with 100 µl of buffer by vortexing for 30 seconds with 2 ml of redistilled benzene-hexane solvent (1:2). This produced a benzene-hexane layer containing the $P_4$ at the top of the tube and an aqueous lower layer. The solvent layer has a lower freezing point than the aqueous layer, allowing separation by placing the tubes in a freezer for 1 hour and then pouring off the solvent layer containing the $P_4$ into assay tubes. Three sets of standards (.01 to 1.0 ng) were included in each assay.

Solvent was evaporated from the unknowns and standards in a vacuum oven.

Progesterone antibody (MSU anti-progesterone #74) was diluted 1:5,000 in PBS-0.1% Knox gelatin containing 1:100 normal rabbit serum. Two hundred microliters of the diluted antibody was added to each assay tube and vortexed. Then 200 $\mu l$ of $^3H-P_4$ (5,000 cpm/200 $\mu l$) was added to each tube. After 24 hours of incubation at 5 C, 400 $\mu l$ of 2nd antibody (sheep anti-rabbit gamma globulin) in PBS-0.1% Knox was added to each tube. After 48 hours of incubation the assay tubes were centrifuged at 3,000 rpm for 30 minutes and 0.5 ml of the supernatant fluid was diluted with scintillation fluid (4.5 ml) for radioactive quantification.

Estradiol-17β ($E_2$-17β)

The technique used for assaying $E_2$-17β has been described (Oxender et al., 1977a). The assay utilized mare's serum in triplicate. Estradiol was extracted from 0.5 ml serum by vortexing for 1 minute with 5 ml of redistilled benzene. This produced a benzene layer containing the estradiol at the top of the tube. The lower serum layer was frozen in dry ice-methanol bath and the benzene layer was decanted into assay tubes. Three sets of standards (0 to 50 pg estradiol-17β) were included in the assay. Benzene was evaporated from the unknowns and standards in a vacuum oven.

Estradiol antibody (MSU anti-estradiol #74) was diluted 1:5,000 in PBS-0.1% Knox gelatin and 1:100 normal rabbit serum and vortexed. After 24 hours of incubation at 5 C, 400 $\mu l$ of 2nd antibody (sheep anti-rabbit gamma globulin) in PBS-0.1% Knox was added to each tube. After 48 hours of incubation, the assay tubes were centrifuged at

3,000 rpm for 30 minutes and 0.5 ml of the supernatant fluid was diluted with scintillation fluid for radioactive quantification.

## Preparation of Treatments

Dr. J. W. Lauderdale of the Upjohn Company provided the $PGF_{2\alpha}$-Tham salt. The salt was reconstituted and diluted in 0.85% saline to the desired concentration of $PGF_{2\alpha}$ free acid (10 mg/3 ml) for individual mare treatment.

Estradiol-17β was purchased as a desiccated powder from Sigma Laboratories. Peanut oil was used as a diluent to obtain a final concentration of 3 mg estradiol-17β per 3 ml peanut oil for treatments.

The gonadotropin releasing hormone (GnRH) used was an analogue (D-Leu-6) supplied by Dr. S. Webel of Abbott Laboratories. The desiccated powder was dissolved in saline to provide 200 μg of GnRH per 3 ml saline.

## Statistical Analysis

Four separate 3 x 3 Latin squares were utilized with a one-way analysis of variance to test for significant effects of periods, treatments and residual effects of treatments. With this technique, any residual effects of treatments and/or period effects could be compensated for by using adjusted treatment means when testing treatments. Then, to qualify significant effects of treatments on the intervals from $PGF_{2\alpha}$ to estrus and $PGF_{2\alpha}$ to 1st ovulation, orthogonal contrasts were performed using a one-sided t-statistic.

A Latin square design with repeat measure was used to test the significance of changes in serum hormones utilizing a split-plot analysis of variance followed by orthogonal contrasts among treatments and contrasts over time when indicated.

A one-way analysis of variance was used to test for significant differences between ovulation intervals within treatment groups when looking at the effects of follicle size at the time of prostaglandin $F_{2\alpha}$ administration. A one-way analysis of variance was also used to compare the mean intervals for estrus duration within treatment groups for mares having 1 or 2 ovulations.

Distribution of multiple ovulations among the 3 different treatment groups A, B and C was evaluated utilizing Chi square analysis.

## RESULTS AND DISCUSSION

### Estrus and Ovulation after $PGF_{2\alpha}$, $E_2$-17$\beta$, and GnRH Treatments in Mares

The interval from $PGF_{2\alpha}$ (10 mg sc) treatment to estrus averaged 3.4 $\pm$ 0.2 days for the control mares (treatment A). Mares in treatment B had a similar interval of 3.2 $\pm$ 0.3 days; however, the interval from $PGF_{2\alpha}$ to estrus for treatment C mares, 2.3 $\pm$ 0.1 days, was significantly shorter ($p<0.5$) than for the other 2 groups (Table 3).

Table 3. Estrus and ovulation during a control cycle (A) and following treatments B and C in 12 mares[a]

| Interval | Treatment | | |
|---|---|---|---|
| | A[b] | B[c] | C |
| | (days) | | |
| From $PGF_{2\alpha}$ to estrus | 3.4 $\pm$ 0.2 | 3.2 $\pm$ 0.3 | 2.3 $\pm$ 0.1 |
| From $PGF_{2\alpha}$ to 1st ovulation | 7.6 $\pm$ 0.7 | 6.7 $\pm$ 0.6 | 6.2 $\pm$ 0.3 |
| From onset of estrus to 1st ovulation | 4.6 $\pm$ 0.6 | 3.5 $\pm$ 0.6 | 3.9 $\pm$ 0.4 |
| From last ovulation to end of estrus | 1.8 $\pm$ 0.3 | 1.9 $\pm$ 0.2 | 2.3 $\pm$ 0.2 |
| Duration of estrus | 6.6 $\pm$ 0.4 | 5.8 $\pm$ 0.7 | 6.3 $\pm$ 1.0 |

[a]Values are means $\pm$ SEM.

[b]n = 11

[c]n = 11

Treatment C mares received 3 mg of estradiol-17β on 2 consecutive days following $PGF_{2\alpha}$ and it seems likely the exogenous estradiol-17β was responsible for the earlier detection of behavioral estrus in these mares.

The behavioral response to exogenous estrogens appears to vary with the reproductive state of the animal. The administration of 5 mg of estradiol-17β to mares with an active corpus luteum will not induce estrus. However, ovariectomized mares or anestrous mares respond to 1 mg of estradiol-17β and show behavioral estrus within 3 to 6 hours (Hughes and Stabenfeldt, 1977). The response observed in $E_2$-17β treated mares in this experiment following induced luteolysis may be similar to mares in anestrus or ovariectomized mares, since low progesterone levels have also been reported for ovariectomized and anestrous mares.

The 1st ovulation in the treatment A mares was detected on the average 7.6 $\pm$ 0.7 days after $PGF_{2\alpha}$ administration or 4.6 $\pm$ 0.6 days from the onset of estrus. The signs of behavioral estrus ended 1.8 $\pm$ 0.3 days following the last ovulation and the mean duration of estrus was 6.6 $\pm$ 0.4 days for control mares (Table 3). Data for these control mares compare favorably with results reported by previous researchers for mares receiving prostaglandin $F_{2\alpha}$ (Douglas and Ginther, 1972; Allen and Rowson, 1973; Noden et al., 1973, 1974; Oxender et al., 1975a,b).

The interval from $PGF_{2\alpha}$ to 1st ovulation in treatment B mares averaged 6.7 $\pm$ 0.6 days and, in treatment C, 6.2 $\pm$ 0.3 days (Table 3). Although these intervals averaged nearly 1 day shorter than the controls, the differences were not statistically significant. There were no significant differences in the interval from last ovulation to the end of estrus, or duration of estrus among the 3 different treatment groups.

Previous work reported by Ginther and Wentworth (1974) showed that single injections (400 mg) of synthetic GnRH in pony mares on day 2 of estrus did not significantly alter follicular development or time of ovulation. Subsequent work by Garcia and Ginther (1975) tested the effects of several different doses and treatment regimes of GnRH including pretreatment with estradiol. In one experiment continuous intravenous infusion of GnRH sufficient to maintain an ovulatory LH concentration for 24 hours on day 2 of estrus did not alter ovulation time in the mare. Although pretreatment with estradiol-17β enhanced the LH response to a dose of GnRH, this treatment also failed to influence the time of ovulation.

Oxender et al. (1977b) conducted 2 experiments utilizing $PGF_{2\alpha}$ and GnRH in an attempt to predict or synchronize ovulation in mares. The 1st experiment demonstrated that mares treated subcutaneously with 2 or 5 mg of GnRH 96 hours after $PGF_{2\alpha}$ treatment had a 2.5-fold increase in serum LH; however, time of ovulation and duration of estrus were not significantly altered from controls. In a 2nd experiment, mares received GnRH (5 mg sc) daily (starting 96 hours after $PGF_{2\alpha}$) until ovulation occurred. Again the interval from $PGF_{2\alpha}$ treatment to ovulation and duration of estrus were not significantly shortened nor synchronized.

In contrast to the experiments with GnRH which failed to change the time of ovulation in mares, Irvine et al. (1975) reported a shorter duration of estrus for GnRH treated mares. Both intravenous and subcutaneous treatments with GnRH on day 2 of estrus significantly shortened duration of estrus and time to ovulation; the subcutaneous treatments were repeated daily until ovulation. The success of Irvine and his co-workers is not easily explained, since several authors have reported that GnRH treatment failed to change the time of ovulation in mares. However, 1 or 2 factors may account for the differences. One series of GnRH treatments was completed during April when the mean duration of estrus for the control mares was reported as 11.8 $\pm$ 2.1 days, and this is, it appears, significantly longer than the mean duration of estrus reported for mares in June and July of 5.2 $\pm$ 0.5 days (Noden et al., 1975). Secondly, Irvine et al. (1975) treated mares on day 2 of estrus rather than 96 hours after $PGF_{2\alpha}$ and not all $PGF_{2\alpha}$-treated mares were in estrus at the time of GnRH treatment in the latter experiments. Thus, it appears the time that GnRH is used during estrus may also be a factor.

