ENDOCRINE AND REPRODUCTIVE RESPONSE OF THE BOVINE TO PREGNANT MARE SERUM GONADOTROPHIN (PMSG)

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CHAPTER I

INTRODUCTION

Ranchers and stockmen are continually faced with the problem of maximizing efficiency in their operations. One area that offers great promise as a means of accomplishing this is that of increasing the reproductive efficiency of the beef cow. Improved feeding, breeding, and management techniques have made a definite contribution to increasing the number of live calves at birth and weaning. However, even with all the advances made in the livestock industry in the recent years, the producer can still only expect, at best, little more than a 90% calf crop. For this reason, much research effort has been devoted in recent years to trying to increase calf numbers per cow unit through the use of exogenous gonadotrophins to induce multiple births.

One of the primary hormones that has been used in such studies is pregnant mare serum gonadotrophin. Many of these experiments have been quite successful in inducing superovulation and superfetation, in many cases too successful in that the cows give birth to too many calves or ovulate so many eggs that they do not become pregnant, or the resulting pregnancy cannot be maintained. While it is true that other problems have also been implicated in the multiple birth studies, such as retained placentas, lowered birth weights, reduced fertility and freemartinism in the heifer calves, uncontrolled superovulation still ranks as one of the most important. If these problems could be solved, the induction of

multiple births would not only prove beneficial to the commercial beef operator but might also be a great boon to the purebred producer in that he could conceivably progeny test his females.

In addition, knowledge of the endocrine changes occurring during the reproductive cycle is essential if maximum progress in reproductive efficiency is to be made. In this regard, little data are available concerning hormonal fluctuations after PMSG treatment. This study was, therefore, primarily concerned with investigating the endocrine response of bovine female when PMSG is administered either during the late luteal phase or both during the early luteal and late luteal phases of the estrous cycle. Such information might yield clues to the relationships between plasma hormone concentrations after PMSG and subsequent ovulation rates and fertility. These data might then demonstrate which endocrine responses should be controlled in order to optimize the superovulatory response.

CHAPTER II

LITERATURE REVIEW

Introduction

The induction of multiple births in beef cattle has been the subject of a number of research studies since Casida et al. (1943) reported that exogenous gonadotrophic hormones could be used to induce superovulation in the bovine. Cole and Hart (1930) first described gonadotrophic activity in the serum of pregnant mares, and pregnant mares' serum gonadotrophin (PMSG) has since become the most commonly used hormone for inducing superovulation and superfetation in beef cattle. Many reviews (Hafez et al., 1965; Bellows and Short, 1972; Laster, 1971; Johnson, 1972) are available concerning the topic of multiple births in the bovine.

Most investigators have employed a single injection of gonadotrophin given in the late luteal phase (day 16-18) of the cycle. However, Schilling and Holm (1963) suggested that a series of two injections gave better control of the superovulatory response. They reasoned that 1500 I.U. PMSG given on day five of the estrous cycle might prevent atresia of all but one of the early developing follicles and the second injection given later in the cycle would cause these follicles to mature rather than resulting in uncontrolled follicular growth. Using this treatment schedule, these investigators reported that 70% of the treated cows had the desired two or three ovulations.

This report by Schilling and Holm (1963) stimulated a series of investigations at the Fort Reno Research Station to determine the effectiveness of two PMSG injections in inducing multiple births in beef cows. Turman et al. (1971) reported 23 multiple births from 52 cows conceiving at the first estrus following the second PMSG injection. Similarly, Laster et al. (1971) and Johnson et al. (1975) have shown that multiple births may be induced from a synchronized estrus.

The results obtained in these previous studies stimulated the present trial which was designed to examine the endocrine response of the beef female to injections of PMSG. However, since very little information is available concerning the endocrine status of the cow after she has been treated with PMSG, this review will also be concerned with the hormone levels found in the nonpregnant normal cow during the estrous cycle.

Bovine Estrous Cycle

The bovine estrous cycle may be divided into four basic periods; namely, proestrus, estrus, metestrus and diestrus. Proestrus (the follicular phase) is characterized by rapid growth of the follicle and usually lasts two or three days. Proestrus is followed by the period of estrus, the time in which the female is sexually receptive to the male and mating occurs. The period of estrus usually lasts only a short time averaging approximately 17 hours in the cow and slightly less in the heifer (Trimberger, 1948). In the cow, ovulation occurs and the corpus luteum begins to form during metestrus. The uterus prepares for implantation and is primarily under the influence of progesterone during diestrus (the luteal phase). The entire estrous cycle usually spans about 21 days in

the cow, while the cycle of the heifer may be slightly shorter (Desjardins and Hafs, 1968; Swanson et al., 1972).

