ESTRUS, OVULATION AND CHANGES IN SOME HORMONES DURING THE ESTROUS CYCLE OF MARES AND AFTER PROSTAGLANDIN $F_{2\ ALPHA}$

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ABSTRACT

ESTRUS, OVULATION AND CHANGES IN SOME HORMONES DURING THE ESTROUS CYCLE OF MARES AND AFTER PROSTAGLANDIN $F_{2\alpha}$

By

Patricia Ann Noden

The principal purpose of this research was to determine whether $PGF_{2\alpha}$ was luteolytic in horses, as had been reported in other species. Plasma progesterone was measured after intrauterine injection or subcutaneous injection of $PGF_{2\alpha}$ as an indication of luteal function. In the first experiment, I measured duration of estrus, time of ovulation during estrus, and plasma progesterone, estrone, estradiol, androstenedione and LH during four consecutive estrous cycles in six or eight mares. The first and third cycles served as controls for the second which was initiated by 10 mg intrauterine (iu) $PGF_{2\alpha}$. The fourth cycle was initiated by 15 mg subcutaneous (sc) $PGF_{2\alpha}$.

During a control estrous cycle the interestrual interval was 14.5 days, estrus persisted 5.2 days and ovulation was detected 1.5 days before estrus ended. At 7 to 9 days after ovulation during a control cycle, 10 mg $PGF_{2\alpha}$-Tham salt was deposited in the uterus of six mares. After iu $PGF_{2\alpha}$, estrus began 2.2 days later; ovulation occurred 1.6 days before the end of estrus which persisted 7.5 days. These criteria measured during the subsequent

control estrous cycle (cycle III) were similar to those measured during the control cycle which preceded $PGF_{2\alpha}$ treatment; estrus persisted 5.6 days, ovulation occurred 1.8 days before the end of estrus.

In all three cycles, during the 3 days before estrus began, progesterone decreased from values typical of diestrus (13 to 17 ng/ml) to values below 1 ng/ml when estrus began ($P < .01$); and then increased for 7 to 9 days after ovulation to diestrual values. In all three cycles, estradiol increased from low values (2 to 4 pg/ml) during diestrus to a peak (10 to 17 pg/ml) 1 or 2 days before ovulation, and decreased to diestrual concentrations by the time ovulation occurred. Androstenedione also increased to a peak before the time of ovulation, but estrone did not change significantly throughout the three cycles. Plasma LH began to increase 1 day before estrus, had increased 10-fold ($P < .01$) by the time ovulation occurred 6 to 8 days later, and remained high until progesterone increased near the end of estrus. Then LH decreased gradually for 7 to 8 days after the end of estrus.

When sc $PGF_{2\alpha}$ (15 mg) was given to eight mares 7 to 9 days after ovulation, estrus began 2.8 days later, persisted 7.8 days, and ovulation was detected 2.1 days before the end of estrus. Hormone changes resembled those in the first three cycles. In summary, $PGF_{2\alpha}$ was luteolytic in mares, whether given iu or sc, and ovulation occurred with relatively precise synchrony--at 8.0 ± 0.6 and 8.0 ± 1.0 days after $PGF_{2\alpha}$.

In a second experiment designed to determine the minimal effective dose of $PGF_{2\alpha}$-Tham salt, mares were given, 2, 3, 5 or 10 mg $PGF_{2\alpha}$ (sc) 7 to 9 days after ovulation. As determined by changes in blood progesterone and interval to onset of estrus, 2 or 3 mg $PGF_{2\alpha}$ each caused luteolysis in 3 of 4 mares, whereas 5 or 10 mg $PGF_{2\alpha}$ caused luteolysis in all mares treated on day 7 to 9 after ovulation. Among the mares which responded to $PGF_{2\alpha}$, estrus began within 3.2 ± 0.3 days and estrus persisted 6.9 ± 0.4 days. Ovulation occurred 1.8 ± 0.2 days before the end of estrus, about 8.3 ± 0.4 days after $PGF_{2\alpha}$. Therefore, 5 mg $PGF_{2\alpha}$ appeared to be the minimal effective dose to cause luteolysis; 3 mg $PGF_{2\alpha}$ caused luteolysis in some mares.

A third experiment was designed to determine the time after ovulation when the corpus luteum (CL) becomes susceptible to luteolysis by $PGF_{2\alpha}$. On day 1, 3, 5 or 7 after ovulation, mares were given 10 mg $PGF_{2\alpha}$-Tham salt (sc). As determined by changes in blood progesterone and interval to onset of estrus, $PGF_{2\alpha}$ was luteolytic in none of five mares treated on day 1, two of five on day 3, and in all mares treated on day 5 and day 7. Among the mares which responded to $PGF_{2\alpha}$, estrus began 2.6 ± 0.4 days after $PGF_{2\alpha}$ and persisted 7.6 ± 0.6 days. Ovulation occurred 1.9 ± 0.3 days before the end of estrus--8.3 ± 0.8 days after treatment with $PGF_{2\alpha}$. Therefore, while $PGF_{2\alpha}$ induced luteolysis in some mares 3 days after ovulation, it appeared to be luteolytic in all mares by 5 days after ovulation.

The luteolytic property of $PGF_{2\alpha}$ was also tested in non-cycling mares with abnormally extended luteal function. In three mares which had exhibited estrus for an average of 35 days, $PGF_{2\alpha}$ treatment caused luteolysis--estrus and ovulation followed. Estrus began within 2.6 ± 0.4 days after $PGF_{2\alpha}$ and persisted 5.0 ± 1.1 days; ovulation occurred 0.7 ± 0.6 days before the end of estrus, 6.9 ± 0.5 days after $PGF_{2\alpha}$ treatment.

To test fertility, eight mares were mated during the estrus after $PGF_{2\alpha}$ treatment. Six of the eight mares conceived. One aborted in early gestation; while in the remainder, the duration of gestation was normal (332 days) and the foals were healthy.

I conclude that $PGF_{2\alpha}$ is luteolytic in mares, in sc doses $\geq$ 5 mg (Tham salt), when given at least 4 or 5 days after ovulation. The estrus following $PGF_{2\alpha}$ does not differ from control cycles. $PGF_{2\alpha}$ appears to have practical potential for control of estrus and synchronization of ovulation in mares. Possibly mares could be bred once about 7 or 8 days after $PGF_{2\alpha}$ without regard to estrous behavior.

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By

Patricia Ann Noden

A DISSERTATION

Submitted to
Michigan State University
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DOCTOR OF PHILOSOPHY

Departments of Physiology and Dairy Science

1975

To the mind--

it can think if you use it!

BIOGRAPHICAL SKETCH

of

Patricia Ann Noden

I was born in Los Angeles, California, on June 12, 1942, to Winifred and William Fail. My parents had migrated to California 5 years earlier to escape the results of dust bowl and depression in the Kansas farming community in which they had resided. However, in 1948, my parents purchased a farm in Wilson County, Kansas, and I began my grade school education in a one-roomed rural school. After completion of my eighth year, I entered Midway Consolidated High School near Buffalo, Kansas. I received an A.A. degree from Chanute Junior College in 1962 and a B.S. and M.S. in Zoology from Kansas State University in 1964 and 1966. During the next year I worked with Dr. Webb Haymaker, a neuropathologist at the National Aeronautics and Space Administration Base at Moffett Field, California. Then during the next 4 years I taught biology, general science and general mathematics at Sanger High School near Fresno, California.

In September of 1970 I enrolled at Michigan State University in the Physiology Department. During this year I was accepted by Dr. Harold Hafs and the Department of Dairy Science to continue my studies for a Ph.D. degree. The past 4 1/2 years at Michigan State University have been rewarding. I have assisted in teaching

classes in three departments and participated in research projects in four laboratories. These experiences and the association with Dr. Harold Hafs, his associates and fellow graduate students in the Animal Reproduction Laboratory have contributed immensely to not only my professional but also my personal maturation. The completion of this thesis and the Ph.D. degree in June 1975 brings anticipation of what lies ahead: an opportunity to continue to improve as a teacher, a researcher and as a person.

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I am deeply indebted to my major professor, Dr. Harold Hafs, for continued encouragement, support and direction during my graduate studies. I especially appreciate his continued efforts to impart to me his optimism, enthusiasm and intellectual concern for teaching and research and their application to practical situations. I also appreciate his help with technical procedures, statistical analysis, and seminar and publication preparation. I am also grateful for the advice and support of my committee members, Drs. S. D. Aust, J. L. Gill, G. D. Riegle and H. A. Tucker.

This thesis is a summation of the continued effort of several people in addition to myself. Dr. Wayne D. Oxender procured animals, directed and assisted with the rectal palpations, estrus behavior determinations and blood sampling. Additionally, the unselfish help and encouragement of Mary Vomachka is gratefully acknowledged. Rex Payne, Alan Swanson, Steve Wilson during 1972, and David Bolenbaugh and Cherolee Ricky during 1973 helped with

collection of samples, organization of data and afforded encouragement. Providing additional assistance with RIA methods were Drs. Wayne Oxender, Lee Edgerton and Edward Convey. For technical assistance with RIA's, column chromatography and radioiodination of LH I wish to thank Candice Rankin, Janice Mapes, Patty Kaneshiro, David Jolly and Jim Byrne. For advice with statistical analysis, I acknowledge Dr. Roger Neitzel.

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To all of these people and to numerous others I say, "Thank you for your help and encouragement." Without them this thesis would not have been written.

LIST OF TABLES

LIST OF FIGURES

TABLE OF CONTENTS

INTRODUCTION

"The notorious infertility of mares has raised the question of the relationship of the time of service to ovulation" (Asdell, 1946). It is now generally agreed that an insemination or mating which occurs no more than 24 hours prior to ovulation is more fertile than those earlier or later in the estrual period. To ensure this requires either repetitive breeding (or insemination) throughout estrus, or accurate prediction of ovulation time. However, the time of ovulation is difficult to predict in the mare, since it occurs sometime during a 4- to 10-day estrus. Unfortunately, the interval from onset of estrus to ovulation is highly variable while the interval from ovulation to the end of estrus is relatively constant. In addition, estrus in the mare is difficult to detect except in the presence of a stallion.

The prolonged estrus and variable time of ovulation are not the only problems associated with reproductive efficiency and conception in the mare. In addition, the mare is a seasonal breeder, with an anovulatory period during the fall and winter. This limits opportunity for conception. Furthermore, fertility may be decreased by physiological factors common in other species as well as in mares, factors such as ovarian dysfunction or uterine infection. Finally, owing to the birthdate requirements of horse breed associations, the most desirable breeding period

is as near after the first of the year as possible, a time when most mares have just begun to cycle and breedings result in a low fertility.

Because of the low fertility in mares, many researchers have attempted to control the time of ovulation. Gonadotropic hormones which stimulate follicular growth and ovulation, steroid hormones which delay or hasten the onset of estrus and changes in the photoperiod have been used with moderate success, but precise synchronization of ovulation had not been achieved.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), a 20-carbon fatty acid, caused luteolysis (i.e., the death or demise of the corpus luteum) in rats (Pharris and Wyngarden, 1969), sheep (McCracken et al., 1972) and heifers (Louis et al., 1972). Ovulation occurred with reasonable synchrony (99 ± 12 hours) after $PGF_{2\alpha}$ treatment in cows (Louis et al., 1972). If $PGF_{2\alpha}$ induced luteolysis, estrus and synchronous ovulation in horses, it could contribute materially to efficient breeding management of mares.

The major objective of this study was to determine if $PGF_{2\alpha}$ was luteolytic in mares. I compared the luteolysis after deposit of $PGF_{2\alpha}$ in the uterus (iu) with that of various doses of $PGF_{2\alpha}$ injected subcutaneously (sc) during diestrus. Also, I determined how soon following ovulation $PGF_{2\alpha}$ would cause luteolysis. Furthermore, to determine if endocrine events associated with the estrous cycle differed from normal after $PGF_{2\alpha}$-induced luteolysis, I determined blood progesterone, estradiol, estrone, androstenedione and LH, and I estimated the duration of estrus and the time of ovulation in mares given $PGF_{2\alpha}$ and in control mares.

REVIEW OF LITERATURE

Reproductive Patterns of the Mare (Equus caballas)

Seasonal Changes

Walter Heape was one of the first (1900) to state that the mare is a "polyoestrous animal with a tendency toward monoestrum." Eckstein and Zuckerman (1956b) reviewed the evidence in wild species of the genus Equus and concluded that "The breeding season lasts from April to September, ovulation apparently being limited to the period May-August." Observations by John Hammond in 1936 indicated that the "breeding season" was a function of degrees latitude, being less marked nearer the equator than in Canada and Britain (Eckstein and Zuckerman, 1956b). Marshall (1936) concluded that "The results as a whole undoubtedly suggest a correlation between the sexual season and the incidence of daylight," because seasonal breeders (ewes, mares) transferred from the southern hemisphere to the northern hemisphere exhibited two "seasonal seasons" during one calendar year. For example, a pony mare after having exhibited normal estrous cycles in the southern hemisphere during November to February was then transported to Scotland (56°N). This mare adjusted to the increasing ambient photoperiod and exhibited cyclic estrual activity--as did her new herdmates--in the spring. This same phenomena was observed when ewes, ferrets and red deer were transferred from the southern to the northern hemisphere (Marshall, 1936; Eckstein and Zuckerman, 1956b).

Thus, depending on environmental conditions, there is a period of ovarian inactivity sometimes termed the "winter anestrum" in the majority of mares. In Michigan, this period begins in September and continues until April in most mares. In Kentucky, 75 to 85% of mares showed signs of estrus and ovulated about every 19 to 22 days from April through October, but only 20 to 25% of the mares showed evidence of ovarian cyclicity as early as January and February (Hughes et al., 1972) Nishakawa (1959) concluded that on the average, mares in Japan (similar in latitude to Kentucky) exhibited regular estrous cycles from April to October. Among mares in the southern hemisphere, 75% or more ovulated cyclically between mid-November to mid-April, and the lowest incidence of ovulation was from mid-June to mid-September (Osborne, 1966; Van Niekerk, 1967).

The Estrous Cycle

During the time of the year when the majority of mares were exhibiting regular estrous cycles, the estrous cycles ranged from 19 to 23 days and the duration of estrus ranged between 4 and 9 days (Asdell, 1946). Andrews and McKenzie (1941) reported an average estrous cycle of 20.7 days and 5.3 days duration of estrus in 45 draft and 38 light mares over the breeding seasons of 2 years. Data on duration of the estrous cycle and duration of estrus were similar for horses and ponies in Japan (Nishakawa, 1959).

Attempts to characterize vaginal, cervical and external genitalial changes during estrus in the mare indicated most were not reliable predictors of estrus. Color changes in the labia and vaginal mucosa occurred, but they were extremely variable. The

vaginal and cervical mucosa were highly vascular near the time of ovulation, and secretions from these tissues were most abundant during estrus. Changes in vaginal cell types did not correlate consistently with stages of the estrous cycle; however, some cornification of vaginal surface epithelium occurred during estrus presumably due to increased estrogens (Andrews and McKenzie, 1941). Nishakawa (1959) demonstrated changes in viscosity of cervical mucus throughout the cycle, but like Andrews and McKenzie (1941) found no consistent changes in vaginal cytology.

A more reliable predictor of estrus is the muscular tone and position of the cervix in the vaginal canal. During estrus the cervix became flaccid and endematous, and the cervical lumen was easily penetrated. During diestrus the cervix acquired more muscular tone tightly sealing the cervical lumen, and extended as a noticeable protrusion into the vagina. In addition, the vascularity of the cervix decreased during diestrus, resulting in detectable color differences (Andrews and McKenzie, 1941). The cervical changes in color and muscular tone during the estrous cycle could be used to detect the phase of estrus (or diestrus) in mares but they were not specific enough to predict when ovulation would occur.

Even if these criteria accurately reflected stage of estrous cycle, collection of cervical mucus, vaginal cytology and observation of cervical color or tone would require frequent use of a vaginal speculum. The technical difficulty of the above procedures coupled with the increased hazard of uterine infection following

use of the vaginal speculum limit their usefulness as monitors of stage of estrous cycle. In addition, these changes were not sufficiently specific to predict ovulation.

However, estrus behavior can be detected by use of a stallion. Estrus is the only portion of the cycle during which mares respond positively to the attentions of a stallion. Positive responses to the "teaser" stallion included elevation of the tail, spreading the rear legs and squatting. This characteristic stance was accompanied by "winking" (eversion of the clitoris through the labia) and frequent urination (Andrews and McKenzie, 1941). Nishakawa (1959) used a five-point scale to rate intensity of estrual behavior during estrus, while Andrews and McKenzie (1941) rated sexual behavior during the entire cycle on a six-point scale. Both authors reported an intensification of sexual receptivity before ovulation.

Anatomically, the ovary of the mare differs from that of other species in two important ways. Firstly, the ovary is covered by by a fibrous capsule except for the ovulation fossa, a groove in the ventral surface of the ovary. Secondly, it is through this groove that follicular rupture occurs (Asdell, 1946). The small follicles distributed throughout the ovarian stroma migrated to the ovulation fossa as they grew (Eckstein and Zuckerman, 1956a). After ovulation, the ruptured follicle collapsed, filled with blood to form a corpus hemorrhagicum and the corpus luteum began to form from granulosa cells. The corpus luteum (CL), was mushroom-shaped with the stem turned toward the ovulation

fossa. Since the CL was only about 50 to 75% the size of the follicle and buried in the stroma of the ovary, it was difficult to detect via rectal palpation (Day, 1940; Andrews and McKenzie, 1941; Asdell, 1946).

Follicles are detectable (1 to 2 cm) via rectal palpation around the onset of estrus and increase to a maximum size (5 to 6 cm diameter) just before ovulation. Andrews and McKenzie (1941) palpated follicles ranging from 1.0 cm to 7.5 cm during estrus; those of draft mares were larger than those in light mares. Follicles on the left ovary were larger than those on the right, probably because about 60% of the ovulations occurred from the left ovary. Double ovulations were infrequent (3.8%). Although somewhat smaller than follicles of mares, the follicles of ponies also increased in size as estrus progressed (Nishakawa, 1959). Nishakawa (1959) reported that 25% of the 107 ovulations recorded occurred 2 days before the end of estrus and 75% occurred 1 day before the end of estrus. In agreement, estrus ended 24 to 48 hours after ovulation in mares studied by Andrews and McKenzie (1941).

Follicular fluid (Andrews and McKenzie, 1941; Short, 1961) and urine of mares (Loy, 1970; Dohn and Cole, 1971) contain estrogens. Urinary estrone increased from 20 µg/l to 60 µg/l during the week before ovulation, and urinary estradiol increased in smaller amounts from 9 µg/l to 18 µg/l (Loy, 1970). Mayer et al. (1940) reported two urinary estrogen peaks; one during estrus and the other near the time of luteolysis 10 to 15 days after estrus.

Asdell (1946) suggested the peak of estrogen which occurred during the luteal phase of the estrous cycle may cause luteolysis, since at about the same time, blood progesterone decreased from 10 to 20 ng/ml to concentrations below 1 ng/ml during estrus (Short, 1959; Smith et al., 1970; Stabenfeldt et al., 1972). During estrus, progesterone remained below 1 ng/ml until after ovulation; it increased significantly by 24 hours after ovulation and reached maximal values (10 ng/ml) by 6 days postovulation (Stabenfeldt et al., 1972). Blood progesterone decreased beginning 3 days before the next estrus, reflecting corpus luteum regression.

Follicular fluid of mares contained progesterone as well as estradiol and estrone (Short, 1960). Presumably, the granulosa and thecal cells secreted these steroids into the follicular fluid. Short (1960) also found other steroids in follicular fluid: cortisol, 17-α-hydroxy-progesterone, androstenedione, epitestosterone and two unidentified compounds (an estrogen and a 17-ketosteroid). The concentration of estrogenic steroids in follicles did not change throughout the cycle, but the total estrogen increased as the follicles increased in size (Mayer et al., 1940). On the other hand, Knudsen and Velle (1961) measured 6- to 10-fold greater concentrations of estradiol in follicles from mares during estrus than in mares at other stages of the cycle. Follicular estrone likewise increased during estrus but was present in quantities only 10% those of estradiol. Knudsen and Velle (1961) pointed out, however, follicular fluid was collected only from one large follicle when present, otherwise several small follicles near the

ovarian surface were used. In contrast, Mayer et al. (1940) collected follicular fluid from all follicles present on the ovary throughout the cycle. It seems feasible that estradiol concentrations may increase during estrus only in the actively growing follicles and the steroid metabolism in small follicles may differ from that of an ovulatory follicle. This would explain the apparent discrepancy between the two studies. Short (1961) concluded that larger follicles had higher estrogen and androstenedione concentrations than small follicles. Younglai (1971) always found the highest steroid concentrations associated with the most vascular follicles.

Reports in the 1930s indicated that the equine pituitary was rich in gonadotropins (Fevold, 1939; Chance et al., 1939). Reproductive organs of animals given equine gonadotropins respond differently than those of animals given gonadotropins of some other species. For example equine luteinizing hormone (LH) had higher specific activities than did LH from other species tested by rat ventral prostate assay (Hutchison et al., 1968). Increasing the frequency of injections of pituitary powder (Meyer and McShan, 1941), augmentation of gonadotropic activity by heme (McShan and Meyer, 1941) or use of 5% beeswax in sesame oil (Hutchison et al., 1968) failed to potentiate the action of equine LH, whereas LH or FSH preparations from other species were potentiated with the above treatments. Equine pituitary extracts increased the weights of rat uteri (Armstrong and Greep, 1965) or immature rat ovaries (Meyer and McShan, 1941) in lower doses than did ovine or bovine

preparations. Parlow (1963) ranked the potencies of LH activity as measured by rat ventral prostate assay of the following gonadotropins: monkey, human and horse crude pituitary powder > rat pituitary or castrate rat serum > ovine, bovine, or porcine pituitary.