A few researchers have treated estrous mares with GnRH to induce ovulation. Heinze and Klug (1975), in a clinical trial, reported that 38 of 39 mares ovulated a mature follicle within 48 hours following an injection of 4 mg GnRH during a period of estrus lasting more than 9 days. In contrast, only 11 of 16 nontreated control mares ovulated within the same period. Thus, GnRH appears to have the ability to induce ovulation in mares under certain circumstances.

## Serum Hormone Concentrations after Subcutaneous $PGF_{2\alpha}$

Serum progesterone averaged 5.4 $\pm$ 0.7 ng/ml in treatment A mares when 10 mg $PGF_{2\alpha}$-Tham salt was injected sc 7 days after the previous ovulation (Tables 4 and 7). Progesterone decreased rapidly ($p<.01$) to 1.3 $\pm$ 0.3 ng/ml within 24 hours and, by 48 hours, progesterone concentrations averaged below 1 ng/ml, where they remained

Table 4. Serum hormone concentrations during the estrous cycle following treatment with $PGF_{2\alpha}$ (10 mg sc) in 12 mares (control treatment A)[a]

| Day | Serum Hormone Progesterone | LH | Estradiol |
|---|---|---|---|
| | ----------(ng/ml)---------- | | --(pg/ml)-- |
| Day of $PGF_{2\alpha}$ treatment | 5.4 ± 0.7 | 141 ± 36 | 6.8 ± 1.6 |
| Two days after $PGF_{2\alpha}$ | 0.4 ± 0.0 | 197 ± 63 | 8.0 ± 1.5 |
| Onset of estrus[b] | 0.2 ± 0.0 | 298 ± 61 | 8.6 ± 1.4 |
| One day before ovulation | 0.2 ± 0.0 | 1035 ± 216 | 15.1 ± 2.3 |
| Ovulation detected | 0.3 ± 0.1 | 875 ± 164 | 6.3 ± 0.1 |
| End of estrus | 2.5 ± 0.3 | 1072 ± 224 | 3.7 ± 0.5 |

[a]Values are means ± SEM.

[b]Mares stood firmly when mounted by teaser stallion.

until ovulation occurred (Figure 1). Following ovulation, progesterone increased ($p<.05$) to 2.5 ± 0.3 ng/ml within 48 hours, indicating rapid luteinization of the corpus hemorrhagicum and signaling termination of estrus behavior. Progesterone levels continued to rise ($p<.05$) to a peak diestrus concentration of 6.2 ± 0.7 ng/ml 7 days later. The relative changes in serum progesterone following the induced luteolysis were comparable to those reported by Allen and Rowson (1973), Noden et al. (1974) and Oxender et al. (1975a,b).

The LH changes during estrus in these mares were similar to those previously reported by Pattison et al. (1974), Whitmore et al. (1973), Noden et al. (1974) and Oxender et al. (1975b). Serum LH averaged 141 ± 36 ng/ml when $PGF_{2\alpha}$ was given during mid-diestrus

Figure 1. Serum progesterone following $PGF_{2\alpha}$ (10 mg sc) in mare no. 10, showing 2 ovulations within 24 hours.

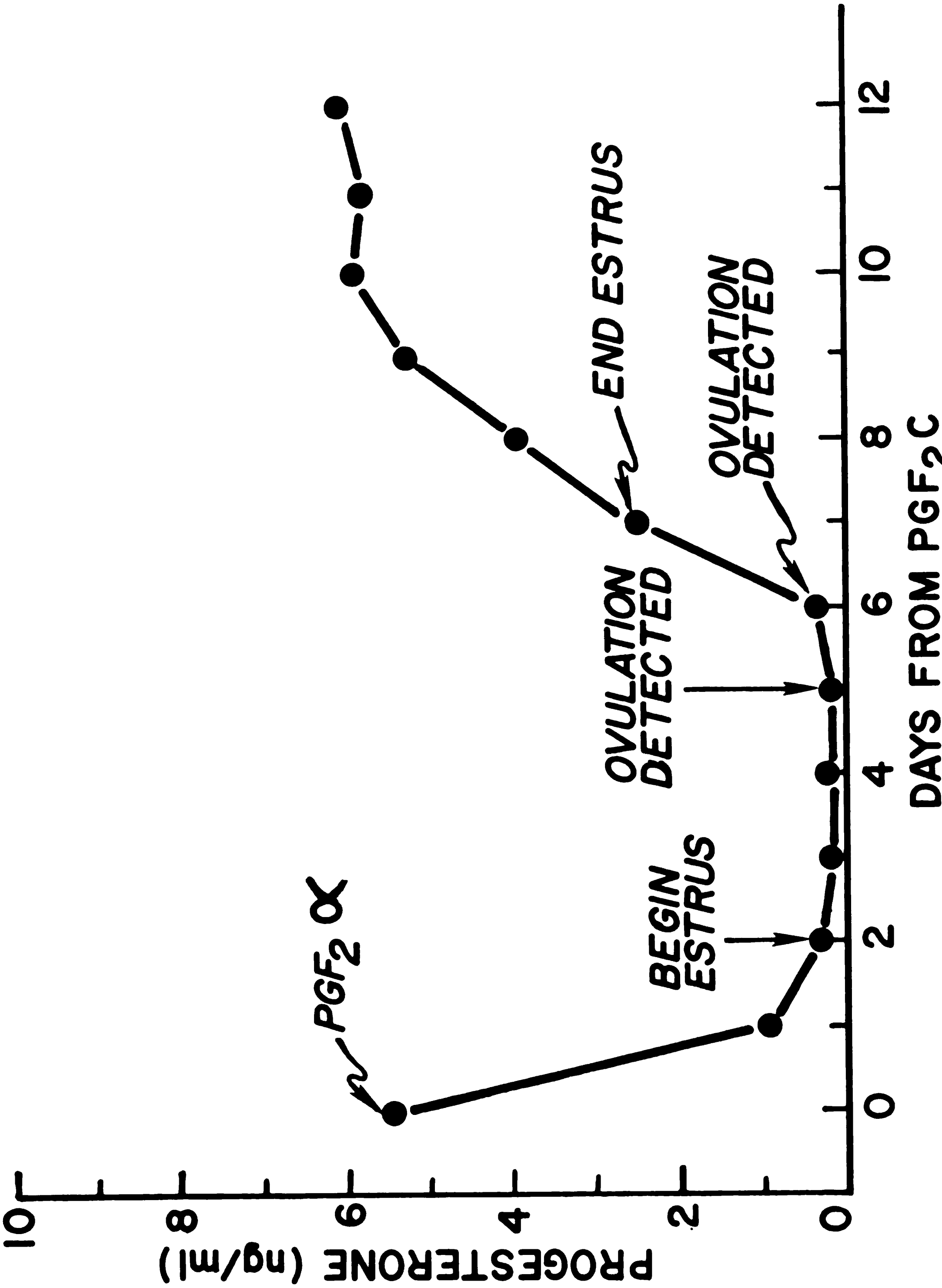
PROGESTERONE (ng/ml)
DAYS FROM $PGF_2C$
$PGF_2\alpha$
BEGIN ESTRUS
OVULATION DETECTED
OVULATION DETECTED
END ESTRUS
0
2
4
6
8
10
12

Figure 1

(Tables 4 and 7). The LH concentration increased to 298 $\pm$ 61 ng/ml by the onset of estrus and continued to increase throughout estrus to 875 $\pm$ 164 ng/ml by the time of ovulation, with a peak of 1072 $\pm$ 224 ng/ml by the end of estrus.

Estradiol concentrations for mares during the control estrus increased to a peak prior to ovulation. On the day of $PGF_{2\alpha}$ treatment, estradiol averaged 6.8 $\pm$ 1.6 pg/ml and had increased slightly to 8.6 $\pm$ 1.4 pg/ml at estrus onset (Tables 4 and 7). During estrus, estradiol levels increased ($p<.05$) to 15.1 $\pm$ 2.3 pg/ml 1 day prior to ovulation and then rapidly decreased by the day of ovulation to 6.3 $\pm$ 0.1 pg/ml ($p<.05$). The levels continued to decrease, averaging 3.7 $\pm$ 0.5 pg/ml by the end of estrus ($p<.05$). These results are in agreement with estradiol levels reported from the same laboratory by Noden et al. (1975) for mares during normal estrous cycles and Oxender et al. (1975b) for mares treated with prostaglandin $F_{2\alpha}$.

## Serum Hormone Concentrations after $PGF_{2\alpha}$ and GnRH

Serum progesterone values in treatment B mares followed a similar pattern to that of the control mares (Tables 5 and 7). At the time of $PGF_{2\alpha}$ administration, progesterone concentration averaged 5.3 $\pm$ 0.5 ng/ml and decreased to 0.3 $\pm$ 0.0 ng/ml within 48 hours. The serum concentration remained less than 1 ng/ml throughout estrus, increasing to 2.0 $\pm$ 0.3 ng/ml by the end of estrus. Diestrus values averaged 4.5 $\pm$ 0.6 ng/ml by the 7th day postovulation.

Serum LH averaged 176 $\pm$ 46 ng/ml on the day of $PGF_{2\alpha}$ treatment and had increased slightly to 249 $\pm$ 44 ng/ml 2 days after $PGF_{2\alpha}$ (Tables 5 and 7). These values were not different than those in the control mares, nor would they be expected to be different since GnRH

Table 5. Serum hormone concentrations during the estrous cycle following treatment with $PGF_{2\alpha}$ (10 mg sc) and GnRH (200 μg/injection) in 12 mares (treatment B)[a]

| Day | Serum Hormone: Progesterone | LH | Estradiol |
|---|---|---|---|
| | (ng/ml) | | (pg/ml) |
| Day of $PGF_{2\alpha}$ treatment | 5.3 ± 0.5 | 176 ± 46 | 8.6 ± 1.7 |
| Two days after $PGF_{2\alpha}$ | 0.3 ± 0.0 | 249 ± 44 | 10.7 ± 2.7 |
| Onset of estrus | 0.3 ± 0.0 | 389 ± 71 | 12.0 ± 2.8 |
| One day before ovulation | 0.5 ± 0.3 | 563 ± 104 | 16.8 ± 3.2 |
| Ovulation detected | 0.3 ± 0.1 | 549 ± 92 | 6.0 ± 0.9 |
| End of estrus | 2.0 ± 0.3 | 832 ± 195 | 4.6 ± 2.7 |

[a]Values are means ± SEM.

treatment was not begun until 3 days (72 hours) after $PGF_{2\alpha}$ injection. Onset of estrus varied in its time relation to $PGF_{2\alpha}$ treatment but averaged 3.2 ± 0.3 days after $PGF_{2\alpha}$ (Table 3). Serum LH at estrus onset averaged 389 ± 71 ng/ml, which was not significantly different than the 298 ± 61 ng/ml for control mares. The treatment effect of GnRH on serum LH at estrus onset was not evident when comparing data from Tables 4 and 5, since several mares would not yet have received their GnRH treatments. However, in comparing treatments A and B in Table 7, the GnRH treated mares showed a significant ($p<.01$) 2- to 3-fold increase in LH levels versus little or no change in the controls. An average concentration of 729 ± 80 ng/ml was obtained 8 hours following the 1st GnRH injection, and levels remained elevated throughout the GnRH treatment period.

