HORMONAL PATTERNS IN THE BEEF COW TREATED WITH MELENGESTROL ACETATE AND PREGNANT MARE'S SERUM

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Master of Science

by

Suchint Simaraks

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ABSTRACT

Twenty one post-partum and three open Charolais X Angus cows, maintained under dry conditions, were individually fed 1 mg MGA (6α-methyl, 6-dehydro, 16α-methylene, 17-acetoxy progesterone) per day for 16 days beginning an average of 35.8 days from calving. PMS (Pregnant Mare Serum) was injected subcutaneously 16 days after estrus or 20 days after last MGA feeding if estrus was not observed. HCG (Human Chorionic Gonadotrophin) was given by intramuscular injection 48 hrs following PMS treatment. Cows in group I received PMS while those in group II received PMS + HCG. All cows were moved to pasture in the presence of a bull of proven fertility after treatment except nine cows, three from each different treatment group, and three control cows from which daily blood collections were made by venipuncture. Blood sampling started one day after last MGA feeding or 89 days after calving in the control cows. Serum samples were obtained for determination of progesterone, estrogens and luteinizing hormone (LH) by radioimmunoassay.

Four out of 24 cows expressed estrus within 13 days after the last MGA feeding. From group I, ten cows responded to PMS by expressing estrus an average of 5.5 days after injection. Five of these cows later returned to heat. In group II, nine cows responded to PMS + HCG treatment averaging 5.8 days to estrus. Among these cows,

eight later returned to estrus. Control cows were in estrus within ten days after being transferred to pasture.

Control cows showed a hormonal pattern similar to that of normal cycling cows. After MGA treatment progesterone concentration of all cows remained lower than 1 ng/ml for a period of time varying from nine to 20 days. None of these cows showed similar patterns of progesterone levels in response to PMS or PMS + HCG treatment. Injection of PMS on the day before progesterone dropped to baseline levels appeared to produce a greater response in terms of progesterone secretion (15.25 ng/ml) in one animal.

LH levels in cows treated with MGA + PMS remained lower than 1 ng/ml until after PMS injection when LH levels increased in all cows.

Estrogen levels were high (varying from 6-16 pg/ml) on the day after MGA feeding and increased in all cows the next day. High levels were maintained about nine days in four cows, then decreased. The other two cows had levels which dropped on the third day and remained low until gonadotrophin injection. After gonadotrophin injection, estrogen levels increased greatly in all cows except one, the highest value determined being 184 pg/ml.

Average interval from calving to first estrus in treated cows was 74.23 days while in control cows it was 122.3 days. Two out

of 19 cows which responded to gonadotrophin treatment appeared to be pregnant at the induced estrus and one of them produced twins. Six cows did not conceive and the rest produced one calf each.

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INTRODUCTION

Although estrous synchronization has been successfully achieved in normal cycling beef and dairy cattle by the use of progesterone compounds, the fertilization and conception rates (CR) are low at the first synchronized estrus. Various gonadotrophic hormones have been employed in conjunction with the various synchronizing agents to overcome this depressed fertility but without completely satisfactory results. Gonadotrophins alone have been used to produce multiple births in cattle with some degree of success. Failure to obtain expected outcome may involve many factors, such as fertilization failure, improper time of mating or insemination, defective transport of sperm, failure to detect estrus and abnormal uterine environment. Recently, there have been suggestions that high progesterone levels at the time of fertilization has caused this reduced fertility rate and that the proper ratio of estrogen to progesterone is lacking. Similarly, synchronization or induction of estrus in the post partum cow has not been very successful and only limited information is available.

The purpose of this study was to determine the effectiveness of MGA in estrous synchronization of post partum beef cows. To eliminate the problem of depressed fertilization, gonadotrophins were also administered. Blood levels of the hormones, luteinizing hormone (LH), progesterone and estrogen were measured to determine if the treatment had any effect on the normal endocrine balance.

LITERATURE REVIEW

Effects of progestogens on the estrous cycle of cattle

The effects of progestogens on the estrous cycle of the farm animals are variable, depending on such factors as route of administration, dosage level, stage of estrous cycle during which the hormone is applied and type of hormone used.

Cycling animals

Trimberger and Hansel (1955) subcutaneously injected 50, 75 or 100 mg of progesterone daily into cows at various times in the estrous cycle for periods of time varying from 3 to 13 days in length. Estrus was satisfactorily controlled in most of the animals and they returned to heat an average of 4.6 days after treatment was stopped. The cycle length increased from 20.8 to 26 days and more ovarian abnormalities were detected following treatment. Conception rate (CR) at first synchronized estrus was low (12.5%) but improved at second estrus to what was considered to be a normal level (82.5%).

Nellor and Cole (1956) used a crystalline progesterone implant to eliminate the necessity of daily injection. The dosages ranged from 540-1120 mg and were started at different stages of the estrous cycle. Estrus occurred 15 to 19 days after progesterone implantation in 89% of the heifers which received 540-560 mg and 15 to 23 days after implantation

of 700-1120 mg of progesterone and ovulation was detected in 95% of the animals. When equine gonadotrophin was injected (750-2140 I.U.) 15 days after progesterone treatment 90% of the animals came into heat 1 to 4 days after gonadotrophin injection, and a higher dose of gonadotrophin seemed to produce more multiple ovulations. The CR was low when both hormones were used in combination.

Ulberg and Lindley (1960) administered progesterone subcutaneously daily for 14 days at dosages ranging from 12.5 to 50 mg alone or with 0.5 to 10 mg of estradiol benzoate given 96 hr after the last progesterone injection. Eighty three out of 101 animals which received 25 mg progesterone were observed in estrus within 10 days, averaging 4.8 days after cessation of treatment, while those that received 50 mg returned to heat at an average of 5.7 days. All estrogen treated animals were in heat 4 days after the last progesterone injection or 24 hr after the estrogen injections. Estrus was observed significantly more often in animals treated with estradiol. However, the higher dosages of progesterone tended to reduce the pregnancy rate.

Lamond (1964) removed the corpus luteum (CL) from 48 Hereford heifers, gave each of them 20 mg of progesterone daily for 6 days, with some receiving a subcutaneous injection of 1000 I.U. or 2000 I.U. of Pregnant Mare Serum (PMS) one day after the final progesterone administration. Some were also given an intravenous injection of either 72, 360 or 1800 I.U. of Human Chorionic Gonadotrophin (HCG) at the time of insemination which was 2 days after PMS administration.

Fertility was highest with HCG treatment, but the highest level (1800 I.U.) did seem to depress fertility. Enucleation of the CL before treatment seemed to result in higher fertility.

Vanblake et al. (1963) compared the reproductive performance of 20 untreated heifers with 60 heifers treated by feeding varying levels of 6α-Chloro, 17α-acetoxy progesterone (CAP), ranging from 0.02 to 0.3 mg per lb body weight for 15 or 20 days. The hormone proved to be extremely potent in inhibiting estrus and ovulation. Cycles of heifers fed 0.02 mg per lb body wt. per day were synchronized into a period of 4 to 6 days after hormone withdrawal and 6 to 9 days for those receiving 0.12 mg per day per animal. The conception rate at the first synchronized estrus, although slightly lower was comparable to the control group. Wagner et al. (1968) gave animals 10 mg of CAP per head per day for 14 days, followed by 5 mg/head/day for 4 days. All heifers were artificially inseminated 12 hr after the onset of estrus. Fertilization rate at the first breeding period following treatment was lower than in controls but was normal when heifers were bred at the second heat. It was felt that CAP may have exerted its effect on fertilization by modifying either; (1) the reproductive tract environment or (2) the ovum prior to ovulation. Baker and Coggins (1968) treated 50 beef cows in a similar manner to that done by Wagner et al. (1968). Twenty five of the animals were injected with 1000 I.U. of HCG 48 hr after the last feeding of CAP and they were artificially inseminated 12 hr after this injection or after the onset of heat. CAP suppressed estrus in 94% of the cows

during the feeding period. HCG treatment had no influence on the incidence of estrous or conception rate. Fulton et al. (1971) fed 50 heifers 10 mg of CAP daily for 9 days and gave an intramuscular injection of 5 mg of CAP and 5 mg estradiol valerate on day 1 of the treatment. Within 5 days after treatment 98% of the cows came into heat and 46% conceived to the first service.

Wiltbank et al. (1967) observed low fertilization rates when heifers were fed with 500 mg of 16α, 17-dihydroxy progesterone acetophenide (DHPA) per animal daily for 20 days. However, when a dosage of 400 mg was fed for 9 days with intramuscular injection of 0.5 mg estradiol valerate on the second day of the feeding period the fertilization rate was equal to that of controls. Wiltbank and Kasson (1968) fed heifers 400 mg of DHPA per animal daily for 7 days. Estradiol injection given on the second day of DHPA treatment to cause regression of the corpus luteum resulted in a short estrous cycle in some heifers. The authors felt that it was due to the prolonged action of estradiol valerate. Therefore, in a subsequent trial, the DHPA feeding period was extended to 9 days and 95% of heifers showed estrus within 96 hr after treatment and no short estrous cycles occurred. Fifty four percent of the heifers bred by artificial insemination at the synchronized estrus conceived as compared to 52% of the controls. In a later trial when 400 mg or 75 mg of DHPA was used the CR after breeding at the synchronized estrus was 62% and 59%, respectively, which was significantly ($P<0.01$) lower than in the controls (83%).

Liang and Fosgate (1971) established effective dosages for 17α-ethynyl, 19-nortestosterone (SC 4640) and 17α-ethyl, 19-nortestosterone (Nilevar) to be 7.5 and 5.0 mg/day, respectively, when given by daily injection for 12 days. Estrus was synchronized within 2 to 6 days post-treatment in 87.5% of heifers treated with SC 4640 and 100% of those treated with Nilevar and the pregnancy rates were 87.5 and 75.0%, respectively, from artificial insemination at the first synchronized estrus. Some of the SC 4640-treated heifers appeared to experience embryonic death and it was suggested that SC 4640 may have in some way altered the normal sequence of events between conception and placentation.

Hansel et al. (1961) reported the first successful synchronization of the estrous cycle in cattle by feeding 6α-methyl, 17α-acetoxy progesterone (MAP). The compound effectively inhibited estrus and ovulation until 3 to 4 days after treatment was stopped. MAP was mixed with soybean oil meal and fed once daily. Cows with an average weight of 1000 lbs received approximately 986 mg per head per day for the first 10 days and 50 mg for the second 10 days. All cows were artificially inseminated 3 to 5 days after treatment, with half of them taken at random receiving a subcutaneous injection of 0.5 mg estradiol at the time of insemination. The conception rate at first service was decidedly low. Only 25% conceived to this breeding and estradiol injection did not have any effect on fertility.

Nelms and Combs (1961) reported that 10 out of 15 cows conceived when bred at the synchronized estrus after being treated with 220 mg of MAP per head per day for 15 days. When the dosage was increased to 250 mg per animal per day for 14 days and these animals were inseminated without regard to estrus on the third, fourth, fifth day after the end of treatment, 40% became pregnant which was comparable to the conception rate of 60 control heifers during the first 21 days of insemination. Dhindsa et al. (1964) fed cattle with MAP at a range of 180 mg per head daily for 18 days. The animals were artificially inseminated at the first synchronized estrus and bred naturally later. Estrus was synchronized within 3.5 days after the withdrawal of MAP. The CR at the first estrus was higher than that of the controls. Zimbelman (1963) reported on the minimum effective dosages of MAP for estrous synchronization in dairy and beef cattle. MAP feeding started on day 15 of the cycle in dairy heifers and lasted for 16 days or 20 days. The period of feeding in beef cows was 18 days started at random stages of the cycle. The minimal effective dosage of MAP in dairy heifers fed individually twice daily when started on day 15 of estrous cycle, was above 135 mg. In group feeding, dosages of 150, 180, 210 and 400 mg were capable of inhibiting estrus and ovulation during treatment. In beef heifers, group feeding 120 or 180 mg per animal daily inhibited and synchronized ovulation in 94% of the animals. Considering both beef and dairy heifers 93% were in estrus on the second, third and fourth days after the last MAP feeding. Fahning et al. (1966) fed

0.4 mg MAP/lb. body weight per day to Holstein heifers for a minimum period of 11 days and a maximum of 18 days. Eighteen out of 19 animals showed estrus within 2 to 4 days following MAP treatment. The CR of the treated animals was 26.3% and 57.9% following first and second service, respectively.

Dhindsa et al. (1967) treated beef heifers by orally giving 180 mg of MAP daily per animal for 18 days. Eighty seven percent of the heifers showed estrus within 90 hr after the last MAP feeding. The CR following breeding at the first and second estrus after treatment was 94% in the treated animals and 63% in the controls.

Zimbelman (1961) gave 5 mg MAP/head/day and 75% of animals were in heat between 48 and 84 hr after treatment. Conception rates in control, individual and groups feeding lots were 75, 25, 75%, respectively at first service, while at second service the CR was 81% for all treated animals.

Collins et al. (1961) successfully synchronized estrus in cattle by feeding 0.5 mg of MAP for 20 days, whether begun on day 3, 7, 10, 15 or 19 of the estrous cycle.

Hansel et al. (1966) compared the effectiveness of MAP (240 mg/head/day) and CAP (10 mg/head/day). MAP treated animals came into estrus slightly sooner than did those receiving CAP. The CR at

the first service of MAP-fed cows was significantly higher than those fed CAP, (64.4 vs. 35.6). The slightly longer time for the CAP-fed group to come into estrus following treatment may have been due to the greater potency or longer half life of CAP.

Zimbelman and Smith (1966), in determining the orally effective levels of 6α-methyl, 6-dehydro, 16α-methylene, 17-acetoxy progesterone (Melengesterol Acetate or MGA) in synchronizing estrus, found that dosages of 0.25 to 8 mg per animal per day inhibited ovulation and estrus in virtually all animals. Dosages of 0.125 and 0.0625 mg were not effective in preventing ovulation, and it appeared that certain dosages may suppress estrus but allow ovulation to occur. Heifers showed estrus from 1.5 to 4.5 days after last feeding at the 0.2 mg dosage and 4 to 9 days at the 2.0 mg level. The longer intervals encountered at daily dosage level of 1 to 2 mg apparently resulted from overdosing. The CR for various groups at first service varied from 25 to 88%. Considering the total, 42% conceived from one service and 82% from two services.

Tripathe and Howell (1969) reported that feeding 0.45 mg MGA per beef heifer daily for 18 days produced a comparable CR to that of the controls under natural service, while the same treatment in a later trial produced a significantly lower CR than the controls when artificial insemination was used. The CR at the estrus subsequent to the first synchronized estrus was not significantly different from the controls. Roussel and Beatty (1969) synchronized

heifers with 1 mg of MGA per animal per day for 14 days, during either spring, winter or summer months of the year. Most of the heifers showed estrus after treatment and the mean interval from the last feeding to the first and second estrus was 4.9 and 21.9 days, respectively. Overall CR for the control group was 53% as compared to 60% in the treated group. Estrus synchronization in hot summer months tended to have slight adverse effect on estrus and fertility. The duration of estrus was shorter in the summer group as compared to spring and winter. The interval from the last feeding to estrus in summer, winter and spring was 4.0, 3.5 and 3.0 days and to second estrus was 23.6, 21.5, 20.5 days, respectively. The CR were 40, 60, 80%, respectively.

Chakraborty et al. (1971) successfully synchronized estrus by feeding MGA (10 mg/animal/day) for 14 days to dairy cows, beginning at any stage of the estrous cycle. Estrus occurred 2 to 6 days after treatment; within 24 hr in 83% of animals. The CR was reduced after artificial insemination at first service and double insemination at this synchronized estrus did not improve conception. The CR at the first synchronized estrus was 8.33%, which was significantly ($P<0.05$) lower than in the controls. Fifty four percent of the animals conceived at the second estrus which was similar to the conception rate at first estrus of the controls (58.3%). Zimbelman et al. (1970) summarized the data concerning the use of MGA on conception rate. Conception rates from breeding at first synchronized estrus were about 70% of the rate of controls while at the second synchronized estrus

it was not depressed. Zimbelman and Smith (1966) used MGA in conjunction with many compounds for the purpose of precisely timing the induction of ovulation since it has been suggested that the reduced CR is due to ovulation failure or improper time of insemination. Estradiol cyprionate (ECP), estradiol-17β, carbestrol, HCG, PMS, oxytocin, neostigmine, amphetamine, nafoxidine hydrochloride and chlomiphene citrate were employed and were administered between day 8 and 13 of MGA-feeding. Estradiol -17β and ECP were effective at certain dosages, while gonadotrophins gave varying results. HCG at certain levels induced ovulation as detected by palpation of the ovaries for corpora lutea (CL). PMS at a level of 2000 I.U. administered intravenously caused extensive follicular development which made CL detection impossible. Other compounds tested gave no effect on estrus or ovulation. When ECP was injected on the last or on day 1 or day 2 of MGA feeding it increased the overall incidence of estrus. Ovulation, however, was not affected as compared to those that received only MGA. It appeared to reduce the CR at first service. The authors suggested that reduced CR following MGA or MGA plus ECP treatment was due to multiple causes, viz., ovulation failure, ovum loss, fertilization failure and embryonic death.

Zimbelman and Smith (1966) injected ECP, HCG and Luteinizing Hormone (LH) during MGA treatment (day 8 to 12 of MGA feeding) and observed ovulation by laparotomy on days 2, 5, 8 and 11 after injection of each. They found that ECP, HCG and LH were effective in inducing

ovulation in most of the animals but CL development after day 5 was not considered normal. They suggested that early regression of the CL was due to a deficiency of gonadotrophin, probably LH brought about by the inhibitory action on the pituitary gland of estrogen or progesterone treatment continued after the estrogen injection. It was felt that enough endogenous or exogenous gonadotrophin was probably available for the initial stages of CL formation in each of the injected group, since the induced CL appeared normal from heifers laparotomized on day 5.

Boyd and Tasker (1971) treated dairy heifers with 1 mg of MGA per animal per day for 14 days or gave it in conjunction with HCG, PMS or estradiol injections. All animals were inseminated at 12 and 24 hr after treatment. Estrus was synchronized but the CR at first service was low. The CR at first and second estrus was 66.6% for control, 33.3% for MGA treatment alone, 88.8% for MGA plus HCG and 22.2% for MGA plus estradiol plus PMS plus HCG. The latter treatment upset regular cyclic activity and subsequent fertility with MGA plus HCG producing the best results.

Hill et al. (1971) started treating heifers on either day 4 or 14 of the cycle with 0.5 mg or 1.0 mg of MGA for 14 days. The interval from the last feeding of MGA to estrus in the 0.5 mg group was 3 to 7 days and in the 1.0 mg-group it was 5 to 10 days with an average of 4.75 to 7.26 days, respectively. Thus, synchronization was

better when the treatment began on day 4 of the cycle. After mating the heifers were slaughtered and ova were recovered. More nucleated ova were obtained from treated heifers than in the controls, suggesting that the incidence of fertilization failure was higher than in untreated animals.

Post-partum cows

Wiltbank and Cook (1958) compared the reproductive efficiency between nursed cows and milked cows (milked twice daily). The interval from calving to first estrus was approximately 84 days in nursed cows and 54 days in milked cows, while the interval from calving to first detectable CL was 53 days and 36 days, respectively. Conception rate in nursed cows was lower and more services per conception were required. They suggested that the difference in reproductive performance between the two groups might be the decreased supply of gonadotrophin or an insensitivity of the ovaries to the usual levels of gonadotrophin resulting from suckling action.

Tilton et al. (1966) reported that the interval from calving to involution of uterus, first estrus, ovulation and conception in beef heifers ranged from 43 to 46, 46 to 53, 38 to 50 and 55 to 87 days, respectively. Intervals from calving to ovulation in beef heifers and dairy heifers were 35 (range 20-51) and 30 (range 15-44) days, respectively, Hill et al. (1972).

Zimbelman (1963) fed post-partum heifers and cows (9 to 22 days after calving) with MAP for 17 days. The data indicated that MAP feeding had no significant effect on the average interval from calving or from the end of treatment to either post-treatment ovulation or conception. Average CR of all treated animals bred within 2 days of last MAP feeding was 51%.

Britt et al. (1972) fed 180 mg of MAP per animal per day for 10 days to post-partum cows. All cows started cycling after treatment and the CR was 88%, compared to 74% in those that received no MAP. When MGA was employed instead of MAP, starting from day 48 post-partum, the interval to first service did not differ among treatments. Therefore, MGA feeding for 10 days did not affect time of estrus. There was no great differences in conception rate at first service. In a further experiment, the duration of treatment was extended to 14 days and started from 35 to 55 days post-partum. The conception rate during days 1 to 10 after last MGA feeding (first service) varied from 13 to 18% in the different herds, and during 21 to 30 days after MGA withdrawal (second service) varied from 21 to 71%.

Fosgate (1961) reported that 21 intramuscular injections of 17α-hydroxy progesterone caproate (Delalutin) at the rate of 100 mg given bi-weekly from parturition reduced the interval from calving to uterine involution in Holstein and Jersey cows. However, the average

interval from calving to first ovulation was significantly longer in treated animals than the controls (62.3 vs. 40.2 days). The average interval from calving to first estrus was also longer (70 vs. 47.9 days). Delalutin had no effect upon conception rate in treated animals as compared to the control (71.4 vs. 69.2%).

Oxenreider and Melampy (1966) noticed that induction of ovulation by PMS or HCG reduced the post partum interval to ovulation and estrus but had no influence on uterine involution.

Foote et al. (1960a) gave a single subcutaneous injection of progesterone to cows 30 days after calving and some of these animals were given a 10 mg injection of estradiol 20 days after progesterone administration. All cows were bred at the first estrus occurring after 50 days post-calving. Neither treatments affected time from calving to first service. Average time to first service for untreated, progesterone and progesterone plus estradiol treatment were 76.8, 79.1 and 76.2 days, respectively. The interval from progesterone treatment to first ovulation and estrus was 24.3 and 27.3 days for progesterone alone, and 30.2 and 22.5 days for those that received both hormones. The CR in the latter group was low, while the progesterone treated animals did not differ from untreated cows at first service. Foote et al. (1960b) instead of using a single injection gave daily injections of 1 mg progesterone for 14 days to post partum cows starting on the day after calving. Duration for uterine involution

was not affected by treatments, being 41.2 and 41.4 days for treated and untreated groups, respectively. The average interval to first ovulation and first estrus was 61.1 and 83.0 days, respectively, for the treated group, while the intervals were 41.7 and 65.0 days, respectively in the control animals. Progesterone treatment did not decrease the variation in ovulation time or affect the conception rate.