Hartree et al. (1968) and Reichart and Wilhelmi (1965) each isolated an equine LH fraction with potency of 0.73 units/mg and 0.96 units/mg, respectively, when assayed by the ovarian ascorbic acid depletion method (OAAD) against NIH-LH-S1 (National Institutes of Health standard preparation of sheep LH). Equine follicle stimulating hormone (FSH) was isolated and purified by Saxena et al. (1962) and Hartree et al. (1968): relative potency measured 7 units/mg of NIH-FSH-S1. That is, the FSH isolated was seven times as active as the NIH-FSH-S1 standard. Subsequently, Braselton and McShan (1970) isolated a purer preparation of each hormone than those isolated by Hartree et al. (1968) or Reichart and Wilhelmi (1965). Their LH preparation measured 5.3 units/mg NIH-LH-S1 and the FSH was equal to 90 units/mg NIH-FSH-S1. They suggested that equine LH and FSH had higher molecular weights than LH and FSH molecules of other species. Equine LH also had a 5- to 10-fold higher sialic acid content than gonadotropins of other species (Landefield et al., 1972), as well as a longer biological half-life in rats (Parlow, 1963).

The biological potency of glycoproteins as well as other plasma proteins partially depends on their sialic acid content. Desialylated human FSH and human chorionic gonadotropin (HCG)

had low biological potencies *in vivo* and were rapidly removed from the circulation and destroyed by the liver (Morrell et al., 1971). Therefore the high sialic acid content of equine LH may account for its higher potency than LH of other species in biological assays.

To my knowledge, peripheral plasma concentrations of equine gonadotropins had not been reported prior to initiation of this project. Ovulatory surges of LH occurred coincidentally with onset of estrus in the cow (Swanson et al., 1972), during early estrus in the ewe and sow (Hansel and Echternkamp, 1972) and on the second day of estrus in the bitch (Boynes et al., 1972). The ovulatory surge of LH persisted for a period of less than 24 hours and was followed by ovulation in about 30 hours in the cow, sow and ewe (Hansel and Echternkamp, 1972).

Pregnancy

The duration of gestation in the mare varies among breeds from 329 to 345 days (Asdell, 1946). The first estrus period occurred about 1 week following parturition; sometimes called the "foal heat," it persisted an average of 11.4 days (Andrews and McKenzie, 1941). In addition to progesterone produced by the primary corpus luteum (CL), secondary ovulations and corpra lutea were formed during the first 70 days of pregnancy. These corpra lutea presumably also secrete progesterone (Cole et al., 1931). Short (1959), using paper chromatography, found progesterone in the blood of pregnant mares during the first 90 days of gestation;

after this time it was nondetectable (< 4 ng/ml). However, the placenta of the mare produces 20-α-hydroxypregn-4-en-3-one (Short, 1957).

Gonadotropic activity increased in pregnant mare serum (PMSG) beginning about 40 days postconception, reached a maximum between 50 and 80 days of gestation and was not detectable after 180 days (Eckstein and Zuckerman, 1956b). The endometrial cups of the mare produce PMSG (reviewed by Rowlands, 1963), but it is not of maternal origin. Allantochorion girdle cells of the fetal trophoblast stimulate endometrial cup formation and PMSG production (Allen and Moor, 1972). PMSG, a glycoprotein (58,000 M.W.) composed of two subunits (Gospodarowicz, 1972), had both LH and FSH activity throughout purification procedures (Raacke et al., 1957), and a high sialic acid content compared to other glycoproteins (13.5%). PMSG probably stimulates accessory CL formation (Cole et al., 1931) but this is supposition. Although the secondary corpra lutea formation and PMSG production both occur around 40 days of gestation, massive doses of PMSG did not stimulate follicular development in mares (Nishakawa, 1959).

In addition to PMSG and progestogens, the placenta of the pregnant mare also synthesized large quantities of equilin and equilienin (Girard et al., 1932). These are estrogens with an unsaturated B ring (Girard et al., 1932) as well as the saturated A ring found in ovarian estrogens.

Anestrus

The term anestrus is a source of confusion in animals which are seasonal breeders. The seasonal anestrus for mares occurs during the short daylight period of the year (Hughes et al., 1972). Anestrus can also refer to conditions during the breeding season which cause failure of the estrous cycle. Irregularities of the cycle included (1) long periods of diestrus (termed anestrus by some), (2) irregular periods of estrus and (3) silent estrus (no estrus period detectable although ovulation occurred). Some causes of anestrus other than seasonal anestrous were (1) diseases such as ovarian hypoplasia, pituitary tumors or uterine infections, (2) pregnancy, (3) failure to exhibit estrual behavior ("silent" estrus) and (4) lactation (Roberts, 1971).

Attempts to Control the Estrous Cycle of Mares

The horse is increasingly popular for recreation in many countries; other countries require large numbers of horses for work. Therefore, recently, increased research efforts have been aimed to estrous cycle and/or ovulation control in mares. Unfortunately, until recently, attempts to control the estrous cycle with exogenous hormones, photoperiod control and uterine infusions of saline were only partially successful in the mare.

Gonadotropic Hormones

Induction of follicular growth and/or ovulation would be one means of estrous cycle control. Sequential injection of extracts containing FSH activity followed by LH caused ovulation

in rats (Casida, 1934). This experimental result was followed by attempts to induce ovulation in cows (Casida et al., 1943), in sheep and sows (Nishakawa, 1959), and in mares (Day, 1940; Nishakawa, 1959). PMS or FSH failed to induce follicular growth in the mare (Day, 1940; Bain, 1957; Nishakawa, 1959). However, ovulation in the mare can be hastened if follicles are already present.

In mares treated with 1000 to 2000 R.U. (rat units) of human pregnancy urine at various times during estrus, ovulation occurred 20 to 40 hours later providing a follicle was present during treatment (Day, 1940). Davidson (1947) attempted to monitor maturity of the follicle; when the follicles were large and thin-walled he injected 500 IU of pregnancy urine to induce ovulation. Nineteen of the 23 barren mares (i.e., those that had failed to conceive and foal during 1 or more previous years) ovulated within 24 to 48 hours. Estrus ended 24 hours later. Although there were no control animals mentioned, Davidson (1947) apparently compared the duration of estrus in these mares to stable mates or to their prior records since he claimed the treatment shortened the duration of estrus.

Not only did pregnancy urine induce ovulation during estrus but LH was also partially successful. Bain (1957) administered LH to 134 mares that had a history of ovulation failure and 65 to 80% ovulated. Similarly, the proportion of mares pregnant to breeding after induced ovulation (65%) was similar to the proportion of control or treated mares pregnant after HCG treatment (Loy and Hughes, 1966).

In controlled experiments, administration of Prolan (a commercial preparation of urinary LH; Nishakawa, 1959) or human chorionic gonadotropin (HCG; Loy and Hughes, 1966; Ginther, 1971; Sullivan, 1972) to mares in early estrus hastened ovulation and reduced the duration of estrus. However, Sullivan (1972) indicated successive HCG treatment may have caused antibody formation since mares treated with HCG during three successive estrous periods did not ovulate as early following the third treatment as did control mares; because ovulation occurred near the end of estrus the treated mares then had increased duration of estrus compared to controls. Repetitive use of gonadotropic hormones to stimulate follicular growth and ovulation in mares will require research to obviate antihormone formation.

Steroid Hormones

Exogenous steroids have been used to attempt control of the estrous cycle in mares, since steroids may suppress or induce release of pituitary gonadotropins in mares as they do in other animals. Progesterone or progestogens administration may block estrus, follicular growth and/or ovulation; estrogens may cause estrual behavior and/or cause pituitary gonadotropin release resulting in follicular growth.

Progesterone (100 or 400 mg in oil) injected every other day during the luteal phase of the cycle for 15 days blocked estrus and ovulation in 12 mares (Loy and Swan, 1966). Return to estrus following progesterone withdrawal was dependent on the

dose of progesterone and the season of the year. Small doses (50 mg/day) blocked estrus but not ovulation. Stabenfeldt et al. (1972) detected occasional ovulation during the luteal phase of the cycle in untreated mares, and suggested that progesterone had less influence in suppression of ovulation in the mare than in other animals. Moreover, oral progestogens in doses much higher than the effective dose in cattle or sheep did not block estrus or ovulation in mares (Loy and Swan, 1966; Britt and Ulberg, 1971). Use of progestogens to synchronize estrus in mares awaits the development of more efficacious compounds.

In addition, attempts to induce estrus and ovulation with estrogens have failed. Although therapeutic use of estrogens to treat noncycling mares (Burkhart, 1947b; Bain, 1957) induced a receptive period, results were not clear since there were no observations on "normal" or control mares. In addition, the effect of estrogen treatment on follicle growth was confused with concurrent administration of pregnancy urine to induce ovulation. Mares exhibited a receptive period after stilbestrol treatment during winter anestrus, but without follicular development and ovulation (Nishakawa, 1959).

Artificial Light

The "sexual season" of the equine female is correlated with increasing duration of daylight. Burkhart (1947a) placed a 1000 watt Sollux lamp above four mares to increase the photoperiod by 2 to 6 hours over the normal daylight period in Cambridge, England.

These anestrus mares began estrous cycles 38 to 64 days after the first addition of light on January 1. Control mares did not begin estrous cycles until 21 or more days later than the last of the treated mares. Similar observations were reported by Nishakawa (1959) who, in addition, suggested that the breeding season can be extended until January by addition of 5 hours of artificial light during August through December. Hormonal changes occurring at or before the first estrus in these mares are unknown.

Uterine Infusion With Saline

Irritation of the uterine endometrium modified luteal function in the cow and ewe (reviewed by Anderson et al., 1969) and irrigation of the uterus with saline has been used to treat noncycling mares in an attempt to reinitiate estrous cycles or increase "intensity of display" (Bain, 1957). For example, after intrauterine infusion of saline into 148 noncycling mares, estrus began within 2 to 12 days (Bain, 1957). Ginther (1971) confirmed Bain's observation: saline infused on the sixth or seventh day of diestrus was followed by onset of estrus in 4 days, significantly earlier than control mares.

Induction of Luteolysis With Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)

Utero-Ovarian Relationships

Loeb (1923) demonstrated that the uterus was involved in the regulation of luteal function; hysterectomized guinea pigs had greatly prolonged estrous cycles (Loeb, 1927). Removal of the

entire uterus during the luteal phase of the cycle caused maintaince of the CL for a period longer than (ewe and sow) or nearly equal to pregnancy (cow; Anderson et al., 1969). If only a portion of the uterus remained, the cow continued to cycle about every 21 days; the sow required about one-quarter of one uterine horn and the guinea pig needed nearly one uterine horn to maintain normal estrous cycles (Anderson et al., 1969). The influence of the uterus on luteal function in mares was substantiated by Ginther and First (1971). When mares were killed 30 days after hysterectomy, the CL marked with India ink at the time of hysterectomy was still functional; meanwhile, the marked CL on the ovaries of control mares regressed.

In some species such as the guinea pig, cow, sow and ewe, the uterus exhibited a local control over the CL (Anderson et al., 1969). That is, the luteal function was controlled by the horn of the uterus nearest the ovary containing the CL. Removal of the uterine horn adjacent to the CL resulted in maintaince of the CL, whereas removal of the contralateral horn had no effect in these species (Anderson et al., 1969). If one-half of the uterus (ewe and guinea pig) was removed and at the same time the adjacent ovary removed, cycling continued. However, if one uterine horn and the contralateral ovary were removed, luteal function was prolonged (Anderson et al., 1969). In contrast, 2 of 5 mares with a unilateral hysterectomy (on the side adjacent to the corpus luteum) continued to undergo normal estrous cycles; 3 of 5 mares with a corpus luteum on the ovary contralateral to the unilateral

hysterectomy continued to cycle (Ginther and First, 1971). This suggested that the corpus luteum of the mare is not controlled by local transfer of a substance from the uterus as in some species.

Integrity of the uterine vein is essential to luteal regression: when the uterine vein was ligated on the side of the uterus adjacent to the CL, the estrous cycle was prolonged in ewes (Anderson et al., 1969). In contrast, ligation of the artery alone or of the artery and vein on the contralateral side of the uterus did not effect luteolysis. However, no direct link between the uterine vein and the blood supply to the corpus luteum has been found (Anderson et al., 1969). A counter-current transfer mechanism has been proposed (McCracken et al., 1972). In the ewe the ovarian artery is closely associated with the utero-ovarian vein, and substances might be passed directly between them in some manner, since separation of the two vessels prevented luteolysis at the expected time (McCracken et al., 1972).

Luteolytic Agents

Insertion of foreign bodies and infusion of various toxic materials into the uterus as well as administration of oxytocin modified luteal function (Anderson et al., 1969). Insertion of an inert foreign body into the uterus may reduce or prolong the cycle depending on the stage of the cycle at the time of insertion. A glass or plastic bead, or plastic coil inserted into the uterus of a cow, ewe or guinea pig during the early part of the estrous cycle caused premature luteal regression (Anderson et al., 1969).

This premature regression was probably caused by a local luteolytic influence from the stimulated portion of the uterus.

Irritation of the uterine endometrium by infusion of ethanol, water, iodine, phenol, silver nitrate or saline also modified luteal function (Anderson et al., 1969). Infusion of these compounds during the early luteal phase of the cycle resulted in an early return to estrus in cows, ewes and mares (Bain, 1957; Anderson et al., 1969). In contrast, infusion of these or other compounds later in diestrus caused prolonged luteal function.

In the cow, oxytocin caused premature luteal regression. The duration of the estrous cycle was reduced following daily injection of large amounts of oxytocin during the first week after estrus (Armstrong and Hansel, 1959). Oxytocin treatment on day 15 to 22 or in hysterectomized cows did not alter the estrous cycle. Epinephrine and atropine blocked the luteolytic effects of oxytocin described above (Black and Duby, 1965). In contrast to these results in cows, oxytocin did not alter the estrous cycle in mares (Ginther, 1971).

That the uterus produces a luteolytic factor which normally causes the demise or death of the CL is widely accepted. However, to my knowledge, the uterine luteolysin has not been isolated or identified. Active luteolytic materials isolated from uterine flushings of sows were thought to be high molecular weight protein; whereas active material in the ewe was thought to have a low molecular weight of approximately 1500 (Anderson et al., 1969). Anderson et al. (1969) suggested that either the uterine luteolysin

had a short half-life, or was utilized by the target organ because of its local action in some species.

$PGF_{2\alpha}$

Discovered in the early 1930s, in the semen of rams by Goldblatt and von Euler (reviewed by Oesterling et al., 1972), prostaglandins were purified and identified during the 1950s and 1960s in Sweden by Bergstrom, Samuelsson and van Dorp (Oesterling et al., 1972). They are unsaturated 20-carbon fatty acids, with a molecular weight of about 350. It was apparent very early that seminal extracts had powerful effects on the uterine and vascular smooth muscle.

The relatively recent discovery, that $PGF_{2\alpha}$ was luteolytic in pseudopregnant rats (Pharriss and Wyngarden, 1969), various laboratory animals (Pharriss et al., 1972) and sheep (McCracken, 1970) has prompted a renewed effort to find the uterine luteolysin. Pharriss et al. (1972) reviewed the action of $PGF_{2\alpha}$ on luteal function in various species and concluded that "$PGF_{2\alpha}$ has the qualities and effectiveness to be the long sought uterine luteolytic factor and fits the current hypothesis for the mechanism of uterine directed luteolysis." Several lines of evidence supported his conclusion. (1) $PGF_{2\alpha}$ was luteolytic in species in which the uterus controls luteal function, such as guinea pigs, rabbits, rats and sheep. (2) Prostaglandin caused luteolysis in intact animals when administered systemically; vaginal, intravenous, subcutaneous and intracardiac as well as intrauterine treatments

were effective. I assume if $PGF_{2\alpha}$ is the uterine luteolysin, it would also be luteolytic in hysterectomized animals. Local infusion of $PGF_{2\alpha}$ into the ovarian artery and uterine vein of sheep caused luteolysis in doses lower than systemic treatment (McCracken et al., 1972). This may be because $PGF_{2\alpha}$ was cleared rapidly from systemic circulation (Piper et al., 1970). (3) $PGF_{2\alpha}$ was present in or released by the endometrium at a time when the uterus is known to induce luteolysis. $PGF_{2\alpha}$ was released after distention of the uterus in guinea pigs and after estradiol administration. In addition to $PGF_{2\alpha}$ release, both uterine distention and estradiol treatment were luteolytic in guinea pigs; estradiol worked via the uterus since it was ineffective in hysterectomized guinea pigs (Blatchley et al., 1971). Bland et al. (1971) reported increased $PGF_{2\alpha}$ activity in the endometrium on day 14 in the ewe. An IUD inserted into the uterus increased $PGF_{2\alpha}$ in the endometrium of the ewe; and caused luteolysis in sheep (Wilson et al., 1972). (4) Pregnancy should counteract the presence or action of $PGF_{2\alpha}$ since pregnancy results in prolonged luteal function. Although the luteotropic factor of pregnancy is unknown, various gonadotropins or luteotropic complexes can overcome $PGF_{2\alpha}$-induced luteolysis (Pharriss et al., 1972). (5) $PGF_{2\alpha}$ caused biochemical changes (a shift from progesterone to 20α-hydroxy-pregn-4-en-3-one), as well as morphological changes (irregular shape, pycnotic nuclei, hypoplasia and atrophy of luteal cells) in corpora lutea of rats and hamsters (Pharriss et al., 1972).

All five of the above observations supported $PGF_{2\alpha}$ as a candidate for the uterine luteolysin. McCracken et al. (1972) presented evidence that $PGF_{2\alpha}$ was the uterine luteolysin in the ewe; $PGF_{2\alpha}$ caused luteolysis with a concomitant fall in progesterone secretion in ewes. In addition, $PGF_{2\alpha}$ was released from the uterus of the ewe in high concentrations during day 15 and 16 of the estrous cycle (McCracken et al., 1972) and furthermore, $^{3}H\text{-}PGF_{2\alpha}$ was transferred from the uterine vein to the ovarian artery in support of the proposed counter-current transfer mechanism for the uterine luteolysin in ewes (McCracken et al., 1972).

However, Lukaszewaska and Hansel (1970) and Caldwell et al. (1968) reported that the uterine luteolysin in bovine and porcine endometrial preparations was of a high molecular weight, probably a protein. This would eliminate $PGF_{2\alpha}$ as the uterine luteolysin unless, as Shaw suggested to Pharriss et al. (1972), prostaglandins bind to proteins. It is possible that the active fraction of the bovine endometrium was a protein bound $PGF_{2\alpha}$.

These reports of the luteolytic action of $PGF_{2\alpha}$ led to the discovery in our laboratory that intrauterine $PGF_{2\alpha}$ treatment was followed by estrus at 72 hours and ovulation at 99 hours in cattle (Louis et al., 1972). If $PGF_{2\alpha}$ would induce luteolysis, estrus and ovulation as precisely in mares as it did in cattle, it could obviate the most important breeding management problem in horses. Mares could be bred at a predetermined time after $PGF_{2\alpha}$. Consequently, the overall goal of this thesis was to test whether $PGF_{2\alpha}$ was luteolytic and to characterize the luteolysis induced by $PGF_{2\alpha}$

in mares. Since uterine control of luteal function operates systemically in mares rather than locally as in cows and ewes, part of this research compared luteolysis after systemic or local (uterine) administration of $PGF_{2\alpha}$. Secondly, because the response of the CL to various treatments depended on the age of the corpus luteum, part of the research involved treatment of mares with $PGF_{2\alpha}$ at 1, 3, 5 and 7 days after ovulation. Finally, I administered 2, 3, 5 or 10 mg of $PGF_{2\alpha}$ at 7 days after ovulation to determine minimal effective dose.

METHODS AND MATERIALS

General Methods and Data Collection

Animals

We conducted these experiments during June, July and August of 1972 and 1973. The mixed-breed mares weighted 350 to 500 kg and ranged from 3 to 20 years of age. Except during periods of data collection, all mares grazed on 40 acres of pasture adjoining the Michigan State University Veterinary Research Barn No. 3. A pony stallion, that served as "teaser," was housed in a box stall in the barn.

Blood Collection and Sample Storage

Blood (40 ml) was collected by jugular puncture (16 gauge needle). During the 1972 project, blood was mixed with powdered oxalic acid (41 mg/tube) to prevent coagulation and placed on ice until transferred to the laboratory centrifuge 1 to 6 hours later. After centrifugation (3000 rpm for 30 min.), the plasma was decanted into a plastic vial and stored at -20°C. Blood was taken four times daily during estrus, and daily during diestrus. During 1973, the blood was allowed to coagulate, and after centrifugation the decanted serum stored at -20°C.