It had not been anticipated that 3 injections of GnRH (200 μg/ injection) at 8-hour intervals would cause elevated serum LH for longer than 24 to 48 hours following treatment. And indeed, the serum LH was 563 $\pm$ 104 ng/ml 1 day before ovulation and 549 $\pm$ 92 ng/ml on the day of ovulation (which was significantly less than control values [$p<.01$]). Preliminary work done by Oxender et al. (1977b) had shown that serum LH was increased greater than 3-fold within 60 minutes after 5 mg of GnRH was given as a single dose or multiple doses repeated at 24-hour intervals. The serum LH had decreased within 8 hours after each GnRH injection and was similar to the control group by 24 hours (Figure 2). In those experiments ovulation was not hastened by the GnRH treatments. Based on this work, the present experiment was designed to test the hypothesis that repeated injections of GnRH given before serum LH levels had fallen back to baseline levels might produce an additive effect similar to that presented in Figure 3. Creating a sustained high serum LH level over a 24-hour period coupled with increasing endogenous LH release during estrus might have provided the stimulus for an earlier ovulation and a shortened period of estrus. Success was achieved in elevating serum LH 2- to 3-fold over the 24-hour duration of treatment and the interval from $PGF_{2\alpha}$ to 1st ovulation was approximately 1 day shorter for mares in this group (Table 3).

The pattern of estradiol-17β serum levels in treatment B mares during estrus closely resembled that of the control mares. Serum levels averaging 8.6 $\pm$ 1.7 pg/ml at the time of $PGF_{2\alpha}$ treatment, increased to a peak of 16.8 $\pm$ 3.2 pg/ml 24 hours prior to ovulation and then fell rapidly to 6.0 $\pm$ 0.9 pg/ml by the time ovulation

Figure 2. Average serum LH in mares given multiple 5 mg doses of GnRH at 24-hour intervals. Adapated from Oxender et al. (1977b).

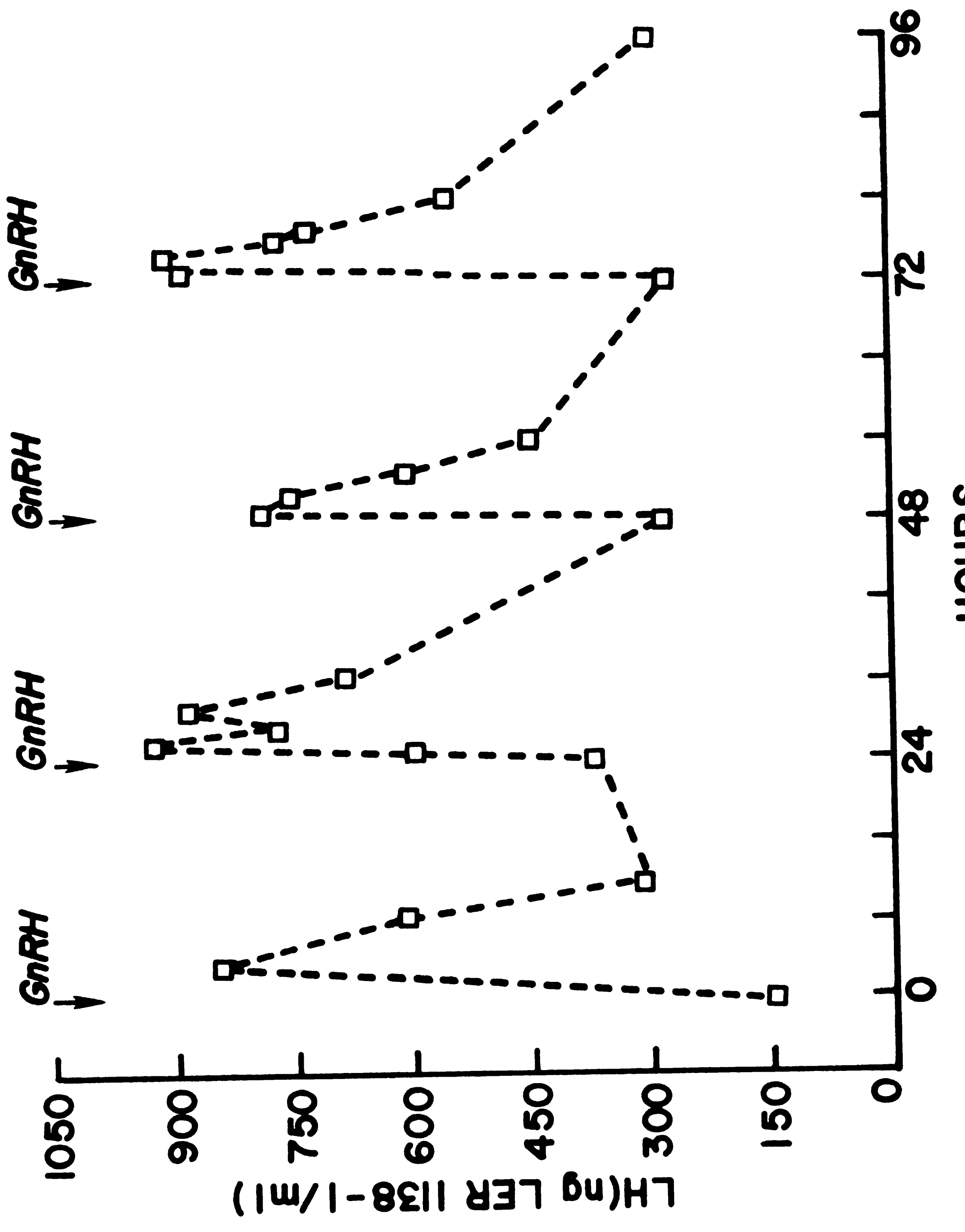
GnRH
GnRH
GnRH
GnRH
LH(ng LER 1138-1/ml)
1050
900
750
600
450
300
150
0
0
24
48
72
96
HOURS

Figure 2

Figure 3. Hypothetical serum LH curve expected following 3 injections of GnRH (200 μg/injection) at 8-hour intervals.

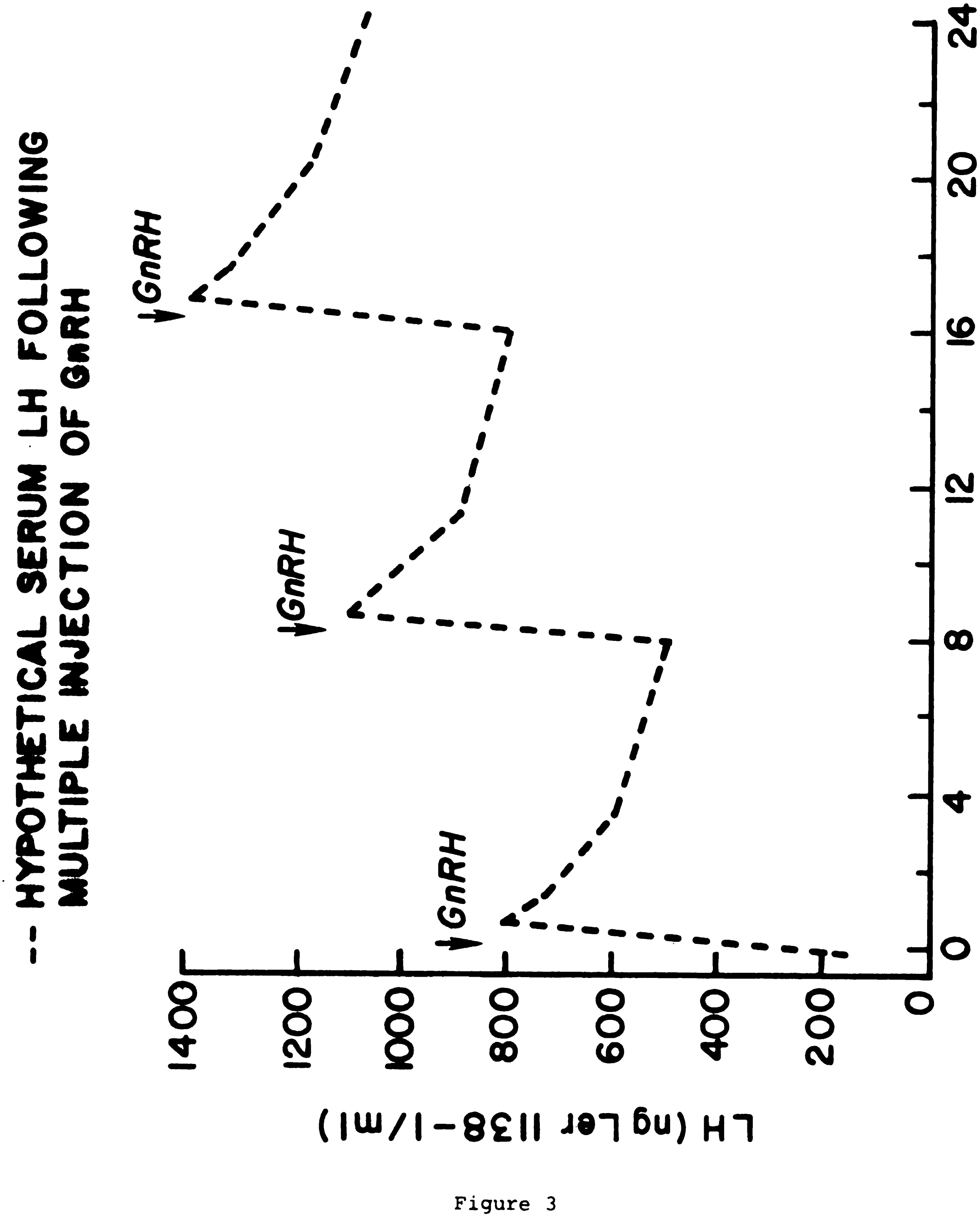
-- HYPOTHETICAL SERUM LH FOLLOWING MULTIPLE INJECTION OF GnRH
GnRH
GnRH
GnRH
HOURS
0
4
8
12
16
20
24
LH (ng Ler 1138-1/ml)
0
200
400
600
800
1000
1200
1400

Figure 3

occurred. The estradiol level had decreased to 4.6 ± 2.7 pq/ml by the end of estrus (Table 5).