Endocrine Status During the Estrous Cycle

The development of specific and sensitive radioimmunoassays for the protein and steroid hormones has greatly stimulated the study of endocrine changes associated with the bovine estrous cycle.

Follicle-Stimulating Hormone (FSH)

Follicle-stimulating hormone is a glycoprotein with a molecular weight of approximately 30,000. The carbohydrate content of the molecule amounts to 27% of the FSH molecule and sialic acid comprises 5% of its weight. The carbohydrate moiety seems to be essential for the biological activity. FSH is produced by the basophilic staining cells of the anterior pituitary gland and its release is probably mediated through hypothalamic releasing factors. The activity of FSH in the female is to promote growth and development of follicles in the ovary. In addition, it will also stimulate increased oxygen uptake and protein synthesis in the theca cells (Baird, 1972).

Early investigations into the functioning of FSH dealt primarily with pituitary levels of the hormone since no assay was available for quantifying it in the peripheral circulation. The greatest fluctuation in pituitary FSH occurs near the time of estrus. Rahka and Robertson (1965), using the ovarian weight augmentation method of Steelman and Pohley (1953), found that the pituitary content of FSH decreased approximately 27% between the beginning and the end of estrus in the cow. These results were substantiated by Desjardins and Hafs (1968). In

addition, Hackett and Hafs (1969) showed a 49% decrease in pituitary FSH between days 18 and 20 of the cycle. More recently Akbar et al. (1974) have reported a specific radioimmunoassay for bovine FSH using rabbit anti-serum to ovine FSH and radioiodinated human FSH. These workers reported that serum FSH values for cycling heifers tended to be higher on the day of estrus but was not significantly different from levels observed during other stages of the cycle when samples were drawn at daily intervals. However, when samples were collected at two hour intervals, the highest level of FSH coincided with the LH peak and was significantly different from the levels measured before or after the LH peak.

Luteinizing Hormone (LH)

As was the case with FSH, luteinizing hormone is a glycoprotein molecule containing less sialic acid than that reported for FSH. LH is also produced by the basophilic staining cells of the anterior lobe of the pituitary gland. LH activity in the female involves several distinct actions including the stimulation of steroid synthesis from all cell types in the ovary. Hoffman et al. (1974) have suggested that LH may be the primary luteotrophic factor in the bovine species. LH also promotes a rapid and large increase in ovarian blood flow probably increasing the supply of the critical metabolites needed by the ovary for its proper functioning. Also, if follicles have been properly primed with FSH, LH will induce ovulation (Baird, 1972).

The primary activity of LH is focused around the time of estrus but occurs about two days after the primary effects of FSH on the ovary have been exhibited. Rahka and Robertson (1965), Anderson and McShan (1966) and Desjardins and Hafs (1968) observed decreased pituitary LH during

estrus which suggested an elevation in plasma LH. Hackett and Hafs (1969), using the ovarian ascorbic acid depletion (OAAD) method of Parlow (1961), demonstrated a rapid reduction in pituitary LH around estrus which coincided closely with increased levels of LH in the blood reported by Hansel and Snook (1970) and Swanson and Hafs (1971). Since the advent of a specific radioimmunoassay for LH (Niswender et al., 1969), several studies have been reported concerning the amounts of LH in the blood of cows during the estrous cycle. Notably among these, Swanson and Hafs (1971) conducted an extensive trial whereby they evaluated LH and prolactin in blood serum from estrus to ovulation in Holstein heifers. Their work showed that serum LH began a continuous but nonsignificant rise five days before estrus which was followed by a highly significant peak of LH. The peak preceeded the onset of standing estrus by about three hours. LH remained elevated for six to eight hours, and ovulation occurred 32 hours after the LH peak. They also demonstrated that serum LH returned to pre-estrual levels within four to six hours after the peak. Similar findings for LH values around the time of estrus have been reported by Hendricks et al. (1970) and Akbar et al. (1974). Similarly, Lemon et al. (1975) reported that plasma LH remained at low levels (2 to 4 ng/ml) until it peaked at 30 to 35 ng/ml before decreasing again to basal levels over a 10 to 12 hour period. The LH peak was closely synchronized with the onset of sexual receptivity.