Foote (1962) divided 80 Hereford cows into the four following treatments: (1) control, (2) subcutaneous injection of 50 mg progesterone from day 12 post-partum through day 23, (3) same as (2) plus single intramuscular injection of 10 mg estradiol 17-β on day 25, and (4) only estradiol on day 25. Average interval from calving to uterine involution was 47, 39, 36 and 38 days; to first estrus was 49, 41, 27 and 27 days; to first ovulation was 44, 33, 31 and 30 days and to conception 57, 49, 48 and 48 days, respectively. All intervals except calving to conception were significantly shorter in treated animals than the controls. Estrogen treatment alone or with progesterone seemed to reduce the CR at first service.

Fosgate et al. (1962) used Delalutin (an ester of 17α - hydroxy progesterone) as an estrous suppressing agent in post-partum cows; giving 22 injections of 100 mg each on alternate days from the day after calving. The interval from parturition to ovulation was significantly ($P<0.01$) longer in the treated cows than in the controls

(62.3 vs 40.7 days). Treated cows had more quiet ovulations before showing estrus (57 vs 44%). The average interval from parturition to first estrus was 70.0 days in treated cows as compared to 47.9 days in the controls. The CR at first service was not affected by treatment.

Saiduddin et al. (1968) treated post-partum cows with 50 mg progesterone injections daily for a period of 10 or 15 days and an intramuscular injection of 10 mg of estradiol-17 was given alone or two days after the progesterone treatment. Cows given estradiol alone or after progesterone resumed ovulation earlier after calving than untreated or progesterone treated alone. Although estradiol was effective when given alone, when preceeded by progesterone it hastened the onset of estrus and ovulation, and conception occurred earlier in cows given both hormones. Progesterone and estradiol both tended to decrease variation among cows in the interval to estrus, ovulation and conception.

Foote et al. (1972) implanted progesterone for 23 days in post-partum beef cows, and some of these animals were also injected with 1800 I.U. of PMS at the time of implant removal, followed by 6000 I.U. of HCG 27 to 34 hr later. Animals treated with progesterone alone had the highest incidence of ovulation within 2 or 6 days after the implant removal. This group also had the highest conception at synchronization and the shortest interval to conception. PMS + HCG treatment tended to help to synchronize estrus and ovulation and

conception to some degree. However, conception rate at first service was decreased. There was a tendency, especially in gonadotrophin treated animals, to have both prolonged and split estrous periods with one to three days between periods of standing heat.

Darwash et al. (1965) fed MGA to cows at levels of 0.4 to 1.0 mg for 18 days starting on day 7 or day 32 post-partum. Animals that received an initial treatment on day 7 came into heat an average of 8.6 days after treatment, which was significantly ($P<0.05$) longer than the average interval in the other groups. Cows treated with 0.4, 0.5 or 1.0 mg/head/day started on day 32 came into estrus an average of 4.3, 4.7 and 5.2 days after treatment, respectively. Seven out of the 49 cows that were treated developed follicular cysts and failed to ovulate at the end of treatment.

Boyd (1969) fed 0.5 mg of MGA per animal per day for 18 days, beginning on day 43 of lactation. Reproductive efficiency, though not different between the control and treated groups, was poor. First insemination was made at an average of 73 days post-partum in treated cows and 74 days in the control animals. Tilton (1966) used 180 mg of MAP for the same duration, but starting on day 20 post-partum and found that this increased the interval to first estrus (74.9 vs. 58 days) but gave a comparable CR to that of control animals (64.7 vs. 56.0%). Zimbelman (1963) reported that post-partum beef cows were synchronized by feeding MAP prior to occurrence of the first post-partum

ovulation. The treatment caused a significant reduction in the variability, but not in the average interval from calving to first post-partum ovulation.

Veenhuizen and Wagner (1964) reported successful synchronization of estrus in post-partum beef cows with CAP (10 mg/head/day for 18 days). Thirty cows averaging 227 days post-partum were 90% synchronized with a 50% CR at first service and 70% CR following two services by artificial insemination.

Spahr et al. (1970) treated post-partum cows as follows: 1) control, bred at first heat after 60 days post-partum; 2) fed MAP to produce estrus at 55 days and bred at following heat; 3) fed MAP to produce heat at 75 days post-partum and bred at that estrus (these animals received 500 I.U. of HCG two days after an 18-day MAP treatment). Percentage fertility at first service, service interval, days open and percent pregnant at 100 days post-partum were 56.6, 76.9, 97.3, 52.8; 52.2, 85.3, 110.3, 43.8 and 31.2, 87.9, 118, 35.5 for groups 1, 2 and 3, respectively.

Brown et al. (1972) fed beef cows 120 mg DHPA daily for 9 days, with 5 mg of estradiol valerate being injected on the second day of progestogen treatment. Some of these animals received additional estradiol on day 10 (one day after stopping DHPA treatment) with no further treatment or injection of 1000 I.U. of PMS and 750 I.U.

of HCG at the time of insemination. Treatment began 5 to 10, 10 to 15, 20 to 25, 30 to 35 or 40 to 45 days post-partum. All cows were artificially bred 12 hr after post-partum estrus and again 12 hr later. Progestogen and two estradiol injections significantly reduced ($P<0.05$) the interval from calving to first estrus, ovulation and conception, especially when treatment started on days 5 to 10 post-partum. Gonadotrophin injection did not decrease the intervals from calving to estrus, ovulation or conception. Treatment later in the post-partum period (40 to 45 days) prolonged the same intervals. The degree of synchronization (within 6 days after treatment) was highest in the animals which received progesterone followed by estrogen, especially when treatment began 20 to 25 days post-partum. Conception rate was highest in the group given estrogen on the second day of progestogen feeding (84%). This was particularly true in the group which was treated on days 30 to 35 post-partum (86%).

Hill et al. (1972) treated beef cows with 10 mg CAP/animal/day for 10, 20, 30 or 40 days following calving and gave an injection of 0.5 mg of estradiol -17β 72 hr after CAP was stopped. Treatment was not effective in hastening the onset of estrus. The interval from calving to first estrus was not shortened significantly when compared with the control except in the case of the 10 day treatment group (51.4 vs. 60.5 days). Conception rate at the first service was not significantly affected by any treatment, and no great effect on the interval to conception was observed.

Foote and Radmall (1972) reported on the different responses between beef and dairy cows to a single injection of 10,000 I.U. of HCG 12 days after calving. In beef cows the treatment did not influence the interval from calving to uterine involution (48 days in the treated group and 43 days in the controls). Intervals from calving to ovulation, estrus and conception were longer in treated animals than in the control. In dairy cows the average interval to first ovulation decreased from 30 days in untreated animals to 14 days in treated animals. The difference between beef and dairy cows in response to HCG treatment may be due to one or more factors including genetics, milk production, method and frequency of milk removal and nutrition.

Tilton et al. (1966) reported unsuccessful results in synchronization of post partum cows with MAP, started 25 days after calving. Foote et al. (1960b) also reported unsatisfactory results.

Superovulation

Gordon et al. (1962) reported on the use of PMS for superovulation induction in a large number of cattle. The dosages used varied from 800-2000 I.U. and the injection was given on days 15, 16, 17, 18 or 19 of the estrous cycle. The response in terms of number of ovulations was linearly related to the dosage and increased from an average of 1.43 at 800 I.U. to 3.97 at 2000 I.U. It was also

evident that animals injected with the low dosage on day 15 of the cycle did not respond to the treatment as well as when the injection was given on days 16, 17 or 19. There was a tendency for those cattle which came into estrus earlier than 3 days from the time of PMS injection to have fewer ovulations than those that took a longer interval. The response in the short-interval animals was particularly poor at dosage levels below 2000 I.U.

Turman et al. (1969) injected cows with 1500 I.U. of PMS on days 3, 4, 5 or 6 of the cycle and 2000 I.U. on days 16, 17, or 18. This was followed by 2500 I.U. of HCG given immediately after breeding by natural service. Sixty four percent of the cows conceived at first breeding post-PMS and produced 29 single and 23 multiple births (12 twins, 8 triplets, 2 quadruplets and 1 quintuplet). Cows not conceiving at first service were bred during a subsequent 90-day breeding period. The cows which subsequently calved produced a calving percentage of 134%. Calving difficulty did not increase with multiple births, but calf losses at birth increased as litter size increased. An increase in the incidence of retained placenta was also observed in cows producing multiple births.

Schwartz and Schally (1969) induced superovulation by injecting PMS or ovine Follicle Stimulating Hormone (FSH). Treatments were as follows: 1) control; 2) 1500 I.U. PMS on day 5 and 2000 I.U. on day 16 of estrous cycle; 3) 2000 I.U. PMS on day 16 of the cycle;

and 4) 2.5 mg of FSH on day 5 and 3.0 mg on day 16. The percentage of heifers which ovulated, the ovulation rate and the range in ovulations were 100%, 1.06 , 1-2; 89%, 6.56, 0-16; 89%, 4.72, 0-13; and 100%, 1.39, 1-6 for treatments 1 ro 4, respectively. The percentage of animals becoming pregnant was 55, 11, 22 and 44% for the same respective groups. It seemed that PMS injection depressed the pregnancy rate and gave excessive ovulations, while FSH treatment appeared to give somewhat better results.

Scanlon et al. (1968) treated heifers and cows with 3000 I.U. of PMS on day 16 of the estrous cycle, followed by an injection of 2000 I.U. HCG on the day of estrus or on day 21 if the animals did not show estrus. A number of animals failed to show estrus after treatment, while 74% shed more than one egg with a maximum of 55 ovulations. It was clear that there was a relationship between the average ovulatory response and the time interval from PMS to the onset of estrus; as the interval increased the mean ovulation rate rose markedly.

Laster et al. (1971) reported that the injection of 2500 I.U. HCG 3 days following the second 2000 I.U. PMS injection on day 17 of the estrous cycle (first injection on day 5) resulted in more twin and fewer triplet ovulations than when HCG was injected on the day of estrus. No significant difference in ovulation rate was noted when the PMS injections were timed from non-synchronized or synchronized estrus. They also found that ovulation did not always occur in

follicles which were stimulated by the first injection of PMS and that increased dosages of HCG stimulated more ovulations. Refractoriness was observed when PMS was used repeatedly; a lower percentage of multiple ovulations occurred in animals treated at a second sequence of PMS injections. The reason for the low CR and high embryonic mortality in PMS treatment could not be determined.

Bellows and Short (1972) indicated that cows showing estrus within 3 to 5 days from the last PMS injection had the most desirable superovulation and multiple birth response. This suggested the need of being able to accurately predict when estrus would occur in cows treated with gonadotrophic hormones. Therefore, experiments were conducted to determine if PMS treatment could be successfully used to induce superovulation in cows whose estrous cycle had been synchronized with an orally active progesterone. This was done by feeding 180 mg of MAP/head/day for 11 days combined with an injection of 5 mg estradiol valerate on day 2 of the treatment period. Various dosages of PMS were used (800-2000 I.U.) given either on day 7, 8, 9, 11 or 12 of MAP treatment. Their results were variable. Dosages below 800 I.U. or 800 I.U. given as two 400 I.U. injections gave no response while 800 I.U. given at a single injection resulted in a superovulation response in only one heifer. When 1200 I.U. was given as single dose, the average ovulation rate was 8.4 with all of the animals having multiple ovulations. Overall, the ovulation rate ranged from 0-20 with 0-100% of the heifers having multiple ovulation. It was also found

that synchronized heifers or cows often ovulated two or more ova but produced only single or no calves.

Reynolds et al. (1969) gave twice daily injections of 0.625 mg of FSH on days 8, 9, 10, 11 and 12 of an 11-day MAP or MGA treatment and an injection of 0.5 mg of estradiol on day 2. The ratio of cows which came into estrus within 5 days after the end of progestogen feeding, and the pregnancy rate at the first and the second estrus was 9/11, 5/9, 3/3 and 11/14, 5/11, 1/5 for the combined MAP and MGA treatments, respectively. Percentage of pregnancies at first service with twin embryos was 33% for MAP and 20% for MGA treatments. Embryo degeneration appeared to occur with both multiple and single embryos.

Vincent and Mills (1972) studied the effect of FSH on ovulation rate and multiple births. Cows were treated 11 to 18 days after the previous estrus with 5 mg of Norethandrolone alone per animal daily for 4 days or with daily injections of FSH for 5 days with the total dosages ranging from 6.3 - 12.5 mg by once or twice daily injection. More cows treated with FSH had multiple ovulations (averaging 1.9 ovulations per cow) and the higher level of FSH produced more multiple births. No significant differences were observed among treatments on conception rate. Total CR for the control group was 73% for Norethandrolone alone it was 46% and was 55% for the FSH-treated group. A higher percentage of cows having multiple ovulations following treatment with FSH became pregnant than cows with a single ovulation (62% vs. 48%). The overall CR was considered satisfactory (57%). When

the period of Norethandrolone treatment was increased to 10 days and the injection of 5 mg estradiol valerate was given on the second day of the treatment in combination with FSH injection, the CR was low although 73% of these cows showed estrus within 72 hr following treatment.

Bellows et al. (1969) studied the dose-response relationship in synchronized beef heifers treated with FSH. Heifers which received a single injection of 75 mg of FSH on the last day of a 9-day MGA treatment did not respond well. FSH injected on days 9, 10, and 11 (to a total of 75 mg) resulted in marked follicular stimulation as indicated by increased diameter and follicular fluid weight. Levels between 12 and 25 mg produced a good ovarian response and little difference in response was observed between dosages of 25, 50 or 75 mg. This was interpreted as evidence of a plateau in the dose-response relationship. Animals treated with 12.5 or 6.25 mg gave ova recovery which ranged from 65 to 81.8% and a fertilization rate that was comparable to the controls, indicating that ova were potentially fertile. Synchronization tended to depress the ova recovery rate and resulted in a drop in the number of sperm seen surrounding each ova.

Hormonal Patterns in the Peripheral Blood of Cattle During Normal and Synchronized Estrous Cycles

Progesterone

Gomes et al. (1963) determined progesterone in bovine CL using a spectrophotometric technique. The values increased significantly during the first 14 days of the estrous cycle, then precipitously declined to the next estrus. Progesterone concentration in the plasma from the jugular vein did not reflect the stage of cycle.

Plotka et al. (1967) assayed progesterone in jugular blood by using a double isotope derivative technique. Average progesterone levels were lowest 2 days post-estrus and were nearly as low during estrus. The concentration was doubled by 12 to 14 days post-estrus, then declined by approximately one-half by 1 to 2 days before the next observed estrus. The average progesterone concentration was significantly higher at day 12 than during estrus or 2 days post-estrus.

Donaldson et al. (1970) determined the progesterone concentration in peripheral blood by a competitive protein binding (CPB) technique. The concentration which was lowest on days 0 to 2 increased to a maximum between days 12 and 15 of the cycle. In many cycles the values after day 8 were variable and in some animals decreased markedly before increasing to a second peak around day 14.

The concentration decreased rapidly during the 4 days before the subsequent estrus.

Kazama and Hansel (1970) employed thin layer chromatography to measure progesterone in peripheral blood collected every 6 hr from the onset of estrus until ovulation from five Holstein heifers. There was little if any preovulatory production of progesterone in the animals. Less than 60 ng/100 ml plasma during the 2 days before estrus was detected in three heifers. The other two had 235 and 263 ng/100 ml of plasma 2 days before estrus. The levels declined sharply to 11 and 15 ng/100 ml 1 day before estrus. The concentration was not detectable in two out of the five animals from the onset of estrus until 1 day after ovulation and was non-detectable in four of five heifers just before ovulation occurred.

Hendricks et al. (1971) obtained blood at 6.30 and 16.00 hr everyday for 4 days during proestrus and estrus, and analysed for progesterone concentration by a CPB method. The values were at a peak which averaged 7.2 ng/ml on the third and fourth day prior to estrus in most cows. Hendricks et al. (1970) reported that plasma progesterone concentration was less than 0.5 ng/ml from 0 to 15 hr after the beginning of estrus when collected four to five times during estrus at a 3 hr interval. In 25 of 37 determinations made during estrus, progesterone was not detected (less than 0.3 ng/ml). The level increased 0.69 ng/day through the eixth day after estrus. From days

8 to 14 the mean concentration continued to increase at an average rate of 0.15 ng/day, and then decreased after day 16.

Swanson et al. (1972) noticed that the corpus luteum size during the first three estrous cycles in heifers were significantly larger than the average of CL of later cycles ($P<0.01$). Corpus luteum size was smallest 2 days after estrus, increased continually to 2.2 cm on day 11 and then subsequently decreased to day 2 size. This pattern was comparable to the progesterone level in the blood determined by the CPB technique. Levels increased between day 4 and day 7 followed by further increase on day 11 to a peak of 6.9 ng/ml which occurred 3 days before estrus. Then it increased to 2.5 ng/ml on day 2 and continued to decline through day 2 following ovulation. At estrus, serum progesterone averaged 0.2 ng/ml; ranging from undetectable levels up to 1 ng/ml.

Wattemann et al. (1972) determined peripheral blood progesterone levels in normal estrus cycles of cows by radioimmunoassay (RIA). Blood was collected on day 2, 4, 7 and 11 and daily from day 18 until estrus. Progesterone decreased rapidly during the 3 days before estrus, remained low from day 1 through day 2 and increased from 0.92 ng/ml on day 4 to 4.57 ng/ml by day 11; it then declined before the next estrus.

Garverich et al. (1971) reported that plasma progesterone, measured by CPB, in normal cycling cows differed significantly during

the cycle, increasing approximately 2.5 ng/ml during the 5 day interval from day 11 to day 15. Levels averaged 12.6 ng/ml during the later period as compared to 5.1 ng/ml for the day of estrus. The luteal phase ended about 5 days before esturs as indicated by a gradual regression of the CL and a decrease in peripheral progesterone level. Robertson and Sarda (1971) also obtained high progesterone levels up to day 15 which then declined sharply to the next estrus. Christensen et al. (1974) determined progesterone concentration in peripheral blood serum during the estrus cycle of three non-lactating beef cows. Progestin levels rose and fell coincident with growth and regression of the CL. Serum progestins were lowest on day 0 (1.30 ng/ml) and remained low until day 4, when levels began to increase, reaching a peak on day 15 (6.15 ng/ml). Thereafter, the levels declined until the next cycle.

Britt and Ulberg (1972) measured progesterone levels by a CPB technique, during the estrus cycle before treatment with MGA for 14 days (fed at 1 mg/day). The levels rose from 0.5 ng/ml at estrus (day 0) to 4.4 ng/ml on day 15, then dropped to 0.5 ng/ml during the 3 days preceeding the next estrus. The levels fluctuated between 3.7 and 7.7 ng/ml during and for 2 days subsequent to the MGA treatment. These levels then decreased from 6.8 to 3.1 ng/ml during the 3 days preceeding the first post-treatment estrus. During the cycle subsequent to MGA synchronization, the peripheral progesterone concentration increased from 0.4 ng/ml on day 0 to 3.7 ng/ml on day 15. This was

followed by a drop from 3.4 to 0.8 ng/ml during the 3 days preceeding the second post treatment estrus. A fluctuation in progesterone concentration occurred during MGA treatment, even though all CL had regressed to less than 10 mm in diameter by the third day of MGA administration.

Hendricks et al. (1970) observed a higher incidence of fertilization failure and a retardation of the ova cleavage rate in heifers which had an average progesterone concentration more than 2.8 ng/ml of blood during the 4 days before estrus.

Hill et al. (1971) reported progesterone levels during MGA treatment for 14 days. The mean progesterone concentration was similar to the control value obtained on comparable days of the estrus cycle. They indicated that MGA did not influence either the life-span or progesterone secretion of the CL present when treatment began. Lamond et al. (1971) using the same CPB method as Hill et al. (1971) measured progesterone concentration in 10 beef heifers during and after MGA treatment (0.5 mg/head/day). The treatment commenced on day 15 of the cycle and continued for 16 days. Blood was collected at 8:00 hr on day 15 and thereafter at 3-day intervals until day 30 and also 2 days after MGA feeding was stopped. The concentration in four heifers declined between days 15 and 20, the expected time of CL regression, and remained low until the end of treatment. In the remaining heifers, plasma progesterone concentrations fluctuated to a minor degree in three heifers and a major degree in the other three.

Two days after the final MGA treatment progesterone in the plasma varied from non-detectable levels to 1.3 ng/ml. It was noticed that in two heifers that did not show estrus after the MGA regime, the progesterone levels rose from 0.3 to 1.3 ng/ml between days 30 and 32 in one and to 5.7 ng/ml in the other 3 days before the end of MGA treatment. The other two heifers which were in estrus on days 36 and 39, had progesterone concentrations of 1.7 ng/ml and 2.2 ng/ml, respectively on day 30.

Randel et al. (1972) treated dairy heifers with either 0.5 or 1.0 mg of MGA for 14 days and 62 hr later half of the heifers in each group were injected with 200 mg of estradiol-17β. Progesterone levels were measured by a RIA technique. The average progesterone levels did not significantly differ between the groups fed either 0.5 or 1 mg of MGA from days 7 to 14 of the treatment. Progesterone levels at 24 to 56 hr after the last feeding or at 5 to 9 days after ovulation were comparable to the control group on days 7 and 14 of the cycle. Irrespective of the estrogen treatment, plasma progesterone increased after MGA feeding from 3.8 ng/ml at 24 hr to 8.6 ng/ml at 80 hr and remained high for 88 to 120 hr.