## Serum Hormone Concentrations after $PGF_{2\alpha}$, $E_2$-17β and GnRH

Serum concentrations of progesterone for mares in treatment C did not differ significantly from mares in treatments A and B (Tables 6 and 7). The LH curve followed the trend of a gradual increase,

Table 6. Serum hormone concentrations during the estrous cycle following treatment with $PGF_{2\alpha}$ (10 mg sc), $E_2$-17β (3 mg sc) and GnRH (200 μg/injection) in 12 mares (treatment C)[a]

| Day | Serum Hormone Progesterone | LH | Estradiol |
|---|---|---|---|
| | ----------(ng/ml) | ---------- | --(pg/ml)-- |
| Day of $PGF_{2\alpha}$ treatment | 4.7 ± 0.4 | 141 ± 25 | 5.2 ± 1.0 |
| Two days after $PGF_{2\alpha}$ | 0.4 ± 0.1 | 189 ± 28 | 7.2 ± 1.5 |
| Onset of estrus | 0.3 ± 0.1 | 223 ± 30 | 7.6 ± 1.6 |
| One day before ovulation | 0.1 ± 0.0 | 268 ± 41 | 13.4 ± 1.9 |
| Ovulation detected | 0.2 ± 0.0 | 369 ± 74 | 6.5 ± 1.4 |
| End of estrus | 2.4 ± 0.1 | 569 ± 112 | 5.9 ± 0.7 |

[a]Values are means ± SEM.

peaking near the time of ovulation, similar to treatments A and B (Table 6). Concentrations of LH on the day of $PGF_{2\alpha}$ treatment averaged 141 ± 25 ng/ml, which was not different from treatments A and B, which had levels of 141 ± 36 ng/ml and 176 ± 46 ng/ml, respectively. Three injections of GnRH following pretreatment with $E_2$-17β induced an approximate 2-fold increase in serum LH levels in the

Table 7. Serum hormone concentrations in the 3 treatment groups following $PGF_{2\alpha}$[a]

| Treatment | Day | Progesterone | LH | Estradiol |
|---|---|---|---|---|
| | | Serum Hormone | | |
| | | ---------(ng/ml)---------- | | --(pg/ml)-- |
| Treatment A | 7 | 5.4 ± 0.7 | 141 ± 36 | 6.8 ± 1.6 |
| | 8 | 1.3 ± 0.3 | 119 ± 22 | 6.1 ± 2.0 |
| | 9 | 0.4 ± 0.0 | 197 ± 63 | 8.0 ± 1.5 |
| | 10 (1630 hrs) | 0.3 + 0.1 | 183 ± 29 | 8.5 ± 0.9 |
| | 10 (2400) | 0.3 ± 0.0 | 239 ± 35 | 8.2 ± 1.7 |
| | 11 (0800) | 0.5 ± 0.1 | 291 ± 101 | 13.4 ± 4.0 |
| | 11 (1630) | 0.3 ± 0.1 | 272 ± 63 | 14.1 ± 3.9 |
| Treatment B | 7 | 4.2 ± 0.4 | 176 ± 46 | 8.6 ± 1.7 |
| | 8 | 1.3 ± 0.3 | 182 ± 29 | 7.9 ± 1.9 |
| | 9 | 0.3 ± 0.1 | 249 ± 44 | 10.7 ± 2.7 |
| | 10 (1630) | 0.2 ± 0.1 | 352 ± 72 | 6.7 ± 1.1 |
| | 10 (2400) | 0.2 ± 0.1 | 729 ± 80 | 7.6 ± 1.5 |
| | 11 (0800) | 0.3 ± 0.0 | 868 ± 112 | 10.2 ± 2.2 |
| | 11 (1630) | 0.2 ± 0.1 | 615 ± 127 | 9.2 ± 2.0 |
| Treatment C | 7 | 6.0 ± 0.6 | 141 ± 25 | 4.8 ± 1.0 |
| | 8 | 1.3 ± 0.2 | 128 ± 20 | 4.8 ± 1.0 |
| | 9 | 0.3 ± 0.1 | 189 ± 28 | 6.1 ± 1.6 |
| | 10 (1630) | 0.5 ± 0.3 | 286 ± 42 | 5.8 ± 1.7 |
| | 10 (2400) | 0.4 ± 0.1 | 462 ± 97 | 12.2 ± 2.3 |
| | 11 (0800) | 0.4 ± 0.1 | 417 ± 74 | 9.1 ± 1.4 |
| | 11 (1630) | 0.4 ± 0.1 | 355 ± 79 | 9.6 ± 1.3 |

[a]Values are means ± SEM.

treatment C mares compared to control (treatment A) mares (Table 7). The LH response to GnRH appeared less in treatment C versus treatment B. One day before ovulation, the serum LH level was 268 $\pm$ 41 ng/ml, which was significantly less ($p<.01$) than treatment A (1035 $\pm$ 216 ng/ml) or treatment B (563 $\pm$ 104 ng/ml). The lower LH concentrations continued for this group through ovulation, averaging 569 $\pm$ 112 ng/ml at the end of estrus compared to 1,072 $\pm$ 224 ng/ml for control mares and 832 $\pm$ 195 ng/ml for mares in treatment B.

Estradiol levels were not different in the treatment C mares compared with the other 2 treatment groups (Tables 6 and 7). A level of 5.2 $\pm$ 1.0 pg/ml at the time of $PGF_{2\alpha}$ administration increased to 13.4 pg/ml 1 day prior to ovulation, then decreased to 6.5 $\pm$ 1.4 pg/ml when ovulation was detected. The serum concentrations of estradiol recorded in Tables 6 and 7 do not reflect the effects of the exogenous administration of 3 mg $E_2$-17β on 2 consecutive days following $PGF_{2\alpha}$ injection. Perhaps this is due to rapid transformation by the liver or excretion. The rationale for pretreating the mares with $E_2$-17β is based on data suggesting that estrogen plays a significant role in regulating gonadotropin secretion by the pituitary in several species. Reeves et al. (1971b) reported that pretreatment of anestrous ewes with estradiol facilitated an increased response of LH release induced by GnRH. It has also been demonstrated in the cow and the ewe that exogenous administration of estradiol would elicit a significant rise in plasma LH in the absence of high levels of progesterone. Progesterone has been shown to have a negative feedback effect on plasma LH in these species (Hansel and Echternkamp, 1972).

Garcia and Ginther (1976a) used ovariectomized mares to demonstrate that injecting 1 mg estradiol daily for 15 days caused plasma LH to also increase on a daily basis. Daily injections of 100 mg progesterone for 15 days caused a daily decrease in plasma LH, suggesting a similar regulatory role of estradiol and progesterone on gonadotropin secretion in mares as reported for the cow and ewe. In addition, previous work by Garcia and Ginther (1975) had shown that treatment of pony mares on day 2 of estrus with $E_2$-17β alone increased plasma LH concentration after 24 hours and that the LH response to a dose of GnRH given on day 3 was enhanced when compared to mares not pretreated with estradiol-17β.

In contrast, the results of this experiment do not demonstrate an augmented LH release following $E_2$-17β pretreatment. In fact, there may have been a negative effect on serum LH by the time of ovulation. The results might be related to the fact that the mares in this study were not treated on days 2 and 3 of estrus as in the above study, but rather 1 to 2 days prior to estrus. Another study by Keye and Jaffe (1974) suggests that the positive feedback effect of estradiol may be both dose and time related. They showed a decreased gonadotropin response to administered GnRH in women in the presence of midcycle levels of estradiol which was not consistent with their hypothesis that the midcycle surge of gonadotropins was due to increased sensitivity of the pituitary to GnRH brought about by increasing levels of estradiol.

In conclusion, it appears reasonable to assume that a time and dose relationship exists in the regulation of gonadotropin secretion by estradiol. What that relationship is awaits further elucidation in the mare.

## Effects of Ovarian Follicle Size on the Interval to First Ovulation

During this study, rectal palpation of mares in diestrus often detected large follicles (≥20 mm diameter) that seemed to persist from the preceding estrus or in other mares follicles appeared to develop rapidly during the early diestrus period. It appears that large follicles present at the time of $PGF_{2\alpha}$ treatment may ovulate within 48 hours (Table 8).

Table 8. The influence of ovarian follicle size at the time of $PGF_{2\alpha}$ administration on the interval in days to 1st ovulation in 12 mares

| Size of largest ovarian follicle (mm) | Treatment A | Treatment B | Treatment C |
|---|---|---|---|
| | (days) | | |
| 10-19 | 8.8 ± 0.7[a] (5)[b] | 5.8 ± 0.5 (8) | 6.7 ± 0.8 (7) |
| 20-29 | 6.7 ± 0.9 (3) | 5.0 ± 0.0 (2) | 4.5 ± 0.5 (2) |
| ≥30 | 4.5 ± 0.5 (2) | 6.0 ± 0.0 (1) | 3.3 ± 1.9 (3) |

[a]Mean interval in days ± SEM.

[b]Number of mares in each category.

Preliminary observation of the palpation records caused concern that mares having a large palpable follicle when given $PGF_{2\alpha}$ had a shorter interval to ovulation regardless of the treatment group. Four mares ovulated within 48 hours of $PGF_{2\alpha}$ treatment, and in these cases the interval to ovulation had to be discarded in favor of estimated values determined from the Latin square for purposes of statistical analysis of data.