Estrual Behavior Rating

The mares were tested once daily for estrus by exposure to a pony stallion (teasing). For each encounter, both the stallion

and the mare were rated on a 6-point scale similar to that used by Andrews and McKenzie (1941). Six was recorded for a mare if she stood unrestrained and allowed the stallion to mount (estrus). Winking, urination, tail raise and stance which accompanied estrus were recorded, but if these behavioral displays were not accompanied by standing to be mounted, the mare was rated 4 or 5 depending on the number of signs displayed. Kicking or striking, squealing and/or biting and caudad orientation of the ears were considered negative reactions and the mare was rated 1 or 2 depending on the degree of these negative responses. A passive reaction toward the stallion (rated 3) was the absence of the above signs except occasional caudad orientation of the ears. Because the mares were teased once daily, the onset of estrus was considered to be 12 hours previous to first rating of 6, and the end of estrus was estimated to be 12 hours previous to the first rating less than 6.

Ovulation Detection

Follicular changes were monitored by palpation of the ovaries, daily during estrus or after $PGF_{2\alpha}$ treatment, and every third day during diestrus. The diameter of follicles greater than 1.0 cm was estimated to the nearest 0.5 cm. Ovulation was estimated to have occurred 12 hours previous to first detection of a collapsed follicle or corpus hemorrhagicum.

Preparation of Treatments

$PGF_{2\alpha}$-Tham salt (475.6 M.W.) was supplied in a powdered form by Dr. J. W. Lauderdale of the Upjohn Company. It was diluted

to the desired concentration in 0.85% saline and stored at -20°C until it was used. For intrauterine (iu) administration, 10 mg of $PGF_{2\alpha}$ was thawed, aspirated into a sterile disposable 5 ml syringe and injected through a sterile plastic insemination catheter which had been inserted asceptically through the cervix. Then 2 ml of air was injected through the insemination catheter to force the remainder of the $PGF_{2\alpha}$ into the uterus. In other experiments $PGF_{2\alpha}$ was given subcutaneously (sc) in the neck.

Because cows responded to 5 mg of $PGF_{2\alpha}$ (Louis et al., 1972) deposited into the uterus, I decided to use 10 mg $PGF_{2\alpha}$ for iu treatment in mares because of their larger size and the lack of local control of CL function by the uterus in mares. For sc treatment, 15 mg of $PGF_{2\alpha}$ was selected as the initial dose because 20 mg $PGF_{2\alpha}$ caused luteolysis in a 550 kg test mare.

Radioimmunoassays (RIA)

The antisera for my research, supplied by Dr. Gordon D. Niswender, Colorado State University, Fort Collins, Colorado, were tested for specificity, sensitivity and recovery of added mass. The steroid antisera were prepared in rabbits or sheep against a 6β-succinyl or 6-oxime steroid conjugated to bovine serum albumin.

The assay for progesterone was described by Louis et al. (1973) and the specificity of the progesterone antiserum (#869) was reported by Niswender (1973). To further validate the progesterone assay for equine blood, 22 samples representing all stages of the estrous cycle were extracted with benzene: hexane

(1:2) and chromatographed on LH-20 Sephadex with chrloroform: ethanol (96:4) to isolate progestogens from estrogens and glucocorticoids (Swanson et al., 1972). The progestogens were rechromatographed on another LH-20 Sephadex column with heptane: chloroform:ethanol:water (200:200:1:saturated) to isolate progesterone from other progestogens (Gwazdauskas et al., 1972). Progesterone eluted (fraction 8) well in advance of 17α-hydroxyprogesterone (fraction 12) and 20α-hydroxy-pregn-4-en-3-one (fraction 20) in the second solvent system. The RIA values for the chromatographically isolated progesterone were highly correlated ($r = 0.98$; $P < 0.01$) with values for the same samples determined by RIA directly on benzene:hexane (1:2) extracts without chromatography (avg. 4.5 vs 4.6 ng/ml, respectively). In addition, extraction of tritiated progesterone from serum with benzene: hexane (1:2) was high (80 ± 1%; Louis et al., 1973) compared with other tritiated steroids; estrone (56%), estradiol (27%), cortisol (1.4%) and corticosterone (8%) with the same solvent ($n = 6$ each). Therefore, progesterone was quantified in the blood of nonpregnant mares by RIA directly on solvent extracts without chromatographic isolation from other steroids.

The androstenedione assay was described by Mongkonpunya et al. (1975). Various steroids were tested for their ability to displace antibody (#866) bound tritiated androstenedione in this RIA; estradiol had 11% activity, estrone 1% and progesterone, cortisol and corticosterone less than 0.3%. Validation of the assay for mare blood paralleled that described above for

progesterone. Androgens were isolated in the 10th fraction from the first column (chloroform:ethanol) and androstenedione eluted well before (fractions 5 to 7) dihydrotestosterone (fractions 14 to 16) and testosterone (fractions 17 to 19) on the second column (heptane:chloroform:ethanol:water). Values for 10 samples assayed after direct extraction with benzene:hexane (1:2) or after double column isolation were not highly correlated ($r = -0.1$) and the averages differed ($P < .01$, 160 ± 17 vs 68 ± pg/ml, respectively). Consequently, androstenedione was assayed in another 12 samples after direct extraction, after double column chromatography or after one chromatography on mini-columns. The mini-column (Thatcher, 1974) was a 2.5-ml disposable glass syringe containing LH-20 Sephadex with benzene:methanol (85:15; Carr et al., 1971) solvent. Androstenedione (0.2 to 1.2 ml) eluted prior to estrone (1.2 to 1.5 ml) and estradiol (3.0 to 5.0 ml). The average value for androstenedione in the 12 samples was 137 ± 14, 94 ± 15 and 126 ± 14 pg/ml, respectively, when measured after isolation by the three methods. Androstenedione values after mini-column isolation were highly correlated ($r = .8$, $P < .01$) with those after direct extraction, but values from the double column were not highly correlated ($r = .11$, $r = .04$) with either of the other procedures. Therefore, recovery of various amounts of androstenedione added to mare blood plasma was determined after isolation from the mini-column and after direct extraction (Table 1). Androstenedione was highly recoverable by both methods (90.7% vs. 104.5%). Therefore, androstenedione was quanitified by RIA without chromatographic

TABLE 1.--Recovery of androstenedione added to 0.5 ml mare plasma as determined by direct extraction or after chromatography with benzene:methanol (85:15) on LH-20 Sephadex.

Androstenedione	Isolation System	
	Direct Extraction	Mini-Column
(pg)	----------(pg/ml)----------	
0	55	103
50	106	---
100	149	173
200	---	310
500	650	516
1000	1108	---
Overall % recovery[a]	104.5±4.6%	90.7±5.7%

[a] $\frac{\text{pg androstenedione observed}}{\text{pg androstenedione expected}} \times 100\%$; expected was calculated from the value when no androstenedione was added, plus the amount added to the mare serum.

isolation in blood from the experimental mares. The cross reactions of estradiol (10.7%) or estrone (.8%) in the RIA should not have influenced the results, since quantities of estradiol and estrone are estimated to be only about 2 to 10% of androstenedione in mare blood. In 13 assays (n = 639 samples), an average of 84.4% of the androstenedione was extracted with benzene:hexane (1:2); RIA values were corrected for procedural loss of hormone.

The estradiol (antiserum #825) assay was similar to that reported by Wettemann et al. (1972) and Britt et al. (1974). To validate the estradiol assay, estradiol in 18 samples of mare blood

plasma (representing all stages of the estrous cycle) were quantified by RIA after extraction with benzene (direct extraction) or after benzene extraction and chromatographic isolation on the mini-columns described for the androstenedione RIA. Estradiol averaged 4.7 ± 0.8 pg/ml after direct extraction and 3.7 ± 3.8% pg/ml after mini-column chromatography and results from the two methods were highly correlated ($r = .91$, $P < .01$). In addition, 87% of the estradiol added to 1 ml of mare serum was recovered by direct extraction whereas only 64% was recovered after mini-column chromatography (Table 2).

Ten plasma samples were processed similarly to validate the estrone assay (antiserum #84; Britt et al., 1974) for mare plasma. Estrone averaged 9.5 pg/ml after direct extraction and only 4.9 pg/ml after mini-column chromatography, and the two methods were not highly correlated ($r = .28$). Higher values after direct extraction may have been caused by failure to isolate estrone from other cross-reacting steroids; because RIA after direct extraction recovered more estrone (121 ± 7.6%) added to 1 ml of mare serum prior to quantification than RIA after chromatographic isolation (66.5 ± 6.0%; Table 2). Substantial estrone was lost during mini-column chromatography.

To test for interference of androstenedione and/or estradiol on the estrone assay, estrone (0, 10 or 20 pg) was added to 1 ml of mare plasma (Table 3) to which estradiol (0 or 50 pg) and/or androstenedione (0 or 500 pg) had been added. Estradiol (50 pg) increased the estrone values by 2.1 to 3.6 pg, whereas

TABLE 2.--Recovery of estrone or estradiol added to 1 ml mare plasma as determined by direct extraction or after chromatography with benzene:methanol (85:15) on LH-20 Sephadex mini-columns.

Added Estrogen	Estradiol		Estrone	
	Direct Extraction	Mini-Column	Direct Extraction	Mini-Column
(pg)	----------(pg/ml)----------			
0	1.7	2.1	2.0	1.7
5	6.3	4.6	9.7	5.5
10	9.3	8.6	15.6	7.6
20	19.7	12.9	24.6	14.5
50	---	---	55.1	27.9
Overall % recovery[a]	87.0±4.2%	64.0±3.8%	121±7.6%	66.5±6.0%

[a] $\frac{\text{observed}}{\text{expected}} \times 100\%$.

TABLE 3.--Recovery of estrone added to 1 ml of mare plasma with or without competition of added androstenedione and/or estradiol.

Estrone Added	Picograms Added Estradiol (E_2) or Androstenedione (A)			
	0 E_2 +0 A	50 E_2 +0 A	0 E_2 +500 A	50 E_2 +500 A
(pg)	----------(pg/ml)----------			
0	1.23	3.32	3.08	4.35
10	11.34	14.91	11.03	14.51
20	19.52	21.92	23.21	23.08

androstenedione inflated estrone values by 0 to 2.7 pg. However, the inflation of estrone values by estradiol and androstenedione would be maximal when the hormones are at maximum concentrations, since androstenedione and estradiol increased simultaneously in women (Moghissi et al., 1972; Abraham, 1974). Therfore, if these three hormones increase simultaneously in mares, peak values of estrone would be most inflated and estrone changes would therefore not be masked, but enhanced, by estradiol and/or androstenedione.

Specificity of the estrone and estradiol RIA were accessed by determining competition of several different steroids with tritiated (^3H) estradiol or estrone for their respective antisera (Table 4). Relative activity (RA), used to determine specificity, was calculated by comparing the amount of a compound which displaced a constant amount of the estradiol or estrone from their respective antisera. The estrone antisera was relatively specific for estrone; none of the hormones tested had more than 1.4% the relative activity (RA) of estrone (estradiol = 1.38%). However, the estradiol antisera bound testosterone (7.67% RA) and estrone (9.17% RA) in relatively large amounts. Androstenedione which is present in larger quantities than either estrogen in mare blood had $\leq$ 1.5% RA (Table 4) on either RIA. I decided to quantify estrone and estradiol by their respective RIA following direct extraction of the unknowns with benzene (without chromatography), because (1) estrogen recovery was reduced by column chromatography, (2) cross reaction of either RIA with other steroids extracted by benzene was negligible (benzene extracted 65% of added

TABLE 4.--Relative activity[a] of selected steroids in the radioimmunoassay for estradiol and estrone.

Crossreacting Steroid or Sterol	Relative Activity in RIA for	
	Estradiol	Estrone
Estrone	.0917	1.0000
Estradiol	1.0000	.0138[b]
Cholesterol	.0033[c]	.0008[c]
Corticosterone	.0033[c]	.0008[c]
Cortisol	.0033[c]	.0008[c]
Progesterone	.0033[c]	.0008[c]
20-α-hydroxyprogesterone	.0033[c]	.0008[c]
Androstenedione	.0150	.0011[b]
Testosterone	.0767	.0008[b]

[a]Relative activity = $\frac{\text{pg of estrogen at 50\% displacement of } {}^{3}\text{H-estrogen}}{\text{pg of steroid at 50\% displacement of } {}^{3}\text{H-estrogen}}$.

[b]Interpolation from slope since 50% depression was not achieved with highest amount tested.

[c]10,000 ng was the maximum amount of steroid tested; therefore 10,000 was used for the denominator in the above fraction although 50% depression was not achieved.

^{3}H-androstenedione, 82% ^{3}H-progesterone, 71% ^{3}H-corticosterone) and (3) I expected relatively low plasma concentrations of unidentified estrogens or androgens in plasma from nonpregnant mares.

Plasma LH was determined using a double antibody RIA similar to that described (Niswender et al., 1968). Purified ovine LH (LER-1056 C2, supplied by Dr. L. E. Reichert, Emory University, Atlanta, Georgia) was iodinated (^{125}I) and diluted to 20,000 cpm per 100 µl in 0.01 M phosphate buffered (pH 7.0) saline (PBS) with

0.1% Knox gelatin. Rabbit anti-ovine LH (GDN #15, supplied by Dr. Gordon Niswender, Colorado State University, Fort Collins, Colorado) was diluted 1:32,000 in 0.01 M PBS (pH 7.0) with 1:400 normal rabbit serum. Equine LH (LER 1138-1, 0.27 U NIH LH-S1/mg, supplied by Dr. L. E. Reichert) was used as a standard, and PBS (pH 7.0) with 0.1% gelatin was used to dilute unknowns. Otherwise, details of this RIA for equine LH resembled those for the rat LH RIA (Niswender et al., 1968).

The RIA values for LH in four dilutions (10 to 300 µl) of plasma from each of three mares were parallel to the standard curve (Figure 1). Ten to 100 ng of equine FSH (supplied by Mr. L. Nuti, University of Wisconsin, Madison, Wisconsin) and 25 to 300 ng of highly purified bovine TSH (supplied by Dr. J. Pierce, UCLA, Los Angeles, California) cross reacted 8% and 9%, respectively in the LH assay (Figure 1). To further test for TSH cross reactivity, two mares at about 190 days of pregnancy were given intravenously 100 µg thyrotrophin releasing hormone (TRH, supplied by Dr. R. Rippel, Abbott Labs., Chicago). Jugular blood was taken by venipuncture hourly for 6 hours, and plasma thyroxine was determined by the Tetrasorb method (Hernandez et al., 1972) as a reflection of serum TSH. After injection of TRH, serum thyroxine more than doubled at 60 min. ($P < .01$) and remained elevated for 6 hours (Figure 2, on page 37). Although serum thyroxine (and presumably TSH) was increased markedly after TRH, LH measured by my RIA was unchanged during this period. This observation indicates that the 9% cross reactivity of bovine TSH

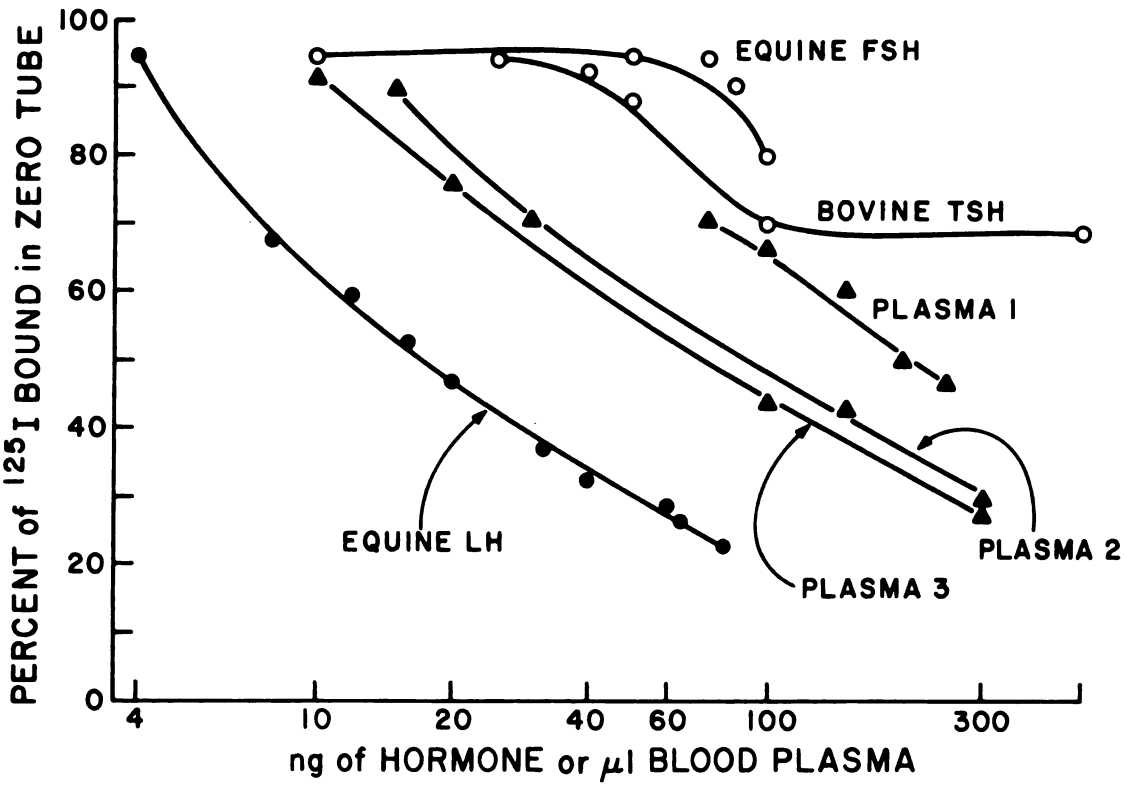

Figure 1.--Radioimmunoassay of standard equine LH (LER 1138-1) and relative activities of equine FSH, bovine TSH and equine blood plasma.

in the LH RIA (Figure 1) may have been associated with LH contamination in the bovine TSH preparation. Alternatively, the antiovine LH may cross react with bovine TSH and not equine TSH.

Experimental Design

Luteolysis, Estrus, Ovulation and Plasma Hormones after $PGF_{2\alpha}$ in Mares

In June 1972, six mares with normal ovaries and uterus were selected for the initial experiment to quantify luteolysis induced

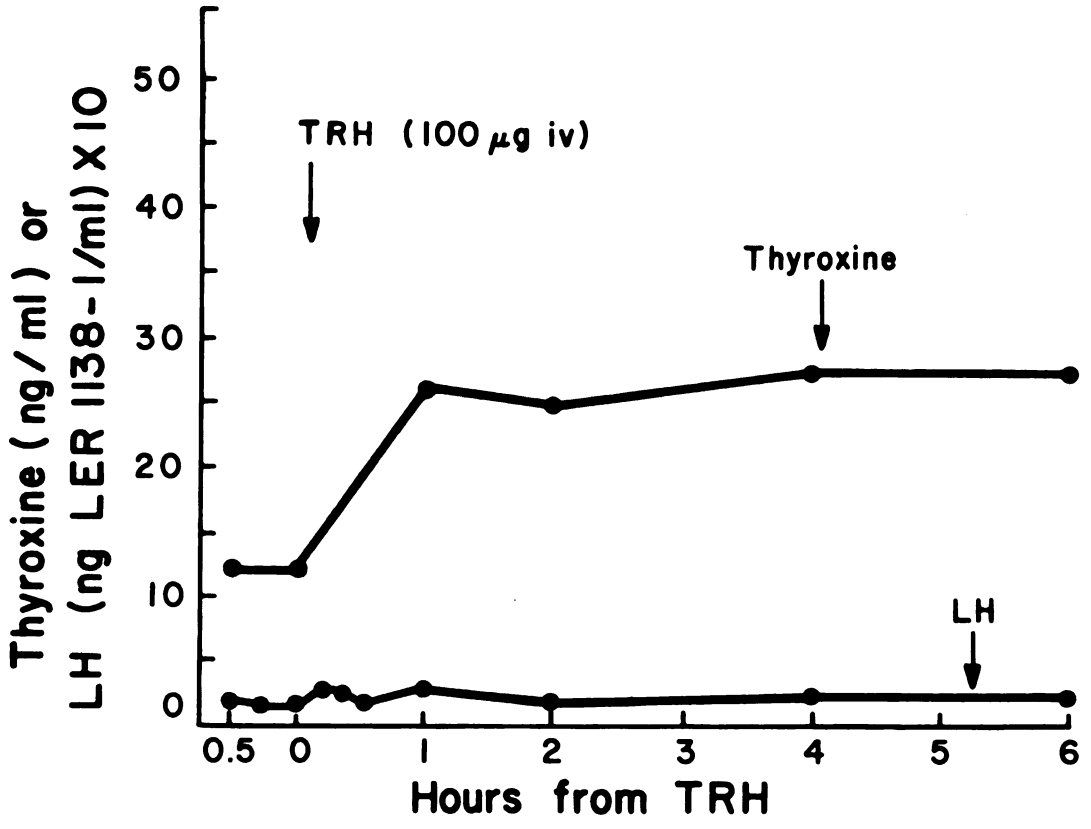

Figure 2.--Plasma LH and thyroxine after thyrotropin releasing hormone (TRH) in two mares.

by $PGF_{2\alpha}$. Seven days after the ovulation which occurred during estrus of a control cycle (cycle I), 10 mg of $PGF_{2\alpha}$-Tham salt in 1 ml of saline was deposited in the uterus. Estrus and ovulation followed $PGF_{2\alpha}$ (cycle II) and the mares were allowed a second control estrous cycle (cycle III). Then, 7 days after ovulation during the second control cycle, 15 mg of $PGF_{2\alpha}$ was injected sc in eight mares; blood sampling, palpation, and estrual behavioral observations were continued (Table 5) through the estrus which

TABLE 5.--Frequency of bleeding, teasing and rectal palpation of reproductive organs in mares given $PGF_{2\alpha}$.