Prolactin

Prolactin is a single polypeptide chain containing about 211 amino acid residues and having a molecular weight of approximately 24,000 (Baird, 1972). It is generally considered to be produced by the

acidophilic staining cells of the anterior pituitary, and its role in reproductive physiology remains obscure. Prolactin has been implicated along with ACTH, growth hormone, estrogen and progesterone in the initiation and maintenance of milk secretion. It has also been suggested that prolactin is involved in the maintenance of the corpus luteum in some species, notably the rat; however, Hoffman et al. (1974) have suggested that prolactin may not serve this function in the bovine. Highly variable levels of prolactin have been reported during the bovine estrous cycle. Sinha and Tucker (1969), using the pigeon crop sac "micro" method of Reece and Turner (1937), noted a significant decrease in pituitary prolactin from estrus until two days later. Swanson and Hafs (1971) reported that serum prolactin began a sustained rise during the four days preceding estrus and then decreased. They noted, however, that due to the large variation between heifers, this increase only approached significance. This simultaneous decrease in prolactin in the serum and the pituitary would seem to indicate that both synthesis and release of prolactin must be affected during and immediately following estrus (Swanson and Hafs, 1971). In addition, these same workers noted that both LH and prolactin increased during the morning and afternoon and were lower at noon and during the night suggesting a diurnal change in these two hormones.

Progesterone

Progesterone is a steroid hormone produced in large amounts by the corpus luteum and in smaller quantities by the granulosa cells of the ovarian follicle before ovulation and by the adrenals. Progesterone is generally considered to be of prime importance in the maintenance of

pregnancy. Early studies on this steroid hormone dealt primarily with examining the corpora lutea of cycling cows as evidenced by the work of Mares et al. (1962) in which they reported a significant increase in the corpus luteum concentrations of progesterone from day seven to day 15 which was followed by a significant drop at day 17 of the estrous cycle. These workers assayed progesterone according to the method of Loy et al. (1960) involving column and paper chromatography followed by ultra-violet spectrophotometry. In an extensive review, Gomes and Erb (1965) noted that luteal progesterone was lowest at estrus and gradually increased to peak levels at days 14 to 15. The concentration of progesterone in the ovarian vein followed the same pattern (Gomes et al., 1963). Similar results were also reported for progesterone in the peripheral blood by Plotka et al. (1967), Stabenfeldt et al. (1969), and Sprague et al. (1971). In addition, Swanson et al. (1972) and Lemon et al. (1975) reported that the peak level of progesterone in heifers (8 to 10 ng/ml) occurred three days prior to estrus; while at the time of estrus, progesterone ranged from undetectable to 1.0 ng/ml. These results compare favorably with those reported by Kazama and Hansel (1970), Wettemann et al. (1972), and Glencross et al. (1973).

Estrogen

Estrogen is a collective term used to describe a class of steroid hormones which includes estradiol, estrone and estriol. This group of hormones is generally considered to have profound effects on the psychological manifestations of heat as well as the development of secondary sex characteristics in the female. Estrogens can be synthesized by several tissues including the ovary, adrenal and placenta with the

primary source being the ovary during the bovine estrous cycle. Short (1962) demonstrated that estradiol 17B was the major estrogenic steroid in the follicular fluid of the cow. He also noted that estrone was present but in much smaller amounts; however, estriol has not been found in the bovine ovaries (Mellin and Erb, 1965) but may be found in the plasma and urine and is thought to be an oxidation product (Kutsky, 1973). Short (1962) also noted that higher levels of estradiol-17B were found in preovulatory follicles than in follicles at other stages of the estrous cycle. In addition, England et al. (1973) have reported the presence of estradiol 17α in some bovine follicles.

Early studies on estrogens were largely concerned with urinary concentrations since adequate laboratory techniques were not available to evaluate serum levels. Estrogens do not appear to be stored in the body but rather are excreted as metabolites with a major route of excretion being the feces (Mellin and Erb, 1965). Since the feces:urine excretory ratio seemed to be quite constant, most investigators measured urinary levels. However, since the advent of radioimmunoassay, several investigators have reported estrogen levels in bovine plasma throughout the estrous cycle. In a rather extensive study, Wettemann et al. (1972) evaluated the estradiol content in the plasma of Holstein heifers. They found that estradiol was lowest on the second day of the cycle and remained at this low level through day 11. Estradiol then increased slightly three days before estrus and continued to rise to 9.7 pg/ml 0.5 days before estrus. Estradiol remained high on the day of estrus and then declined to baseline levels. Similar findings were reported by Echternkamp and Hansel (1973) who noted that the estradiol concentration began to rise two to three days prior to estrus but declined rapidly