When mares were grouped according to follicle size within each treatment group, mares in control treatment A showed a significantly shorter interval to ovulation ($p<.05$) when they had a follicle in the category 20-29 mm or 30 mm or greater (Table 8) at the time of $PGF_{2\alpha}$ treatment. The ovulation interval in days was $6.7 \pm 0.9$ and $4.5 \pm 0.5$, respectively, for the above follicular size groups, in contrast to the $8.8 \pm 0.7$-day interval for mares having follicles 10-19 mm in diameter. No significant differences between follicular size categories were observed in treatment B mares. Although not statistically significant because of small numbers of animals, mares in treatment C having a follicle 30 mm or larger had an ovulation interval ($3.3 \pm 1.9$ days) approximately one-half as long as the $6.7 \pm 0.8$-day interval recorded for mares having follicles 10-19 mm in diameter. Because the number of mares in each category was small, a meaningful interpretation of the results was not possible. Further study of the influence of follicular size at the time of treatment on the interval to ovulation is needed because this is an area of clinical importance for purposes of predicting the time of ovulation in mares.

Exogenous $PGF_{2\alpha}$ has been reported to cause a surge of LH in sheep (Carlson et al., 1973), cows (Hafs et al., 1975) and bulls (Kiser et al., 1976). The mechanism of action of the $PGF_{2\alpha}$ is not known. Recent work by Noden et al. (1978) demonstrated a transient 1.5- to 2.5-fold increase in serum LH 30 to 60 minutes following $PGF_{2\alpha}$ injection. The levels then declined toward pretreatment values during the next 3 hours. The reported transient increases in blood LH after $PGF_{2\alpha}$ treatment in mares, ewes, cows and bulls does not appear to be related to the endogenous LH surge occurring near the time of ovulation. Whether $PGF_{2\alpha}$ is exerting a direct or indirect (Drouin et al., 1976) effect on pituitary LH release is not known. However, exogenous progesterone prevented the LH increase observed at 3 to 9 hours after $PGF_{2\alpha}$ treatment in cows (Hafs et al., 1975), suggesting that the increase may be due to decreased progesterone and not to a direct effect on the hypothalamic-pituitary axis.

Additional experimental evidence in laboratory animals indicates the prostaglandins may be involved in the ovulation mechanism. Labhsetwar (1973) reported the occurrence of ovulation after injection of $PGF_{2\alpha}$ in pregnant hamsters, and Graafian follicles in rabbits were found to contain PGE and PGF, whose levels significantly increased as ovulation approached (Yang et al., 1974). Data also demonstrated that levels of $PGF_{2\alpha}$ in the rat ovary significantly increased before ovulation (Iseaka et al., 1975). In addition, Grinwich et al. (1972) blocked ovulation in rabbits using indomethacin, a prostaglandin inhibitor. Thus, it appears that prostaglandins in plasma and the ovary may be involved with follicular maturation and the process of ovulation in some species. In mares, exogenous $PGF_{2\alpha}$ treatment caused a transient surge in serum LH and a limited number of mares appeared to ovulate (based on rectal palpation) within 48 hours following injection of $PGF_{2\alpha}$. This ovulation may be triggered by the direct effect of exogenous $PGF_{2\alpha}$ on the follicle or the transient LH surge. However, ovulation does not occur within 48 hours in mares with small follicles at the time of $PGF_{2\alpha}$ treatment.

## Effect of Multiple Ovulations on Estrus and on the Luteolytic Response to $PGF_{2\alpha}$

There appeared to be a frequent occurrence of multiple ovulations during estrous cycles in this study. In 17 of the 36 (47%) estrous cycles studied, multiple ovulations were observed. Two ovulations were observed in most cases; however, 3 mares had 3 ovulations during an estrous cycle. There was no significant difference in the number of multiple ovulations among the 3 treatment groups (Table 9).

Table 9. The number of multiple ovulations in 12 mares during 36 total estrous cycles categorized according to treatment group

| Treatment | Number of Mares with 2 or More Ovulations |
|---|---|
| A | 4 |
| B | 7 |
| C | 6 |

It is difficult to compare results on frequency of ovulation with the previously reported literature because of the different experimental treatments. However, multiple ovulations are known to occur, and reported frequencies were 3.8% (Andrews and McKenzie, 1941), 25% (Stabenfeldt et al., 1972) and 42% (Warszawsky et al., 1972). Stabenfeldt et al. (1972) reported that mares with 2 ovulations averaged 1 day longer in estrus ($6.0 \pm 1.2$ days) than mares with 1 ovulation ($5.1 \pm 1.9$ days), although the results were not statistically significant. A similar pattern was noted in this study when ovulations and length of estrus were compared within treatment groups

(Table 10). Mares having 2 ovulations during estrus in treatment B had an estrus duration of 7.4 $\pm$ 1.3 days, which was longer than mares having only 1 ovulation (4.7 $\pm$ 0.5 days), and this difference approached ($p<0.10$) statistical significance. Likewise, mares in

Table 10. The influence of the number of ovulations during an estrus on the duration of estrus in 12 mares

| No. of ovulations/ estrus period | Treatment A | Treatment B | Treatment C |
|---|---|---|---|
| | (length of estrus [days]) | | |
| 1 | 6.7 $\pm$ 0.5[a] (7)[b] | 4.7 $\pm$ 0.5 (7) | 5.1 $\pm$ 0.8 (8) |
| 2 | 7.0 $\pm$ 0.7 (5) | 7.4 $\pm$ 1.3 (5) | 8.8 $\pm$ 2.3 (4) |

[a]Mean $\pm$ SEM.

[b]Number of mares in each treatment group.

treatment C with double ovulations also had a longer estrus (8.8 $\pm$ 2.3 days) than mares with single ovulations (5.1 $\pm$ 0.8 days).

The influence of multiple ovulations on the length of estrus should not be overlooked when comparing treatment effects among groups. The effects of double or multiple ovulations may also provide a partial explanation for the variation in estrus duration among mares. Although not analyzed statistically, the interval between ovulations occurring during estrus seemed to correlate with the length of estrus (compare Figures 1 and 4). Mare no. 10 (Figure 1) shows the typical progesterone curve for a mare having 2 ovulations during estrus within a 24-hour interval. Progesterone decreased (<1 ng/ml) approximately 24 hours following $PGF_{2\alpha}$ induced luteolysis and remained below 1 ng/ml throughout estrus, then increased again 24 hours after the 2nd ovulation. Stabenfeldt et al. (1972) also reported that mares ovulating 2 follicles usually did not have a progesterone rise until 24 hours after the 2nd ovulation. Mare no. 14 (Figure 4) received the same treatment as mare no. 10; however, the progesterone pattern was unusual because this mare ovulated a 45 mm follicle shortly after $PGF_{2\alpha}$ treatment. Following the 1st ovulation the progesterone level increased (>1 ng/ml) 3 days later, then decreased again. During this time the mare was not observed in estrus. Following the 2nd progesterone decrease to less than 1 ng/ml, the mare began showing estrus behavior which lasted 8 days. During estrus 2 more ovulations were detected in this mare, with progesterone finally increasing 24 hours after the last ovulation. After the 1st ovulation, functional luteal tissue apparently formed for a short period causing the transient rise in the progesterone concentration and the suppression of estrus behavior. A similar phenomenon was also reported by Stabenfeldt et al. (1972).

## Effect of Diestrus Ovulations on the Luteolytic Response to $PGF_{2\alpha}$ Administration

Ovulations were also detected during the diestrus period in mares on this study. However, following these ovulations no increase in progesterone levels was observed. A typical progesterone curve for a mare having a diestrus ovulation is depicted in Figure 5. Whether the diestrus ovulations are true ovulations or a form of follicular atresia remains debatable. Follicular atresia may be occurring without formation of luteal tissue, or they may be soft palpable corpora lutea that rapidly regress in size.

Figure 4. Serum progesterone following $PGF_{2\alpha}$ (10 mg sc) in mare no. 14 with 3 ovulations during an estrus.

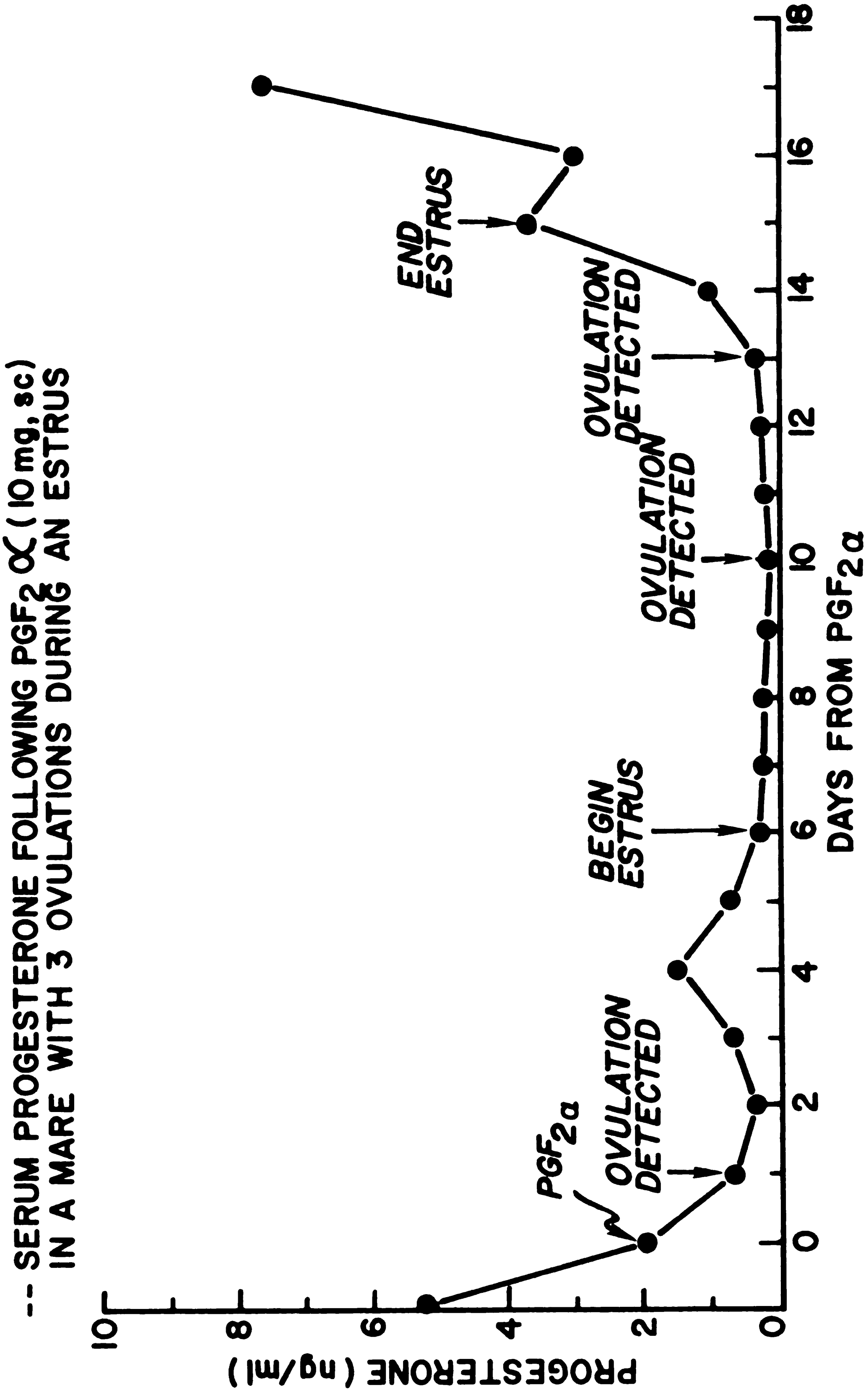
-- SERUM PROGESTERONE FOLLOWING PGF$_2$α (10 mg, sc)
IN A MARE WITH 3 OVULATIONS DURING AN ESTRUS
PROGESTERONE (ng/ml)
DAYS FROM PGF$_{2\alpha}$
PGF$_{2\alpha}$
OVULATION DETECTED
BEGIN ESTRUS
OVULATION DETECTED
OVULATION DETECTED
OVULATION DETECTED
END ESTRUS
0
2
4
6
8
10
12
14
16
18