Lamond and Gaddy (1972) injected 1500 to 3000 I.U. of PMS to induce superovulation 4 days after an 18-day MGA or CAP treatment ended. Cows were fasted for 3 days after PMS injection to prevent excessive follicular development. Jugular blood was obtained on days

15, 20, 25 and 30 after mating and progesterone concentration was assayed by a CPB technique. The cows with multiple CL had plasma progesterone concentrations that were considerably in excess of normal levels. However, the relationship between number of CL and progesterone levels could not be clearly defined because of the scatter of values.

Rich et al. (1971) observed no significant difference of progesterone levels in non-, single- and multiple ovulating heifers treated with FSH on days 10, 12 or 14 during a 14-day MGA treatment. Total progesterone decreased during the early days of MGA feeding and this decline was most rapid in heifers which received 5.0 mg of estradiol valerate on day 2 of the MGA treatment. Subsequent to MGA feeding but prior to estrus, progesterone levels were generally less than 1.5 ng/ml. Hill et al. (1971) reported levels of 2.4 to 6.1 ng/ml during MGA feeding while Britt et al. (1972) reported them to be 3.7 to 7.7 ng/ml.

Chow et al. (1972) using a CPB technique, observed that plasma progesterone value (days 4 to 0) after MGA treatment was significantly lower than in control cycles and also did not present the characteristic precipitous decline of plasma progesterone values seen in the control animals. Thus, ovarian secretory activity may be altered immediately following MGA treatment resulting in a lowered progesterone peak.

Dobson et al. (1973) measured progesterone and estrogen by RIA from the peripheral blood taken from animals that were treated as follows: 1) controls; 2) MGA for 14 days; 3) MGA + 2500 I.U. HCG at 72 hr after last MGA feeding; 4) MGA + 0.5 mg estradiol benzoate on day 1 of MGA treatment + 2500 I.U. PMS at 36 hr + 2500 I.U. HCG at 72 hr after last MGA feeding. Blood was collected over an estrous cycle in the control animals and daily after the last dose of MGA until the day after estrus in the hormone-treated cows. The progesterone level in the control group decreased from 9.0 ng/ml at 4 days before estrus to 0.35 ng/ml on the day of estrus. Progesterone concentration in group (2) was at a basal level (less than 1.0 ng/ml) after withdrawal of MGA. In group (3), the concentration was very low in six of the eight animals. One other had a concentration of 5 to 8 ng/ml and did not return to estrus for another 38 days, while the other had a concentration of 8 ng/ml on days 2 and 3 of MGA feeding which then fell to 1 ng/ml after treatment. In group (4), all animals had a very low concentration, except one which had concentration of 9 ng/ml, in each sample after MGA treatment and came into estrus 10 days after MGA treatment. Only one of this group conceived. The progesterone was low for several days in those animals which received MGA, as compared to the control group, which was low on only the day of estrus. The concentration was higher than normal basal levels before estrus for those animals which had MGA treatment. These workers suggested that low fertility may be caused by an alteration in the ratio of progesterone to estrogen before estrus.

Estrogen

Wettemann et al. (1972) reported that estradiol in the peripheral blood of the cow was lowest and relatively constant during the luteal phase of the estrous cycle, averaging about 3.6 pg/ml from day 2 through day 11. The values increased to 4.8 pg/ml at 3 days before heat, continued to increase to 9.7 pg/ml about 12 hr before the onset of heat and remained high on the day of estrus (8.4 pg/ml). Christensen et al. (1971) found that estrogen levels were highest (176 pg/ml) approximately 24 hr before the LH peak and were also elevated (141 pg/ml) on days 5 and 6 of the cycle. Levels during the remainder of the cycle fluctuated between 98 and 133 pg/ml. Hendricks et al. (1971) observed that the estrogen level fluctuated within a range of 0.5 - 10.0 pg/ml for the first 3 days of a 4-day period prior to estrus in heifers. A major peak (15-25 pg/ml) occurred on the day prior to estrus. The estrogen concentration rose to a peak several hours prior to the release of LH.

Echternkamp and Hansel (1971) using a RIA technique, found the mean estrogen level of estrus to be 809 pg/100 ml and the levels for 3 days before and 3 days after estrus were 147, 422, 546 and 179, 176 and 169 pg/100 ml, respectively. Shemesh et al. (1972) determined estrogen levels by a CPB procedure from peripheral blood which was collected once daily, except that blood was collected every 4 hr from 24 hr before expected estrus until the time of ovulation. Levels increased gradually from 1.5 to 7.6 mg/100 ml during the 3 days

preceeding estrus and showed a sharp peak of 17 ng/100 ml about 4 hr before the onset of estrus. The estrogen level had already begun to decline by the time the first signs of heat were detected and reached its nadir (0.8 ng/100 ml) 12 hr later. Minimum values were inevidence at the time of ovulation (20 to 32 hr after onset of estrus). A minor rise was observed on day 4 of the cycle, and a more sustained increase occurred on days 10 to 13 with a peak on day 11. The pre-estrus estrogen peak is short-lived and may be missed if the sampling of blood was not frequent enough. This peak appeared to precede the pre-ovulatory surge of LH. Blockey et al. (1973) and Nancarrow et al. (1973) employing the same procedure as Shemesh et al. (1972) found that the levels of estrogen were much higher than those reported by Shemesh et al. (1972). Blockey et al. (1973) determined estrogen from peripheral plasma to be 325 pg/ml on the day before estrus while Nancarrow et al. (1973) reported a value of 6425 pg/ml 2 days before estrus in plasma obtained from the ovarian vein. Christensen et al. (1974), who employed a solid phase RIA technique to measure estrogen in normal cycling beef cows, reported that estrogen levels were highest (<150 pg/ml) at 25.6 hr prior to the LH peak and 18.1 hr prior to the onset of estrus. They then decreased at 8 hr prior to ovulation but rose again on day 5 of the cycle. Estrogen levels then did not differ significantly between other days of the estrus cycle.

Hachett et al. (1972) measured estrogens by RIA during and after MAP synchronization. All synchronized heifers had a surge in

plasma estrogens (65 pg/ml) 2 days after MAP withdrawal regardless of whether they were treated with estradiol benzoate or observed in estrus. However, the values in treated heifers were several times higher than those observed in the plasma of normal untreated cows at the time of estrus. Thus, high estrogen levels may be a cause of reduced fertility often found in cattle synchronized by progesterone and estrogen treatments. Rodeffer et al. (1972) determined estrogen levels during and after synchronization by progesterone injection (either given daily for 18 days or every 2 days for nine injections). Estradiol valerate was injected on day 1. The mean estrogen peak preceeded the mean LH peak by about 5 days in normal and synchronized heifers (treated for nine injections). However, in heifers given 18 daily injections, the estrogen peak values were observed 0.5 day after the LH peak. This abnormal estrogen pattern may indicate the cause of the fertility problem often encountered following an 18-day treatment of synchronizing agents. On the other hand, Hendricks and Lamond (1972) observed that estrogen levels increased to a maximum or near maximum levels on the day before estrus in all cows they sampled.

Chow et al. (1972) reported that plasma levels of estradiol were non-significantly elevated during MGA feeding. Daily estradiol levels during the synchronized estrus were significantly ($P<.01$) higher than during the estrus following the synchronized heat and than in the control group. No difference was detected between control and second estrus estradiol values. Thus ovarian activities may be altered

immediately following MGA treatment resulting in elevation of estradiol levels.

Dobson et al. (1973) reported on estrogen levels in control and hormone treated animals. In the control group the concentration increased from 7.0 pg/ml 4 days before estrus to 14.0 pg/ml on the day of estrus, then decreased to 9.0 mg/ml 1 day after heat. MGA treatment alone did not alter the pattern of estrogen at the synchronized heat and the animals which received MGA + HCG showed a similar concentration of estrogen to the follicular stage of the control and MGA-treated groups. However, HCG appeared to depress estrogen level on day 4. The estrogen values were also comparable to those of the control group in those that received MGA + estradiol + PMS and HCG.

Luteinizing Hormone (LH)

Rakha and Robertson (1965) investigated the timing of LH release from the pituitary gland during the bovine estrus cycle and found a significant drop in both FSH and LH content in the pituitary during a period of 0 to 18 hr after the onset of estrus. Varian et al. (1967) measured LH in the blood of cattle by the ovarian ascorbic acid depletion (OAAD) technique and found a LH peak as high as 35 mug/100 ml plasma 4 hr after the onset of estrus. The duration of the peak was limited to less than 3 hr and ovulation occurred 24 hr to 32 hr after

the LH surge. In late estrus about 2-5 mug/100 ml was detected, and by day 7 of the cycle the level of LH increased about four-fold and remained high until the mid-to late-luteal phase (days 12 to 16). Hendricks et al. (1970) employing an RIA technique, reported that LH increased markedly at estrus to an average of 40 ng/ml in eight of ten estrous periods studied. The rise coincided with the initiation of estrus and reached its peak 3 to 6 hr later. Ovulation occurred 22 and 26 hr after the LH peak in two cows, suggesting that it required some time for LH to complete its action on the follicle to cause ovulation. A slight increase in serum LH concentration was also observed on day 8 of the cycle. Christensen et al. (1971) collected blood at 2 hr intervals and observed a preovulatory peak of LH (18-86 ng/ml) 24 hr prior to ovulation and 9 hr after the onset of estrus for each cycle. The elevated level lasted 12.1 hr and levels during the rest of the cycle varied from 0.6 to 1.6 ng/ml.

Swanson and Hafs (1970) reported that LH increased to 8.07 ng/ml on the day of estrus and decreased to 1.10 ng/ml 1 day after. During estrus, LH concentration was highest (25.9 ng/ml) from 4:00 to 6:00 A.M. in four heifers but it was at 6:00 P.M. in a fifth animal. LH was elevated for about 6 hr in each animal. Estrus was first observed from 8 hr before to 6 hr after the peak in serum LH. The heifers ovulated 31 hr after peak serum LH and 30 hr after the onset of estrus. Gaverich et al. (1971) observed a three-fold increase in plasma LH which changed linearly from day 5 through day 1.

The peak lasted for 4 to 8 hr. From daily sampling it was found that on the day of estrus the average LH concentration was 24 ng/ml, from days 1 to 16, it was from 0.4 to 0.6 ng/ml, while from days 17 to 19 it was from 0.8 to 1.2 ng/ml.

Hackett and Lyons (1971) reported that LH concentration were below 1 ng/ml during most of the cycle but levels increased around estrus and around mid-cycle in some animals. The highest value of estrus was 7.8 ng/ml while it was 2.5 ng/ml near mid-cycle. Niswender et al. (1969) detected a range of 12-60 ng/ml of LH in the serum of five heifers bled on the day of estrus and an average of 1.5-2.5 ng/ml was found during the luteal phase.

Carr (1972) collected blood at 2 hr-intervals during two successive estrous periods and found peak values of approximately 100 ng/ml of serum. There was evidence of a mid-cycle peak which occurred about 11 days before the onset of the next heat.

Hansel and Snook (1970) reported that corresponding peaks in LH and progesterone were seen in some but not in all animals during the luteal phase of the cycle, i.e., days 5 to 17. These seemed to occur at about a 4 to 5 day interval and one peak usually correspond to the development of a large mid-cycle non-ovulated follicle. An ovulatory peak of LH occurred shortly before the beginning of heat.

Echternkamp and Hansel (1971) reported that the peak of LH at estrus varied from 5.7 to 55 ng/ml. Snook et al. (1971) collected blood every other day throughout the estrus cycle and observed basal levels of serum LH throughout most of the cycle of about 2 to 4 ng/ml. Fluctuations were observed in all heifers and a small luteal phase rise was noted in all animals studied. This rise occurred between days 9 and 13. The mean value for the peak was 4.6 ng/ml, while a second small rise in the level was observed 4 to 7 days before ovulation with an average value of 5.3 ng/ml. The major peak occurred at the time of estrus and ranged from 7 to 50 ng/ml. The luteal phase rise of LH level was thought to be correlated with growth and atresia of anovulatory follicles.

Christensen et al. (1974) found that during the estrous cycle the mean values of LH ranged from 0.6 ng/ml on day 15 to 1.8 ng/ml on days 3, 4 and 13. An average peak of 58.9 ng/ml occurred at 7.4 hr after the onset of estrous and 24.1 prior to ovulation. High levels of LH lasted about 12 hr.

Rich et al. (1971) reported that LH levels were less than 1 ng/ml throughout a 14 day MGA-treatment with slight elevations occurring after FSH injection on days 10, 12 or 14 of the treatment. This might have been due to contamination of LH in the FSH preparation. There was no significant difference in plasma LH in single or multiple ovulations. Randel et al. (1972) observed LH levels of less than

1 ng/ml on days 7 and 14 of MGA treatment. Plasma LH was significantly ($P<.005$) higher 24 and 56 hr after cessation of MGA than during the MGA regime. A further increase was evident 5 and 9 days after ovulation.

Hendricks and Lamond (1972) injected PMS on the last day of a 16 or 20 day progesterone treatment. Plasma LH concentration exceeded 1 ng/ml before estrus, and an LH peak occurred in most of the treated cows. There was a clear relationship between the number of ovulations and the length of the period which LH remained above what was considered the normal level (<1 ng/ml).

Rodeffer et al. (1972) synchronized heifers with progesterone by giving a daily injection for 18 days or every 2 days for nine injections. The LH surge occurred 24 hr prior to ovulation and 54.5 hr after the onset of estrus, with no marked difference between the treated or control animals. Estrogen peaks were detected 10 to 30 hr following the LH release in three of four heifers in the 18-day treatment group while in five control heifers the estrogen peak was detected in three heifers prior to the LH release while one heifer showed an elevated estrogen level 16 hr following the LH peak. The estrogen peak was detected prior to or coinciding with the LH surge in the animals receiving progesterone every other day.

Hackett et al. (1972) reported that LH levels varied from 0.5 to 4.6 ng/ml during MAP treatment. Estrus was synchronized within

a 2 to 4 day period after MAP withdrawal and the animals had peak levels of LH which ranged from 65 to 46.8 ng/ml and corresponded closely with estrus.

The current knowledge concerning the normal levels of progesterone, estrogen and LH throughout the estrous cycle of the cow are summarized in Tables 1, 2 and 3, respectively. The profiles of what may be considered as the normal levels of LH, progesterone and estrogen in the peripheral blood throughout the estrous cycle of the cow are presented in Figure 1.

TABLE 1. Peripheral plasma progesterone levels during the estrous cycle (ng/ml)

References	Days of cycle																				
	11	12	13	14	15	16	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10
Donaldson et al. (1970)	5.8	6.3	6.7	6.8	6.6	6.3	5.8	4.1	1.5	0.6	0.4	0.4	0.4	0.8	1.4	2.2	2.9	4.2	4.8	5.1	5.6
Hendricks et al. (1970)	-	3.5	-	3.7	-	6.5	-	-	-	-	-	-	0.9	-	2.5	-	3.2	-	3.8	-	2.5
Robertson & Sarda (1971)	4.7	5.3	5.0	4.3	5.0	2.5	0.8	0.4	0.1	0.1	0.1	0.5	0.2	0.4	2.3	1.0	2.6	2.4	4.0	3.5	4.0
Sprague et al. (1971)	3.4	3.9	1.7	-	2.2	1.9	2.0	2.0	2.0	1.4	1.5	1.3	1.3	1.6	3.2	1.8	2.8	3.5	2.8	3.9	3.3
Swanson et al. (1972)	3.7	-	-	-	-	-	-	7.0	2.5	0.5	0.3	-	0.2	-	0.5	-	-	2.5	-	-	-
Wettemann et al. (1972)	4.5	-	-	-	-	-	-	3.2	2.2	0.4	0.3	-	0.2	-	0.9	-	-	2.5	-	-	-
Christensen et al. (1974)	5.0	4.8	5.3	4.1	6.0	5.3	4.0	3.0	2.2	1.5	1.2	1.6	1.5	1.6	2.4	2.7	3.2	3.5	3.2	3.8	3.9
$\bar{x}$	4.5	4.7	4.6	4.7	4.9	4.5	3.1	3.2	1.7	0.7	0.6	0.9	0.6	1.1	1.8	1.9	2.9	3.1	3.7	4.1	3.8
S.D. ±	0.8	1.1	2.1	1.4	1.9	2.1	2.2	2.2	0.8	0.5	0.5	0.6	0.5	0.6	0.9	0.7	0.3	0.7	0.7	0.7	1.1
S.E. ±	0.3	0.5	1.05	0.7	0.9	0.9	1.1	0.9	0.3	0.2	0.2	0.3	0.2	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.5
n	6	5	4	4	4	5	4	6	6	6	6	4	7	4	7	4	5	6	5	4	5

TABLE 2. Peripheral plasma estrogen levels during the estrous cycle (pg/ml)

References	Days of cycle																				
	11	12	13	14	15	16	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10
Hendricks et al. (1971)	-	-	-	-	-	-	-	-	-	20.0	20.0	10.0	-	-	-	-	-	-	-	-	-
Shemesh et al. (1972)	79.5	45.0	40.0	27.0	36.0	31.5	15.0	39.0	48.0	152 0	34.0	9.0	9.0	16.5	48.0	19.5	10.5	15.0	21.0	18.0	45.0
Van der Walt et al. (1972)	30.6	28.2	38.8	18.1	18.7	28.8	36.2	26.6	99.8	25.4	25.2	40.4	28.0	28.2	18.3	-	-	-	-	-	-
Wettemann et al. (1972)	3.6	-	-	-	-	-	-	4.8	7.7	9.2	8.4	-	3.0	-	3.9	-	-	3.8	-	-	-
Echternkamp et al. (1971)	-	-	-	-	-	-	-	1.5	5.6	7.0	10.6	3.0	2.7	2.8	-	-	-	-	-	-	-
Dobson et al. (1973)	-	-	-	-	-	-	6.6	9.0	9.3	11.1	13.8	8.9	-	-	-	-	-	-	-	-	-
*Christensen et al. (1974)	22.0	38.0	10.0	27.0	-	12.0	-	58.0	7.0	80	23.0	26.0	35.0	17.0	14.0	57.0	46.0	38.0	34.0	14	20.0
$\bar{x}$	33.9	37.0	29.6	24.0	27.3	24.1	19.2	23.1	29.5	43.5	19.2	16.2	15.5	16.1	21.0	38.2	28.2	18.9	27.5	16	32.5
S.D. ±	32.4	8.4	16.9	5.1	12.2	10.5	15.2	22.2	38.0	54.1	9.0	14.1	14.9	10.3	18.9	26.5	25.1	17.4	9.1	2.82	17.6
S.E. ±	16.2	4.8	9.8	2.9	8.6	6.1	8.8	9.0	15.54	20.43	3.4	5.7	6.7	5.1	9.4	-	-	10.0	6.5	-	-
n	4	3	3	3	2	3	3	6	6	7	7	6	5	4	4	2	2	3	2	2	2

Progesterone determined by CPB technique except Wettemann et al. (1972) by RIA
Estrogen and LH determined by RIA except that of Shemesh et al. (1972) by CPB
*Due to very high value, the lowest (93 pg/ml) was subtracted from all.

TABLE 3. Plasma LH levels during the estrous cycle (ng/ml)

References	Days of cycle																				
	11	12	13	14	15	16	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10
Swanson et al. (1972)	1.5	-	-	-	-	-	-	1.6	2.2	2.8	11.8	-	1.9	-	1.7	-	-	1.4	-	-	-
Christensen et al. (1974)	0.8	1.5	0.5	0.5	0.5	0.5	0.6	1.0	1.0	1.0	58.0	0.5	0.8	0.9	1.1	0.8	0.7	0.6	0.5	0.8	0.8
Carr (1972)	1.2	4	7	4.2	2.1	2	2.0	2.2	3.5	2.5	35.0	1.8	1.8	1.8	1.9	1.8	1.8	1.9	1.9	5.0	3.0
Wettemann and Hafs (1973)	1.2	-	-	-	-	-	-	-	-	2	12	-	1.3	1.2	1.4	-	-	1.1	-	-	-
Wettemann et al. (1972)	-	-	-	-	-	-	-	-	-	-	8.4	-	3.0	-	3.9	-	-	3.8	-	-	-
Snook et al. (1971)	3.3	-	4.3	-	2.6	-	5.6	-	4.2	-	2.3	4.2	-	3.5	-	3.1	-	3.0	-	2.8	-
Gaverick et al. (1971)	0.4	0.4	0.4	0.4	0.3	0.4	0.7	0.9	1.1	0.7	33.6	0.4	0.5	0.5	0.5	0.5	0.4	0.4	0.3	0.5	0.4
$\bar{x}$	1.4	1.9	3.1	1.7	1.3	0.9	2.2	1.4	2.4	1.8	25.9	1.7	1.5	1.5	1.7	1.5	0.9	1.8	0.9	2.2	1.4
S.D. ±	1.0	1.8	3.1	2.1	1.1	0.8	2.3	0.6	1.4	0.9	17.6	1.7	0.8	1.1	1.1	1.1	0.7	1.3	0.8	2.0	1.4
S.E. ±	0.4	1.1	1.5	1.2	0.5	0.5	1.1	0.3	0.6	0.4	6.6	0.8	0.3	0.5	0.4	0.5	0.4	0.5	0.5	1.0	0.8
n	6	3	4	3	4	3	4	4	5	5	7	4	6	5	6	4	3	6	3	4	3

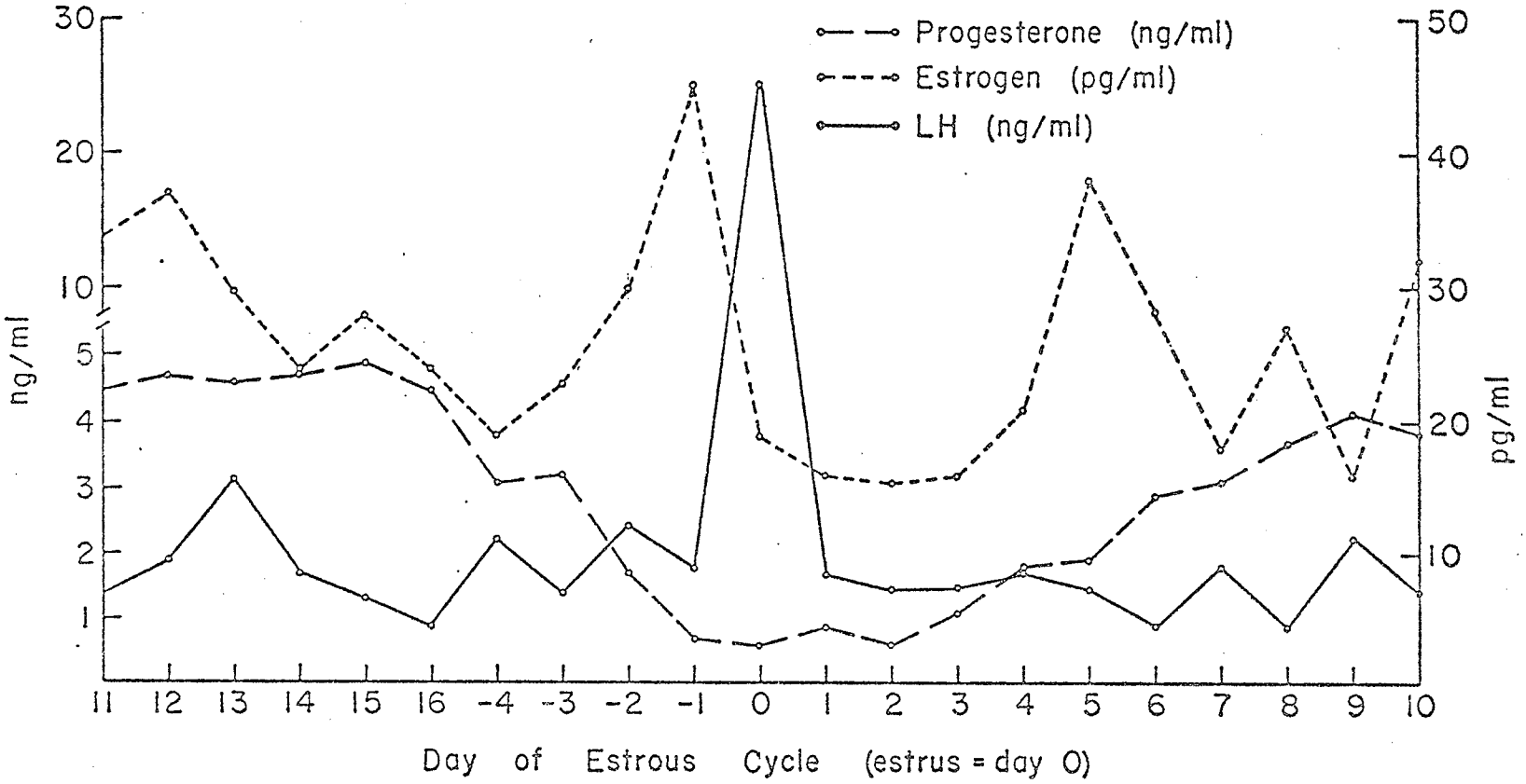


Figure 1. Patterns of progesterone, estrogen and LH during estrous cycle.