Cycle	n	Bleeding	Teasing	Palpation
		--------(daily)-------		(weekly)
I Control	6	1-4X	1X	3-7X
II $PGF_{2\alpha}$ (10 mg, iu)	6	2-4X	1X	3-7X
III Control	8	1-4X	1X	3-7X
IV $PGF_{2\alpha}$ (15 mg, sc)	8	2-4X	1X	3-7X

followed the sc administration of $PGF_{2\alpha}$ (cycle IV). After each $PGF_{2\alpha}$ administration, mares were observed for side effects.

Statistical analysis of differences in intervals between cycles (e.g. duration of estrus, interval from ovulation to end of estrus, interval from onset of estrus to ovulation) and follicle size were analyzed using analysis of variance (ANOVA). For analysis of hormone changes various segments of the cycle were selected for analysis. For example, the decrease in blood progesterone for 3 days after $PGF_{2\alpha}$ in treated mares was compared with that during luteolysis in untreated mares by ANOVA. In addition, values for all five hormones on 6 selected days of the cycle were compared by least squares ANOVA within and among cycles. The values used for analysis were from samples taken at (1) 5 days before estrus (cycle I and III) or on the day $PGF_{2\alpha}$ was given (cycle II and IV), (2) onset of estrus, (3) 1 day before ovulation,

(4) day of ovulation, (5) end of estrus and (6) 7 days after ovulation (cycle I, II, III or IV) which was the day of $PGF_{2\alpha}$ administration that began cycle II or IV. Several other analyses for specific changes in specific hormones will be described in the Results and Discussion section.

Statistical analysis among all four cycles was not possible, because although 6 mares were included in all four cycles, two additional mares were included in cycle III and IV that had not been treated with iu $PGF_{2\alpha}$. Thus some mares were nested within cycles III and IV, others were factorially related to all four cycles. Therefore, for purposes of statistical analysis the two parts of the experiment were considered separately. That is, for the first three cycles data for 6 mares were compared by ANOVA on selected days. Then a separate set of ANOVA was used to compare cycle III and IV for 8 mares each. Thus in each analysis mares were considered to be random effects; treatments and selected days were fixed effects. The lines in the ANOVA table were cycles (C), mares (M), selected days (D; where applicable) and the various two-way interactions (CM, CD, MD) and the three-way interaction (CMD). To test for treatment differences, the F ratio of the mean squares for C and CM was compared (C/CM) with critical values of the F distribution (Rohlf and Sokal, 1969). The difference between various days was tested by D/DM and the pattern of the hormone changes was tested by the F ratio of the mean squares CD/CMD. In an experiment such as this in which blood samples were taken sequentially the error may be correlated and not independently

distributed. This violation of the assumptions of the ANOVA (that the error is randomly and independently distributed) would result in overestimation of significance.

Estrus, Ovulation and Serum Progesterone After 2, 3, 5 or 10 mg of $PGF_{2\alpha}$

A second experiment, started in June 1973, was an attempt to determine the minimal luteolytic dose of $PGF_{2\alpha}$. Mares were given (sc) 2, 3, 5 or 10 mg of $PGF_{2\alpha}$-Tham salt in 1 ml of saline at 7 to 9 days after ovulation. On the first day (after initiation of the project) they exhibited estrual behavior, the 12 nonpregnant mares available for this experiment were assigned to be given one of the various doses of $PGF_{2\alpha}$ until each treatment was given to a minimum of four mares. For example, the first mare exhibited estrual behavior on June 6th; she was assigned to be given 5 mg $PGF_{2\alpha}$ on the seventh day after ovulation was detected. Two more mares exhibited estrus for the first time on June 15; they were assigned to be given 3 mg and 2 mg, respectively. The mares were not allowed control cycles between treatments for two reasons. There were only a small number of mares available for the experiment and it was necessary to confine the experiment to the months of June, July and August. One mare was given 3 treatments, five mares were given 2 treatments and six mares were given 1 treatment. Although the possibility exists of residual effects from one treatment to the next, it was not considered to be a major problem since there was no indication of residual

effects for the criteria measured in the previous experiments (1972). The mares were teased daily, palpated daily during estrus and serum samples were taken daily beginning 1 day prior to $PGF_{2\alpha}$ treatment and continuing until 3 days after treatment for RIA of serum progesterone.

Estrus, Ovulation and Serum Progesterone After $PGF_{2\alpha}$ on 1, 3, 5 or 7 Days After Ovulation

A third experiment was designed to test the interaction between stage of CL development and luteolysis induced by $PGF_{2\alpha}$. Ten mg of $PGF_{2\alpha}$-Tham salt was administered to each of 4 to 6 mares on day 1, 3, 5 or 7 after ovulation. On the first day the estrual behavior of a mare was rated a 6, she was assigned to a treatment group. The mares were given 10 mg $PGF_{2\alpha}$; the first on 1 day after ovulation, the second mare on day 3, the third on day 5 after ovulation *et cetera* until a minimum of four mares was treated on each of the selected days after ovulation. A total of 12 mares was treated; one mare was treated 3 times (three different treatments), seven mares were treated twice and four mares were treated once. No mare was included in the same treatment group twice. The mares were checked for estrual behavior once daily, palpated daily during estrus and 10 ml of blood was drawn once daily from the day before $PGF_{2\alpha}$ was administered until 3 days after treatment.

In both the experiment to determine minimal luteolytic dose of $PGF_{2\alpha}$ and in the experiment to test luteolysis induced by $PGF_{2\alpha}$ at various days after ovulation, I used one-way ANOVA to

detect differences in intervals from (1) $PGF_{2\alpha}$ to onset of estrus, (2) $PGF_{2\alpha}$ to ovulation, (3) onset of estrus to ovulation, (4) ovulation to end of estrus and (5) onset of estrus to end of estrus. A two-way ANOVA designed to test repeat measurements (Gill and Hafs, 1972) was used to test for differences among treatments and the interaction of treatment and time.

Breeding, Pregnancy and Foaling After $PGF_{2\alpha}$

To determine fertility of mares during estrus following sc $PGF_{2\alpha}$, beginning on the second day of estrus eight mares were mated every other day to a fertile stallion until ovulation occurred. Pregnancy was detected by palpation at 30 to 60 days after breeding and by foaling.

RESULTS AND DISCUSSION

Luteolysis, Estrus, Ovulation and Plasma Hormones After $PGF_{2\alpha}$ in Mares

Control Estrous Cycle (I)

Behavioral response of the mare to a teaser stallion during the first control cycle averaged 2.7 ± 0.3 (on an arbitrary 6-point scale) 5 days before onset of estrus (Table 6) indicating negative and/or passive responses. The numerical rating of respone increased to 6.0 ± 0.0 on the day estrus began when the mares allowed the stallion to mount. During estrus all mares were given a rating of 6 (by definition), then on the day of ovulation one mare did not exhibit estrual behavior and the estrual behavior rating for six mares averaged 5.7 ± 0.2. Behavior of the mares during mid-diestrus was characterized by aggressive hostility or passive reaction toward the teaser stallion. The average rating was 2.4 ± 0.2 (Table 6).

During the first control estrous cycle, estrus persisted 5.2 ± 0.5 days; ovulation occurred 3.8 days after the onset of estrus or 1.5 days before the end of estrus (Table 7 on page 45). For two mares in which an earlier estrus had been observed the interestrual interval was 14.5 days (Table 7). Similar data for duration of estrus and time of ovulation during estrus were reported earlier (Andrews and McKenzie, 1940; Stabenfeldt et al., 1970).

TABLE 6.--Estrual behavior rating[a] for mares during four consecutive estrous cycles.

Day	Cycle			
	I Control	II iu PGF_2	III Control	IV sc PGF_2
Five days before estrus	2.7±0.3[b]	---	2.4±0.2	---
or on day of $PGF_{2\alpha}$	---	2.4±0.2	---	3.0±0.5
Two days before estrus	3.6±0.4	---	3.1±0.1	---
or one day after $PGF_{2\alpha}$	---	2.4±0.2	---	3.1±0.3
One day before estrus	4.7±0.4	---	3.0±0.4	---
or two days after $PGF_{2\alpha}$	---	4.5±0.7	---	2.9±0.5
Onset of estrus	6.0±0.0	6.0±0.0	6.0±0.0	5.9±0.1
One day before ovulation	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
Ovulation detected	5.7±0.2	6.0±0.0	5.4±0.6	6.0±0.0
End of estrus	3.8±0.6	2.6±0.4	2.9±0.5	3.0±0.4
Mid-diestrus	2.4±0.2	2.5±0.2	3.0±0.5	2.7±0.3

[a]The behavior of a mare exposed to a stallion was rated on a scale from 1 to 6. Negative responses were rated 1 if accompanied by kicking, squealing, and switching of the tail; passive responses were rated 3; and positive responses were rated 6 if the mare allowed the stallion to mount. (See page 25 for details.)

[b]Arbitrary units of estrual behavior. Mean ± SEM.

TABLE 7.--Estrus and ovulation during two control cycles and after $PGF_{2\alpha}$ (10 mg, iu) in six mares.[a]

Interval	Control Cycle (I)	$PGF_{2\alpha}$ Cycle (II)	Control Cycle (III)[b]
	----------(days)----------		
From $PGF_{2\alpha}$ to estrus	---	2.2±.3	---
From $PGF_{2\alpha}$ to ovulation	---	8.0±.6	---
From onset estrus to ovulation	3.8±.5[b]	5.8±.7	3.8±.4
From ovulation to end estrus	1.5±.5	1.6±.2	1.8±.6
Duration of estrus	5.2±.5[b]	7.5±.9	5.6±.8
Interestrual interval	14.5±2.5[c]	8.8±.9	14.2±.4

[a]Estrus and ovulation were estimated at 12 hours previous to time first detected. Values are means ± S.E.M.

[b]n = 5. [c]n = 2.

Plasma progesterone averaged 17.1 ± 2.3 ng/ml during diestrus (Table 8; Figure 3); it decreased from 9.5 ± 2.7 ng/ml 3 days before onset of estrus to 0.7 ± 0.2 ng/ml the first day of estrus ($P < .01$), and remained below 1 ng/ml until ovulation occurred. With formation of the corpus luteum, progesterone increased nearly linearly from 5.0 ± 1.2 ng/ml the first day the mares did not exhibit estrus (defined as end of estrus in the following discussion) to 13.6 ± 2.2 ng/ml about 7 to 9 days after ovulation (mid-diestrus). Similar changes of progesterone during the estrous cycle were reported in mares (Smith et al., 1970;

TABLE 8.--Plasma hormone concentrations during a control estrous cycle in six mares (cycle I).

Day	Plasma Hormone				
	Progesterone	LH	Estradiol	Estrone	Androstenedione
	-------(ng/ml)-------		----------(pg/ml)----------		
Five days before estrus	17.1±2.3[a]	71±20	2.7±1.1	11.0±2.7	190±40
Two days before estrus	5.3±1.9	102±11	5.6±0.4	9.2±0.9	230±76
One day before estrus	1.4±0.2	236±75	5.9±0.9	10.9±2.1	147±20
Onset of estrus	0.7±0.2	323±78	7.1±1.7	9.7±0.9	182±30
One day before ovulation	1.2±0.8 (0.4±0.1)[d]	661±113	11.5±2.7	12.5±2.3	380±73
Ovulation detected	1.9±1.2 (0.8±0.5)[d]	918±199	6.9±1.8	11.2±1.7	218±30
End of estrus[b]	5.0±1.2	806±124	4.8±0.9	11.9±1.7	188±18
Mid-diestrus[c]	13.6±2.2	123±35	4.4±0.8	10.0±1.1	207±36

[a]Values are means ± S.E.M.

[b]First day mares were not in estrus.

[c]7 to 9 days after ovulation; day of $PGF_{2\alpha}$ administration for cycle II.

[d]These values omit data for one mare with abnormal progesterone pattern during estrus (see page 83).

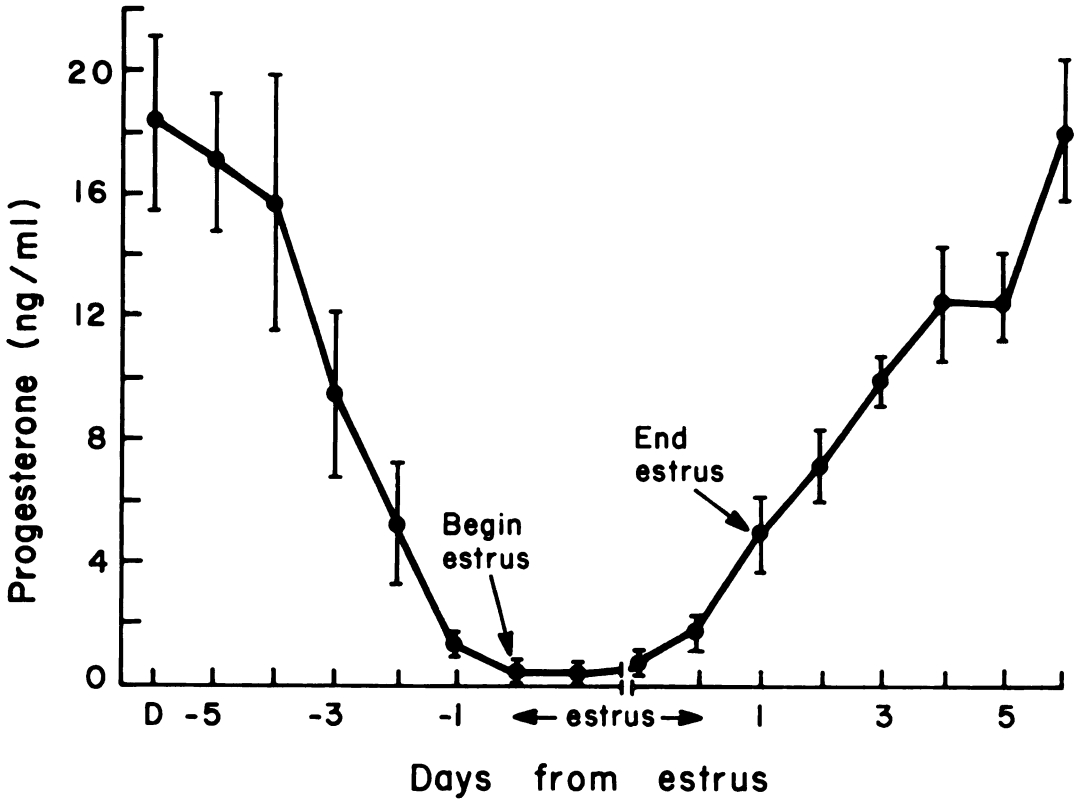

Figure 3.--Plasma progesterone during the control estrous cycle (I) in six mares. Values (means ± S.E.M.) are centered on the onset of estrus and the end of estrus.

Plotka et al., 1972; Stabenfeldt et al., 1972) and in pony mares (Squares et al., 1970; Sharp, 1972; Allen and Hadley, 1973). Stabenfeldt et al. (1972) suggested that the progesterone decline which began 3 days before onset of estrus (Figure 3) reflected the end of full luteal activity, and reported a significant increase in progesterone the day after ovulation. In the first control cycle in my mares, progesterone increased from 0.4 ± 0.1 ng/ml approximately 12 hours before ovulation, to 2.7 ± 1.3 ng/ml

36 hours after ovulation suggesting rapid formation of luteal tissue following ovulation.

As blood progesterone declined during the 3 days before estrus, plasma estradiol increased 2-fold from 2.7 ± 1.0 pg/ml 5 days before estrus to 5.6 ± 0.4 pg/ml 2 days before estrus and to a maximum of 17.7 ± 3.7 pg/ml ($P < .01$) 2 days prior to ovulation (Table 8; Figure 4). Follicular growth accompanied increased estradiol production; follicles averaged 1.7 ± 0.4 cm in diameter prior to estrus and increased to 4.2 ± 0.2 cm the day ovulation was detected. Estradiol decreased to 4.8 ± 0.9 pg/ml by the end of estrus and then ranged from 3 to 6 pg/ml until mid-diestrus. The peak in plasma estradiol 2 days before ovulation (Figure 4) is in agreement with the peak of urinary estrogen near ovulation in mares (Loy, 1970). Nett (1974) reported increased blood total estrogens during the foal estrus; Pattison et al. (1974) obtained 10-fold higher values for blood estradiol, but the relative changes during the cycle resembed those in Figure 4. However, in marked constrast to estradiol, estrone did not change significantly throughout the cycle (Table 8).

The lack of parallel change in estrone and estradiol concentrations is not clear to me, particularly in light of the significant changes in urinary estrone concentrations in mares (Loy, 1970). Urinary estrone increased near ovulation in women (Moghissi et al., 1972); plasma estrone and estradiol both increased near estrus in cows (Hansel and Echterncamp, 1972;

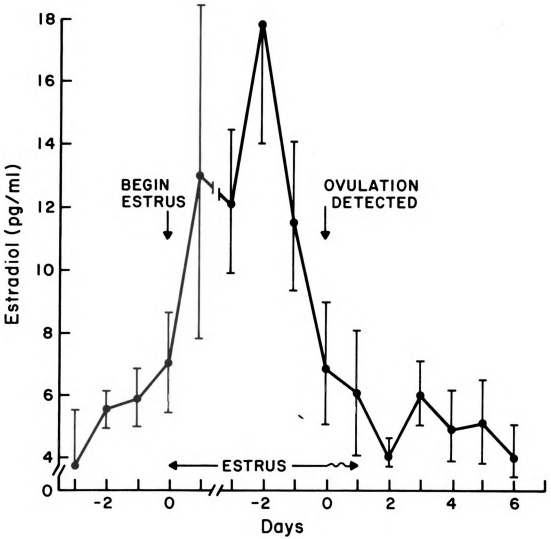

Figure 4.--Plasma estradiol during the control estrous cycle (I) in six mares. Values (means ± S.E.M.) are centered on the onset of estrus (0) and the day ovulation was first detected (0).

Wettemann et al., 1972). Hansel and Echterncamp (1972) also reported peaks of estrone during diestrus, but these were highly variable. In contrast, others report that estrone did not change significantly during the estrous cycle of cows (Glencross et al., 1973). Adrenal production of estrone may have contributed to the plasma estrone pattern observed in my mares.

The changes in urinary estrone throughout the estrus cycle may reflect metabolism of plasma estradiol, because the quantity of urinary estrone exceeded the estradiol in mares (Loy, 1970) and in humans (Moghissi et al., 1972); urinary estriol was even more abundant than estrone or estradiol in women (Moghissi et al., 1972). In contrast, Younglai (1970) and Knudsen and Velle (1961) found greater quantities of estradiol than estrone in follicular fluid of mares. Thus the ratio of estradiol to estrone apparently changes between the time estrogen is produced by the follicle and when it is excreted into the urine; estradiol predominates in the follicle and estrone predominates in urine.

During mid-diestrus, LH ranged from 53 to 70 ng/ml (baseline); it increased ($P < .01$) by the day before estrus (236 ± 75 ng/ml), and continued to increase to a broad peak of 800 to 1000 ng/ml which persisted until 2 days after ovulation (Figure 5). Then it decreased gradually during the next 7 or 8 days to 123 ± 35 ng/ml when $PGF_{2\alpha}$ was deposited into the uterus to begin cycle II. The broad elevation of LH during estrus (Figure 5; Table 8) agrees with comparable data reported by Whitmore et al. (1973) and Pattison et al. (1974). In all three studies, blood

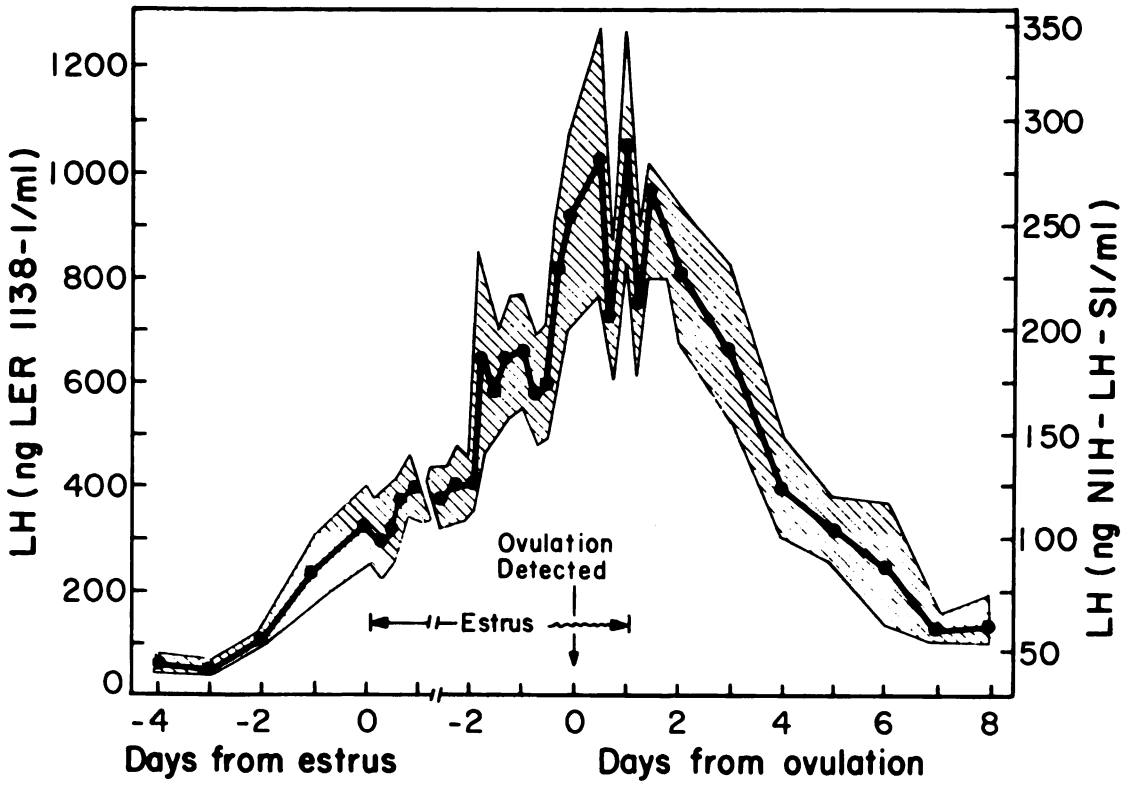

Figure 5.--Plasma LH during the control estrous cycle (I) in six mares. Values are centered on onset of estrus (0) and the day ovulation was first detected (↓). The shaded area represents the S.E.M. around each plotted point.