Figure 4

Figure 5. Serum progesterone following $PGF_{2\alpha}$ (10 mg sc) in mare no. 2, showing an early ovulation with a short estrus and a diestrus ovulation.

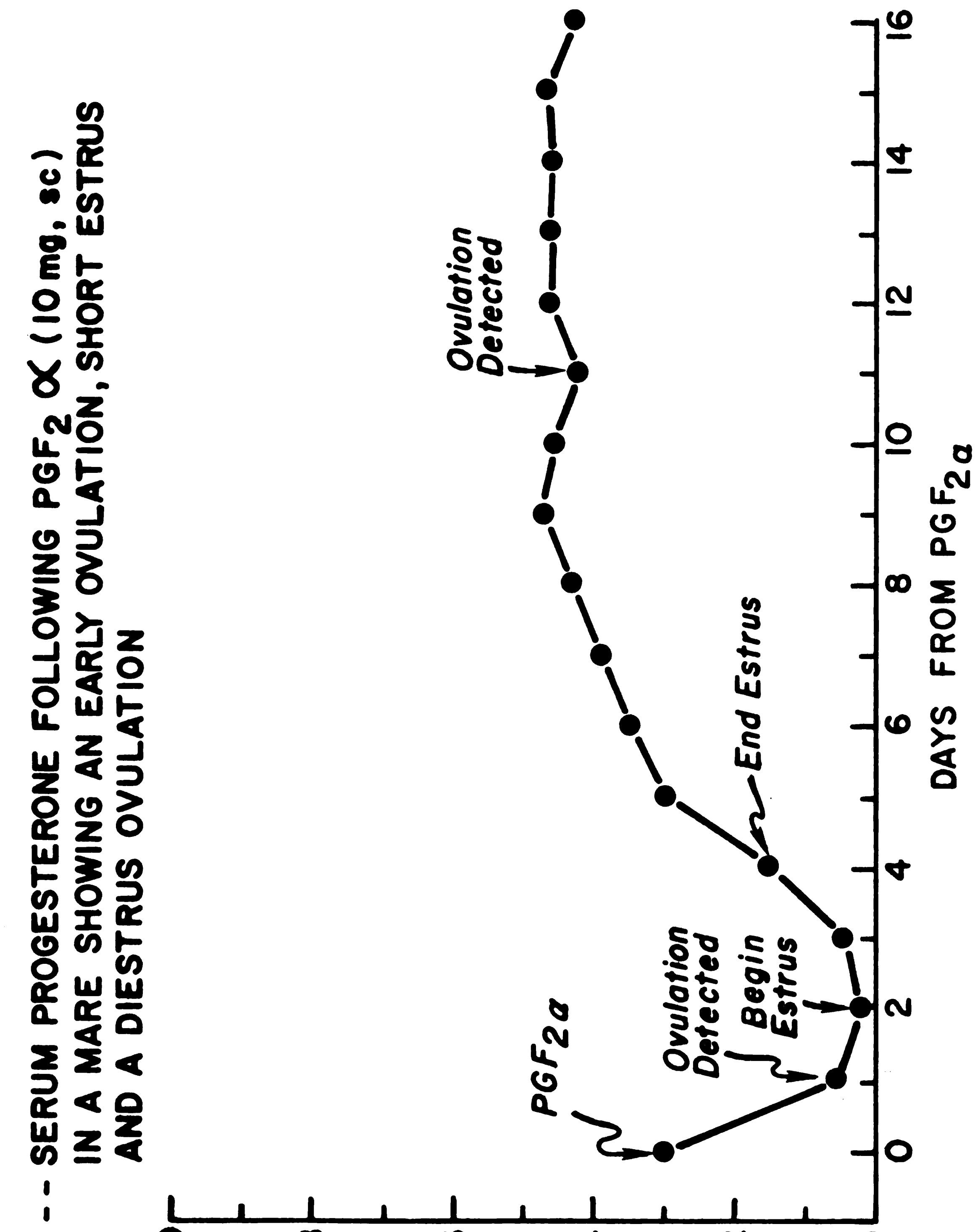
-- SERUM PROGESTERONE FOLLOWING PGF2 α (10 mg, sc)
IN A MARE SHOWING AN EARLY OVULATION, SHORT ESTRUS
AND A DIESTRUS OVULATION
PROGESTERONE (ng/ml)
DAYS FROM PGF2a
PGF2a
Ovulation Detected
Begin Estrus
End Estrus
Ovulation Detected
0
2
4
6
8
10
12
14
16

Figure 5

Evidence exists that ovulation of a follicle can occur in the presence of a functional corpus luteum. Hughes and Stabenfeldt (1977) reported on a mare that was artificially inseminated 24 hours after HCG was administered to facilitate ovulation of a diestrous follicle. Progesterone values at the time of HCG administration and insemination were greater than 1 ng/ml plasma. Ovulation occurred within 24 hours of the insemination and the mare was determined pregnant at 40 days. She subsequently delivered a live foal 368 days following insemination.

If following a diestrus ovulation functional luteal tissue forms, it does not appear to cause a detectable increase in serum progesterone concentrations. Ovulation during the diestrus period just prior to their scheduled $PGF_{2\alpha}$ injection was detected in 4 mares on this study.

It has been demonstrated that immature corpora lutea of less than 5 days' duration do not respond to the luteolytic effects of $PGF_{2\alpha}$ (Allen and Rowson, 1973; Douglas and Ginther, 1975a,b; Oxender et al., 1975a). If these diestrus ovulations had resulted in formation of functional corpora lutea, these mares would not have returned to estrus following $PGF_{2\alpha}$ treatment due to formation of new luteal tissue (Table 11). Luteolysis of the mature corpus luteum (CL) of 7 days' duration would be expected, but the immature CL theoretically should continue to develop and produce progesterone. All 4 mares with diestrus ovulations returned to estrus in 4 days or less, which compares favorably with the mean $PGF_{2\alpha}$ to estrus interval of $2.9 \pm 0.2$ days for all mares in the study and indicates the diestrus ovulation did not result in formation of a functional CL.

Table 11. Effect of diestrus ovulations on the luteolytic response to $PGF_{2\alpha}$ administration in 4 mares

| Mare No. | Age (days) of CL at Time of $PGF_{2\alpha}$ Injection | $PGF_{2\alpha}$ to Estrus Interval (days) |
|---|---|---|
| 4 | 7 and 3 | 4 |
| 6 | 7 and 2 | 3 |
| 10 | 7 and 4 | 3 |
| 15 | 7 and 1 | 2 |

## CONCLUSIONS

Serum hormone changes during the estrous cycle after $PGF_{2\alpha}$ differed only slightly among the 3 treatment groups except for the transient increase in LH following GnRH treatments. After 10 mg $PGF_{2\alpha}$-Tham salt, a significant decrease in progesterone occurred within 24 hours, verifying that $PGF_{2\alpha}$ was an effective luteolytic agent in mares. During estrus, progesterone remained below 1 ng/ml, and following ovulation serum concentrations increased above 1 ng/ml, indicating the end of estrus. Estradiol concentrations began increasing at a time when progesterone levels were decreasing. The estradiol increase preceded the LH increase, peaking 1 or 2 days before ovulation. Serum LH reached peak values near ovulation.

Mares which received 3 injections of GnRH at 8-hour intervals had a 2- to 3-fold increase in serum LH concentrations during the 24-hour treatment period. However, maximum LH concentrations near the time of ovulation were not greater than those of control mares. Although the interval from $PGF_{2\alpha}$ to the 1st ovulation in the GnRH treated mares averaged nearly 1 day shorter, this difference was not statistically significant.

Mares receiving 3 mg of estradiol-17β on 2 consecutive days after $PGF_{2\alpha}$ had a significantly shorter interval to estrus, averaging 1 day less than for the other treatment groups. Duration of estrus and the interval from ovulation to the end of estrus were not different among the 3 treatment groups. The failure to obtain a significant treatment effect on the interval from $PGF_{2\alpha}$ to ovulation and estrus duration indicates that the treatment regime probably did not provide a sufficient stimulus to affect follicular development and ovulation.

In conclusion, $PGF_{2\alpha}$ was an effective luteolytic agent in mares and GnRH treatment caused a transient increase in serum LH concentrations. Although only limited success was achieved in inducing ovulation in mares during this study, the information gained from this study will aid future studies on ovulation control. Perhaps a greater understanding of the interrelationships between the sex steroids and the gonadotropins will provide an answer to the regulatory mechanism of ovulation in the mare.

# BIBLIOGRAPHY

## BIBLIOGRAPHY

Allen, W. R. and M. J. Cooper. 1975. The use of synthetic analogues of prostaglandins for inducing luteolysis in mares. Ann. Biol. Anim. Bioch. Biophys. 15: 461-469.

Allen, W. E. and J. C. Hadley. 1974. Blood progesterone concentrations in pregnant and nonpregnant mares. Equine Vet. J. 6: 87-93.