MATERIALS AND METHODS

Twenty one post-partum and three open Charolais X Angus cross bred cows were randomized into two groups of 12 cows each. All cows were housed in the barn at the Glenlea Research Station, University of Manitoba and were allowed good quality hay and water ad libitum. They were individually fed 1.0 mg of MGA per day (in 1.0 lb of soybean oil meal) for 16 days. Animals were observed twice daily, morning and evening, for signs of estrus during and after MGA treatment. Group I was given an injection of PMS following MGA treatment, while group II animals also received an injection of HCG in addition to the PMS.

PMS was injected 16 days after the estrus following last MGA feeding in the cases where estrus was observed; otherwise it was given 20 days after the last MGA feeding. HCG was injected 48 hrs following PMS treatment. PMS was given at a level of 2000 I.U. by subcutaneous injection to all cows; and 2500 I.U. of HCG was given by intramuscular injection to cows in group II. MGA, PMS and HCG were obtained from the Upjohn Company, Haver-Lockhart Laboratories and Ayerst Laboratories, respectively. The experimental plan and the interval from calving to the various treatments for each cow is given in Table 4.

After treatment, all cows were moved to pasture and bred

TABLE 4 Experimental Plan

Group	Animal No.	No. of days from calving to: Start of MGA Treatment	End of MGA Treatment	PMS Injection	HCG Injection	1st Estrus
I	*1	41	56	76	-	81
	2	37	52	72	-	76
	3	36	51	71	-	74
	4	36	51	71	-	74
	*5	35	50	70	-	75
	6	35	50	79	-	63
	7	56	71	91	-	98
	*8	27	42	62	-	68
	9	23	38	58	-	65
	[b]10	-	-	-	-	-
	[b]11	-	-	-	-	-
	[b]12	-	-	-	-	-
$\bar{x}$		36.2	51.2	72.2	-	74.8
II	13	41	56	78	80	62
	14	39	54	74	76	92
	15	38	53	73	75	82
	16	37	52	72	74	81
	*17	36	51	71	73	81
	18	35	50	70	72	80
	*19	40	55	75	77	77
	20	37	52	72	74	83
	21	35	50	70	72	73
	22	32	47	72	74	56
	*23	30	45	69	71	53
	24	27	42	62	64	65
$\bar{x}$		35.5	50.5	71.5	73.5	73.7
TOTAL $\bar{x}$		35.8	50.8	71.8	73.5	74.2
Cont -rol (C)	*C1	**90	[a]113	-	-	119
	*C2	**89	[a]112	-	-	125
	*C3	**88	[a]111	-	-	123
$\bar{x}$		89.0	112.0	-	-	122.3

** No. of days from calving to the start of blood collection.
* Animals which were sampled for hormone analysis.
a End of blood collection.
b Open cow.

by natural service to a Devon bull of proven fertility. Treatments were staggered so that only one group of six cows was presented for service at a time.

Jugular blood was obtained by venipuncture from three cows from group I and from three cows in group II. Blood also was obtained from three post-partum non-treated control animals to provide normal values. Daily blood collection began on the day after the last MGA feeding, while twice daily collection was done on the day of estrus or suspected estrus. About 33-40 ml of blood was drawn into a polyethylene tube and allowed to clot overnight at $4^{o}C$. Serum was separated by centrifugation at 4-$5^{o}C$, and kept frozen at -$20^{o}C$ until assayed. Collection continued until four days after the estrus induced by gonadotrophin treatment, or if the cows did not show estrus, collection continued for eight to ten days after the gonadotrophin administration.

Since control cows did not express heat for up to 90 days after calving, blood collection was made once daily for 24 days beginning at an average of 89 days after calving.

Steroid Hormone Assays

Reagents and materials

1. Ether: freshly opened cans of analytical grade anhydrous ether from Baker Chemical Company were used for extraction of the steroids from the serum.

2. Dextan-coated charcoal: prewashed Norit A charcoal (Matheson Coleman and Bell) 250 mg and 2 mg of Dextran T70 (Pharmacia) were mixed in 100 ml of phosphate buffer saline (PBS) and kept at $4^{o}C$. During use, the mixture was maintained in suspension at $4^{o}C$ by a magnetic stirrer. This preparation was employed to remove the unbound steroids. Approximately 90% of the labelled steroid in the absence of any antiserum was removed by this preparation.

3. Radioactive Steroids: progesterone - 1, 2 - ^{3}H (Sp. Act. 50.3 ci/mM) and estradiol - 17β - 6, 7 - ^{3}H (Sp. Act. 46.6 Ci/mM) were obtained from New England Nuclear and were diluted to a concentration of 40 uCi/ml in benzene: ethanol (ratio of 9:1) and stored at $4^{o}C$. The radio-chemical purity was greater than 97% as indicated by the supplier. Working radioactive solutions were prepared by evaporating an appropriate volume of the stock solution under a stream of nitrogen (N_2) and then redissolving the residue with PBS to a final concentration of ca. 10,000 CPM and 1,000 CPM, respectively.

4. Nonradioactive steroids: progesterone and estradiol-17β obtained from Mann Research Laboratories were used for preparation of standards. They were diluted in absolute ethanol and stored at -20°C. Working solutions were prepared by evaporating similarly to that described for the radioactive steroids and then diluting to desired concentration. The working solutions of radioactive and nonradioactive steroids were kept at 4°C and were freshly prepared each week.

5. Antisera: the progesterone antiserum (generously provided by Dr. G. D. Niswender, Colorado State University) was prepared by immunizing rabbits with progesterone -6- hemisuccinate-BSA (bovine serum albumin) and used at a dilution of 1:3000. The estradiol antiserum (#029-14, kindly supplied by Dr. B. V. Caldwell, Yale University) was prepared by immunizing sheep with estradiol-17β - 17-hemisuccinate-BSA and used at a dilution of 1:100,000.

6. Phosphate buffer saline (PBS): containing 0.1% gelatine was prepared by dissolving 1 gm gelatine (Knox, unflavored), 1 gm Sodium Azide (NaN3), 9 gm Sodium Chlorine (NaCl), 5.38 gm Sodium Phosphate, monobasic (NaH2PO4) and 16.35 gm Sodium Phosphate, dibasic (Na2HPO4.7 H_2O) in 1 liter of double-distilled water and the pH was adjusted to 7.00.

7. Scintillation fluid: one gallon of scintillation grade toluene

(Beckman) was mixed with 242 ml of concentrated liquid scintillation, spectraflour PPO-POPOP (Amersham/Searle) to give a concentrate of 6 gm/ml of PPO and 75 mg/ml of POPOP.

8. Other reagents and materials: Glass-distilled benzene was obtained from Burdick and Jackson Lab. and U.S.P. grade absolute ethanol from Canadian Industrial Alcohols and Chemical Ltd. and used without further distillation. Disposable 20 x 150 mm culture tubes (Kimble) were used for extraction of the steroids from the sera and 12 x 75 mm disposable tubes were used for the radioimmunoassay. A two-phase counting system, which contained 1.2 ml of sample in the aqueous phase together with 10.0 ml of scintillation fluid in the scintillation vial was employed after overnight equilibration at counting temperature. All samples were counted in a Nuclear-Chicago Mark II liquid scintillation counter with an efficiency for ^{3}H of 59% and a background of 16 CPM.

Progesterone Assay Procedure

In general, the procedure is similar to that described by Abraham et al. (1971) with some minor modifications and outlined as follows:

1) Extraction of Serum

1. An appropriate volume of PBS was added to a 20 x 150 mm culture

tube containing enough sample of make up to 1.0 ml.

2. 0.1 ml of radioactive progesterone was added (containing about 1,000 CPM). This tracer served for estimation of recovery.

3. From a newly opened can, 8.0 ml ether was added to the tube and mixed for 30 sec.

4. The tube was placed in a freezer at -20° until material was frozen (about 2-3 hr).

5. The unfrozen supernatant was then decanted into a correspondingly-labelled tube.

6. The extract was dried under a gentle stream of N_2 in a water bath at $39^{\circ}C$.

7. The dried ether extract was dissolved in 1.5 ml PBS by shaking for at least 10 min.

2) Preparation of Standard and Sample

a) Standard

1. 0.1 ml antibody was added to each standard tube.

2. 0.5 ml of a standard preparation to provide 0, 25, 50, 100, 200, 400 and 800 pg of progesterone was added to appropriately labelled duplicate tubes.

3. 0.1 ml of progesterone-^{3}H (containing 10,000 CPM) was added to all tubes and stirred.

4. Tubes were equilibrated overnight at $4^{o}C$.

b) Sample

1. 0.1 ml antibody was added to all sample tubes.

2. 0.5 ml of the dissolved ether extract was added to the sample tube.

3. 0.1 ml of progesterone-^{3}H (containing 10,000 CPM) was added to all tubes and stirred.

4. Tubes were equilibrated overnight at $4^{o}C$.

c) Standard and Sample

1. 0.5 ml charcoal suspension was added to each standard and sample tube, mixed and incubated for 20 min.

2. Tubes were centrifuged for 10 min. at 3,000 RPM.

3. The above two steps were done at $4^{o}C$.

4. Supernatant from each tube was decanted into a scintillation vial.

5. 10.0 ml of scintillation fluid was added to each of the vials and thoroughly shaken.

6. All the vials were equilibrated overnight in the scintillation spectrometer and each was then counted for 4 min.

d) Recovery

1. 0.5 ml of the dissolved ether extract was added directly into a scintillation vial (designated as recovery tube).

2. 0.1 ml of progesterone-^{3}H (containing 1000 CPM) was added to a second scintillation vial with PBS to make a total of 0.5 ml (designated as R-CPM and used in calculation for percent recovery).

3. 10.0 ml of scintillation fluid was added to both recovery and R-CPM vials and thoroughly shaken.

4. Vials were then placed in the scintillation spectrometer and were counted for 4 min. after overnight equilibration.

e) Calculations

$$\text{Percent recovery} = \frac{\text{Activity counted in recovery vial} \times 100}{\text{Activity in R-CPM vial}}$$

Only 0.5 ml from the total of 1.5 ml was used.

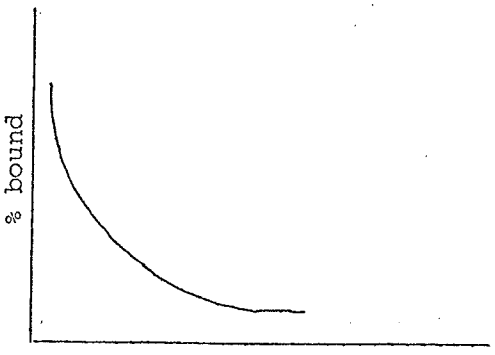
Therefore, percent recovery for 1.5 ml of total serum used =

$$\frac{\text{Activity in recovery vial} \times 100 \times 3}{\text{Activity in R-CPM vial}}$$

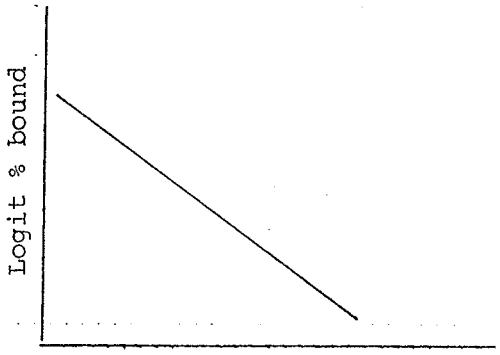
Percent Bound

Activity counted from 0 pg of standard preparation (i.e., the absence of unlabelled hormone) was considered to be 100% bound. Hence, percent bound for each standard or assay sample = $\frac{\text{Activity in standard or assay sample} \times 100}{\text{Activity in 0 pg vial}}$

Percent bound from known standards may be plotted on ordinary graph by using the Y axis as % and the X axis as concentration in pg (Fig. 2a). When the same data is transformed to a plot of logit (Y) vs log (X) as suggested by Rodbard and Lewald (1970), a linear relationship is observed (Fig. 2b). The results reported herein were obtained by using the logit vs log plot.



conc. (pg)
Fig. 2a



log conc. (pg)
Fig. 2b

All standards and samples were assayed in duplicate. The percent bound of the unknown sample was compared with the percent bound of the known standard and the value (in pg/ml) was calculated as follows:

$$\frac{\text{xpg} \times 100}{\% \text{ Recovery}} = \text{pg/ml of serum used}$$

Estrogen Assay Procedure

The general procedure is similar to that of progesterone, but due to the extremely low values of estrogen in blood the volume of serum used and the method to obtain percent recovery was modified. Approximately 4.0 ml of serum was used and 0.5 ml of buffer was added to dissolve the extract. Recovery was obtained by adding 1000 CPM of estradiol-17β-^{3}H to the serum and 1000 CPM also was used in the R-CPM tube. The calculations were the same as for progesterone. For both estrogen and progesterone, accuracy and precision were determined by adding a known amount of the steroid to double distilled water and proceeding as was done for the unknown sera.

Precision, accuracy and sensitivity of RIA for steroid hormones

Precision - According to Ekins and Newman (1970) precision is related to the reproductivility of the measurement and is usually represented by the standard deviation of replicate estimates, assuming they are

normally distributed about the mean. From Fig. 3a the measurement of precision of any quantity h is related both to precision of the estimate of the response (R) and the slope of the response curve $(\frac{dR}{dh})$ at the corresponding point.

Sensitivity - As applied to an assay technique, refers to the ability of the system to measure the smallest amount with acceptable precision (Ekins and Newman, 1970). Thus the technique is considered to be more sensitive if it can measure a smaller amount with the same precision or with even greater precision. This concept may be formalized by defining the sensitivity of an assay as the precision of measurement of a 0 quantity (Fig. 3b). It is clear that sensitivity merely represents a limiting case of the concept of precision in the particular case where h, in quantity is equal to zero (Ekins and Newman 1970).

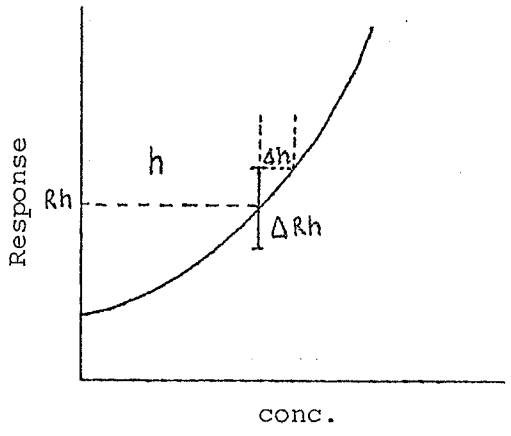


Fig. 3a. Precision

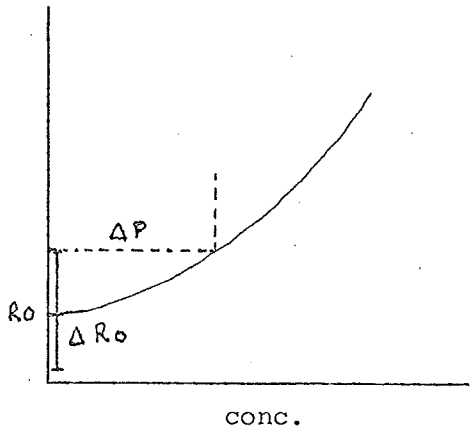


Fig. 3b. Sensitivity

a) Precision of measurement of h - Δh

$$\Delta Rh = \text{S.D. of response Rh}$$

$$\Delta h = \frac{Rh}{dR/dh}$$

b) Sensitivity = precision of measurement of h when h = 0.

Accuracy - Yield of a value close to the real value.

Precision and Sensitivity of progesterone standard curve

Data accumulated from five different assays produced the precision and sensitivity of the assay. At the 25 and 50 pg levels, the precision was 4.4 and 4.3 pg, respectively. It was within 11.0 to 18.4 pg in the range from 100 to 400 pg, and 50.0 pg at the 800 level (Table 5). Intra-assay data obtained from quadruplicate determinations indicated that the precision was within 1.46 to 11.55 pg in the range from 25 to 800 pg (Table 5).

Accuracy and Precision of Progesterone Assay

This was done by quantitating a series of known amounts of progesterone added to double distilled water, anestrus ewe serum, or ether-extracted cow serum. The mean, the standard deviation and the coefficient of variation are presented in Table 6 for the determinations

TABLE 5. Precision and sensitivity of progesterone standard curve

	Inter-assay (n=5)				Intra-assay (n=4)			
	Evaluation based on % bound			Precision + Sensitivity	Evaluation based on % bound			Precision + Sensitivity
	$\bar{x}$	S.D.	C.V.		$\bar{x}$	S.D.	C.V.	
25	84.86	2.49	2.93	4.44 (.56)	87.50	.91	1.04	1.46 (.62)
50	74.30	1.74	2.34	4.35 (.40)	72.98	1.44	1.97	3.13 (.46)
100	60.68	2.97	4.89	11.00 (.27)	58.51	.52	.88	1.79 (.29)
200	43.84	1.98	4.52	11.64 (.17)	42.18	.94	2.22	5.87 (.16)
400	28.64	1.47	5.13	18.37 (.08)	31.39	.39	1.24	6.19 (.06)
800	18.68	1.25	6.69	50.00 (.02)	19.33	.26	1.34	11.56 (.02)

C.V. Coefficient of variation (expressed as %)

S.D. Standard deviation

made from each of the materials. The coefficient of variation was usually well below 20% in all assays with the exception of the zero level in double distilled H_2O when the coefficient of variation reached 26.5%. Similar quantitations were observed whether the progesterone was added to water, anestrus ewe serum (both inter-and intra-assay) or cow's serum.

The precision of the progesterone determinations declined as the concentration increased from 25 pg to 800 pg. At higher levels (400 and 800 pg) the measurement became less acceptable especially in inter-assay (Table 5).

Although the standard curve suggests the precision and sensitivity to be as low as 25 pg, the accuracy and precision determined by adding known amounts of progesterone to water (Table 6) did not agree with the standard curve. Therefore, to get a compromise between precision and accuracy, the sample should contain at least 65 pg in order to get a result with acceptable precision and accuracy. A comparable situation is found in the precision and accuracy of estrogen determination and to justify both the sample should at least contain 12.5 pg of estrogen.