LH began to increase near the onset of estrus to maximal levels near ovulation and remained elevated until after ovulation; the subsequent decrease appeared nearly symmetrical to the increase. However, plasma LH values in this study are higher than the others. A major portion of this difference may be due to RIA differences; Whitemore et al. (1973) used an anti-PMS first antibody and an equine LH standard of approximately 10-fold higher potency than that used in our study. Pattison et al. (1974) used the same anti-ovine LH used in the present study, but they

used ovine LH (LER-1056-C2) for a standard which is of higher potency than my equine LH standard. Consequently, the LH standards may account for most of the quantitative differences among these three reports.

Sustained elevations of plasma LH throughout estrus with maximum concentrations after ovulation in mares is in contrast to the brief 8-hour surge of LH prior to ovulation in most other species. The LH surge occurs coincidentally with onset of estrus in the cow (Swanson et al., 1972; Hansel and Echternkamp, 1972), during early estrus in the ewe and sow (Hansel and Echternkamp, 1972), and on the second day of estrus in the bitch (Boynes et al., 1972; Smith and McDonald, 1974). The significance of the prolonged elevation of LH during estrus in mares is not apparent to me. That this may be an artifact of cross reaction with other pituitary hormones seems unlikely. The data in Figure 2 discounted any significant interference of TSH in the equine LH RIA, because while plasma thyroxine doubled within 1 hour after TRH injection, LH did not change. Furthermore, in addition to TSH release, TRH injections also released prolactin and growth hormone (GH) in several other species (Convey, 1973). If TRH released prolactin and/or GH in my mares, these hormones did not interfere with my RIA of LH because LH did not change after TRH injection.

As outlined in the Review of Literature, the duration of estrus in mares is 2 to 5 times longer than in most other species. During estrus, the ovulatory follicle migrates through the ovarian stroma to the ovulation fossa where rupture occurs (Asdell, 1946).

It is possible that enzymes necessary for follicular migration and/or thinning of the follicular wall prior to ovulation may require prolonged LH stimulation. Rondell (1970) suggested that hydrolytic enzymes in the follicle wall require LH to trigger their ovulatory function.

When the follicles of mares were manually ruptured on day 2 of estrus, luteinization did not occur whereas luteinization followed follicle rupture on day 5 (Asdell, 1946). This suggested that LH or a product produced in response to LH was not present in sufficient quantities to support luteinization before day 5 of estrus. Equine granulosa cells from large follicles luteinized when grown in vitro (Channing, 1969) whereas granulosa cells from small follicles did not, indicating changing metabolic capability of the granulosa cells as the follicle grew. Greater amounts of LH may bind to each equine granulosa cell in large follicles than to these cells in small follicles. Large granulosa cells of sows bind more HCG/cell than do granulosa cells of small follicles (Channing, 1974). Possibly LH directly or indirectly stimulates the number of LH receptors, thus affecting the ability of the granulosa cells to undergo luteinization.

Equine LH has a slower disappearance rate than LH of other species when disappearance of exogenous LH is monitored in a horse (Ginther et al., 1974) or in a rat (Parlow, 1963). As discussed in the Review of Literature, the long half-life of equine LH is probably due to a high sialic acid content. The slow disappearance

rate of equine LH may in part contribute to the slow decrease in endogenous LH after ovulation in the mare.

Plasma androstenedione (Figure 6; Table 8) increased ($P < .05$) from 182 ± 30 pg/ml at onset of estrus to a peak of 380 ± 73 pg/ml the day before ovulation, and decreased to 188 ± 18 pg/ml the first day of diestrus. Androstenedione was present in

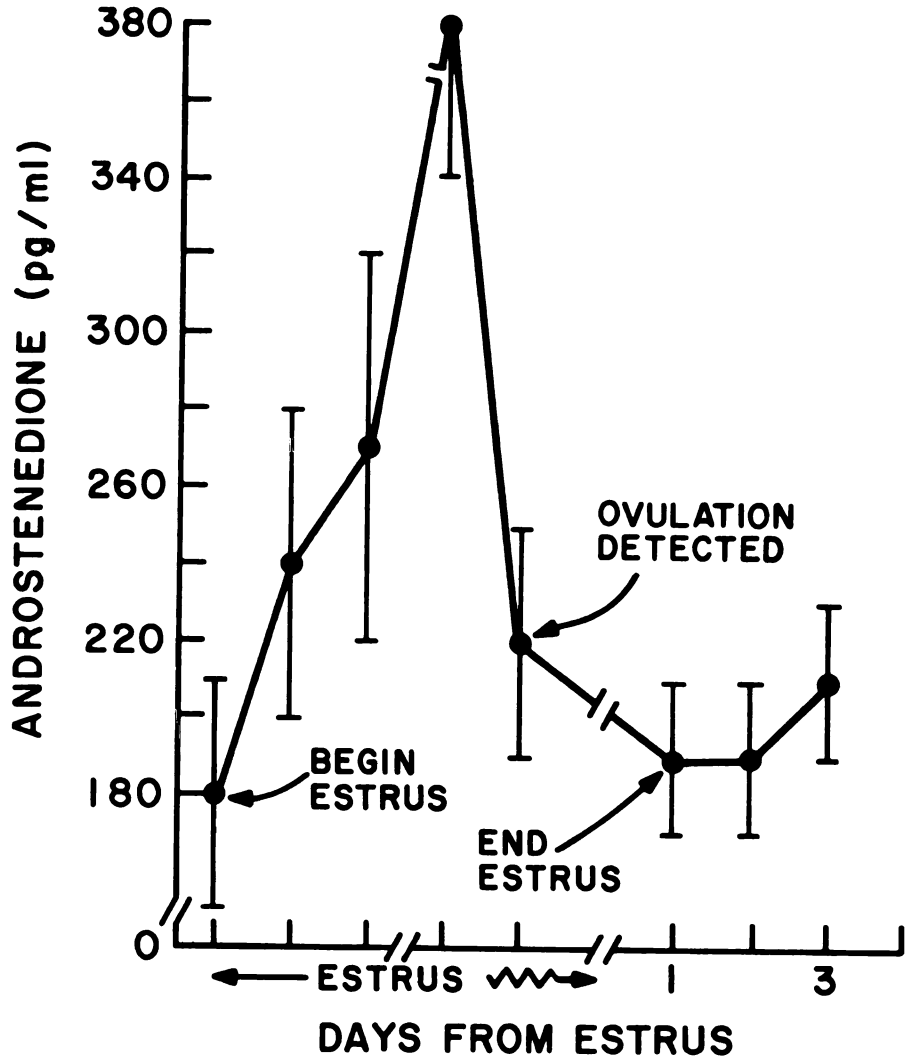

Figure 6.--Plasma androstenedione during the control estrus cycle (I) in six mares. Values (means ± S.E.M.) are centered on onset of estrus, ovulation and end of estrus.

follicular fluid of mares (Short, 1960; 1961). Younglai (1971) reported highest concentrations of androstenedione in vascular follicles present during estrus. Similar to the peak of androstenedione before ovulation in mares (Figure 6), peripheral blood androstenedione peaked prior to ovulation in rats (Dupon and Kim, 1973) and in women (Abraham, 1974) where the ovary was the origin of 45 to 70% of the androstenedione. The function of androstenedione in the female is unknown; perhaps it affects estrual behavior, follicular maturation or ovulation, or it may reflect estrogen synthesis. The reason for the changes in androstenedione before ovulation and the lack of changes in estrone concentrations are not apparent.

The composite of the changes in progesterone, estradiol, and LH for control mares in Figure 7 indicates that progesterone decreased to near basal values before the LH surge began near the onset of estrus. Furthermore, the LH surge persisted until progesterone began to increase at 1 or 2 days after ovulation. These observations suggest increasing progesterone may cause a decrease in LH release in mares. On the other hand, the LH decrease may be a delayed response to cessation of estradiol production several days earlier, since estradiol may stimulate LH production in mares. Estradiol increases preceded or accompanied the increase in LH (Figure 7). However, neither LH nor estradiol increased until progesterone had decreased by $\geq$ 50%. These facts suggest that estradiol and progesterone both influence LH release in the mare.

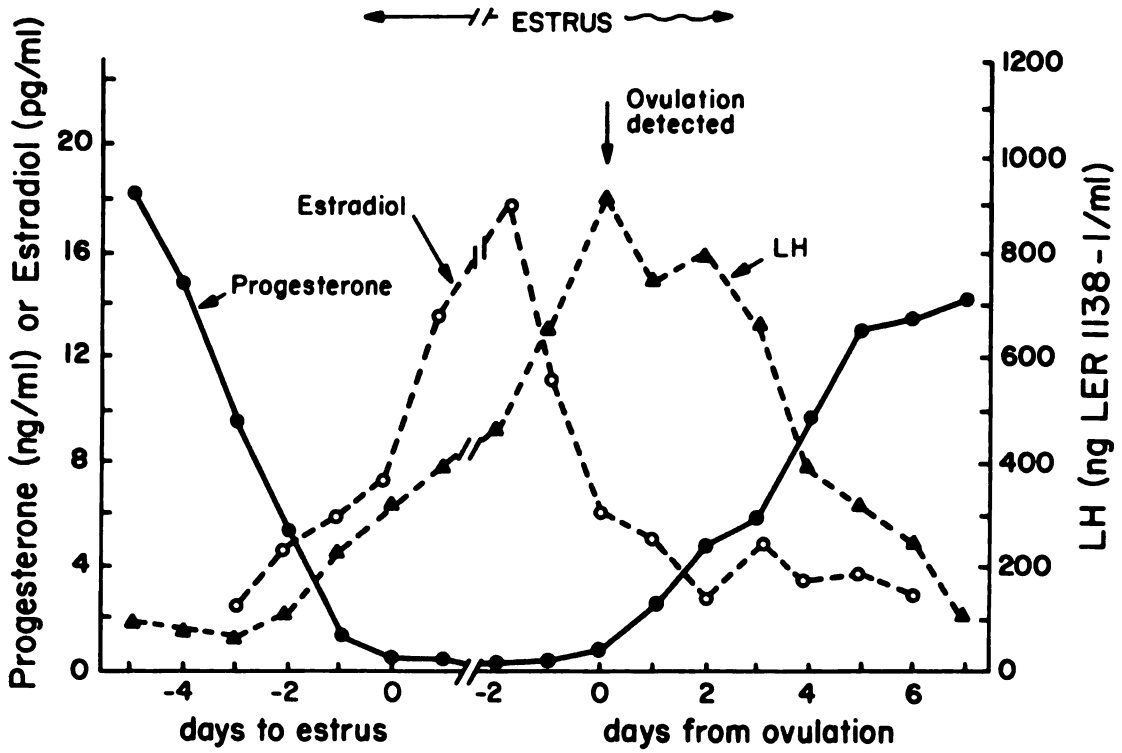

Figure 7.--Relative changes of plasma progesterone, LH and estradiol during the control estrous cycle (I) in six mares. Values (means ± S.E.M.) are centered on the onset of estrus (0) and the day ovulation was first detected (↓).

Estrous Cycle After Intrauterine $PGF_{2\alpha}$ (II)

Seven to nine days after the ovulation which occurred during the first control estrus, 10 mg $PGF_{2\alpha}$-Tham salt was deposited into the uterus of each of six mares. An average of 2.2 days later, the mares exhibited signs of behavioral estrus (Table 6); and ovulation occurred 5.8 days after the onset of estrus (Table 7). Therefore after $PGF_{2\alpha}$, ovulation occurred 2 days later

($P < .05$) after onset of estrus than in the preceding control estrus (Table 6). However, the interval from ovulation to the end of estrus (1.6 days) resembled that in the control estrus, and the duration of estrus after $PGF_{2\alpha}$ was not significantly longer. The interestrual interval (8.8 days) was reduced 5 days ($P < .01$) by comparison with the previous interestrual interval (Table 7) owing to luteolysis precipitated prematurely by the $PGF_{2\alpha}$. Estrual behavior rating during this estrous cycle resembled those in cycle I (Table 6).

Plasma progesterone averaged 13.6 ng/ml when $PGF_{2\alpha}$ was infused into the uterus (Table 9; Figure 8, on page 59). Progesterone decreased to 5.8 ng/ml within 12 hours ($P < .01$), and the decrease continued to 2.6 ng/ml and 0.9 ng/ml by 24 and 48 hours (Figure 8). The precipitous decrease in progesterone after $PGF_{2\alpha}$ resembled that after $PGF_{2\alpha}$ in heifers (Louis et al., 1973) and although it appeared to be more rapid, the decrease in progesterone for 3 days after $PGF_{2\alpha}$ did not differ significantly from that during the 3 days prior to estrus during normal luteolysis in mares (Figures 3 and 8). Progesterone in blood plasma increased rapidly after ovulation averaging 4.1, 11.5 and 13.7 ng/ml on days 1, 4 and 6 after estrus as in the control cycle (Figure 3).

Similar to changes after normal luteolysis, plasma estradiol ($P < .06$) and androstenedione ($P < .01$) increased during the estrus after $PGF_{2\alpha}$-induced luteolysis to maximum values before ovulation and had begun to decrease by the time ovulation was

TABLE 9.--Plasma hormone concentrations during the estrous cycle following treatment with prostaglandin $F_{2\alpha}$ (10 mg, intrauterine) in six mares (cycle II).

Day	Plasma Hormone				
	Progesterone	LH	Estradiol	Estrone	Androstenedione
	-------(ng/ml)-------		----------(pg/ml)----------		
On day of $PGF_{2\alpha}$ treatment	13.6±2.2[a]	123±35	4.4±0.8	10.0±1.1	207±36
One day after $PGF_{2\alpha}$	2.6±0.5	188±49	4.4±0.4	9.6±1.5	163±10
Two days after $PGF_{2\alpha}$	0.9±0.2	284±61	5.7±1.0	7.9±0.8	130±15
Onset of estrus	0.7±0.2	317±64	6.9±0.8	8.0±0.8	143±11
One day before ovulation	0.3±0.1	870±221	9.7±2.3	9.4±0.9	238±28
Ovulation detected	0.6±0.1	952±158	6.0±1.4	9.0±0.8	232±36
End of estrus[b]	4.1±0.8	1025±186	4.9±1.1	8.8±1.1	162±20
Mid-diestrus[c]	13.5±2.3	208±60	4.0±0.9	8.1±0.6	132±17

[a]Values are means ± S.E.M.

[b]First day mares were not in estrus.

[c]7 to 9 days after ovulation.

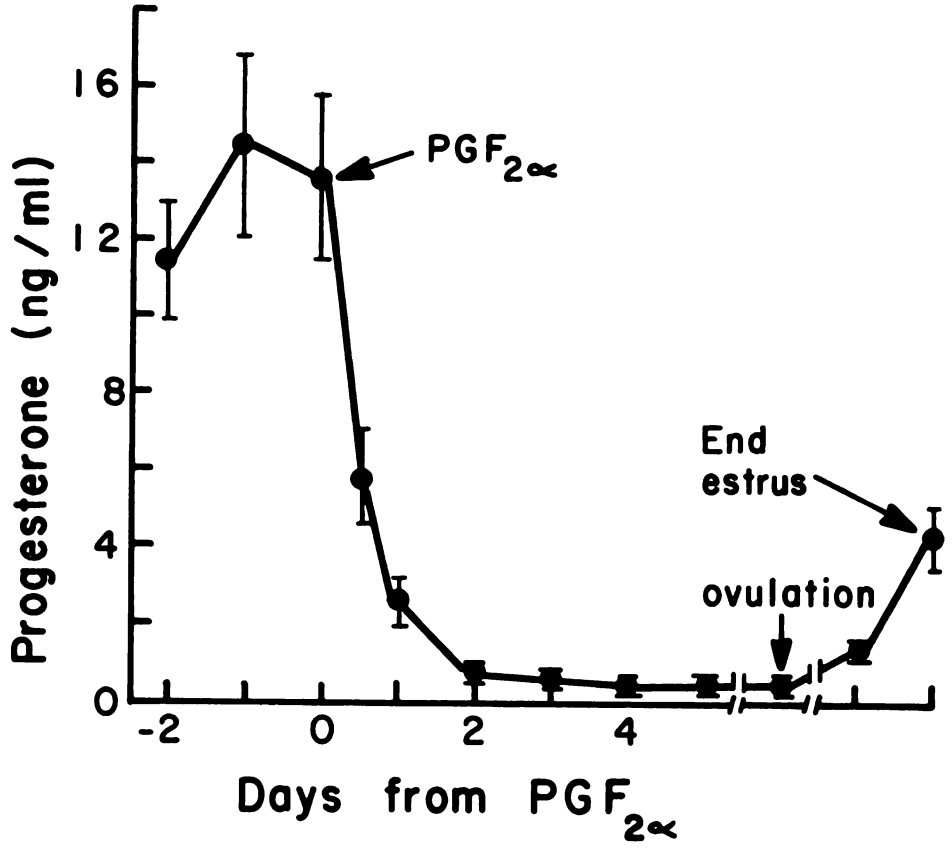

Figure 8.--Plasma progesterone after $PGF_{2\alpha}$ (10 mg, iu) in six mares. Values are means ± S.E.M. and are centered on $PGF_{2\alpha}$ treatment (0), the day ovulation was detected (↓) and the end of estrus.

detected (Table 9). Estradiol and androstenedione did not achieve peak values after $PGF_{2\alpha}$ in cycle II equal to those observed in the first control cycle (Figure 6; Tables 8 and 9). Follicles increased from a diameter of 1.6 ± 0.3 cm at onset of estrus to 3.4 ± 0.3 cm the day before ovulation. As in the control cycle, blood estrone did not change significantly during the $PGF_{2\alpha}$-induced cycle (Table 9).

After the control estrus, LH gradually returned toward baseline; it averaged 123 ± 35 ng/ml when $PGF_{2\alpha}$ (10 mg) was deposited in the uterus at 7 to 9 days after ovulation. On the first day of the estrus after $PGF_{2\alpha}$, LH averaged 317 ± 64 ng/ml ($P < .01$); it increased to a broad peak of about 900 to 1100 ng/ml during the 3 days near ovulation (Table 9). Then LH declined nearly continuously for 8 days, roughly symmetrically with the previous increase, and averaged 69 ± 8 ng/ml for 5 days during mid-diestrus; these changes in LH after $PGF_{2\alpha}$ in cycle II generally resembled those during the first control cycle.

The slightly longer duration of estrus after $PGF_{2\alpha}$ treatment may have been due to additional time necessary for immature follicles to mature; the diestrum was terminated prematurely by $PGF_{2\alpha}$ and average follicular size was smaller ($P < .05$) at onset of estrus during cycle II than during cycle I (1.6 ± 0.3 vs 2.8 ± 0.2 cm). However, the differences in follicle size between these two cycles may be attributable to relative experience of the individuals doing rectal palpation or due to chance alone, because the size of follicles at onset of estrus and at ovulation for cycles III and IV were similar to those for cycle II. ANOVA of the size of follicles for six mares during the first three cycles indicated a difference ($P < .01$) in size of follicles at onset of estrus, but not at ovulation among cycles. In addition, follicle size is probably not a very objective measure of follicular maturity, because the largest follicle is not necessarily the

ovulatory follicle and ovulation does not occur when a given size is achieved.

Control Estrous Cycle (III)

Although eight mares were originally assigned for observation during this estrous cycle, only the six mares also assigned to cycles I and II will be considered for comparisons to the first control cycle and the estrous following iu $PGF_{2\alpha}$. However, one of these six mares did not exhibit estrual behavior during this cycle although an ovulation was detected. Therefore, the data from this mare could not be included in the data for cycle III. None of the intervals measured during this cycle differed from those during cycle I (Table 7); estrus persisted 5.6 days and ovulation occurred 3.8 days from the onset of estrus. During this control cycle, diestrus lasted 14.2 ± 0.4 days (Table 7) similar to the diestrum during cycle I.

Like the estrual intervals, the changes in hormones during the second control cycle (III) resembled those during the first control cycle (Table 8); and because these hormone changes were similar to those during cycle I they are not separated from the other two mares included in cycle III (Table 10) as a control for cycle IV. However as previously stated, for strictly legitimate statistical analysis, the hormone changes during the first three cycles for six mares were done separately from changes during cycle III and IV for eight mares.

With respect to progesterone changes during cycle III: (1) the significant decrease in progesterone for the 3 days before

TABLE 10.--Plasma hormone concentrations during a control estrous cycle after a cycle induced by $PGF_{2\alpha}$ in seven mares (cycle III).