Allen, W. R. and L. E. A. Rowson. 1973. Control of the mare's estrous cycle by prostaglandins. J. Reprod. Fert. 33: 539-543.

Anand, A. S., B. C. Wentworth, E. D. Plotka, J. L. Sullivan and N. L. First. 1973. Blood LH and progesterone level changes during the estrous cycle of the mare. J. Anim. Sci. 37: 299 (abst.).

Andrews, R. W. and F. F. McKenzie. 1941. Estrus, ovulation and related phenomena in the mare. Univ. of Mo. Agric. Exp. Stat. Res. Bull. 329: 1-117.

Arthur, G. H. 1958. An analysis of the reproductive function of mares based on post-mortem examination. Vet. Rec. 70: 682-686.

Bailey, V. E. and R. H. Douglas. 1977. Induction of ovulation in seasonally anovulatory mares with GnRH. Abstr. 69th Ann. Mtg. Am. Soc. Anim. Sci., Madison, WI.

Carlson, J. C., B. Barcikowski and J. A. McCracken. 1973. Prostaglandin $F_{2\alpha}$ and the release of LH in sheep. J. Reprod. Fert. 34: 357-361.

Convey, E. M., T. W. Beck, R. R. Neitzel, E. F. Bostwick and H. D. Hafs. 1977. Negative feedback control of bovine serum luteinizing hormone (LH) concentration from completion of the preovulatory LH surge until resumption of luteal function. J. Anim. Sci. In press.

Day, F. T. 1940. Clinical and experimental observations on reproduction in the mare. J. Agr. Sci. 30: 244-261.

Douglas, R. H. and O. J. Ginther. 1972. Effect of prostaglandin $F_{2\alpha}$ on length of diestrus in mares. Prostaglandins 2: 265-268.

Douglas, R. H. and O. J. Ginther. 1973a. Effect of prostaglandin $F_{2\alpha}$ in ewes and pony mares. J. Anim. Sci. 37: 308 (abst.).

Douglas, R. H. and O. J. Ginther. 1973b. Luteolysis following a single injection of prostaglandin $F_{2\alpha}$ in sheep. J. Anim. Sci. 37: 990-993.

Douglas, R. H., M. C. Garcia and O. J. Ginther. 1975. Luteolysis with prostaglandin $F_{2\alpha}$ or an antiserum against an equine pituitary fraction in hysterectomized mares. Am. J. Vet. Res. 36: 1793-1795.

Douglas, R. H. and O. J. Ginther. 1975a. Effects of prostaglandin $F_{2\alpha}$ on estrous cycle or corpus luteum in mares and gilts. J. Anim. Sci. 40: 518-522.

Douglas, R. H. and O. J. Ginther. 1975b. Effects of prostaglandin $F_{2\alpha}$ on the oestrous cycle and pregnancy in mares. J. Reprod. Fert., Suppl. 23: 257-261.

Douglas, R. H. and O. J. Ginther. 1976. Concentration of prostaglandins F in uterine venous plasma of anesthetized mares during the estrous cycle and early pregnancy. Prostaglandins 11: 251-260.

Drouin, J., L. Ferland, J. Bernard and F. Labrie. 1976. Site of the *in vivo* stimulatory effect of prostaglandins on LH release. Prostaglandins 11: 367-375.

Evans, M. J. and C. H. G. Irvine. 1975. Serum concentrations of FSH, LH and progesterone during the oestrus cycle and early pregnancy in the mare. J. Reprod. Fert., Suppl. 23: 193-200.

Evans, M. J. and C. H. G. Irvine. 1976. Measurement of equine follicle stimulating hormone and luteinizing hormone: Response of anestrous mares to gonadotropin releasing hormone. Biol. Reprod. 15: 477-484.

First, N. L. 1973. Synchronization of estrus and ovulation in the mare with methallibure. J. Anim. Sci. 36: 1143-1148.

Ganjam, V. K. and R. M. Kenney. 1976. Androstenedione, total estrogens and progesterone in the peripheral blood plasma of mares following prostaglandin $F_{2\alpha}$ administration. Abstracts of 9th Ann. Mtg. of Soc. for the Study of Reprod., Philadelphia, PA.

Garcia, M. C. and O. J. Ginther. 1974. Unpublished data. University of Wisconsin. Personal communication.

Garcia, M. C. and O. J. Ginther. 1975. Plasma luteinizing hormone concentration in mares treated with gonadotropin-releasing hormone and estradiol. Am. J. Vet. Res. 36: 1581-1584.

Garcia, M. C. and O. J. Ginther. 1976a. Effects of ovariectomy and season on plasma luteinizing hormone in mares. Endocrinology 98: 958-962.

Garcia, M. C. and O. J. Ginther. 1976b. LH control by estradiol and progesterone in mares. J. Anim. Sci. 43: 285 (abst.).

Geschwind, I. I., R. Dewey, J. P. Hughes, J. W. Evans and G. H. Stabenfeldt. 1975. Plasma LH levels in the mare during the oestrous cycle. J. Reprod. Fert., Suppl. 23: 207-212.

Ginther, O. J. 1971. Some factors which alter the estrous cycle in mares. J. Anim. Sci. 33: 1158 (abst.).

Ginther, O. J. 1974. Occurrence of anestrus, estrus, diestrus, and ovulation over a 12-month period in mares. Am. J. Vet. Res. 35: 1173-1179.

Ginther, O. J. 1977. Unpublished data. University of Wisconsin, Madison, WI. Personal communication.

Ginther, O. J. and B. C. Wentworth. 1974. Effect of a synthetic gonadotropin-releasing hormone on plasma concentrations of luteinizing hormone in ponies. Am. J. Vet. Res. 35: 79-81.

Ginther, O. J., H. L. Whitmore and E. L. Squires. 1972. Characteristics of estrus, diestrus and ovulation in mares and effects of season and nursing. Amer. J. Vet. Res. 33: 1935-1939.

Greenhoff, G. R. and R. M. Kenney. 1975. Evaluation of reproductive status of nonpregnant mares. J. Am. Vet. Med. Assoc. 167: 449-458.

Greenwald, G. S. 1970. Development of ovulatory refractoriness in the rabbit to cyclic injections of human chorionic gonadotropin. Fertil. Steril. 21: 163.

Grinwich, D. L., T. C. Kennedy and D. T. Armstrong. 1972. Dissociation of ovulatory and steroidogenic actions of luteinizing hormone in rabbits with indomethacin, an inhibitor of prostaglandin biosynthesis. Prostaglandins 1: 89.

Hafs, H. D., T. M. Louis, J. N. Stellflug, E. M. Convey and J. H. Britt. 1975. Blood LH after $PGF_{2\alpha}$ in diestrous and ovariectomized cattle. Prostaglandins 10: 1001-1009.

Hansel, W. and S. E. Echternkamp. 1972. Control of ovarian function in domestic animals. Amer. Zool. 12: 225-243.

Heinze, H. and E. Klug. 1975. The use of Gn-RH for controlling the oestrous cycle of the mare (preliminary report). J. Reprod. Fert., Suppl. 23: 275-277.

Holtan, D. W., R. H. Douglas and O. J. Ginther. 1974. Ovulation control in mares with progesterone or $PGF_{2\alpha}$. J. Anim. Sci. 39: 211 (abst.).

Holtan, D. W., R. H. Douglas and O. J. Ginther. 1977. Estrus, ovulation and conception following synchronization with progesterone, prostaglandin $F_{2\alpha}$ and human chorionic gonadotropin in pony mares. J. Anim. Sci. 44: 431-437.

Hughes, J. P. and G. H. Stabenfeldt. 1977. Conception in a mare with an active corpus luteum. J. Am. Vet. Med. Assoc. 170: 733-734.

Hutton, C. A. and T. N. Meacham. 1968. Reproductive efficiency on fourteen horse farms. J. Anim. Sci. 27: 434-438.

Iesaka, T., T. Sato and M. Igarashi. 1975. Role of prostaglandin $F_{2\alpha}$ in ovulation. Endocrinol. JPN. 22: 279-285.

Irvine, D. S., B. R. Downey, W. G. Parker and J. J. Sullivan. 1975. Duration of oestrus and time of ovulation in mares treated with synthetic GnRH (AY-24,031). J. Reprod. Fert., Suppl. 23: 279-283.

Kenney, R. M., V. K. Ganjam and R. V. Bergman. 1975. Non-infectious breeding problems in mares. Scope 19: 16-24.

Keye, W. R., Jr. and R. B. Jaffe. 1974. Modulation of pituitary gonadotropin response to gonadotropin-releasing hormone by estradiol. J. Clin. Endocrinol. Metab. 38: 805-810.

Kimball, R. A. 1976. Prostaglandin $F_{2\alpha}$ specific binding in equine corpora lutea. Soc. for the Study of Reprod., Abstracts of 9th Ann. Mtg., Philadelphia, PA.

Kiser, T. E., H. D. Hafs and W. D. Oxender. 1976. Increased blood LH and testosterone after administration of prostaglandin $F_{2\alpha}$ in bulls. Prostaglandins 2: 545-553.

Kooistra, L. H. and O. J. Ginther. 1976. Termination of pseudopregnancy by administration of prostaglandin $F_{2\alpha}$ and termination of early pregnancy by administration of prostaglandin $F_{2\alpha}$ or colchicine or by removal of embryo in mares. Am. J. Vet. Res. 37: 35-39.

Labhsetwar, A. P. 1973. Neuroendocrine basis of ovulation in hamsters treated with prostaglandin $F_{2\alpha}$. Endocrinology 92: 606-610.

Lauderdale, J. W. and P. A. Miller. 1975. Regulation of reproduction in mares with prostaglandins. Proc. 21st Ann. Conv. of Am. Assoc. Equine Practitioners, Boston, MA.

Loy, R. G. and J. P. Hughes. 1966. The effects of human chorionic gonadotropin on ovulation, length of estrus and fertility in the mare. Cornell Vet. 56: 41-50.

Loy, R. G. and S. M. Swan. 1966. Effects of exogenous progestogens on reproductive phenomena in mares. J. Anim. Sci. 25: 821-826.

Miller, K. F., L. J. Freedman, M. C. Garcia and O. J. Ginther. 1977. FSH, LH and progesterone during the estrous cycle in mares. Abstracts of 69th Ann. Mtg. of the Amer. Soc. of Anim. Sci., Madison, WI.