Precision and Sensitivity of Estradiol-17β Standard Curve

Results presented from five different assays (Table 7) indicate that the precision was within 0.5 to 6.5 pg in the range from 6.2 to

TABLE 6. Accuracy and precision of progesterone determination

Test Medium*	Progesterone conc. (pg.)	No. of determinations	Amount quantitated		
			$\bar{x}$	S.D.	C.V.
Double distilled H_2O (Intra-assay)	0	4	44.75	11.80	26.50
	25	4	64.50	8.34	12.90
	100	4	153.75	9.53	6.19
	200	4	273.00	11.22	4.10
	400	4	439.00	20.08	4.50
	800	4	816.75	27.20	3.33
Anestrous ewe serum (Intra-assay)	0	4	186	16.6	8.9
	100	4	290	30.5	10.5
	200	4	396	27.1	6.8
	400	4	618	35.0	5.6
	800	4	910	109.0	11.9
Anestrous ewe serum (Inter-assay)	0	4	180	26.0	14.4
	100	4	296	29.0	9.7
	200	4	418	24.7	5.9
	400	4	564	34.7	6.2
	800	4	878	39.8	4.5
Ether extracted cow serum	0	4	265.16	27.64	10.42
	50	4	327.41	8.03	2.45
	100	4	362.48	18.41	5.01
	200	4	441.93	29.80	6.74
	400	4	563.57	44.47	7.89
	800	4	878.10	13.21	1.50

*1.0 ml in all cases

C.V. - Coefficient of variation (expressed as %)
S.D. - Standard deviation

TABLE 7. Precision and sensitivity of standard curve for estradiol-17β

	Inter-assay (n=5)				Intra-assay (n=4)			
	Evaluation based on % bound			Precision + Sensitivity	Evaluation based on % bound			Precision + Sensitivity
	$\bar{x}$	S.D.	C.V.	pg (slope)	$\bar{x}$	S.D.	C.V.	pg (slope)
6.2	90.96	1.20	1.31	1.25 (.96)	93.04	1.01	1.08	1.05 (.96)
12.5	81.22	0.83	1.02	0.86 (.96)	86.28	1.15	1.33	1.30 (.88)
25	66.84	0.50	0.74	0.53 (.94)	71.77	0.88	1.22	1.00 (.97)
50	48.22	1.52	3.15	2.23 (.68)	52.23	1.04	1.99	1.40 (.74)
100	31.04	2.09	6.73	6.53 (.32)	33.50	0.39	1.16	1.08 (.36)
200	19.48	1.74	8.93	13.38 (.13)	20.69	0.43	2.07	3.30 (.13)
400	11.72	1.06	9.04	25.25 (.04)	13.43	0.54	4.02	21.60 (.02)

100 pg and was within 13.4 pg to 25.2 pg at the 200 and 400 pg levels, respectively. Quadruplicate determinations within an assay gave the following results: in the range of 6.2 to 200 pg the precision was within 1.0 to 3.3 pg and was 21.6 pg at the 400 pg level (Table 7).

Accuracy and Precision of Estradiol-17β assay

Table 8 presents data obtained from quantitative analysis of a series of known amounts of estradiol-17β (E_2) added to double distilled water, ovariectomized ewe serum, or ether extracted cow serum. The coefficient of variation of intra-assay, when a known amount of E_2 was added to distilled water varied from 2.0 to 9.4% at levels ranging from 12.5 to 400 pg. It increased to 15.8% at the 6.25 pg level and 55.0% at the 0 pg level. In inter-assay the coefficient of variation was well under 20% at levels between 12.5 to 400 pg. Likewise, it increased to 51.0% and 40.3% at the 6.25 and 0 pg levels, respectively. Since ovariectomized ewe serum and ether extracted cow serum already contained some initial amount of estradiol, the coefficient of variation was well below 20%.

Specificity

The cross reactions of selected steroids with the progesterone 6β-BSA antibody as reported by Niswender (1973), is given in Table 9, while those for the estrogen antiserum, as given by Wu and Lundy (1971)

TABLE 8. Accuracy and precision of estrogen assay

Test Medium*	Progesterone conc. (pg.)	No. of determinations	Amount quantitated		
			$\bar{x}$	S.D.	C.V.
Double distilled H_2O (Intra-assay)	0	4	2.18	1.20	55.04
	6.25	4	5.63	.89	15.80
	12.5	4	11.63	1.10	9.45
	25	4	22.76	.45	1.97
	50	4	49.78	1.96	3.93
	100	4	94.60	3.12	3.29
	200	4	187.85	5.98	3.18
	400	4	340.31	13.52	3.97
Double distilled H_2O (Inter-assay)	0	5	2.4	0.57	23.7
	6.25	5	4.54	2.50	51.04
	12.5	5	11.00	1.89	17.10
	25	5	23.11	2.15	9.30
	50	5	41.76	3.31	7.92
	100	5	84.74	5.67	6.69
	200	5	174.83	5.42	3.10
	400	5	372.76	15.24	4.08
Ovariectomized ewe serum (Intra-assay)	0	4	27.76	0.83	2.98
	6.25	4	36.53	2.52	6.89
	12.5	4	43.51	4.59	10.54
	25	4	49.84	3.84	7.69
	50	4	68.02	2.63	3.86
	100	3	108.61	4.62	4.43
	200	4	175.04	13.70	7.82
	400	4	326.20	14.01	4.29
Ether extracted cow serum	0	5	20.88	3.82	18.29
	6.25	5	30.92	2.75	8.9
	12.5	5	40.96	2.03	4.95
	25	5	52.97	2.18	4.11
	50	5	78.36	7.49	9.55
	100	5	128.81	10.24	7.95
	200	5	242.23	19.53	8.06

*4.0 ml in all cases

TABLE 9. Cross reaction of various steroids with anti-progesterone 6-β-BSA serum (Niswender 1973)

Steroid	% Cross Reaction
C_{21} Steroids	
Progesterone	100.0
Pregnenolone	3.4
11α-Hydroxyprogesterone	0.9
17α-Hydroxyprogesterone	0.3
Δ4 - Pregnene-20β-ol-3-one	0.1
Δ4 - Pregnene-20α-ol-3-one	0.1
5β - Pregnene-3α, 20α-diol	< 0.1
5α - Pregnene-3β, 20β-diol	< 0.1
Deoxycorticosterone	0.1
Corticosterone	< 0.1
Cortisone	< 0.1
Hydrocortisone	< 0.1
C_{19} Steroids	
Testosterone	< 0.1
Δ4 - Androstene - 3,17-dione	< 0.1
Δ5 - Androstene - 3β, 17β-diol	< 0.1
Dehydroepiandrosterone	< 0.1
Dihydrotestosterone	< 0.1
C_{18} Steroids	
Estradiol-17α	< 0.1
Estradiol-17β	< 0.1
Estrone	< 0.1
Estriol	< 0.1

are presented in Table 10.

The only progestogen which had considerable cross-reactivity with the anti-progesterone-6β-BSA serum was pregnenolone, 3.4% (Table 9). Two estrogens which had significant cross reaction with the estradiol antibody were estrone (63.7%) and estriol (18.7%), (Table 10).

Luteinizing Hormone Assay

Reagents

1. Phosphate Buffer: 0.188 gm $NaH_2PO_4.H_2O$, 2.643 gm Na_2HPO_4 and 17.53 gm NaCl was dissolved in distilled H_2O and brought up to 2000 ml. Final pH of 7.8.

2. Phosphate Azide Buffer: 2.0 gm Na-Azide was added to 1000 ml of phosphate buffer, final pH 7.8.

3. 1% EW-PO_4-Azide Buffer: 5.0 gm egg white powder (Sigma Chemical Co.) was added to 495 ml of Phosphate-Azide Buffer.

4. 3% Rabbit Serum (RS)-PO_4-EDTA Buffer: 18.6 gm EDTA was put in a 1000 ml beaker, 800 ml Phosphate-Azide buffer was added and the solution was warmed and mixed. The pH was adjusted to 7.6 and distilled H_2O was added up to 1000 ml. 3.0 ml RS was pipetted into 97 ml Phosphate-EDTA buffer.

TABLE 10. Cross-reaction of selected compounds with estradiol antibody (Wu & Lundy 1971)

Compound	% cross-reaction
Estrone	63.7
6-Dehydroestrone	2.8
16α-Hydroxyestrone	2.6
2-Hydroxyestrone	1.3
Estrone 3-Methylether	0.7
1-Methylestrone	0.6
Estrone 3-Methylether	0.2
2-Methoxyestrone	0.00
17β -estradiol	100
Ethinylestradiol	6.7
17α-estradiol	5.1
6-Dehydroestradiol	4.1
17β-estradiol 3-methylether	0.2
2-Methoxyestriol	0.05
Ethinylestradiol 3-methylether	0.00
Estriol	18.7
16,17-Epistriol	3.3
17-Epiestriol	3.3
16-Epiestriol	1.6
Estriol 3-methylether	0.03
Testosterone	0.00
Androstenedione	0.00
Dehydroepiandrosterone	0.00
Progesterone	0.00
17α-Hydroxyprogesterone	0.00
L-Adrenaline	0.00
L-(-)-Tyrosine	0.00
Tyramine	0.00

5. LH Antiserum (#15 Antibody): (obtained from G. D. Niswender, Colorado State University). 10 ml distilled H_2O was added to vial to give a 1:400 dilution in 0.05 M EDTA-PBS and was stored in 0.2 ml quantity in vials in freezer. For assay, 0.2 ml was diluted to 50 ml with 3% RS-PO_4-EDTA buffer to result in a 1:100,000 dilution.

6. LH Standard (NlH-LH-B6): Obtained from the National Institute of Health, Endocrine Study Section, Bethesda, Maryland. Serial dilutions were made in 1% EW-PO_4-Azide Buffer.

7. Labelled LH: I^{125} was purchased from Cambridge Nuclear, Radiopharmaceutical Corp., Billerica, Massachusetts and radioiodination was done in accordance with procedures by Niswender et al. (1969).

Procedure: The method is basically that of Niswender et al. (1969), as modified by Howland (1971). The method in brief is as follows:

1. 0.3 ml 1% EW-PO_4-Azide buffer was added to all tubes (10 x 75 mm culture tubes).

2. 0.2 ml serum was added to appropriate tubes (each unknown sample wad done in duplicate).

3. Following amounts of LH standard and buffer were added to prepare standard curve:

diluent (ml)	LH Std. (ml)	ng LH
0.50	0.00	0.0
0.49	0.01	0.1
0.48	0.02	0.2
0.46	0.04	0.4
0.42	0.08	0.8
0.38	0.12	1.2
0.48	0.02	2.0
0.46	0.04	4.0
0.44	0.06	6.0

4. 0.2 ml LH antibody was added to all tubes (#15 at 1:100,000 dilution).

5. 0.1 ml I^{125}-LH (3000 cpm) was added and mixed. Labelled LH was diluted in 1% EW-PO_4-Azide buffer to approx. 3000 cpm.

6. Tubes were covered with Parafilm and incubated for 5 days at $5^{o}C$.

7. 0.2 ml 2nd antibody was added and mixed. To prepare 2nd antibody, 3 parts Goat Anti-Rabbit α-Globulin (Antibodies Incorp., Davis, California) was diluted with 7 parts PO_4-Azide buffer.

8. Tubes were incubated for 24 hr at $5^{o}C$ during which time a white ppt. forms.

9. Mixed ppt. and added 1.0 ml PO_4-Azide buffer.

10. Centrifuged for 20 min at 2000 rpm.

11. Supernatant was poured into a second culture tube.

12. Both supernatant and ppt. were counted for 2 min in a Packard Tri-Carb gamma Spectrometer (Model 3001).

Calculations

$$\text{Percent Bound} = \frac{\text{activity of precipitate}}{\text{activity of supernatant + precipitate}} \times 100$$

The standard curve was drawn by plotting percent bound against concentration of LH. The results were plotted on ordinary graph or on logit vs log paper. The remaining calculations were the same as for the steroid assays.

RESULTS

Occurrence of estrus

None of the treated animals were observed in estrus during the MGA feeding period. Four of the 14 treated cows expressed estrus within 13 days after MGA withdrawal. However, after PMS treatment 10 of 12 cows in group I showed estrus an average of 5.5 days after injection (Table 11). Five of these cows had recurring estrus which included the three cows which were retained for blood collection (Table 11). Nine of the 12 cows, which also received the HCG injection, in group II, came into estrus an average of 5.8 days following last treatment (Table 11). Eight of them had recurring estrus and this included one cow which was retained for blood collection.

None of the non-treated cows expressed estrus during the time they were kept in the barn for blood collection. However, estrus was observed in all of them within 10 days after they were transferred to the pasture.

Hormone levels

Non-treated control animals

A normal pattern of progesterone as described by Donaldson et al. (1970), Hendricks et al. (1970), Swanson et al. (1972),

TABLE 11. Time interval from PMS or HCG treatment to estrus

Group	Animal No.	No. of days to 1st estrus	No. of days from PMS induced estrus to subsequent estrus
I	[a]*1	5	23
	2	4	-
	3	3	-
	4	3	-
	[a]*5	5	35
	*6	18	17
	7	7	-
	[a]*8	6	27
	*9	7	10
	*10	8	16
	11	7	-
	12	17	-
[b]($\bar{x}$ ± S.D.)		5.5 ± 1.77	
II	*13	7	22
	*14	16	25
	15	7	-
	*16	7	11
	[a]*17	8	21
	*18	8	22
	[a]*19	0	27
	*20	9	18
	*21	1	8
	*22	5	6
	[a]23	26	-
	*24	1	24
[b]($\bar{x}$ ± S.D.)		5.8 ± 2.97	

* Cows observed to have recurring estrus

b Excluding cows not responding to treatment i.e. Nos. 6, 12, 14, 19 and 23.

a Cows retained for blood collection.

Robertson and Sarda (1971), Wettemann et al. (1972), Sprague et al. (1971) and Garverick et al. (1971), of estrogen as reported by Hendricks et al. (1971), Shemesh et al. (1972), Van der Walt et al. (1972), Wettemann et al. (1972), Echternkamp et al. (1971), Dobson et al. (1973) and Christensen et al. (1974) and of LH as indicated by Swanson et al. (1972), Christensen et al. (1974), Carr (1972), Wettemann and Hafs (1973), Snook et al. (1971) and Garverick et al. (1971) was observed in the three non-treated cows that were sampled (Fig. 4, 5 and 6). Although a definitive ovulatory LH peaks were not obtained by the regime of blood collection in this study, rises were seen during the period of low progesterone levels. Similarly, estrogen levels increased during this same time. Although these animals did not show overt estrus during the period of blood collection it is suggested from the hormone profiles that all had ovulated at least once prior to the beginning of blood sampling. The progesterone patterns also suggest that ovulation probably occurred at approximately day 14, day 11 and day 16 of the sampling period in cows C1, C2 and C3, respectively (Fig. 4, 5 and 6).

Group I, MGA + PMS treatment

Progesterone, LH and estrogen patterns in cow's blood sampled from group I (cow Nos. 1, 5 and 8) are shown in Figures 7, 8 and 9. Each of these cows displayed a somewhat different response to MGA as well as to PMS treatment, in terms of progesterone and estrogen secretion. However, the LH pattern appeared to be similar in all three cows.

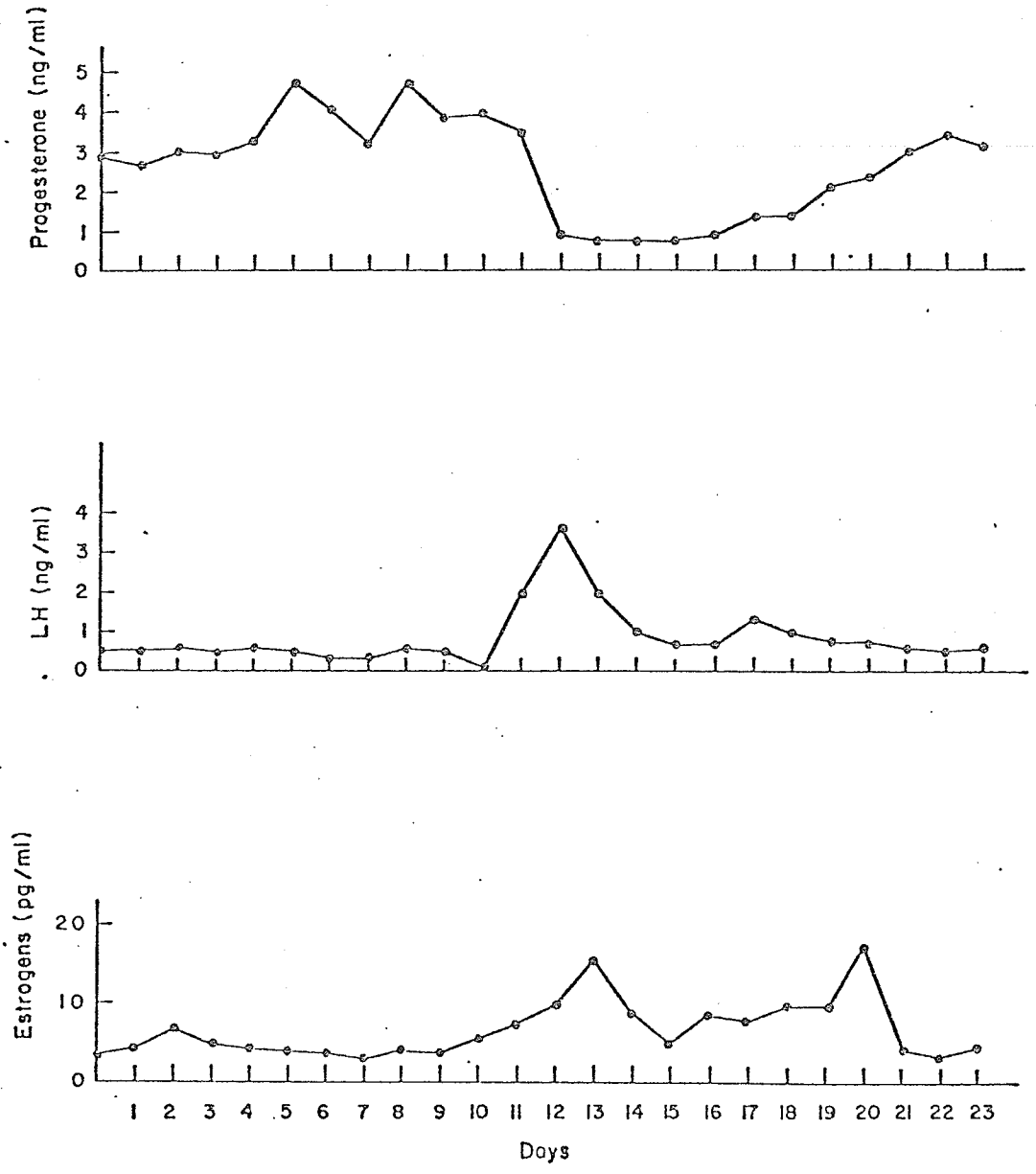


Figure 4. Hormone patterns in non-treated cow No. C1 over a 24-day period beginning 90 days after calving.

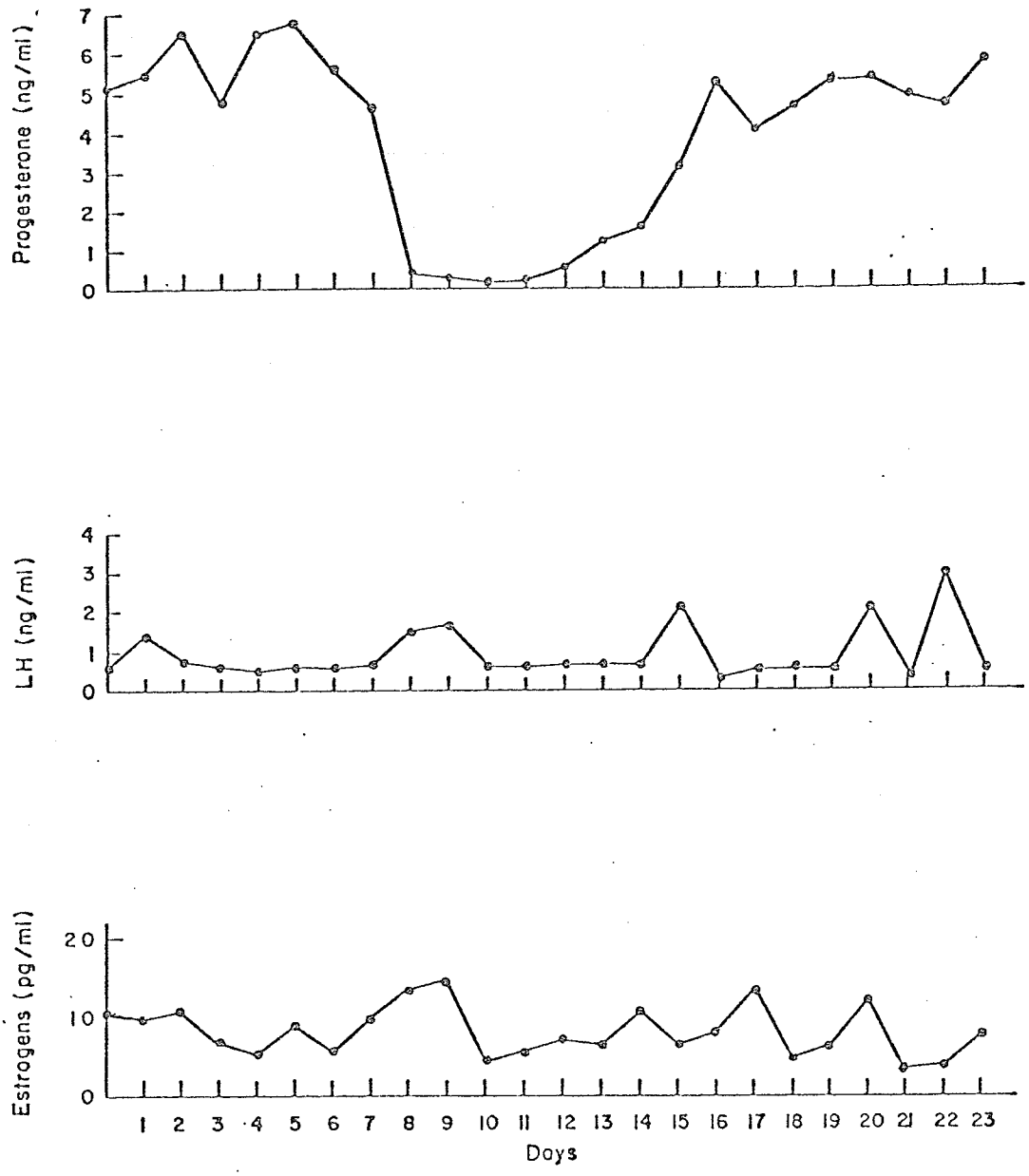


Figure 5. Hormone patterns in non-treated cow No. C2 over a 24-day period beginning 89 days after calving.