Day	Plasma Hormone				
	Progesterone	LH	Estradiol	Estrone	Androstenedione
	-------(ng/ml)------		----------(pg/ml)----------		
Five days before estrus	10.0±0.9[a]	70±14	3.4±1.3	11.2±0.5	132±17
Two days before estrus	3.8±1.3	62±12	5.1±1.1	9.6±1.0	155±27
One day before estrus	1.0±0.2	148±47	6.1±1.2	11.4±1.2	158±33
Onset of estrus	0.4±0.1	316±49	6.7±1.4	10.7±1.0	179±29
One day before ovulation	0.3±0.0	736±118	11.5±3.1	11.4±1.8	226±36
Ovulation detected	0.8±0.3	742±154	15.8±7.2	11.6±2.6	260±40
End of estrus[b]	3.0±0.9	971±156	4.2±0.8	9.1±0.8	227±40
Mid-diestrus[c]	10.7±0.4	161±66	4.1±0.4	12.0±1.1	181±20

[a]Values are means ± S.E.M., n = 7 since one mare did not return to estrus, and two new mares were added.

[b]First day the mares were not in estrus.

[c]7 to 9 days after ovulation; day of $PGF_{2\alpha}$ administration for cycle IV, n = 8.

estrus ($P < .01$) was not significantly different from the progesterone decrease that occurred during luteolysis before the first control estrus; (2) the levels of progesterone during estrus (the day before estrus, 6 days of estrus and the day after estrus) during the two control cycles did not differ; (3) the increase in progesterone during corpus luteum growth (the last day of estrus and the three days following) did not differ between the two control cycles, and finally, (4) the significant ($P < .05$) progesterone changes on 6 days throughout the estrous cycle [(a) 5 days before estrus or day of $PGF_{2\alpha}$ treatment, (b) onset of estrus, (c) 1 day before ovulation, (d) ovulation, (e) end of estrus and (f) mid-diestrus] were not different amont the three estrous cycles.

Estradiol and androstenedione during the second control cycle--as in the first two cycles--increased to peak levels near ovulation. Estradiol averaged 7.3 ± 1.9 pg/ml at onset of estrus, increased to 10.1 ± 3.8 pg/ml the day before ovulation was detected, and averaged 3.9 ± 1.0 pg/ml by the end of estrus (similar to data in Table 10). Androstenedione peaked at 220 pg/ml on the day of ovulation ($P < .05$). Both estradiol and androstenedione increased significantly during estrus before ovulation ($P < .05$) and the production of these steroids was accompanied by increases in follicular size; from 1.6 ± 0.4 cm at onset of estrus to 3.5 ± 0.3 cm before ovulation.

Although estradiol concentrations were highest on the day of ovulation, somewhat later than in the two previous cycles,

estradiol concentrations appeared to be more variable (see SEM's, Tables 8, 9 and 10) near the day of ovulation. Average estrone fluctuated nonsignificantly between 9 and 12 pg/ml throughout the third cycle, as during the first and second cycles.

The pattern of changes in LH around the second control estrus did not differ from that during the first control estrus; LH increased from a baseline of 56 ± 11 ng/ml on the 2 days before estrus to 1237 ± 345 ng/ml 2 days after ovulation and then declined to 200 ng/ml 7 to 9 days after ovulation when $PGF_{2\alpha}$ was given subcutaneously (similar to Table 10). Analysis of variance of LH data from mares on 5 days (the day before estrus, the first day of estrus, the day of ovulation, the first day after estrus and day 7 after ovulation) indicated there was no difference in LH values between the two control cycles and the cycle which began after intrauterine $PGF_{2\alpha}$. In addition, analysis of LH concentrations for six selected days throughout the estrous cycle indicated that (1) during cycle III, LH changed significantly ($P < .05$) and (2) there was no difference in LH patterns (cycle by day interaction) among the three cycles.

The lack of significant differences in all criteria measured between the two control cycles was interpreted to mean that there were no residual effects of $PGF_{2\alpha}$ on the subsequent control cycle. In other words, the ovulation which occurred during the estrus after $PGF_{2\alpha}$ (10 mg, iu) was followed by CL function, luteal regression, estrus and ovulation statistically indistinguishable from those during control cycles and because the second control

cycle did not differ from the first, it was used as a control for changes after sc $PGF_{2\alpha}$ (cycle IV). Two new mares were added to the project during cycles III and IV.

When the hormone values for the seven mares during cycle III are averaged, changes in progesterone, LH, estradiol, estrone and androstenedione were similar to those already detailed for five mares during this cycle (Table 10). They were in estrus 6.0 ± 0.4 days after a 14.5 ± 0.4 day diestrus (Table 11), similar to the length of estrus in the first two cycles. Behavioral responses to the teaser stallion resembled those during cycles I and II (Table 6).

TABLE 11.--Estrus and ovulation during a control cycle and after $PGF_{2\alpha}$ (15 mg/sc) in eight mares.[a]

Interval	Control Cycle (III)	$PGF_{2\alpha}$ Cycle (IV)
	----------(days)----------	
From $PGF_{2\alpha}$ to estrus	---	2.8±0.3
From $PGF_{2\alpha}$ to ovulation	---	8.0±1.0[b]
From onset of estrus to ovulation	4.1±0.4[b]	5.1±0.8[b]
From ovulation to end of estrus	1.9±0.5[b]	2.1±0.3[b]
Duration of estrus	6.0±0.6[b]	7.8±0.8
Interestrual interval	14.5±0.4[c]	9.4±0.4

[a]Estrus and ovulation were estimated at 12 hours previous to time first detected by palpation. Values are means ± S.E.M.

[b]n = 7. [c]n = 6.

Estrous Cycle After Subcutaneous $PGF_{2\alpha}$ (IV)

When 15 mg $PGF_{2\alpha}$-Tham salt was injected sc 7 to 9 days after the ovulation during the second control estrus (cycle III), the eight mares began estrus 2.6 days later, estrus persisted 7.8 days and ovulation occurred 2.1 days prior to the end of estrus (Table 11). None of these intervals differed significantly from comparable intervals during the control cycle (III) or after iu $PGF_{2\alpha}$ in cycle II (Table 7). Changes in estrual behavior resembled the changes during cycles I, II and III (Table 6).

On the day of $PGF_{2\alpha}$ treatment, progesterone averaged 10.7 ± 0.4 ng/ml (Table 12) and had decreased to 4.6 ± 0.3 ng/ml by 12 hours later. As in the cycle following iu $PGF_{2\alpha}$, progesterone continued to decrease and was below 1 ng/ml when the mares first exhibited estrual behavior. Progesterone changes on the 6 selected days throughout the estrous cycle after sc $PGF_{2\alpha}$ were not statistically different than progesterone changes on the 6 days during the control cycle (III). Therefore the decline in progesterone during the 3 days before estrus (cycles I and III) or the 3 days after $PGF_{2\alpha}$ (cycles II and IV) for the six mares represented in all four cycles were compared. The decline in progesterone following sc $PGF_{2\alpha}$ did not differ significantly from that after iu $PGF_{2\alpha}$ (Tables 9 and 12). On the average, the decline in progesterone for 3 days after $PGF_{2\alpha}$ treatment (cycles II and IV) was more rapid ($P \simeq .06$) than that during the 3 days before estrus during the control cycles (Tables 9 and 12).

TABLE 12.--Plasma hormone concentrations during the estrous cycle following treatment with $PGF_{2\alpha}$ (15 mg, sc) in eight mares (cycle IV).

Day	Plasma Hormone				
	Progesterone	LH	Estradiol	Estrone	Androstenedione
	-------(ng/ml)-------		----------(pg/ml)----------		
On day of $PGF_{2\alpha}$ treatment	10.7±0.4[a]	161±66	4.1±0.4	12.0±1.1	181±19
One day after $PGF_{2\alpha}$	2.0±0.1	372±90	5.2±1.3	10.6±1.0	160±10
Two days after $PGF_{2\alpha}$	0.6±0.1	298±64	5.6±1.6	8.6±0.7	146±16
Onset of estrus	0.4±0.1	463±54	5.4±1.1	8.1±0.4	178±18
One day before ovulation	0.4±0.2	585±70	9.9±2.1	9.3±0.5	220±34
Ovulation detected	0.4±0.1	663±71	6.3±1.5	9.8±0.9	196±33
End of estrus[b]	2.7±0.5	936±105	3.8±0.5	8.5±0.3	218±46
Mid-diestrus[c]	7.8±1.5	264±84	3.4±0.4	10.7±0.7	175±19

[a]Values are means ± S.E.M.

[b]First day mares were not in estrus.

[c]7 to 9 days after ovulation.

When sc $PGF_{2\alpha}$ was administered LH averaged 161 ± 66 ng/ml (Table 12); by 24 hours later LH averaged 372 ± 90 ng/ml--a 2-fold increase. LH remained between 300 and 500 ng/ml the next 2 days and then increased to peak values of 936 ± 105 ng/ml near the end of estrus (Table 12). This pattern of LH changes during the cycle after $PGF_{2\alpha}$ differed ($P < .05$) from those during the control cycle (III). Firstly, LH concentrations appear to be higher before estrus and higher when estrus began (Table 12) than at the comparable times during cycle III (Table 10); and secondly, LH concentrations appear to be lower the day before ovulation than during cycle III. However, ovulation occurred normally relative to the end of estrus.

The LH changes after $PGF_{2\alpha}$ appeared to be accompanied by similar changes in estradiol (Table 12); estradiol remained near 5.0 pg/ml for 3 days near the onset of estrus. As in previous cycles, estradiol peaked ($P < .05$) the day before ovulation at 9.9 ± 2.1 pg/ml. The point is this: even though $PGF_{2\alpha}$ may have altered the normal pattern of LH and/or estradiol during estrus, the duration of estrual behavior and time of ovulation were apparently normal (Table 11).

In addition to the alterations of LH changes following $PGF_{2\alpha}$, androstenedione did not change as markedly as during the control cycle, and these changes only approached significance ($P = .16$). However, the highest concentrations (220 ± 34 pg/ml) occurred the day before ovulation, similar to the time of peak

androstenedione concentrations during the first three estrus periods (Tables 8, 9, 10 and 12).

Furthermore, in contrast to the control cycle, estrone decreased significantly before estrus ($P < .01$) during the 2 to 3 days after sc $PGF_{2\alpha}$ (Table 12). Inspection of data from cycle II (Table 9) reveals a similar decrease in estrone during the 3 days after iu $PGF_{2\alpha}$. However the decrease in estrone after iu $PGF_{2\alpha}$ was not statistically significant.

The 2-fold LH increase 1 day after sc $PGF_{2\alpha}$, or the significant decrease in estrone during the 3 days following sc $PGF_{2\alpha}$, or lack of significant changes in androstenedione, may have been a result of altered steroid metabolism after $PGF_{2\alpha}$. However, the mare is a seasonal breeder, and by some manner the ovary becomes quiescent during the winter anestrus, which begins as early as September in some mares in Michigan. The hormonal changes that take place to bring about the onset of seasonal anestrus are unknown. I am suggesting that significant changes observed during cycle IV may have been a result of seasonal changes. There certainly were changes in hormone pattern that appear to occur through the season. For example (Figure 9) the maximal mid-diestrus concentrations of progesterone decreased over the four cycles. Seasonal decrease in luteal progesterone was suggested by Stabenfeldt (1972). Secondly, LH appears to reach maximal concentrations later during estrus as the season progressed; and thirdly, peak androstenedione values were lower as the season progressed (Tables 8, 9, 10 and 12). Whether the differences in LH,

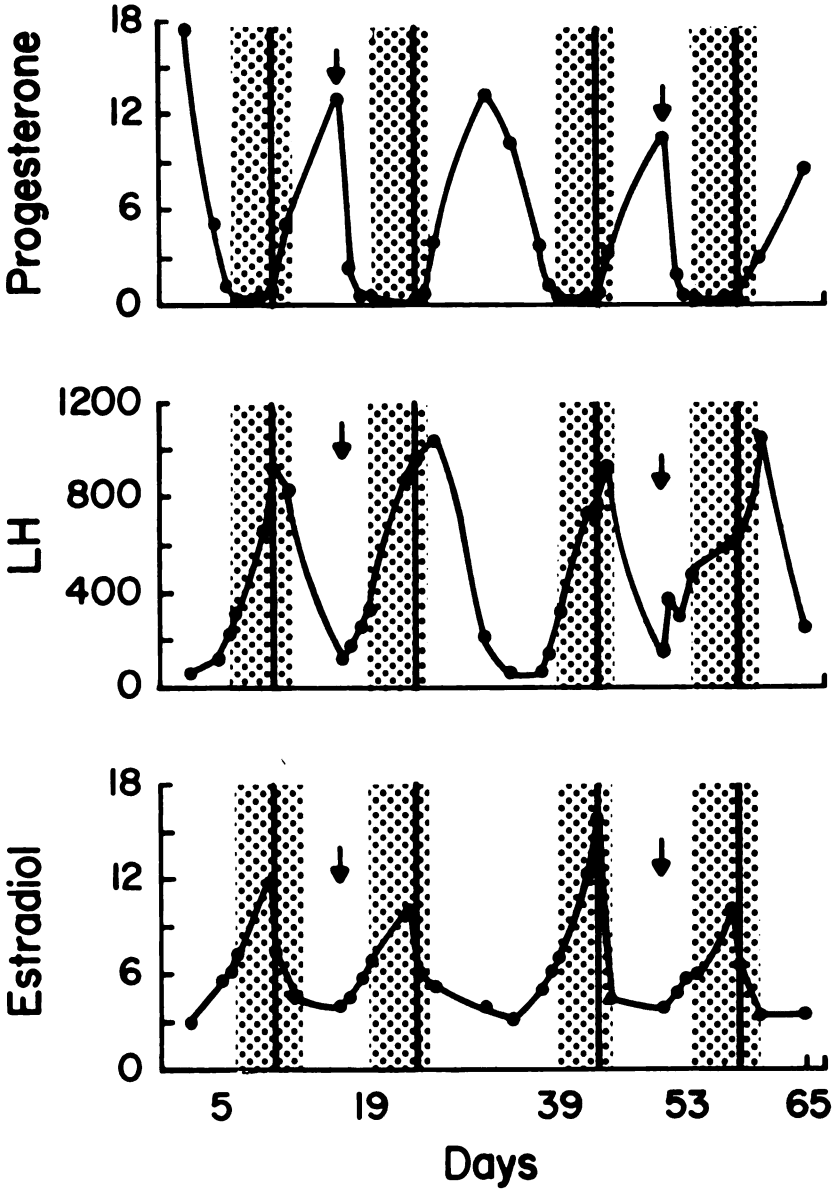

Figure 9.--Relative changes of plasma progesterone, LH and estradiol in mares during four consecutive estrous cycles. The second and fourth cycles were initiated by $PGF_{2\alpha}$ (↓). The patterned bars indicate the period of sexual receptivity; the vertical lines within the patterned bars indicate the day of ovulation.

androstenedione and estrone after sc $PGF_{2\alpha}$ are due to $PGF_{2\alpha}$ altered steroid metabolism, or due to seasonal changes in the pituitary-ovarian relationships or due in part to both is unknown. Nevertheless, during cycle IV, seemingly normal estrual behavior occurred, ovulation took place, and so it follows that the overall changes in hormones associated with these physiological events were also normal.

It was evident from these experiments that $PGF_{2\alpha}$ caused dramatic luteolysis in mares. Each treated mare began estrus within 4.5 days after $PGF_{2\alpha}$ treatment, in agreement with observations for ponies (Douglas and Ginther, 1972). Similarly, an analog of $PGF_{2\alpha}$ (ICI-79939) was luteolytic in Welsh ponies (Allen and Rowson, 1973).

Estrus, Ovulation and Serum Progesterone After 2, 3, 5 or 10 mg of $PGF_{2\alpha}$

Since 15 mg of sc $PGF_{2\alpha}$ resulted in rapid luteolysis when given to mares in the 1972 experiments, 10 mg $PGF_{2\alpha}$ (sc) was tested in one 550 kg mare and proved to be effective to cause luteolysis. In addition, Douglas and Ginther (1972) caused luteolysis in pony mares with the free acid of $PGF_{2\alpha}$ equivalent to about 9 µg/kg $PGF_{2\alpha}$-Tham salt in pony mares. Thus, about 3 to 5 mg $PGF_{2\alpha}$-Tham salt should be near the minimal effective dose in 500 kg mares. Consequently, in this experiment 2, 3, 5 or 10 mg of sc $PGF_{2\alpha}$ was given 7 to 9 days after ovulation to determine if in fact $\leq$ 3 mg $PGF_{2\alpha}$ would be luteolytic in mares.

Most mares during the 1972 experiment returned to estrus 2 to 3 days after $PGF_{2\alpha}$ and all were in estrus by the end of the fifth day. Furthermore, if $PGF_{2\alpha}$ treatment failed to cause luteolysis, normal demise of the CL would occur about 8 days later since the average duration of diestrus in control mares was 13 to 15 days and $PGF_{2\alpha}$ was given 5 to 7 days after the end of estrus. Therefore in this experiment if a mare exhibited estrual behavior within 4 days after treatment, $PGF_{2\alpha}$ was considered to have induced luteolysis. The decrease in blood progesterone after $PGF_{2\alpha}$ was taken as the main indication of luteolysis if the mare began estrus between 4 and 7 days. Luteolysis was not considered induced by $PGF_{2\alpha}$ in mares beginning estrus $\geq$ 7 days after $PGF_{2\alpha}$.

Estrus and Ovulation

After administration of 5 or 10 mg of $PGF_{2\alpha}$ sc, all of the treated mares began estrus within 4 days (Table 13); estrus persisted 6 to 8 days. However, 2 or 3 mg of $PGF_{2\alpha}$ sc failed to uniformly cause luteolysis; one of the four mares in each group did not begin estrus within 7 days after $PGF_{2\alpha}$ and, consequently, they were eliminated from the data in Table 12. However, most mares given 2 or 3 mg $PGF_{2\alpha}$ began estrual behavior by about 4 days after $PGF_{2\alpha}$ treatment (Table 13) as in the previous experiments when mares were given $PGF_{2\alpha}$ iu or sc.

Progesterone

Serum progesterone values, excluding the two mares which did not respond with luteolysis after $PGF_{2\alpha}$, are listed

TABLE 13.--Estrus and ovulation in mares after varying doses of $PGF_{2\alpha}$ (sc) 7 to 9 days after ovulation.[a]

Interval	Dose of Prostaglandin $F_{2\alpha}$ (mg)			
	2	3	5	10
	----------(days)----------			
From $PGF_{2\alpha}$ to estrus	4.5±1.0	2.8±0.4	3.3±0.6	2.5±0.4
From $PGF_{2\alpha}$ to ovulation	8.8±0.3	6.8±1.2	8.3±0.6	9.0±1.1
From onset of estrus to ovulation	4.3±1.2	4.0±0.4	5.0±0.5	6.3±0.8
From ovulation to end of estrus	2.7±0.3	2.0±0.6	1.0±0.0	1.8±0.3
Duration of estrus	7.0±1.2	6.0±1.0	6.0±0.5	8.1±0.7

[a]$PGF_{2\alpha}$ was given subcutaneously in 1 ml of saline; values are means ± S.E.M. Only three of four mares began estrus in response to 2 or 3 mg $PGF_{2\alpha}$, while the data for 5 and 10 mg $PGF_{2\alpha}$ are from five and six mares, respectively.

in Table 14. Similar to progesterone changes after the intrauterine and subcutaneous treatments (Figure 8; Tables 9 and 12), progesterone decreased markedly within 24 hours after 2, 3, 5 or 10 mg $PGF_{2\alpha}$ (sc) and continued to decrease during the 72-hour sampling period. Statistical analysis of the data for treatment effects and differences over time indicated significant differences in the rate of decline of progesterone ($P < .05$) among treatment groups. Serum progesterone decreased more rapidly after 5 or 10 mg of $PGF_{2\alpha}$; the concentration of progesterone fell under 1 ng/ml by 48 hours. However, after 2 or 3 mg $PGF_{2\alpha}$, progesterone had not

TABLE 14.--Mean serum progesterone in mares after varying doses of $PGF_{2\alpha}$ (sc) 7 to 9 days after ovulation.[a]

Hours From $PGF_{2\alpha}$	Dose of Prostaglandin $F_{2\alpha}$ (mg)			
	2	3	5	10
	(ng/ml)			
-24	10.8±1.7	10.8±0.3	11.4±1.2	14.8±3.7
0	9.1±3.2	15.1±2.8	11.2±1.0	11.4±1.9
24	3.2±1.4	3.3±0.5	3.0±0.4	2.4±0.3
48	2.1±1.0	1.5±0.4	0.9±0.1	0.9±0.2
72	1.5±1.0	1.1±0.3	0.6±0.1	0.6±0.1

[a] $PGF_{2\alpha}$ was given subcutaneously in 1 ml of saline; values are means ± S.E.M. Of four mares given 2 or 3 mg $PGF_{2\alpha}$ only three began estrus, while the data for 5 and 10 mg $PGF_{2\alpha}$ are from five and six mares, respectively.

decreased to less than 1 ng/ml by 72 hours when sampling ended (Table 14). Since $PGF_{2\alpha}$ caused luteolysis in an unequal number of mares in the various treatment groups, the results of statistical analysis are only approximate.