Nelson, A. M. R. 1976. The therapeutic activity, post-treatment fertility and safety of prostaglandin $F_{2\alpha}$-tham salt in clinically anoestrous mares: A review. Equine Vet. J. 8: 75-77.

Newton, J., W. Collins, M. Kilpatrick and J. Pyke. 1973. Studies on synthetic LH/FSH-releasing hormone. J. Reprod. Fert. 35: 622-623.

Niswender, G. D., A. R. Midgley, S. E. Monroe and L. E. Reichart, Jr. 1968. Radioimmunoassay for rat luteinizing hormone with antiovine LH serum and ovine $LH^{131}$-I*. Proc. Soc. Exp. Biol. Med. 128: 807-811.

Noden, P. A., H. D. Hafs and W. D. Oxender. 1973. Progesterone, estrus and ovulation after prostaglandin $F_{2\alpha}$. Fed. Proc. Fed. Am. Soc. Exp. Biol. 32: 229.

Noden, P. A., W. D. Oxender and H. D. Hafs. 1974. Estrus, ovulation, progesterone and luteinizing hormone after prostaglandin $F_{2\alpha}$ in mares. Proc. Soc. Exp. Biol. Med. 145: 145-150.

Noden, P. A., W. D. Oxender and H. D. Hafs. 1975. The cycle of oestrus, ovulation and plasma levels of hormones in mares. J. Reprod. Fert., Suppl. 23: 189-192.

Noden, P. A., W. D. Oxender and H. D. Hafs. 1978. Early changes in serum progesterone, estradiol and LH during prostaglandin-$F_{2\alpha}$ induced luteolysis in mares. J. Anim. Sci. In press.

Osborne, V. E. 1966. An analysis of the pattern of ovulation as it occurs in the annual reproductive cycle of the mare in Australia. Aust. Vet. J. 42: 149-154.

Oxender, W. D., P. A. Noden, T. M. Louis and H. D. Hafs. 1974. A review of prostaglandin $F_{2\alpha}$ for ovulation control in cows and mares. Am. J. Vet. Res. 35: 997-1001.

Oxender, W. D., P. A. Noden, D. L. Bolenbaugh and H. D. Hafs. 1975a. Control of estrus with prostaglandin $F_{2\alpha}$ in mares: Minimal effective dose and stage of estrous cycle. Am. J. Vet. Res. 36: 1145-1147.

Oxender, W. D., P. A. Noden and H. D. Hafs. 1975b. Oestrus, ovulation and plasma hormones after prostaglandin $F_{2\alpha}$ in mares. J. Reprod. Fert., Suppl. 23: 251-255.

Oxender, W. D., P. A. Noden and H. D. Hafs. 1977a. Estrus, ovulation, and serum progesterone, estradiol,,and LH concentrations in mares after an increased photoperiod during winter. Am. J. Vet. Res. 38: 203-207.

Oxender, W. D., P. A. Noden and M. C. Pratt. 1977b. Serum luteinizing hormone, estrus and ovulation in mares following treatment with prostaglandin $F_{2\alpha}$ and gonadotropin releasing hormone. Am. J. Vet. Res. 38: 649-653.

Palmer, E. and B. Jousset. 1975a. Urinary oestrogen and plasma progesterone levels in non-pregnant mares. J. Reprod. Fert., Suppl. 23: 213-221.

Palmer, E. and B. Jousset. 1975b. Synchronization of oestrus in mares with a prostaglandin analogue and HCG. J. Reprod. Fert., Suppl. 23: 269-274.

Pattison, M. L., C. L. Chen, S. T. Kelley and G. W. Brandt. 1974. Luteinizing hormone and estradiol in peripheral blood of mares during the estrous cycle. Biol. Reprod. 11: 245-250.

Pineda, M. H. and O. J. Ginther. 1972. Inhibition of estrus and ovulation in mares treated with an antiserum against an equine pituitary fraction. Am. J. Vet. Res. 33: 1775-1780.

Pineda, M. H., M. C. Garcia and O. J. Ginther. 1973. Effect of antiserum against an equine pituitary fraction on corpus luteum and follicles in mares during diestrus. Am. J. Vet. Res. 34: 181-183.

Pineda, M. H., O. J. Ginther and W. H. McShan. 1972. Regression of corpus luteum in mares treated with an antiserum against an equine pituitary fraction. Am. J. Vet. Res. 33: 1767-1773.

Plotka, E. D., D. M. Witherspoon and C. W. Foley. 1972. Luteal function in the mare as reflected by progesterone concentrations in peripheral blood plasma. Amer. J. Vet. Res. 33: 917-920.

Plotka, E. D., C. W. Foley, D. M. Witherspoon, G. C. Schmoller and D. D. Goetsch. 1975. Periovulatory changes in peripheral plasma progesterone and estrogen concentrations in the mare. Am. J. Vet. Res. 36: 1359-1362.

Quinlan, J., S. W. J. Van Rensburg and H. P. Steyn. 1951. The oestrous cycle of the mare when maintained under stabled conditions with restricted exercise at Onderstepoort. Onderstepoort J. Vet. Res. 25: 105-119.

Rance, T. A., B. K. Park, P. H. Rowe and P. D. G. Dean. 1976. A preovulatory rise of dehydroepiandrosterone in the mare measured by radioimmunoassay. J. Steroid Biochem. 7: 677-681.

Reel, J. R., R. R. Humphrey and W. C. Dermody. 1976. Luteinizing hormone-releasing hormone versus human chorionic gonadotropin: Differential effect on the development of ovulatory refractoriness and antibodies. Fertil. Steril. 27: 59-64.

Reeves, J. J., A. Arimura and A. V. Schally. 1971a. Pituitary responsiveness to purified luteinizing hormone-releasing hormone (LH-RH) at various stages of the estrous cycle in sheep. J. Anim. Sci. 32: 89-91.

Reeves, J. J., A. Arimura and A. V. Schally. 1971b. Changes in pituitary responsiveness to luteinizing hormone-releasing hormone (LH-RH) in anestrous ewes pretreated with estradiol benzoate. Biol. Reprod. 4: 88-92.

Rippel, R. H., E. S. Johnson and W. F. White. 1974. Effect of consecutive injections of synthetic gonadotropin releasing hormone on LH release in the anestrous and ovariectomized ewe. J. Anim. Sci. 39: 907-914.

Schally, A. V., A. J. Kastin and D. H. Coy. 1976. LH-releasing hormone and its analogues: Recent basic and clinical investigations. Inter. J. Fert. 21: 1-30.

Schreiber, J. R. and G. T. Ross. 1976. Further characterization of a rat ovarian testosterone receptor with evidence for nuclear translocation. Endocrinology 99: 590-596.

Sharp, D. C. and D. L. Black. 1973. Changes in peripheral plasma progesterone throughout the oestrous cycle of the pony mare. J. Reprod. Fert. 33: 535-538.

Smith, I. D., J. M. Bassett and T. Williams. 1970. Progesterone concentrations in the peripheral plasma of the mare during the oestrous cycle. J. Endocrinol. 47: 523-524.

Squires, E. L., B. C. Wentworth and O. J. Ginther. 1974. Progesterone concentration in blood of mares during the estrous cycle, pregnancy and after hysterectomy. J. Anim. Sci. 39: 759-767.

Stabenfeldt, G. H., J. P. Hughes and J. W. Evans. 1972. Ovarian activity during the estrous cycle of the mare. Endocrinology 90: 1379-1384.

Sullivan, J. J., W. G. Parker and L. L. Larson. 1973. Duration of estrus and ovulation time in nonlactating mares given human chorionic gonadotropin during three successive estrous periods. J. Am. Vet. Med. Assoc. 162: 895-898.

Swanson, L. V., H. D. Hafs and D. A. Morrow. 1972. Ovarian characteristics and serum LH, prolactin, progesterone and glucocorticoids from first estrus to breeding size in Holstein heifers. J. Anim. Sci. 34: 284-293.

Tannen, K. J. and E. M. Convey. 1977. Gonadotropin releasing hormone-induced change in serum luteinizing hormone, testosterone and androstenedione in bulls, steers and steers given testosterone. J. Anim. Sci. 44: 1080-1087.

Trum, B. F. 1950. The estrous cycle of the mare. Cornell Vet. 40: 17.

Van Niekerk, C. H. 1967. Pattern of the oestrous cycle in mares. II. The duration of the oestrous cycle and oestrous period. J. S. Afr. Vet. Med. Assoc. 38: 299-307.

Van Niekerk, C. H., W. H. Gerneke and J. S. Van Heerdon. 1973. Anatomical and histological observations on the reproductive tract of mares with abnormal oestrous cycles. J. S. Afr. Vet. Med. Assoc. 44: 141-152.

Van Niekerk, C. H., J. C. Morgenthal and W. H. Gerneke. 1975. Relationship between the morphology of and progesterone production by the corpus luteum of the mare. J. Reprod. Fert., Suppl. 23: 171-175.

Voss, J. L., J. J. Sullivan, B. W. Pickett, W. G. Parker, L. D. Burwash and L. L. Larson. 1975. The effect of HCG on duration of oestrus, ovulation time and fertility in mares. J. Reprod. Fert., Suppl. 23: 297-301.

Warszawsky, L. F., W. G. Parker, N. L. First and O. J. Ginther. 1972. Gross changes in internal genitalia during the estrous cycle in the mare. Am. J. Vet. Res. 33: 19-26.

Webel, S. K. 1975. Estrus control in horses with a progestin. J. Anim. Sci. 41: 385 (abst.).

Whitmore, H. L., B. C. Wentworth and O. J. Ginther. 1973. Circulating concentrations of luteinizing hormone during estrous cycle of mares as determined by radioimmunoassay. Am. J. Vet. Res. 34: 631-636.

Witherspoon, D. M. and R. B. Talbot. 1970. Nocturnal ovulation in the equine animal. Vet. Rec. 87: 302-304.

Yang, N. S. T., J. M. Marsh and W. J. LeMaire. 1974. Post ovulatory changes in the concentration of prostaglandins in rabbit graafian follicles. Prostaglandins 6: 37-44.

Zolman, J., E. M. Convey, J. H. Britt and H. D. Hafs. 1973. Release of bovine luteinizing hormone by purified porcine and synthetic gonadotropin releasing hormone. Proc. Soc. Exp. Biol. Med. 142: 189.

DIFFERENT METHODOLOGICAL APPROACHES TO
PHILOSOPHY OF SCIENCE