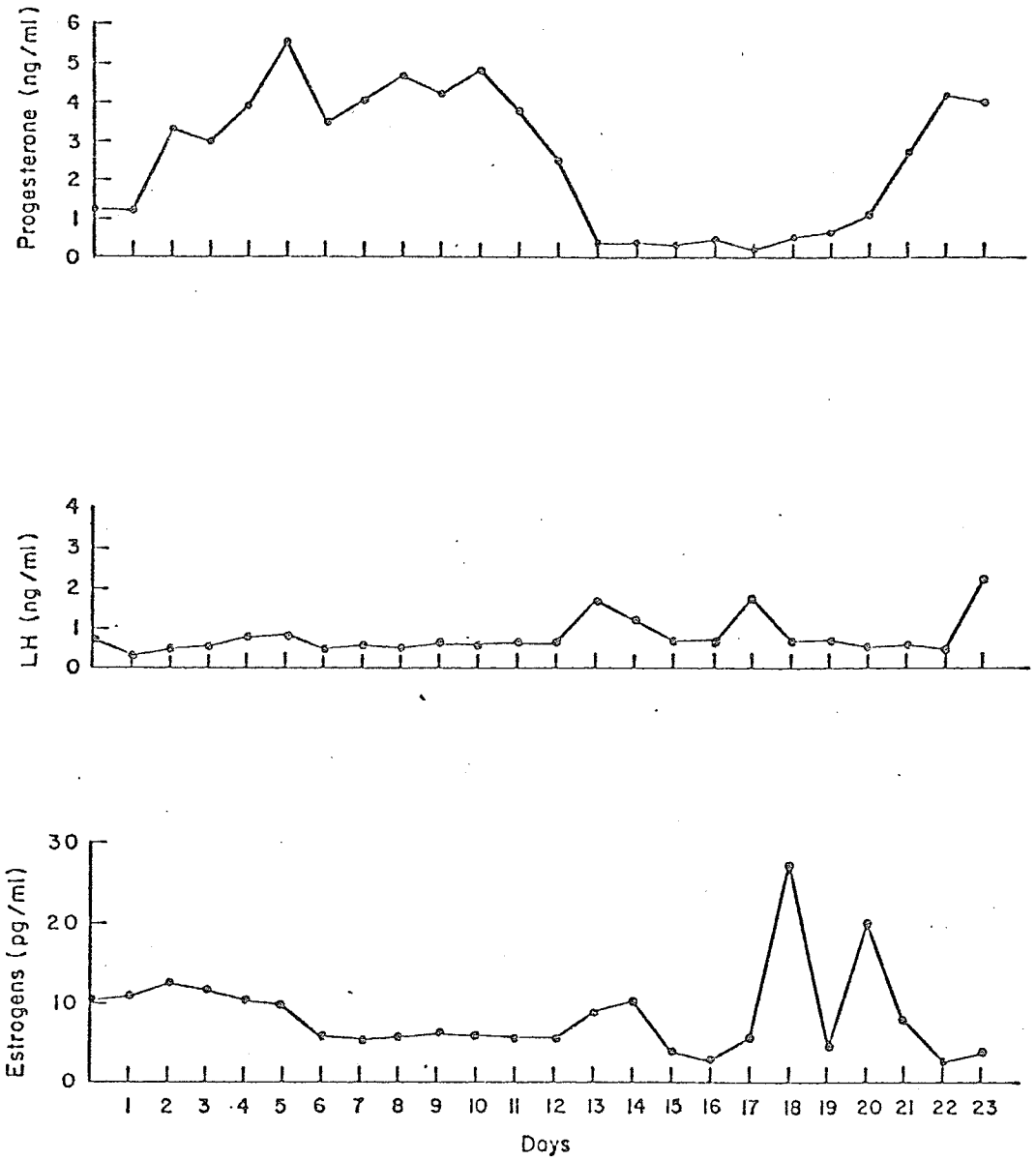


Figure 6. Hormone patterns in non-treated cow No. C3 over a 24-day period beginning 88 days after calving.

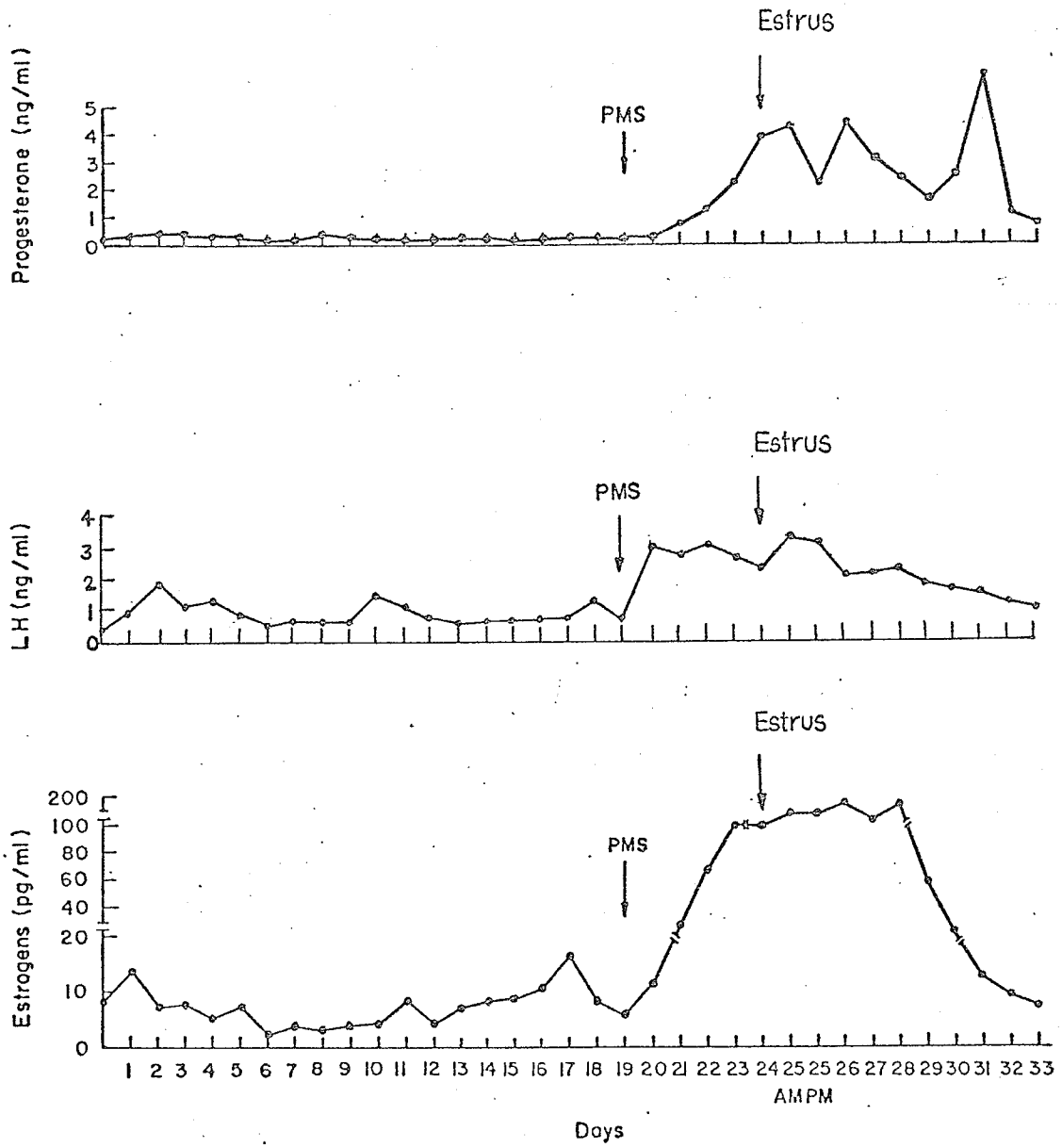


Figure 7. Hormone patterns in cow No. 1 following MGA and PMS treatment. Day 0 = the day after last MGA feeding (estrus was observed on day 24).

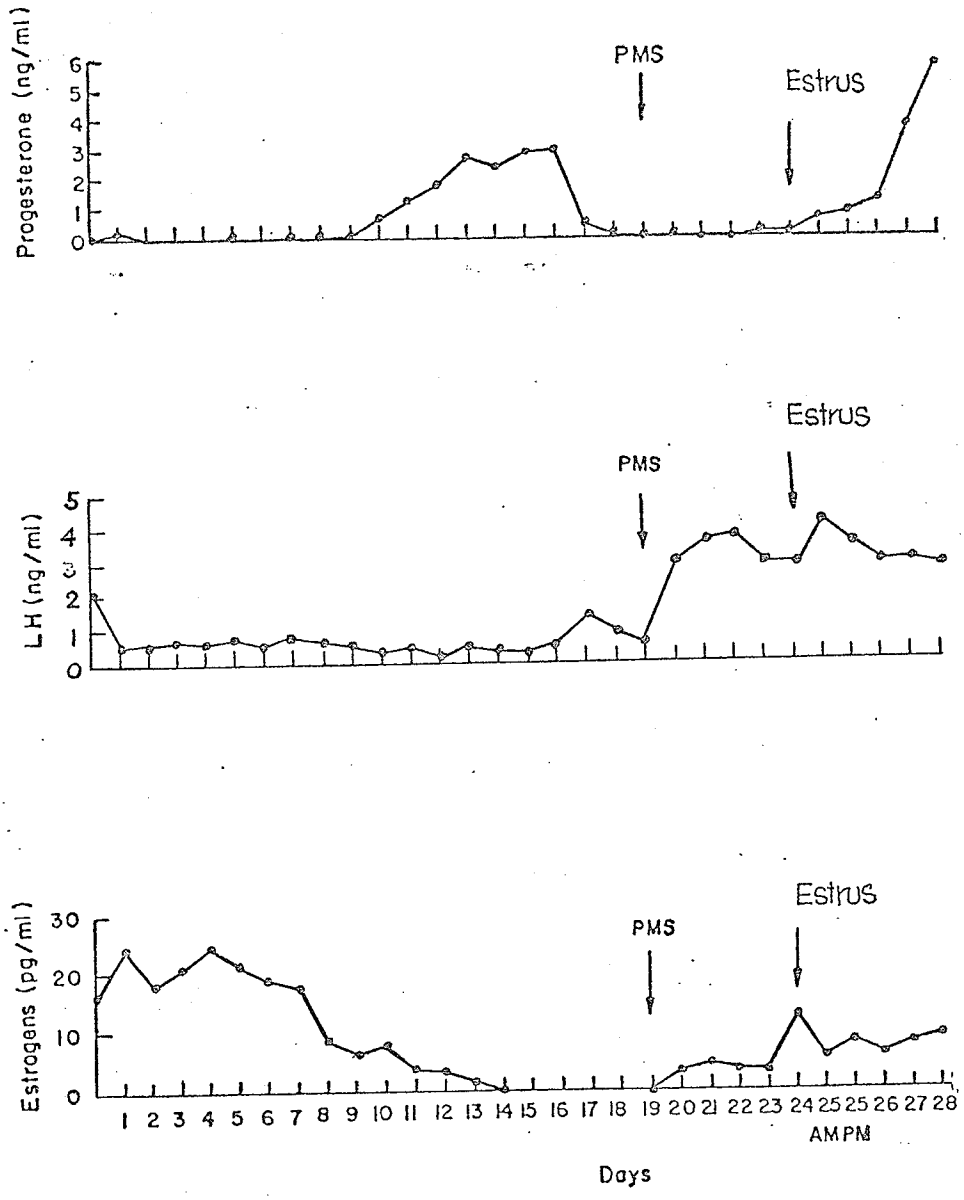


Figure 8. Hormone patterns in cow No. 5 following MGA and PMS treatment. Day 0 = the day after last MGA feeding (estrus was observed on day 24).

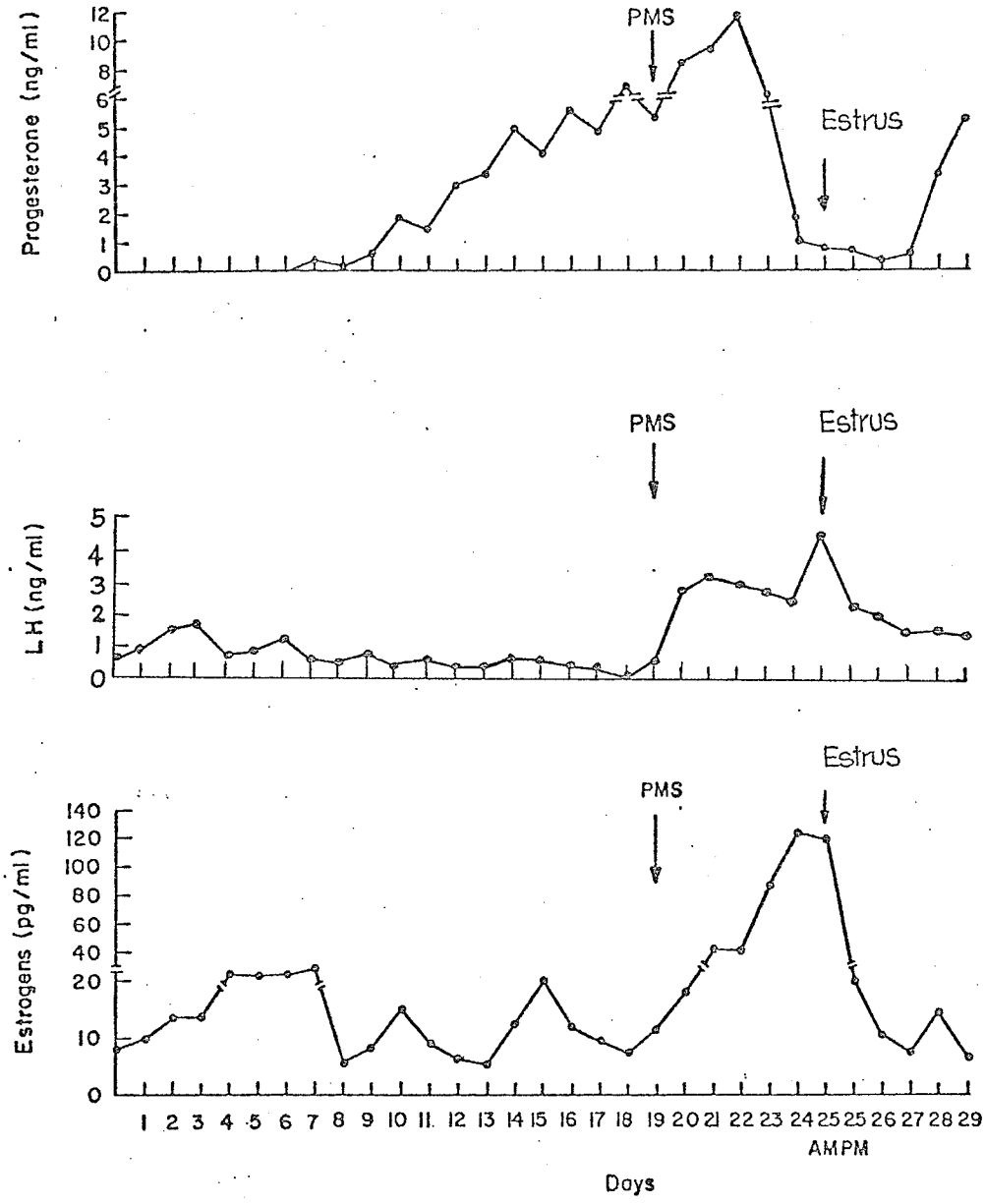


Figure 9. Hormone patterns in cow No. 8 following MGA and PMS treatment. Day 0 = the day after last MGA feeding (estrus was observed on day 25).

After the last day of MGA-feeding (day 0) progesterone in cow No. 1 (Fig. 7) remained low until PMS injection and LH also showed only minor fluctuation. Estrogen also remained relatively low with little fluctuation during this period, indicating little follicular growth. One day after PMS injection progesterone started to rise and remained above baseline levels with some fluctuation for 12 days. Both LH and estrogen rose after PMS injection. LH remained between 2 to 3 ng/ml for several days then declined while estrogen showed a marked response rising up to more than 100 pg/ml for six days before declining. Estrus was observed five days after PMS treatment during which time both progesterone and estrogen were high (Fig. 7).

There was a rise in progesterone of short duration between days 9 and 17 after MGA treatment in cow No. 5 (Fig. 8), perhaps indicating incomplete CL development. LH remained low until PMS injection. Estrogen was very high immediately after MGA treatment (suggestive of excessive follicular development) but then gradually declined to undetectable levels before PMS treatment. LH levels increased immediately following PMS injection but progesterone did not rise until seven days later. There was a peak in estrogen levels of the same magnitude as that of the non-treated cows five days after PMS injection which coincided with the time of observed estrus.

Hormone patterns suggested that ovulation had occurred in cow No. 8 eight or nine days after MGA treatment ended as indicated by the progesterone change (Fig. 9). High estrogen levels were maintained

for four days prior to the rise in progesterone. However, a LH peak was not detected during this time. When PMS was injected (during this presumed luteal phase), it stimulated further secretion of progesterone but did not appear to extend the phase beyond that of normal length. Progesterone dropped to baseline levels five days after PMS injection. At this time both LH and estrogen were high and estrus was observed.

Group II, MGA + PMS + HCG treatment

Progesterone started to rise on day 16 after MGA treatment in cow No. 17 (Fig. 10), indicating probable ovulation. LH declined to baseline levels on day 2 and remained low until after HCG injection. An estrogen peak was observed on day 1, but low levels were then maintained until after HCG injection. There was a small LH peak on day 12 which may possibly be associated with the suggested ovulation that occurred on day 14 or 15. When PMS was injected during the probable early luteal phase a similar response of progesterone was observed as in cow No. 8 (group I). LH did not rise until one day after HCG injection and was maintained between 2 to 4 ng/ml for several days before declining. Estrogen started to rise after PMS injection and reached a peak eight days later and then declined. The magnitude of the response was not as high as in cows No. 1 and 8 (group I).

Cow No. 19 (Fig. 11) had a similar pattern of progesterone secretion to that of cow No. 5 in group I. LH showed fluctuations during the first nine days after MGA treatment while estrogen was high

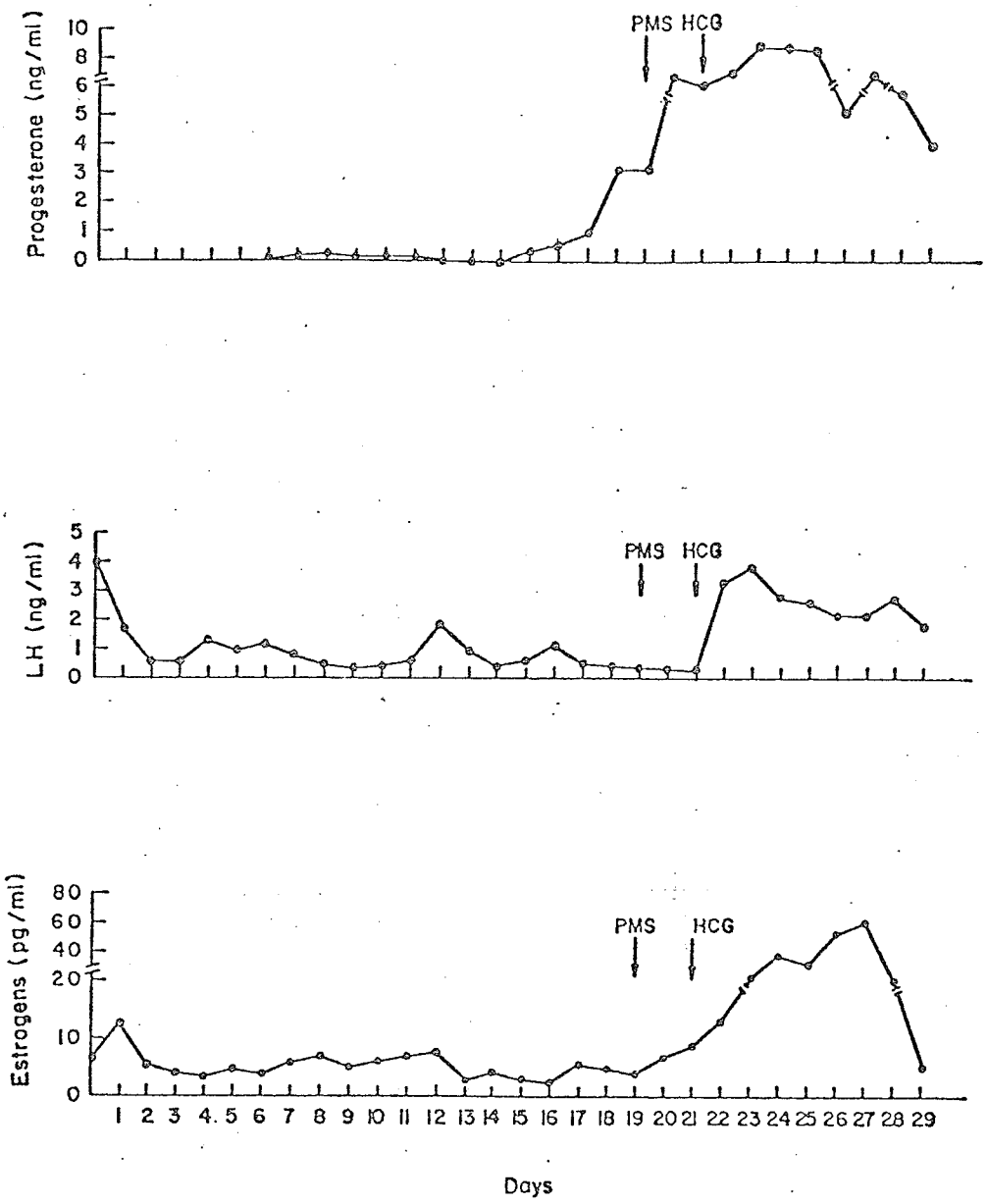


Figure 10. Hormone patterns in cow No. 17 following MGA, PMS and HCG treatment. Day 0 = the day after last MGA feeding.