I conclude that 5 mg $PGF_{2\alpha}$-Tham salt (sc) is adequate to produce luteolysis in mares ($\leq$ 550 kg). Douglas and Ginther (1972) concluded that 1.25 mg of $PGF_{2\alpha}$ (free acid, M.W. = 354.5) was luteolytic in pony mares ($\leq$ 195 kg). Five mg of $PGF_{2\alpha}$-Tham salt (475.6 M.W.) is equivalent to 3.68 mg $PGF_{2\alpha}$ free acid and when compared on a body weight basis the minimal effective dose in my mares (approximately 9 µg/kg) was equivalent to that (8.6 µg/kg) in the pony mares (Douglas and Ginther, 1972).

Estrus, Ovulation and Serum Progesterone After 10 mg $PGF_{2\alpha}$ Administered on 1, 3, 5 or 7 Days After Ovulation

Since 10 mg of sc $PGF_{2\alpha}$ had proved to induce luteolysis in mares on day 7 after ovulation, this dose was chosen to test the relationship between the age of the CL and $PGF_{2\alpha}$-induced luteolysis. In cattle, $PGF_{2\alpha}$ was effective on day 5 or 11 but not on day 3 after ovulation (Louis et al., 1973). But luteal tissue appears to form more rapidly in mares, so I administered 10 mg sc $PGF_{2\alpha}$-Tham salt to 5 mares each on day 1, 3 or 5 after ovulation and to 6 mares on day 7.

Estrus and Ovulation

Some estrual characteristics for the mares which responded to $PGF_{2\alpha}$ are listed in Table 15. The data for mares on day 7 are the same as those included in Tables 11 and 12 under 10 mg. Among the five mares given 10 mg $PGF_{2\alpha}$ 1 day after ovulation none began estrus within 7 days. Only two of five mares treated 3 days after ovulation responded to treatment with a reduced duration of diestrus. All of the mares given $PGF_{2\alpha}$ on 5 or 7 days after ovulation and two mares treated on the third day postovulation began estrus, most within 4 days after $PGF_{2\alpha}$ (Table 15). Average duration of estrus ranged from 6 to 8 days among treatment groups.

Although the interval from $PGF_{2\alpha}$ to ovulation and duration of estrus after $PGF_{2\alpha}$ on day 5 or 7 were somewhat longer than those after treatment on day 3, none of the differences in any of the intervals listed in Table 14 was significant for those mares in

TABLE 15.--Estrus and ovulation in mares given 10 mg $PGF_{2\alpha}$ (sc) on various days after ovulation.[a]

Interval	Days after ovulation			
	1	3	5	7
	----------(days)----------			
From $PGF_{2\alpha}$ to estrus	---	2.0±0.3	2.9±1.0	2.5±0.4
From $PGF_{2\alpha}$ to ovulation	---	5.5±3.0	8.7±1.2	9.0±1.1
From onset of estrus to ovulation	---	3.5±2.5	5.8±0.5	6.3±0.8
From ovulation to end of estrus	---	2.0±0.0	2.0±0.6	1.8±0.3
Duration of estrus	---	5.5±2.5	7.8±1.0	8.1±0.7

[a] $PGF_{2\alpha}$ was given subcutaneously in 1 ml of saline; values are means ± S.E.M. None of the five mares began estrus in response to $PGF_{2\alpha}$ 1 day after ovulation, two of five mares began estrus after treatment on day 3, and the data for day 5 and 7 are for five and six mares, respectively.

which the CL function was terminated. Intervals to estrus and ovulation and duration of estrus in mares given 10 mg $PGF_{2\alpha}$ (sc) on 3, 5 or 7 days after ovulation were comparable to those for mares which responded to 2, 3 or 5 mg of $PGF_{2\alpha}$ on 7 days after ovulation in the previous experiment. Allen and Rowson (1973) also reported that $PGF_{2\alpha}$ was not luteolytic before 3 days after ovulation in mares; and as in this study some animals responded on day 3 while some did not.

Progesterone

Table 16 lists serum progesterone for the mares which responded to 10 mg $PGF_{2\alpha}$ (sc) on 3, 5 or 7 days after ovulation. Progesterone decreased rapidly ($P < .01$) after $PGF_{2\alpha}$ to $\leq$ 1 ng/ml by 48 hours after treatment. As expected from data from control cycles (Figure 3; Tables 8 and 10), serum progesterone averaged 6.7 ng/ml at the time of treatment on day 3 after ovulation, less than the 11 or 12 ng/ml for mares treated on day 5 or day 7. As was true for all mares responding to $PGF_{2\alpha}$ (Figure 8; Tables 9 and 12) as well as for control mares during luteolysis (Figure 3; Tables 8 and 10), estrus began when progesterone was near 1 ng/ml

TABLE 16.--Serum progesterone in mares after 10 mg $PGF_{2\alpha}$ (sc) on various days after ovulation.[a]

Hours From $PGF_{2\alpha}$	Days After Ovulation		
	3	5	7
	----------(ng/ml)----------		
-24	4.9±0.3	11.2±1.9	14.8±3.7
0	6.7±0.4	12.8±3.4	11.4±1.9
24	1.8±0.3	2.9±0.9	2.4±0.3
48	0.7±0.1	1.0±0.2	0.9±0.2
72	1.8	0.7±0.1	0.6±0.1

[a] $PGF_{2\alpha}$ was given subcutaneously in 1 ml of saline; values are means ± S.E.M. None of the five mares began estrus in response to $PGF_{2\alpha}$ 1 day after ovulation, while two of five mares began estrus after treatment on day 3. The data for day 5 and 7 are for five and six mares, respectively.

(Tables 15 and 16). I suspected that the two mares which responded to $PGF_{2\alpha}$ on day 3 may have had a more mature CL than the three mares which did not begin estrus after $PGF_{2\alpha}$. However, serum progesterone was no higher at treatment in the mares that began estrus (6.7 ± 0.4 ng/ml) than in the mares which did not begin estrus (7.6 ± 0.3 ng/ml) within 8 days after treatment on day 3 (Tables 16 and 17).

In contrast to the fall in progesterone after $PGF_{2\alpha}$ treatment on day 5 or 7, serum progesterone continued to increase in mares given $PGF_{2\alpha}$ 1 day after ovulation (Table 17). This increase was comparable to progesterone changes during the 3 or 4 days after ovulation in control cycles (Figure 3).

TABLE 17.--Serum progesterone for mares in which $PGF_{2\alpha}$ did not result in estrus within 8 days of treatment.[a]

Hours From $PGF_{2\alpha}$	Dose of $PGF_{2\alpha}$ (mg)		Days After Ovulation	
	2	3	1	3
-24	17.6	9.0	1.6±1.2	3.9±0.5
0	10.6	6.2	1.7±0.8	7.6±0.3
24	6.3	2.9	2.2±0.3	3.8±1.5
48	5.9	1.7	4.2±0.8	4.1±1.8
72	7.4	2.4	6.3±1.1	4.3±2.5

[a]$PGF_{2\alpha}$ was given subcutaneously in 1 ml of saline; values are means ± S.E.M. Of four mares given 2 or 3 mg $PGF_{2\alpha}$, one mare did not begin estrus, while all five mares given 10 mg $PGF_{2\alpha}$ (sc) on day 1 did not begin estrus and three of five mares treated on day 3 after ovulation did not begin estrus within 8 days.

The transient decrease in progesterone after 2 or 3 mg of $PGF_{2\alpha}$ was given on day 7 or after 10 mg $PGF_{2\alpha}$ on day 3 (Table 17) apparently reflected incomplete luteolysis. Evidently these PGF_2 treatments were not sufficient to complete luteolysis, because although progesterone decreased 50% at 24 hours after $PGF_{2\alpha}$, it began to increase at 72 hours. As a result these five mares did not begin estrus until 8 to 16 days after $PGF_{2\alpha}$ treatment as would have been expected without $PGF_{2\alpha}$ treatment.

In summary, complete luteolysis resulted after 10 mg of $PGF_{2\alpha}$-Tham salt (sc) on or after the fifth day of CL development. Some mares responded with luteolysis after $PGF_{2\alpha}$ on day 3; others had only a transient decline in progesterone and consequently $PGF_{2\alpha}$ did not reduce the duration of diestrus in these mares. $PGF_{2\alpha}$ did not alter the estrous cycle when given on day 1 after ovulation.

Breeding, Pregnancy and Foaling After $PGF_{2\alpha}$

To determine fertility after $PGF_{2\alpha}$, eight mares (four each in 1972 and 1973) were mated during the estrus that followed 10 or 15 mg $PGF_{2\alpha}$-Tham salt (sc). Beginning on the second day of estrus the mares were mated every other day to a fertile stallion until ovulation occurred. Six (75%) of the eight mares mated after sc $PGF_{2\alpha}$ were pregnant (four in 1972, two in 1973) on the basis of palpations around day 40 of gestation. One mare apparently aborted between 50 and 80 days, a period when mares most frequently abort (Allen, 1972) Parturition for five mares (63%) occurred

332 ± 3.0 days after ovulation; all deliveries were unassisted and the foals appeared healthy and normal. Foal heat occurred 6.5 ± 0.4 days later; it persisted 7.0 ± 1.2 days and ovulation occurred 2.2 ± 1.1 days before the end of estrus. The fertility and duration of pregnancies and the characteristics of the foal heats in these mares resembled those reported for untreated mares (Asdell, 1946). That the foals were healthy dispelled fears of abnormal fertilization, implantation or embryogenesis following $PGF_{2\alpha}$ treatment. A Pennsylvania study (Ganjam, 1975) indicated that fertility was normal (65%) in 350 mares mated (or inseminated) following $PGF_{2\alpha}$ (5 mg, sc).

Therefore, $PGF_{2\alpha}$, at least in these two reports, did not decrease conception and pregnancy, because Sullivan (1972) reported that the average pregnancy rate was 51% for the Quarter Horse and 43% for the Thoroughbred. Thus, I am optimistic that $PGF_{2\alpha}$ would not be detrimental to fertility after use for induction of luteolysis. In fact my data suggests the synchrony of ovulation after $PGF_{2\alpha}$ is such that mares could be bred at 7 days after $PGF_{2\alpha}$ without regard to signs of estrus.

Side Effects of $PGF_{2\alpha}$

After infusion of 10 mg $PGF_{2\alpha}$-Tham salt into the uterus of the first mare treated in the first experiment, I noted about 15 minutes later that she was sweating profusely. Her hair and body were wet to the touch, and droplets of water moistened the dry ground beneath her. Three more mares were observed closely

after $PGF_{2\alpha}$ (10 mg, iu) treatment. Sweating began about 10 to 15 minutes after $PGF_{2\alpha}$; first the skin was damp behind and between the front legs and spread until the entire body was damp. Within 15 to 20 minutes after $PGF_{2\alpha}$ droplets of water covered the hairs and dropped from the body. The mares appeared to have experienced a hard run, however all were tied. During the 20 minutes after $PGF_{2\alpha}$ rectal temperature decreased from an average of 100.5°F, by 0.5°F, 1.2°F, and 1.4°F in the three mares. The sweating subsided and the mares had begun to dry off by 35 minutes after treatment. Mares were observed to sweat noticeably after 60% of the $PGF_{2\alpha}$ treatments during the 1972 project. They began to sweat 10 to 15 minutes after $PGF_{2\alpha}$ but were dry by 45 to 60 minutes. They did not appear to be very uncomfortable. For example, often they continued to eat hay. However, modest symptoms associated with colic were noticed in 70% of the mares: dulled eyes, trembling upper lip, involuntary muscle contraction of gut reflected by arched back. Frequent defecation and minor diarrhea were observed once each. These symptoms appeared and disappeared within the 45 to 60 minutes after treatment. During the 1973 experiment, four more mares were observed closely. All four began to sweat 8 to 15 minutes after $PGF_{2\alpha}$; rectal temperatures dropped from 100.8°F to 98.0°F at 35 minutes when sweating had again decreased and the mares began to dry off.

Lauderdale and Miller (1974) rated the sweating in mares on a four-point (0 to 3) scale and found that there was a graded response of sweating and temperature decrease after various doses

of $PGF_{2\alpha}$. The mares receiving smaller doses had less sweating and a smaller change in rectal temperature. In one mare given 200 mg of $PGF_{2\alpha}$-Tham salt, Lauderdale (1973) described profuse sweating, muscle tremors and a dullness of the eyes, but full recovery followed within 3 to 4 hours. Lauderdale and Miller (1974) indicated that mares given epinepherine (10 mg) began sweating within 8 to 10 minutes and had a transient but insignificant (.25°F) decrease in body temperature; shiving in response to epinepherine may have adjusted body temperature back towards normal. Mares given $PGF_{2\alpha}$ were sweating 15 to 20 minutes after treatment, and decreased body temperature (2°F) occurred but shivering did not. The $PGF_{2\alpha}$ mares had a depressed body temperature for 3 to 4 hours until other effects of $PGF_{2\alpha}$ had disappeared. Lauderdale and Miller (1974) also suggested that the mares may not shiver after $PGF_{2\alpha}$ due to depression of the hypothalamic temperature regulating mechanism, because sweating can be caused by stimulus of the anterior hypothalamus; shivering by stimulus of the posterior hypothalamus (Ganong, 1969). It is possible that while $PGF_{2\alpha}$ stimulates sweating, it may inhibit the shivering response. However, this does not appear to be a major problem since even after a mare was given 40-fold higher than the minimal effective dose of $PGF_{2\alpha}$, all of these side effects disappeared within 4 hours.

Similar to the side effects following $PGF_{2\alpha}$ treatment, sweating, hypermotility of the gastrointestinal tract, diarrhea, increased pulse and respiration and slight abdominal pain followed

treatment with a $PGF_{2\alpha}$ analog (ICI 79939) in mares (Allen and Rossdale, 1973). They suggested that it should be possible to develop a $PGF_{2\alpha}$ analog with fewer toxic effects in mares.

Abnormally Prolonged Luteal Function

As previously stated, one mare did not exhibit estrual behavior during cycle III of the first experiment. Inspection of progesterone values for cycle I and cycle III revealed progesterone near 1 ng/ml on 1 day while she was in estrus during cycle I, while her progesterone was never below 5 ng/ml during cycle III. This was in marked contrast to the usual progesterone pattern in mares (Figure 3). This mare did, however, exhibit a normal estrus following $PGF_{2\alpha}$ in cycles II and IV, and progesterone was below 1 ng/ml during estrus after $PGF_{2\alpha}$ in both cases. This observation suggested that $PGF_{2\alpha}$ had potential for induction of luteolysis in mares with abnormally extended CL function. The mare discussed above and two of her herdmates had abnormal estrous cycles. All three of the mares had previously exhibited estrual behavior but only briefly (2 to 3 days) and with less intensity (rated 5+) than normal. Ovulations which occurred in the absence of estrus were detected when progesterone was 5 to 7 ng/ml in all three mares. However, these ovulations were not accompanied by an increase in LH concentrations and may have been atretic follicles or otherwise abnormal follicles. After 35.7 ± 0.9 days of extended luteal function when progesterone was 3 to 11 ng/ml, all three mares were given $PGF_{2\alpha}$. For a total of seven treatments of $PGF_{2\alpha}$ in these

three mares, estrus began in every case in an average of 2.6 ± 0.4 days (Table 18), it persisted 5.4 ± 1.1 days and ovulation occurred 0.7 ± 0.6 days before the end of estrus. The interestrual interval between two consecutive estrus periods following $PGF_{2\alpha}$ treatments was 9.5 ± 1.5 days. One of these mares was mated; she conceived and foaled 330 days later. Kenney et al. (1975) treated 73 maiden, barren and foaling mares having extended periods of clinical

TABLE 18.--Estrus and ovulation after $PGF_{2\alpha}$ in three mares with abnormally prolonged luteal function.[a]

Interval	Control Cycle[b]	$PGF_{2\alpha}$ Cycle[c]
	----------(days)----------	
From $PGF_{2\alpha}$ to estrus	---	2.6±0.4
From $PGF_{2\alpha}$ to ovulation	---	6.9±0.5
From onset of estrus to ovulation	5.0±0.6	4.3±0.5
From ovulation to end of estrus	-1.3±0.3[d]	0.7±0.6
Duration of estrus	3.7±0.7	5.0±1.1
Interestrual interval	35.7±0.9[e]	---

[a]Estrus and ovulation were estimated at 12 hours previous to time first detected. Doses were 10, 15 or 20 mg (sc) or 10 mg (iu). Values are means ± S.E.M.

[b]n = 3. [c]n = 7.

[d]Ovulation occurred after the end of estrus.

[e]The number of days of prolonged luteal function between the end of a previous estrus and the first day of estrus after $PGF_{2\alpha}$.

anestrus (73 days) during the breeding season with $PGF_{2\alpha}$. Estrus began in 73% of the mares 4.4 days after 5 mg of $PGF_{2\alpha}$ (sc) and 55% conceived. Allen and Rossdale (1973) related that a $PGF_{2\alpha}$ analog (ICI 79939) was used as a treatment for nonpregnant mares with no estrual behavior and progesterone levels > 1 ng/ml during the breeding season; 34 of 35 mares began estrus as a result of treatment. It probably is essential for a CL to be present in these mares if $PGF_{2\alpha}$ treatment is to be effective.

$PGF_{2\alpha}$ (sc) treatment of mares with extended luteal function has advantages over infusion of saline or other materials into the uterus to induce luteolysis. Mares had reduced duration of luteal function after infusion of saline (Bain, 1957; Ginther, 1971), but because of the threat of uterine infections from iu treatments, I favor sc $PGF_{2\alpha}$ treatment. Likewise, noncycling mares exhibited estrus after stilbestrol or estrogen treatment, but ovulation did not occur unless a follicle was present (Burkhardt, 1947b; Nishakawa, 1959). Similarly, HCG treatment caused ovulation only when follicles were present (Day, 1940). Therefore, since the luteolysis which follows $PGF_{2\alpha}$ appears to trigger the sequence of events which follow normal uteolysis, it should be a useful treatment for mares with abnormally prolonged luteal function.

GENERAL DISCUSSION

Hormonal Interactions During the Estrous Cycle in Mares

Blood progesterone decreased rapidly in mares during the 3 days before estrus in untreated mares as well as after $PGF_{2\alpha}$ treatment. Similarly, progesterone decreased during the 3 days before estrus (Wettemann et al., 1972) as well as after $PGF_{2\alpha}$ treatment in cows and heifers (Louis et al., 1973; 1974). Furthermore, the decline in progesterone before estrus in ewes and sows (reviewed by Hansel and Echternkamp, 1972) resembles that in mares and cows. Therefore, CL regression and the onset of estrus appear to be temporally related in the mare much like they are in other large domestic animals.

Exogenous progesterone suppresses estrus behavior and/or ovulation in most mammals, including mares (Loy and Swan, 1966). However, quantities of progestogens that blocked estrus did not always block ovulation in mares. For example, 100 mg of progesterone given mares daily blocked estrus and ovulation, but ovulation occurred in the absence of estrual behavior while 50 mg/day was administered. It appears, then, that in the mare ovulation can occur in the absence of estrus, but apparently blood progesterone must be minimal ($\leq$ 1.0 ng/ml) to allow signs of estrual behavior.

Comparatively, maximal progesterone values in the mare were greater (15 ng/ml) than in cattle (4.6 ng/ml; Wettemann et al.,

1972) and sheep (3.0 ng/ml; Thorburn et al., 1969). In addition, much higher doses of exogenous progesterone were required to suppress estrus in the mare than in the cow and ewe (reviewed by Britt and Ulberg, 1971). Similarly, synthetic progestogens in quantities nearly 10- to 20-fold greater than those effective in cattle or sheep were ineffective in mares. It appears that higher levels of exogenous progesterone are necessary to block estrual behavior and/or ovulation in mares than in cows and sheep.

Stabenfeldt et al. (1972) reported that mares occasionally ovulated during early or late diestrus when blood progesterone was at about 50% of maximal diestrual concentrations. In my study, although not accompanied by significant LH changes, four ovulations occurred during periods of abnormally prolonged luteal function; three occurred at times when progesterone was less than maximal for a given mare. Three other ovulations occurred when LH was still elevated during early diestrus. It seems possible that the changes on the ovarian surface called "diestrus ovulations" may in fact be large follicles which subside without ovulation.

Although progesterone remains near basal values during estrus in cows, sows, ewes and mares, the duration of estrus in the mare is much longer than that in the others. In mares, before and during estrus, progesterone remained below 1 ng/ml for 6 to 8 days until about 24 to 36 hours after ovulation. Cows also have progesterone below 1 ng/ml for 5 days (Wettemann et al., 1972), but duration of estrus was less than 1 day. Similar to the cow, progesterone in the ewe remained low for 5 days and estrus persisted

28 hours (Thorburn et al., 1969; Hansel and Echternkamp, 1972). Thus the prolonged period of estrus in the mare does not appear to be due entirely to a prolonged period of low progesterone.

Synthesis of significant quantities of progesterone appears to occur sooner after ovulation in mares than in other species, because blood progesterone increased 7- to 10-fold within 36 hours after ovulation in mares. Formation of luteal tissue occurred in ovaries of mares 24 to 48 hours postovulation (Short, 1964). In contrast, progesterone remained at basal values for 48 to 72 hours after ovulation in the cow and ewe (Thorburn et al., 1969; Swanson et al., 1972). As a result, blood progesterone peaks within about 7 days after ovulation in mares (Table 9, cycle II), but not until 10 or 11 days after ovulation in the ewe and cow.