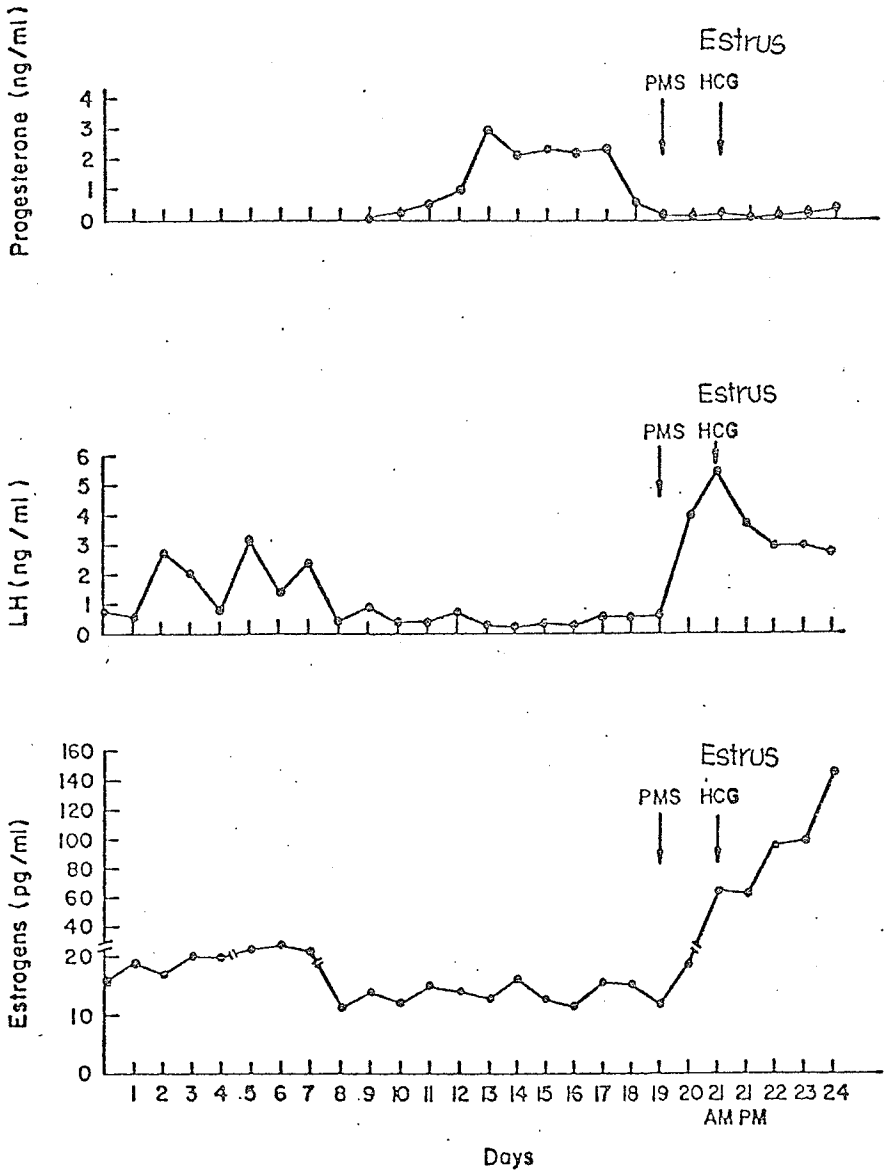


Figure 11. Hormone patterns in cow No. 19 following MGA, PMS and HCG treatment. Day 0 = the day after last MGA feeding (estrus was observed on day 21).

and constant for the same period of time. This occurred prior to the progesterone rise or probable ovulation (Fig. 11). After nine days LH returned to baseline levels until PMS injection. Estrogen declined somewhat but remained more or less constant at relatively high levels. PMS and HCG did not produce an immediate response in terms of progesterone secretion but effectively elevated LH and estrogen. Estrus was observed on the day of HCG injection at which time progesterone was low but LH and estrogen were high.

Cow No. 23 was observed in estrus on day 8 after MGA treatment which was followed by a rise in progesterone for 14 days (Fig. 12). Before the day of estrus high levels of LH and estrogen were observed, but both hormones returned to low levels during the luteal phase. The PMS injection was made on the day before progesterone declined to baseline or day 15 of the estrous cycle. Progesterone did not rise again until three days after HCG injection and it rose to more than two times that of the normal value observed in non-treated cows. Both LH and estrogen were elevated after PMS and HCG injection during which time progesterone was low, however, no estrus was observed.

Interval from calving to first estrus

The overall interval in treated cows was 74.2 days with an average of 74.8 days for group I and 73.7 days for group II. The average for the three non-treated cows was 122.3 days. The average interval from calving to first feeding of MGA was 35.8 days (Table 4).

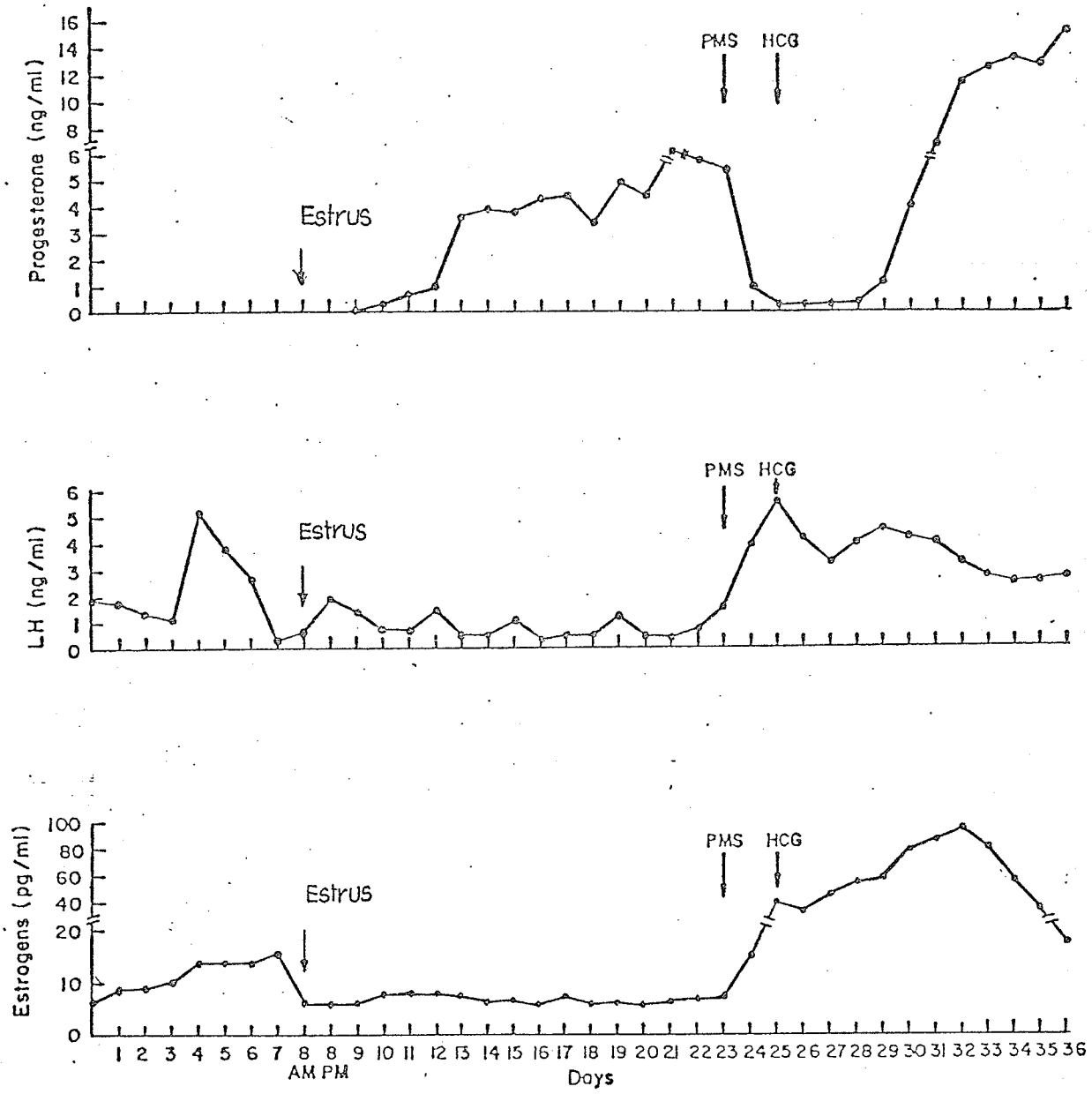


Figure 12. Hormone patterns in cow No. 23 following MGA, PMS and HCG treatment. Day 0 = the day after last MGA feeding (estrus was observed on Day 0).

Calving performance and gestation length

Only one treated cow (No. 3) produced twin calves, while 17 cows had single deliveries and 6 cows did not conceive (Table 12). According to gestation period calculated from first estrus after PMS or HCG treatment only three cows (No. 2, 3 and 23) appeared to conceive at this estrus (Table 12). However, cow No. 23 did not respond to PMS and HCG treatment and was not observed in heat until 26 days following injection.

TABLE 12. Gestation periods and calving performance of treated and control cows

Group	Animal No.	Treatment	No. of calves	Calving date (1973)	Calculated date of conception (1972)	Interval from estrous after treatment to conception (days)
I	1	MGA+PMS	0	-	-	-
	2		1	12 May	6 August	16
	3		2	25 April	20 July	0
	4		1	20 April	15 July	-5
	5		1	29 May	23 August	32
	6		1	14 June	8 September	92
	7		0	-	-	-
	8	MGA+PMS	1	2 July	26 September	44
	9		1	9 June	3 September	20
	10		1	13 June	7 September	24
	11		0	-	-	-
	12		0	-	-	-
II	13	MGA+PMS +HCG	1	30 May	24 August	21
	14		1	13 June	7 September	27
	15		0	-	-	-
	16		1	23 July	16 October	76
	17		1	13 June	7 September	35
	18		1	13 June	7 September	35
	19		0	-	-	-
	20	MGA+PMS +HCG	1	31 May	25 August	12
	21		1	12 June	6 September	34
	22		1	18 July	11 October	73
	*23		1	7 June	1 September	0
	24		1	24 May	18 August	15
Control	C1		1	19 April	14 July	?
	C2		1	8 June	2 September	3
	C3		0	-	-	-

*Cow did not respond to treatment until 26 days later.

DISCUSSION

Effect of exogenous hormones on estrus

Only 16.6 percent of the treated cows came into estrus within 13 days following MGA treatment in this study which is in contrast to the reports by Zimbelman (1963), Britt et al. (1972), Veenhuizen and Wagner (1974) and Darwah et al. (1965). The results are, however, in agreement with Tilton et al. (1966) and Foote et al. (1960b) who reported unsatisfactory results.

Review of the literature revealed that there is some disagreement on the effect of progesterone, relative to the interval from calving to first estrus. Boyd (1969), Foote et al. (1960a) and Hill et al. (1971) found no difference in the interval between treated and untreated animals while Fosgate et al. (1962) reported a delaying effect due to progesterone. The latter stated that progesterone treatment caused the interval from calving to first ovulation to be longer (10 days) and resulted in more frequent "quiet" ovulations before behavioral estrus. Boyd (1969) was able to inseminate first at 73 days after calving when treatment with MAP started at 43 days postpartum and similar results were obtained by Tilton et al. (1966), but treatment was started at 25 days after calving in the latter study.

The differing results are perhaps caused by variation in time after calving at which the progestogens were administered in

the various studies. Saiduddin et al. (1968) reported that treatment started at 18 to 23 days after parturition resulted in a shortened interval to conception. Brown et al. (1972) who used DHPA and gonadotrophin treatment, stated that when the treatment was initiated 5 to 19 days after calving it reduced the interval to first estrus, but when it began 40 to 45 days postpartum it prolonged the interval.

Differing compounds, dosages and routes of administration may also have contributed to the variation previously noted. Variation in environmental conditions and breed of animals used in experiments may also be contributing factors to the deviations in the results.

Seventy-nine percent of the cows responded to gonadotrophin treatment in the present study by showing estrus within 10 days after injection (averaging 5.6 days), which is in agreement with the results of Gordon et al. (1962) and Bellows and Short (1972). However, non-treated cows in the present study did not show overt estrus until an average of 122.3 days after calving which is much longer than that usually encountered. Tilton et al. (1966), Zimbelman (1963), Foote et al. (1960a and b), Foote (1962), Fosgate (1961), Hill et al. (1971), Wiltbank and Cook (1958), Boyd (1969) and Spahr et al. (1970) reported the interval from calving to first estrus (in both dairy or beef cows) ranged from 37 to 97.3 days. The long interval of first estrus in the non-treated

cows and the fact that estrus was observed within 10 days after being released to pasture emphasizes a possible confinement effect on behavioral estrus. Data on progesterone concentration from the non-treated cows suggested that they had undergone "quiet" ovulations.

This gives rise to the question of whether the return of estrus in the treated cows was due to gonadotrophin or to a change in environment. Dutt (1960) found that high ambient temperature delayed the onset of the breeding season in sheep. Stott and Williams (1962), Bond and McDowell (1972) and Gangwar et al. (1965) reported that high temperature also prolonged the estrus cycle in cattle. Neither the temperature in the barn nor outside in the pasture was recorded in this experiment, which was done during the summer months (June - August). However, the temperature recordings obtained from the Winnipeg Weather Reporting Station for the period of the study did not appear to be excessively high. Clearly, other factors are involved, including possible inadequacy of estrus detection while the animals were being maintained in confinement. Since the cows were transferred to pasture immediately following gonadotrophin treatment, a clear cut inference could not be made whether it was the effect of hormone treatment or a change in environment and the possibility of an interaction between these factors can not be overlooked.

Hormone values

The highest progesterone value in the non-treated cows during periods of high levels ranged from 4.7 to 6.7 ng/ml which is comparable to the levels reported by Donaldson et al. (1970), Hendricks et al. (1970), Robertson and Sarda (1971), Sprague et al. (1972), Swanson et al. (1972) and Wettemann et al. (1972) as determined by competitive protein binding (CPB) or by radio-immunoassay (RIA). Most of the early literature reviewed involved the use of the CPB technique to measure progesterone. Niswender (1973) reported comparable results when either RIA, CPB or double isotope derivative methods were compared. Progesterone levels remained below 1 ng/ml for 5 to 7 days in this study which is in agreement with that reported by Donaldson et al. (1970) and Robertson and Sarda (1971). However, Sprague et al. (1971) and Christensen et al. (1974) did not report levels lower than 1 ng/ml throughout the cycle.

Estrogen values obtained around the time of estrus in this study varied from a few pg/ml to almost two hundered pg/ml, as determined by RIA. Although estrous was not detected in the non-treated cows during the sampling period, the peaks seen during the low progesterone period (which are probably the result of increased follicular activity) varied from 10.28 to 27.39 pg/ml. These levels are comparable to those reported by Hendricks et al. (1971), Dobson et al. (1973) but are somewhat

higher than those of Wettemann et al. (1972). Christensen et al. (1971) reported that about 176 pg/ml was present on the day before estrus and 141 pg/ml was found on days 5 and 6. Values obtained by CPB, as reported by Shemesh et al. (1972), were higher than those obtained by RIA. He reported that 152 pg/ml was present a day before estrus, 34 pg/ml on the day of estrus and the lowest value of 9 pg/ml was seen on days 1 and 2 of the cycle. However, Christensen et al. (1974) reported higher values than those of Shemesh et al. (1972) employing the RIA technique (Table 1). Since estrogen has a shorter half-life than progesterone (usually less than a day) Vander Walt et al. (1972) and Shemesh et al. (1972), it is difficult to say which value is relatively closest to the actual peak. Most reports had high levels around estrus with some mid-cycle peaks which is similar to the results obtained in this study.

The same situation is encountered in the LH values. The very short half-life of LH makes it difficult to determine absolute peaks unless very frequent blood collections are made. The basal level of LH was less than 1 ng/ml in this study which is similar to the results obtained by Hendricks et al. (1970), Swanson and Hafs (1970), Hackett and Lyons (1971), Christensen et al. (1971), Gaverick et al. (1971). However, Niswender et al. (1968) and Carr (1972) obtained baseline values greater than 1 ng/ml. The peak values reported in the literature during estrus are extremely

variable, being dependent upon frequency of blood collection. Carr (1972) who collected blood at 30 min intervals reported highest values of 120 ng/ml, while twice daily collection by Snook et al. (1971) resulted in highest values of 50 ng/ml. The peak values obtained in this study with daily collection, ranged from 1.65 to 4.1 ng/ml. Mid-cycle peaks were observed, as reported by Hendricks et al. (1970).

Hormone Patterns

Although estrus was not detected, normal patterns of hormones in the non-treated cows were obtained by comparing values to those in the literature. Estrogen generally increased greatly following a sharp drop in progesterone levels and remained rather low during the period of high progesterone levels. The estrogen peak was of short duration (about 1 day), which was similar to the pattern previously reported by Hendricks et al. (1972), Guthrie et al. (1972), Shearer et al. (1972) in swine; by Scaramuzzi et al. (1970), Pant et al. (1972) and Yuthasastrakosol et al. (1974) in sheep and by Wettemann et al. (1972), Christensen et al. (1971), Hendricks et al. (1971), Echternkamp and Hansel (1971) and Shemesh et al. (1972) in cattle. A few secondary rises in estrogen level were also observed in this experiment, as was also seen by Hendricks et al. (1972) in swine, Scaramuzzi et al. (1970) in sheep; Hendricks et al. (1971) and Shemesh et al. (1972)

in cattle. The secondary peaks are usually reported to be lower than the peaks seen at estrus but in the present experiment the secondary peaks were seen to be higher in two out of the three non-treated cows which were studied. This might be the result of infrequent blood collection and the real absolute peak might have been missed.

The time and size of peak values for LH could not be determined in this study because of the short half life of LH as reported by Carr (1972) and Niswender et al. (1968). Nevertheless, the blood collection interval of 12 or 24 hr in this study indicated a tendency for LH to rise at approximately the same time as estrogen peaked. A tendency for LH to rise again was observed in all non-treated animals, with an interval of 4 to 5 days after the first peak.

Four of the six MGA-treated cows (no. 5, 8, 17 and 19) which were retained for blood collection gave an indication of ovulation or follicular luteinization, and hence CL activity, without behavioral estrus by having progesterone levels higher than baseline after MGA treatment and before PMS injection. Two of the four (Nos. 5 and 19) had progesterone levels which indicated a short life span of the CL, i.e., progesterone increased on day 9 and dropped on days 17 or 18. These two cows also did not readily respond to PMS treatment in terms of progesterone levels. The reason for the short duration of the CL is not clear. It is possible

that there was incomplete development of CL or only partial luteinization of the follicles. One cow (No. 23) was observed in heat and this was followed by increased progesterone levels which remained high for 14 days, while cow No. 1 did not have progesterone above its baseline until after PMS injection. In all cows progesterone was low for at least 9 days after MGA treatment. This has also been reported by Dobson et al. (1973) and Chow et al. (1972). High estrogen levels after MGA treatment were observed in all cows, the duration of these high values varying from one to eight days, followed then by a sharp drop. This indicate the presence of active follicles and four out of six animals ovulated or had luteinized follicles as indicated by a rise in progesterone which occurred a few days after estrogen had dropped.

LH and estrogen levels rose quickly following gonadotrophin treatment, regardless of the level of progesterone (or stage of CL activity) present at the time of injection. Gonadotrophin treatment might have stimulated developing follicles during the luteal phase to ovulate and produce progesterone in addition to the amount already present in the blood or it might have stimulated the already present CL to secrete more progesterone. Lamond and Gaddy (1972) have reported that there is a relationship between the number of CL and the quantity of progesterone in the blood.

In non-treated cows, estrogen levels were low during the luteal phase which was in contrast to the results obtained after

gonadotrophin treatment where estrogen increased even during high progesterone levels. This points out that ovarian tissue is responsive to PMS or HCG treatment at all times. Thus, the action of progesterone in preventing ovulation is probably an inhibition of LH release, rather than inducing insensitivity of the ovary to LH. This would agree with the site of progesterone action as being the pituitary or hypothalamus as reported by Schally et al. (1970). Kanematsu and Sawyer (1965), Kawakami and Sawyer (1961), Docke et al. (1968), Baker et al. (1973) and Labhsetwar and Bainbridge (1971) in the rat; by Exley et al. (1968), Hilliard et al. (1966), Spies et al. (1964) and Hilliard et al. (1971) in the rabbit and by Arimuro and Schally (1970) and Malven and Diaz (1971) in the guinea pig.

Progesterone tends to depress the release of LH from the pituitary and this is dependent somewhat upon the stage of cycle at which the progesterone is applied (Redmond, 1968). However, Ellington et al. (1963), Nellor and Cole (1957) and Labhsetwar et al. (1964) stated that progesterone had no effect on levels of LH and FSH in the pituitary gland. Kanematsu and Sawyer (1965) and Exley et al. (1968) reported that the site of progesterone action is on the hypothalamus in blocking ovulation or release of LH in the rat. On the other hand, Baker et al. (1973), Malven and Diaz (1971), Arimura and Schally (1970) and Hilliard (1971) favoured the pituitary as the site of progesterone action. Stevens et al. (1970) suggested that there is a direct action of progesterone on the ovary.

Many workers, Spies et al. (1969), Hilliard et al. (1966) and Dock et al. (1968), suggested both the pituitary and the hypothalamus as the site(s) of progesterone action. Labhsetwar (1971) concluded that progesterone may exert its action on all three regions, viz., the ovary, pituitary and hypothalamus. Injection of LH could partially overcome the progesterone blocking of LH release, Hilliard et al. (1966) and Redmond (1968). Also primming with estrogen could reduce the progesterone effect, Hilliard et al. (1966) and Spies et al. (1969), in the rabbit. In cattle the action of progesterone or the related steroids is probably not directly on the ovary to any great extent, since in this study cows No. 1, 7 and 8 showed a response to gonadotrophin treatment as indicated by high estrogen and LH even during periods of high progesterone levels.