In the rabbit, before ovulation and following the initial LH release, 20α-hydroxypregn-4-en-3-one (20α-OHP) secretion from the ovarian interstitial tissue acts to prolong LH secretion (Hilliard et al., 1967). Preovulatory increases of progestogens in the human, the rhesus monkey and the rat were reported (reviewed by Miyake, 1974), but no such increase in progesterone has been reported for the cow, ewe or sow (Hansel and Echternkamp, 1972). In agreement with data in cows, I detected no preovulatory increase of progesterone in mares. However, this could be due to infrequent bleeding and/or the inability of the progesterone antibody to bind 20α-OHP. Short (1964) reported the presence of 20α-OHP in the luteal tissue of mares postovulation.

In most mammals, blood estrogen production is related to follicular development. In mares both follicular size and estradiol concentrations increased 2- to 3-fold to a maximum 12 to 36 hours before ovulation (Figures 4 and 7; Tables 8, 9, 10 and 12). Similarly in cows, follicular size increased 2-fold during the 4 to 5 days before ovulation and estradiol was highest 0.5 days before estrus, about 40 hours before ovulation (Hansel and Echternkamp, 1972; Wettemann et al., 1972). Total estrogens in the ewe and sow peaked near the onset of estrus, near the time of maximum follicular development (Scaramuzzi et al., 1971; Hendricks et al., 1972). Thus, the pattern of follicular estradiol production in mares resembles that of other species, peaking 24 to 48 hours before ovulation. However, estrogen may be elevated longer in mares during lowered progesterone, resulting in a longer period of estrus. It is also possible that the longer period of estrus in mares is "due to the longer time taken by the follicle to come to the surface of the ovulation fossa and break through" (Eckstein and Zuckerman, 1956a).

Other than the peak in estradiol just before ovulation, no other significant changes in estradiol were observed during the estrous cycle of mares. However, other estrogens may reflect follicular changes during the remainder of the cycle. Hansel and Echternkamp (1972) reported three elevations of plasma estrone which they suggested coincided with waves of follicular development during the estrous cycle in cows, although Marion and Gier (1971) reported development of follicles during all stages of the

estrous cycle. Urinary estrone was elevated before ovulation in women (Moghissi et al., 1972). However, there were no significant changes in blood estrone during the estrous cycle in my mares (Tables 8, 9, 10 and 12). This is in contrast to reports of increased urinary estrone prior to ovulation in mares (Loy, 1970). Peak urinary estrone, however, may reflect renal metabolism of plasma estradiol.

Estrogens have been implicated as the cause of pituitary LH release in several species. For example, in the cow, ewe and sow estradiol begins to increase 2 to 4 days before LH increases and reaches maximal levels one or two days before the ovulatory surge of LH in the cow and ewe and 4 days before the LH peak in sows (Hansel and Echternkamp, 1972). Furthermore, exogenous extradiol evoked release of LH in ovariectomized heifers (Hobson and Hansel, 1972; Beck and Convey, 1974) in intact heifers after enucleation of the CL (Hobson and Hansel, 1972), and in anestrus (Beck and Reeves, 1973) or ovariectomized ewes (Goding et al., 1970). The increase of estradiol concentrations that preceded and accompanied the LH release in my mares (Tables 8, 9, 10 and 12) suggests that estradiol stimulates release of LH in mares as in the other species. The increase in estradiol began 2 days before estrus, and the LH surge began 1 day later. Then estradiol continued to increase to maximal values the day before ovulation, slightly anticipating the parallel changes in LH.

LH is the ovulatory hormone in all mammals. In most species, an ovulatory surge of LH occurs briefly about 1 day

before ovulation. In cows, for example, a 10- to 20-fold increase in LH persisted 8 hours and preceded ovulation by an average of 32 hours (Swanson et al., 1972). In contrast to the relatively brief ovulatory surge of LH in the cow, LH in mares was elevated for 12 to 14 days. It began to increase near the onset of estrus and continued to a relatively broad plateau persisting for 3 or 4 days near ovulation. To my knowledge, the broad elevation of LH persisting 12 to 14 days is unique to the mare.

LH causes release or synthesis of proteolytic enzymes in the follicle of several species, enzymes which may increase the distensibility (decreased tensile strength) of the follicle wall (Espey, 1974). Perhaps ovulation in mares requires prolonged LH stimulus to produce these enzymes because of unique anatomical features of her ovary. As discussed in the literature review, ovulation nearly always occurs at the ovulation fossa in mares. Therefore, developing follicles must migrate through the ovarian stroma to the fossa before ovulation. Perhaps prolonged LH stimulation is required to complete this migration. However, I have no evidence to support either of these theories.

That continued LH stimulus is a requirement for ovulation in mares is indicated by response of time of ovulation to different amounts of LH released to gonadotropin releasing hormone (GnRH) stimulus. When 400 μg of GnRH was given to pony mares once on day 2 of estrus, LH increased 2-fold and the blood LH was sustained 3 hours (Ginther and Wentworth, 1974); the duration from onset of estrus to ovulation was similar to that in control mares. However,

when 2 mg of GnRH was given to light mares beginning on day 2 of estrus daily until ovulation, the interval from onset of estrus to ovulation was reduced (Downey et al., 1974). This suggests that release of greater quantities of endogenous LH after continued stimulus with exogenous GnRH caused ovulation to occur sooner after the onset of estrus and possibly that release of some minimal quantity of LH may be necessary for ovulation to occur.

Presumably, the brief surge of LH before ovulation and/or the low levels following the LH spike in cows and ewes is sufficient to cause luteinization of the granulosa cells and initiate progesterone secretion. Possibly the granulosa cells of the ruptured follicle of mares requires a prolonged LH stimulus for this to occur. This may be related to the fact that equine granulosa cells have 60% fewer receptors than porcine granulosa cells (Channing, 1974). Assuming LH is luteotropic in the mare as in most species (Hansel and Echternkamp, 1972), the sustained concentration of LH before and after ovulation may account for more rapid development of luteal function after ovulation in mares than in other species.

The anterior pituitary of the mare appears to be sensitive to removal of progesterone, as is true in other species; after progesterone decreased $\geq$ 50%, estradiol increased, and increased LH followed 1 day later. Similar changes were reported for the ewe, cow and sow (reviewed by Hansel and Echternkamp, 1972). However, LH does not decline until progesterone is increased in the mare suggesting progesterone may be required to

stop anterior pituitary release of LH. An alternative, however, is that a longer time is required for metabolism of equine LH (as discussed in the review of literature).

Short (1972) suggested androstenedione may be important in regulating estrous behavior in mares. Although there is some evidence to suggest that androgens are related with sexual behavior in primates (Everitt and Herbert, 1969), to my knowledge it has not been proven. Some authors reported an androstenedione peak before ovulation in women (Abraham, 1974; Baird et al., 1974), while others found no change in androgens at ovulation (Horton, 1965). Similarly, androstenedione was elevated during proestrus in rats (Dupon and Kim, 1973) and ewes (Baird et al., 1968). In my mares, blood androstenedione was elevated during the period when mares exhibited estrual behavior, and peaked 1 or 2 days before ovulation. Since mare follicular fluid and ovarian vein blood contained large quantities of androstenedione (Short, 1964), presumably the blood androstenedione is of follicular origin in mares as reported for women (Abraham, 1974) and may reflect synthesis of estradiol.

The ever increasing levels of estradiol (Figure 4) and androstenedione (Figure 6) were accompanied by ever increasing concentrations of LH in the plasma samples collected 5 to 6 days before ovulation. The continual increase of estradiol was terminated 2 days before ovulation (Figure 4, cycle I) whereas androstenedione levels reached a maximum 1 day before ovulation (Figure 6,

cycle I). LH increased to maximal levels the day of ovulation (Figure 7, cycle I).

It would be interesting to test the idea that the continual increase of LH in some manner first blocked the enzymatic conversion of androstenedione to estradiol 1 to 2 days before ovulation and secondly blocked the formation of androstenedione just before ovulation. According to this scheme, LH would continue to eliminate or block enzymes of the steroidogenic pathway in the ovary resulting in an increase in progesterone. Progesterone in turn might exert a negative influence on the pituitary and/or hypothalamus causing diminution of LH release about 2 days after ovulation (Figure 7). With further increase in progesterone, LH secretion finally reached basal levels 6 or 7 days after ovulation (Figure 7).

Mechanism of Action of $PGF_{2\alpha}$

The uterus of the cow and ewe exert a local control on the ovary (reviewed by Anderson et al., 1969), but uterine control of luteal function acts systemically in the mare (Ginther and First, 1971). A possible explanation may lie in the fact that the utero-ovarian vein and the ovarian artery were closely related anatomically; i.e., the tortuous ovarian artery was adhered to the surface of the utero-ovarian vein in the ewe and cow (Ginther, 1974). This might facilitate the direct transfer of the uterine luteolysin from the uterine vein into the ovarian artery to deliver luteolysin in high concentration directly to the ovary. In the mare,

however, such an intimate relationship does not exist between the two vessels. Therefore, it was not surprising that $PGF_{2\alpha}$ was no more effective as a luteolysin when injected into the uterus than when given systemically (sc) in mares. In contrast, the cow (Hafs et al., 1974; Stellflug et al., 1975) and the ewe (Ginther, 1974) required considerably more $PGF_{2\alpha}$ to cause luteolysis when administered systemically than when $PGF_{2\alpha}$ was injected into the uterus. My data on $PGF_{2\alpha}$-induced luteolysis agree with Ginther's (1974); $PGF_{2\alpha}$ was equally effective whether given systemically or injected into the uterus of mares. Possibly the uterine luteolysin, like $PGF_{2\alpha}$, is inactivated rapidly in the lungs (Piper et al., 1970) and/or other tissues such as the liver, kidney and spleen (Oesterling, 1972). If so, a direct transfer of the uterine luteolysin or $PGF_{2\alpha}$--from the utero-ovarian vein to the ovarian artery--would avoid degradation by the lungs or other tissues before it reached the CL. On the other hand, the tissues of animals without a local transfer mechanism for the transfer of the uterine luteolysin such as the mare may not degrade $PGF_{2\alpha}$ (or the uterine luteolysin) as rapidly (if at all) as in cows and ewes. This would explain not only the phenomena of local vs systemic control but also the differential effect of sc vs iu $PGF_{2\alpha}$ in the two types of animals having different utero-ovarian relationships.

Although luteolysis is interrupted when the uterus is removed, exogenous $PGF_{2\alpha}$ appears to cause luteolysis in the absence of the uterus; sc $PGF_{2\alpha}$ caused luteolysis in hysterectomized pony mares indistinguishable from that in intact mares given $PGF_{2\alpha}$

(Douglas et al., 1974). Similarly, $PGF_{2\alpha}$ caused luteolysis in hysterectomized cows (Stellflug et al., 1975). Thus, the luteolytic effect of $PGF_{2\alpha}$ does not require the presence of the uterus; $PGF_{2\alpha}$-induced luteolysis appears to represent a direct action of $PGF_{2\alpha}$ on the CL.

The CL of mares appears to be more sensitive to $PGF_{2\alpha}$ than the CL of cows and ewes, since the minimal effective sc dose for $PGF_{2\alpha}$ to induce luteolysis was 22-fold greater on a body weight basis for ewes than for mares (Ginther, 1974). Similarly, the minimal effective dose of $PGF_{2\alpha}$ in cows (Stellflug et al., 1975) was at least 3-fold higher than that for mares. The minimal effective dose of $PGF_{2\alpha}$ was 5 mg or 9 μg/kg in the mares of this study, comparable to that in pony mares (Douglas and Ginther, 1972). As an alternative to greater sensitivity to $PGF_{2\alpha}$ in the mare, perhaps the cow and ewe metabolize $PGF_{2\alpha}$ more rapidly.

The CL appears to be more sensitive to lytic agents when full luteal function has been developed than during the formative stages of the CL. In my study the CL of mares became susceptible to the luteolytic action of $PGF_{2\alpha}$ before day 5 after ovulation; $PGF_{2\alpha}$ caused luteolysis in no mares treated on day 1, in three of four mares treated on day 3 and in all mares treated on day 5 after ovulation. Similar results were reported for pony mares (Allen and Rowson, 1973) and cattle (Rowson et al., 1972).

By what mechanism does $PGF_{2\alpha}$ cause the demise of the CL? Originally, Pharriss and Wyngarden (1969) suggested that venoconstriction of the utero-ovarian vein caused by $PGF_{2\alpha}$ might be

responsible for the resultant luteolysis. The venoconstriction would reduce blood flow to the ovary and "starve" the CL. Furthermore, distribution of radioactive microspheres before and after $PGF_{2\alpha}$ demonstrated a redistribution of blood supply to the CL (reviewed by Poyser, 1973, and Pharriss et al., 1972). However, this distribution of blood supply may be a consequence of CL regression and not a cause.

$PGF_{2\alpha}$ also inhibits cholesterol ester synthetase, an enzyme necessary for formation of the cholesterol esters essential for progesterone production (Poyser, 1973). In contast, $PGF_{2\alpha}$ caused increased progesterone production by luteal tissue in vitro during a 3-hour incubation: but in agreement, a longer, incubation of $PGF_{2\alpha}$ with luteal tissue resulted in decreased progesterone production (Poyser, 1973).

Direct infusion of $PGF_{2\alpha}$ into the ovaries of ewes caused an initial increase in progesterone levels in the ovarian vein. This initial increase in progesterone was followed by a pronounced decrease in progesterone (Poyser, 1973). If $PGF_{2\alpha}$ caused an increase in progesterone production in cows it was not detected in a sample of jugular blood taken 10 minutes after $PGF_{2\alpha}$ treatment (Hafs et al., 1974)--progesterone decreased in cows immediately and continued to decrease rapidly for 1 hour.

In conclusion, the mechanism of action of $PGF_{2\alpha}$ on the CL of the mare (or of other species) is unknown. It is possible $PGF_{2\alpha}$ acts initially to cause redistribution of blood and secondly to inhibit cholesterol synthesis. $PGF_{2\alpha}$ may bind directly

to the luteal cells since prostaglandin receptors have been reported in these cells (Rao, 1974).

Practical Applications

Replacement of horses by tractors, at least in the United States, is reflected by the decreased numbers of horses and mules from a peak of about 25 million in 1920 to 2.8 million in 1950 (U.S. Department of Commerce, 1960). Recently, however, the numbers of horses are increasing--principally for recreation and meat production. There was estimated to be 121 million horses, mules and asses in the world (excluding Russia and Red China) and 8 million in the United States (U.S., Food Agric. Orig., 1972a). In 1972 the meat production from slaughtered horses, mules, asses and crosses was estimated to be 386,000 metric tons (U.S., Food Agric. Orig., 1972b), mostly in Europe and South America.

The amount of time spent horseback riding in the U.S.A. in 1973 was equivalent to that spent golfing, waterskiing or snowskiing (U.S. Department of Commerce, 1974a). Horseracing was attended by 73 million people in 1973, comparable to the total number of people attending college and professional football games (U.S. Department of Commerce, 1974b).

Breeding efficiency in mares is low in comparison to cows, due at least in part to the variability in the duration of estrus and thus the time of ovulation is difficult to predict. Pregnancy rate was 43% after Thoroughbred mares were mated once during estrus, and 51% for Quarter Horse mares inseminated artificially or mated naturally several times during estrus (Sullivan, 1972).

In my study, ovulation in mares occurred relatively synchronously at 8 days after $PGF_{2\alpha}$. Thus, possibly mares could be mated (or inseminated) with normal fertility 7 or 8 days after $PGF_{2\alpha}$ without regard to estrus. For such a management regime to be useful, fertility following $PGF_{2\alpha}$ must be at least equivalent to that achieved with present management systems. In the small number of mares mated after $PGF_{2\alpha}$ in this study, 5 of 8 foaled (63%), and Allen et al. (1975) reported 83% fertility when mares were mated after an analog of $PGF_{2\alpha}$ was used to induce luteolysis. $PGF_{2\alpha}$ did not reduce fertility in either study. Therefore, $PGF_{2\alpha}$ should be useful for breeding management of mares, but larger trials are required. In one such trial, of 350 mares bred once 7 or 8 days after 5 mg sc $PGF_{2\alpha}$, 65% conceived (Ganjam, 1975).

Synchronization of ovulation in mares would (1) reduce the number of inseminations or matings, thereby reducing the number of stallions and labor required, and desirable stallions could be used more widely; (2) fertility may be improved if ovulation were more predictable than it normally is; and (3) since conception would require fewer matings the risk of injury to personnel and/or animals would be minimized and reproductive tract infections would be minimized. Hopefully, as a result more effort could be directed toward genetic improvement of horses for characteristics desirable to the race horses, pack animals, riding horses or work horses by selection and artificial insemination as practiced in cattle.

Moreover, there were indications from my data that some mares which had irregular cycles during the breeding season would

respond to $PGF_{2\alpha}$. Mares with prolonged luteal function (36 days) returned to estrus and ovulated after sc or iu $PGF_{2\alpha}$. Similarly, when Kenney et al. (1975) treated barren, maiden or postpartum mares (with an average anestrus period of 73 days during the breeding season) with $PGF_{2\alpha}$ (5 mg Tham salt, sc or im), the mares began estrus in 4.4 days. Of mares checked by palpation, 86% ovulated 8.9 days later whether or not estrual behavior was exhibited. The mares were bred 7 days after $PGF_{2\alpha}$, and 55% of these mares were pregnant after $PGF_{2\alpha}$. Therefore, in addition to potential for synchronization of ovulation, $PGF_{2\alpha}$ has considerable potential to initiate estrus in mares which fail to have luteal regression, thereby resulting in prolonged luteal function and the absence of estrus.

A note of caution is warranted, however. In addition to the luteolytic effect of $PGF_{2\alpha}$, it can cause uterine contractions, bronchoconstriction, increased intraocular pressure, and venoconstriction (Oesterling, 1972). Therefore, use of $PGF_{2\alpha}$ in pregnant mares or mares with respiratory or cardiovascular disease would not be warranted. Care in handling of $PGF_{2\alpha}$ by humans is also advisable. $PGF_{2\alpha}$ is available from Upjohn company on an experimental basis only, and is not available for purchase at the present time.

CONCLUSIONS

Luteolytic Effects of $PGF_{2\alpha}$ in Mares

Abrupt luteolysis occurred when mares were treated with 5 to 15 mg $PGF_{2\alpha}$-Tham salt on day 5 to 9 after ovulation because in all cases blood progesterone decreased significantly within 12 to 24 hours and continued to decrease until the mares exhibited behavioral estrus about 2.5 days later. $PGF_{2\alpha}$ was equally effective whether administered into the uterus or systemically (sc).

After $PGF_{2\alpha}$, the duration of estrus and the interval between ovulation and end of estrus were not different from those of control mares. Ovulation occurred 1 to 2 days before the end of estrus in the estrous cycle of control and $PGF_{2\alpha}$-treated mares alike; in treated mares ovulation occurred relatively synchronously at 7 to 9 days after $PGF_{2\alpha}$.

Less than 5 mg of $PGF_{2\alpha}$-Tham salt on day 7, or 10 mg given on day 3 did not consistently cause complete luteolysis. Although there was a significant decrease in progesterone in all mares 24 hours after treatment, this decrease did not continue in some mares. $PGF_{2\alpha}$ given the day after ovulation did not cause luteolysis, and in these mares progesterone continued to increase as it did in control mares after ovulation, reaching maximal values about 7 days later.

During the control cycle (I) in the first experiment, progesterone decreased from 17.1 to 1.4 ng/ml during the 5 days before

estrus. Two days before estrus when progesterone was 5.3 ng/ml, estradiol had increased 2-fold to 5.6 pg/ml; LH was significantly increased the following day. During estrus while progesterone remained below 1 ng/ml estradiol, androstenedione and LH increased to peak concentrations. Androstenedione and estradiol decreased to diestrual values by the end of estrus; LH remained elevated until 1 to 2 days after ovulation and decreased gradually for 7 to 9 days while progesterone increased. Estrone concentrations did not change significantly during the estrous cycle.

Plasma progesterone changes during the estrous cycle after $PGF_{2\alpha}$ differed only slightly from comparable changes during control cycles. The progesterone decrease after $PGF_{2\alpha}$--although more rapid ($P < .01$) during the first 24 hours--was not significantly different from that which occurred during natural luteolysis during the 3 days before estrus. When luteolysis was complete at about 48 to 72 hours, basal progesterone was below 1 ng/ml and estrus began.

As with progesterone changes, the changes in estradiol and LH were not influenced by iu $PGF_{2\alpha}$ administration. During both cycles estradiol increases preceded or accompanied the LH increase before ovulation, and like estradiol, androstenedione also peaked 1 or 2 days before ovulation. Again LH reached maximal values near ovulation, remained high for 48 to 60 hours after ovulation and did not decrease until progesterone began to increase. Estrone did not change significantly. These hormone changes during the estrous cycle after iu $PGF_{2\alpha}$ were substantially the same as those

that occurred during luteolysis, follicular growth, ovulation and CL function during a control cycle.

The second control cycle was identical to the first in every measured criterion. I therefore believe that for these variables, $PGF_{2\alpha}$-Tham salt had no residual effect on subsequent estrous cycles.

Mares were fertile during the estrus which followed $PGF_{2\alpha}$; and the foals born to these mares were physically normal. Thus, $PGF_{2\alpha}$ did not depress fertility, and has potential for estrus control and synchronization of ovulation in brood mares. Treatment of the noncycling mare with $PGF_{2\alpha}$ also has potential; $PGF_{2\alpha}$ caused luteolysis in mares with prolonged luteal function during the breeding season.

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