Cow No. 23 (Group II) probably showed the most appropriate expected pattern of hormone levels according to treatment. High levels of LH and estrogen were seen in the blood shortly before estrus which occurred at 14 days after the end of MGA treatment. These subsequently were low while progesterone was maintained at high levels. Gonadotrophin treatment was coincidental with the time at which progesterone dropped and this stimulated the greatest levels of LH and estrogen found in the study. The ovulation response was probably the highest in this animal since the highest progesterone level recorded in the study (15.2 ng/ml) was seen on day 36, i.e., 11 days after gonadotrophin treatment (Fig. 12).

PMS injection at 15 days after estrus thus seemed to be the optimal time to produce the greatest response, which is in agreement with the findings of Gordon et al. (1962) and Turman et al. (1969), who also gave PMS on day 16 or 17 of the estrous cycle and obtained a satisfactory ovulatory response.

The maintenance of high estrogen and LH levels for several days after gonadotrophin treatment was noticed as compared to the normal cycles in non-treated cows in which the peak of both hormones usually lasted only a day, or less. Cow No. 17 did not show estrus after gonadotrophin treatment during the luteal phase as was observed in cow No. 1. It may be that there was not a high enough estrogen level in cow No. 17 to induce behavioral estrus. This to some extent supports the thoery that a proper ratio of progesterone and estrogen is necessary to induce estrus, Hilliard et al. (1966) and Spies et al. (1969).

Only one cow produced twin calves. This cow did not return to estrus after the one induced by PMS, while the majority of the cows had recurring estrous periods after PMS induced estrus and produced only single calves. This contributed to the low percentage of twins since conception occurred subsequent to any stimulatory effect. The irregularities in the cycle length and the frequent occurrence of split estrus after PMS or HCG treatment may have contributed to the low fertility rate at the first heat subsequent to gonadotrophin treatment. The appearnce of what might be considered

higher than "normal" values of LH and estrogens immediately after gonadotrophin injection would indicate possible hormonal imbalance at this estrus.

There did not appear to be any differences in hormonal response following PMS injection as compared to treatment with both PMS and HCG.

CONCLUSION

Synchronization of postpartum cows beginning 35 days after calving was not completely effective. The postpartum interval in control cows was longer than usual. Although a normal pattern in hormone levels was observed around 103 days after calving, animals did not consistently exhibit estrus until released to pasture from the dry-lot condition. This result suggests some environmental factor which affects the expression of behavioral estrus. Further studies are needed to fully investigate this point.

Gonadotrophin injections led to a quick response in terms of estrogen and LH secretion even when progesterone was high. This suggests progesterone action by inhibiting LH release is on the pituitary or brain rather than direct action on the ovary itself. Gonadotrophin treatment should be administered about 16 days after estrus to obtain a satisfactory response in terms of ovulation rate. Comparative responses, as effected by PMS or PMS and HCG, can not be made unless the treatment is made at the same stage of the estrous cycle; which is difficult to do in postpartum anestrus cows.

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APPENDIX

TABLE 1 Serum levels of progesterone, estrogens and LH in Cow No. C1

Day	Progesterone (ng/ml)		Estrogens (pg/ml)		LH (mg/ml)	
	$\bar{x}$	± S.D.	$\bar{x}$	± S.D.	$\bar{x}$	± S.D.
0	2.83	0.39	3.52	0.02	0.50	0.00
1	2.66	0.15	4.14	1.25	0.52	0.03
2	2.99	0.07	6.59	1.61	0.55	0.07
3	2.94	0.07	4.90	0.70	0.50	0.00
4	3.24	0.35	4.12	1.57	0.57	0.03
5	4.71	0.39	3.87	0.90	0.55	0.00
6	4.07	0.03	3.47	0.00	0.32	0.14
7	3.19	0.11	2.92	0.11	0.32	0.14
8	4.68	0.11	3.91	0.16	0.57	0.03
9	3.82	0.23	3.69	0.04	0.55	0.07
10	3.88	0.31	5.43	1.55	0.15	0.00
11	3.46	0.27	7.37	0.91	1.95	0.10
12	0.87	0.26	9.52	0.73	3.62	0.38
13	0.70	0.07	15.54	2.75	1.90	0.00
14	0.76	0.00	8.73	1.37	0.95	0.07
15	0.74	0.03	4.90	2.67	0.65	0.07
16	0.88	0.03	8.43	0.08	0.65	0.00
17	1.38	0.00	7.86	1.82	1.30	0.00
18	1.39	0.06	9.64	1.65	0.95	0.14
19	2.10	0.07	9.51	2.59	0.67	0.03
20	2.30	0.19	17.45	1.08	0.67	0.03
21	2.94	0.23	4.29	2.14	0.60	0.00
22	3.38	0.39	3.38	0 53	0.55	0.07
23	3.05	0.00	4.47	1.91	0.57	0.03

#Day 0 = start of blood collection (90 days post calving).

TABLE 2 Serum levels of progesterone, estrogens and LH in Cow No. C2

Day*	Progesterone (ng/ml)		Estrogens (pg/ml)		LH (ng/ml)	
	$\bar{x}$	± S.D.	$\bar{x}$	± S.D.	$\bar{x}$	± S.D.
0	5.10	0.47	10.19	0.16	0.60	0.07
1	5.43	0.00	9.78	0.33	1.40	0.10
2	6.54	0.00	10.66	2.10	0.72	0.03
3	4.77	0.00	6.57	0.65	0.60	0.07
4	6.49	0.39	5.30	0.37	0.52	0.03
5	6.74	0.35	8.44	0.61	0.60	0.07
6	5.55	0.00	5.58	0.77	0.57	0.03
7	4.60	0.00	9.99	1.01	0.65	0.07
8	0.32	0.00	13.46	0.54	1.52	0.10
9	0.25	0.09	14.63	1.66	1.65	0.00
10	0.16	0.22	4.63	2.21	0.60	0.07
11	0.21	0.00	5.60	1.67	0.57	0.03
12	0.51	0.11	7.11	1.93	0.65	0.00
13	1.22	0.15	6.52	1.42	0.65	0.00
14	1.55	0.23	10.73	0.20	0.65	0.00
15	3.08	0.11	6.48	1.33	2.10	0.14
16	5.27	0.70	8.05	0.14	0.32	0.14
17	4.02	0.35	13.54	0.21	0.55	0.00
18	4.63	0.51	4.87	1.73	0.57	0.03
19	5.32	0.78	6.41	0.02	0.55	0.07
20	5.35	0.03	12.03	1.50	2.10	0.14
21	4.88	0.15	3.30	0.30	0.32	0.14
22	4.66	0.07	3.84	0.75	3.07	0.24
23	5.74	0.27	7.69	0.40	0.57	0.03

*Day 0 = start of blood collection (89 days post calving).

TABLE 3 Serum levels of progesterone, estrogens and LH in Cow No. C3

Day*	Progesterone (ng/ml)		Estrogens (pg/ml)		LH (ng/ml)	
	$\bar{x}$	± S.D.	$\bar{x}$	± S.D.	$\bar{x}$	± S.D.
0	1.22	0.01	10.35	0.36	0.65	0.07
1	1.22	0.02	10.71	0.25	0.32	0.24
2	3.36	0.34	12.47	0.06	0.50	0.00
3	3.00	0.08	11.85	0.40	0.57	0.03
4	3.92	0.62	10.48	0.82	0.75	0.07
5	5.57	0.04	9.83	0.91	0.85	0.07
6	3.48	0.00	5.65	0.22	0.52	0.03
7	4.06	0.21	5.40	0.16	0.57	0.03
8	4.66	0.08	5.64	0.69	0.55	0.00
9	4.23	0.14	6.25	0.60	0.60	0.00
10	4.82	0.04	6.29	0.74	0.60	0.00
11	3.79	0.12	5.92	2.75	0.62	0.03
12	2.51	0.05	5.97	1.03	0.65	0.00
13	0.42	0.00	8.99	2.24	1.72	0.31
14	0.42	0.03	10.18	1.75	1.20	0.07
15	0.34	0.04	3.88	0.53	0.67	0.03
16	0.52	0.05	2.54	1.14	0.70	0.00
17	0.26	0.01	5.49	0.13	1.77	0.03
18	0.58	0.00	27.39	0.09	0.70	0.00
19	0.71	0.02	4.50	1.34	0.70	0.14
20	1.16	0.04	20.00	5.11	0.57	0.03
21	2.74	0.12	7.47	3.18	0.55	0.07
22	4.19	0.20	2.74	1.12	0.52	0.03
23	4.08	0.05	3.50	0.00	2.22	0.24

*Day 0 = start of blood collection (88 days post calving).

TABLE 4 Serum levels of progesterone, estrogens and LH in Cow No. 1

Day**	Progesterone (ng/ml)		Estrogens (pg/ml)		LH (ng/ml)	
	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
0	0.24	0.04	8.29	1.03	0.37	0.17
1	0.29	0.07	13.85	2.34	1.05	0.07
2	0.42	0.20	7.49	1.06	1.77	0.10
3	0.40	0.00	7.71	0.16	1.15	0.07
4	0.31	0.07	5.40	0.92	1.25	0.14
5	0.27	0.00	6.90	3.02	0.87	0.03
6	0.15	0.01	2.50	0.53	0.52	0.10
7	0.14	0.02	3.90	0.81	0.45	0.00
8	0.34	0.00	3.56	0.34	0.60	0.00
9	0.26	0.02	3.97	0.28	0.57	0.03
10	0.22	0.01	4.53	1.39	1.45	0.07
11	0.20	0.01	8.10	0.58	1.10	0.07
12	0.17	0.01	4.72	2.16	0.70	0.14
13	0.23	0.01	7.11	0.28	0.57	0.03
14	0.26	0.02	8.91	2.45	0.60	0.00
15	0.14	0.02	9.45	0.04	0.55	0.00
16	0.19	0.00	11.38	0.60	0.72	0.03
17	0.21	0.00	16.83	1.53	0.70	0.00
18	0.21	0.02	7.78	0.21	1.20	0.07
19	0.27	0.02	6.61	1.42	0.60	0.00
20	0.23	0.02	11.83	2.02	2.95	0.21
21	0.73	0.03	32.28	4.70	2.75	0.07
22	1.23	0.11	70.34	2.75	3.05	0.07
23	2.33	0.14	101.16	3.30	2.60	0.17
24	3.88	0.57	112.80	0.83	2.27	0.03
25 A.M.	4.21	0.11	150.13	4.29	3.20	0.56
25 P.M.	3.15	0.00	147.09	23.24	3.10	0.56
26	4.29	0.00	184.83	10.54	2.10	0.06
27	2.99	0.00	133.47	7.57	2.20	0.14
28	2.39	0.00	181.02	16.66	2.25	0.35
29	1.52	0.03	59.89	1.52	1.82	0.10
30	2.34	0.40	23.06	0.40	1.67	0.10
31	6.07	0.00	13.20	1.57	1.57	0.24
32	1.09	0.31	9.87	3.32	1.15	0.00
33	0.64	0.16	7.84	0.45	0.95	0.07

**Day 0 = day after last MGA feeding

TABLE 5 Serum levels of progesterone, estrogens and LH in Cow No. 5

Day**	Progesterone (ng/ml)		Estrogens (pg/ml)		LH (ng/ml)	
	$\bar{x}$	± S.D.	$\bar{x}$	± S.D.	$\bar{x}$	± S.D.
0	0.15	0.01	16.36	0.14	2.05	0.07
1	0.23	0.00	24.05	2.86	0.55	0.07
2	<.1	----	18.33	1.26	0.55	0.07
3	<.1	----	20.8	0.05	0.65	0.07
4	<.1	----	24.67	1.56	0.55	0.07
5	0.12	0.02	21.64	2.22	0.70	0.28
6	<.1	----	19.24	2.52	0.47	0.03
7	0.10	0.01	17.91	1.62	0.75	0.15
8	0.14	0.01	8.77	0.38	0.65	0.07
9	0.14	0.06	6.27	0.74	0.50	0.00
10	0.73	0.06	7.97	1.34	0.27	0.03
11	1.28	0.41	3.89	0.50	0.37	0.10
12	1.82	0.06	3.86	0.31	0.15	0.00
13	2.81	0.00	2.44	0.86	0.40	0.14
14	2.48	0.00	<2	----	0.15	0.00
15	2.93	0.17	<2	----	0.20	0.07
16	3.09	0.29	<2	----	0.55	0.07
17	0.55	0.06	<2	----	1.35	0.07
18	0.16	0.05	<2	----	1.00	0.00
19	0.11	0.02	<2	----	0.77	0.03
20	0.13	0.00	3.95	0.26	2.95	0.21
21	<.1	----	4.83	1.49	3.55	0.07
22	<.1	----	3.65	0.56	3.75	0.35
23	0.21	0.01	3.69	0.14	2.90	0.28
24	0.18	0.02	13.05	2.05	2.92	0.24
25 A.M.	0.67	0.00	5.90	0.54	4.10	0.00
25 P.M.	0.82	0.00	8.41	0.11	3.45	0.91
26	1.26	0.03	6.27	0.37	2.90	0.28
27	3.72	0.23	7.34	0.23	2.95	0.21
28	5.78	0.23	9.06	0.02	2.80	0.00

**Day 0 = day after last MGA feeding

TABLE 6 Serum levels of progesterone, estrogen and LH in Cow No. 8

Day**	Progesterone (ng/ml)		Estrogens (pg/ml)		LH (ng/ml)	
	$\bar{x}$	± S.D.	$\bar{x}$	± S.D.	$\bar{x}$	± S.D.
0	<.1	----	8.16	2.71	0.70	0.07
1	<.1	----	9.93	0.82	0.97	0.17
2	<.1	----	13.67	0.15	1.60	0.63
3	<.1	----	13.95	0.48	1.65	0.07
4	<.1	----	24.11	0.69	0.72	0.03
5	<.1	----	23.91	0.72	0.87	0.03
6	<.1	----	24.83	0.48	1.30	0.14
7	0.38	0.54	29.44	0.77	0.60	0.00
8	0.12	0.17	5.86	1.97	0.55	0.07
9	0.58	0.14	8.15	1.31	0.72	0.03
10	1.88	0.23	15.18	0.75	0.32	0.24
11	1.51	0.17	9.64	0.29	0.52	0.03
12	3.05	0.23	6.81	0.23	0.35	0.28
13	3.38	0.00	5.76	0.02	0.32	0.24
14	4.93	0.23	12.83	0.17	0.55	0.00
15	4.10	0.23	21.54	2.61	0.50	0.00
16	5.60	0.86	12.26	0.04	0.32	0.24
17	4.88	0.62	9.97	0.00	0.27	0.31
18	6.90	0.58	7.94	1.20	<.05	0.00
19	5.38	0.07	11.85	2.22	0.52	0.03
20	8.50	0.49	18.41	0.01	2.80	0.00
21	9.45	0.36	43.31	1.97	3.23	0.31
22	11.62	0.73	42.13	3.88	3.00	0.14
23	6.21	0.00	85.18	1.41	2.80	0.00
24	1.09	0.25	125.00	1.41	2.42	0.17
25 A.M.	0.80	0.14	120.00	5.65	4.60	0.00
25 P.M.	0.64	0.17	20.85	2.16	2.30	0.00
26	0.33	0.03	10.92	0.15	2.12	0.10
27	0.60	0.03	7.83	0.65	1.57	0.17
28	4.35	0.11	14.81	2.58	1.55	0.00
29	5.24	0.05	6.93	0.84	1.50	0.21

**Day 0 = day after last MGA feeding

TABLE 7 Serum levels of progesterone, estrogens and LH in Cow No. 17

Day**	Progesterone (ng/ml)		Estrogens (pg/ml)		LH (ng/ml)	
	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
0	<.1	----	6.42	2.49	4.05	0.77
1	<.1	----	12.45	2.22	1.75	0.35
2	<.1	----	5.62	1.95	0.60	0.00
3	<.1	----	4.30	0.00	0.60	0.00
4	<.1	----	3.42	0.28	1.35	0.07
5	<.1	----	4.61	0.99	1.02	0.03
6	<.1	----	4.07	2.14	1.20	0.07
7	0.17	0.03	5.78	0.22	0.85	0.07
8	0.22	0.01	6.88	0.84	0.50	0.00
9	0.16	0.02	5.06	0.11	0.42	0.03
10	0.15	0.01	6.12	0.61	0.45	0.00
11	0.10	0.01	7.03	0.57	0.65	0.07
12	.1	----	7.26	0.68	1.92	0.10
13	.1	----	3.00	0.88	1.02	0.03
14	.1	----	4.21	0.92	0.50	0.00
15	0.41	0.02	3.02	0.21	0.65	0.07
16	0.70	0.02	3.72	0.13	1.15	0.14
17	1.05	0.01	5.55	1.13	0.55	0.07
18	3.15	0.21	4.82	0.15	0.45	0.00
19	3.20	0.00	4.37	0.48	0.42	0.03
20	6.80	0.00	7.22	0.14	0.37	0.03
21	6.35	0.21	9.12	0.16	0.35	0.00
22	7.00	0.28	13.89	0.43	3.40	0.82
23	8.80	0.56	22.88	0.51	3.87	0.17
24	8.75	0.07	37.45	6.73	2.82	0.38
25	8.55	0.21	33.4	1.78	2.67	0.17
26	5.20	0.00	54.23	7.74	2.22	0.31
27	6.90	0.42	61.29	2.13	2.15	0.21
28	5.80	0.54	21.10	1.06	2.75	0.07
29	4.02	0.82	5.37	1.32	1.85	0.00

**Day 0 = day after last MGA feeding

TABLE 8 Serum levels of progesterone, estrogens and LH in Cow No. 19

Day**	Progesterone (ng/ml) $\bar{x}$ ± S.D.		Estrogens (pg/ml) $\bar{x}$ ± S.D.		LH (ng/ml) $\bar{x}$ ± S.D.	
0	<.1	----	16.00	1.75	0.70	0.00
1	0.31	0.02	18.93	1.24	0.55	0.00
2	<.1	----	16.98	0.92	2.72	0.03
3	<.1	----	20.53	1.02	2.10	0.00
4	<.1	----	19.93	1.23	0.80	0.00
5	<.1	----	25.13	1.86	3.25	0.00
6	<.1	----	28.86	3.72	1.40	0.00
7	<.1	----	23.90	1.90	2.42	0.17
8	<.1	----	11.53	1.96	0.45	0.00
9	<.1	----	14.73	0.69	0.90	0.14
10	0.27	0.00	12.13	0.55	0.50	0.00
11	0.59	0.00	15.10	0.73	0.50	0.00
12	0.97	0.09	14.04	1.35	0.75	0.07
13	3.05	0.15	12.96	0.16	0.37	0.10
14	2.31	0.00	16.31	2.19	0.22	0.10
15	2.44	0.01	12.95	1.20	0.37	0.10
16	2.38	0.10	11.96	0.57	0.30	0.00
17	2.43	0.10	15.66	0.70	0.60	0.00
18	0.62	0.00	15.42	2.26	0.55	0.07
19	0.27	0.01	12.34	0.16	0.70	0.14
20	0.17	0.01	19.09	1.90	4.00	0.00
21 A.M.	0.20	0.02	66.14	2.17	5.50	0.00
21 P.M.	0.16	0.02	65.65	0.33	3.75	0.35
22	0.17	0.02	97.12	2.38	3.00	0.00
23	0.26	0.00	100.00	1.06	3.00	0.00
24	0.37	0.00	147.175	8.80	2.80	0.28

**Day 0 = day after last MGA feeding

TABLE 9 Serum levels of progesterone, estrogens and LH in Cow No. 23

Day**	Progesterone (ng/ml)		Estrogens (pg/ml)		LH (ng/ml)	
	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
0	<.1	----	6.77	0.88	1.90	0.14
1	<.1	----	8.87	0.49	1.75	0.28
2	<.1	----	9.05	0.33	1.35	0.07
3	<.1	----	10.39	1.17	1.15	0.07
4	<.1	----	13.94	0.26	5.20	0.49
5	<.1	----	13.96	0.86	3.78	0.35
6	<.1	----	13.88	0.96	2.70	0.14
7	<.1	----	15.68	1.44	0.31	0.00
8 A.M.	<.1	----	6.11	0.03	0.60	0.00
8 P.M.	<.1	----	5.90	0.14	1.90	0.00
9	<.1	----	6.24	0.58	1.42	0.17
10	0.25	0.01	8.04	2.05	0.77	0.03
11	0.72	0.07	8.23	0.12	0.77	0.10
12	1.05	0.07	7.93	0.98	1.50	0.00
13	3.73	0.28	7.70	0.93	0.52	0.03
14	4.03	0.19	6.51	1.01	0.55	0.00
15	3.94	0.00	7.00	0.16	1.07	0.03
16	4.41	0.24	6.31	0.46	0.32	0.24
17	4.44	0.38	7.71	1.15	0.52	0.03
18	3.45	0.53	6.46	0.84	0.52	0.03
19	5.05	0.19	6.79	0.25	1.27	0.17
20	4.54	0.33	6.44	0.32	0.52	0.03
21	6.14	0.14	6.87	0.50	0.35	0.28
22	5.88	0.24	7.68	0.29	0.77	0.17
23	5.53	0.10	7.74	0.28	1.50	0.28
24	1.10	0.14	15.85	0.78	3.95	0.07
25	0.36	0.01	42.28	4.14	5.55	0.00
26	0.39	0.02	36.62	5.29	4.20	0.42
27	0.37	0.00	48.03	1.61	3.25	0.35
28	0.41	0.01	58.49	7.31	4.00	0.00
29	1.33	0.08	60.69	5.26	4.57	0.03
30	4.15	0.45	82.41	0.81	4.25	0.35
31	6.48	0.23	89.03	3.81	4.00	0.00
32	11.67	0.60	98.08	5.29	3.25	0.70
33	12.61	0.98	83.61	0.79	2.75	0.35
34	13.29	0.48	58.89	2.79	2.52	0.31
35	12.73	0.16	38.27	4.21	2.52	0.31
36	15.23	1.11	17.94	2.43	2.75	0.00

**Day 0 = day after last MGA feeding