after estrus. They also noted that changes in plasma estrone levels paralleled those for estradiol, while Glencross et al. (1973) suggested that estrone levels were much lower and showed less variation than estradiol. Christensen et al. (1971) reported similar trends for total estrogens but their values were much higher. Further, Wettemann et al. (1972) noted that elevated estradiol preceded the ovulatory LH peak by one or two days suggesting that estrogen secretion may regulate LH release in cattle. These data were again substantiated by the findings of Christensen et al. (1971). In addition, Echternkamp and Hansel (1973) have suggested that regression of the corpus luteum appears to be essential for maximal estradiol secretion prior to estrus, since estradiol levels rise only after progesterone values decline. Finally Christensen et al. (1971), Glencross et al. (1973) and Dobson and Dean (1974) observed not only increased estradiol one day before estrus but a second increase six days after estrus which is consistent with the suggestion of Rajakoski (1960) concerning midcycle follicular growth in some cows.

Endocrine Status After PMGS

Pregnant mare serum gonadotrophin is a glycoprotein molecule with a molecular weight of approximately 70,000 (Turner, 1966). Potencies of different sources of PMSG are routinely determined by using the bioassay method of Cole and Erway (1941) whereby ovarian weights from immature rats treated with the unknown substance are compared to weights obtained from animals treated with an International PMSG Standard. Cole and Hart (1930) reported that PMSG first appears in the blood of mares between the thirty-seventh and the forty-second day of pregnancy and reaches peak concentrations between the fiftieth and seventieth days of gestation. It

can be extracted from the blood and endometrial cups of the pregnant mare and has properties of both FSH and LH, being primarily FSH in activity. Turner (1966) reported that PMSG cannot be separated into its two entities; thus, any discussion of its hormonal effects must relate to its dual capabilities. In addition, PMSG remains in the blood and lymph and is practically absent from the urine giving rise to its more prolonged effect than that seen from human chorionic gonadotrophin (HCG) or purified extracts of either FSH or LH (Turner, 1966). Onuma et al. (1969) suggest that PMSG may be more effective than FSH in eliciting a superovulatory response in the calf due to the tough follicular walls noted after FSH treatment which may possibly be caused by insufficient LH in the FSH preparation.

Superovulation of the Prepuberal Female

There are many potential benefits to be gained from superovulation of prepuberal calves. These would include the genetic benefits from ovum transfer to reduce the generation interval and progeny testing of females. Casida et al. (1943) showed that superovulation in calves can be induced by hormone treatment. A recent report by Onuma et al. (1970) has summarized much of the current work in this area.

To dramatize the efficiency with which prepuberal calves can be induced to ovulate using PMSG, one need only to examine the work of Seidel et al. (1971). These investigators found that treatment of calves with 1500 I.U. PMSG followed by 50 mg of LH five days later produced an average of 0, 9.4, and 28.2 ovulations in calves 0, 1, and 2 months old. By five months of age, 1500 to 2000 I.U. PMSG and 75 mg of LH produced 77.2 ovulations per calf. In a similar study using Holstein calves from

9 to 11 weeks of age, Onuma et al. (1969) found that treatment with FSH resulted in 3.8 ovulations while 15.4 ovulations were observed after PMSG injections. These workers also noted that follicular and ovulatory response appeared to decline with repeated administration of gonadotrophin suggesting the development of a refractoriness to the hormones.

However, there is only limited data available regarding endocrine changes in the calf after PMSG. Spilman et al. (1973) injected 47 Holstein calves from two to five months of age with 1250-2000 I.U. PMSG followed five days later by 75mg LH. They then measured pituitary LH by the ovarian ascorbic acid depletion test and plasma LH by radioimmunoassay (RIA), with no cross reactivity being found between PMSG and the LH antibody used in the RIA. Plasma and luteal progesterone were quantified using thin layer and gas liquid chromatography. After superovulation, pituitary LH values decreased significantly compared to control values and remained low. Plasma LH values rose sharply on the day after PMSG injection and declined gradually to pretreatment levels. The suggestion was made that this rise in LH reflects endogenous release of LH following estrogen secretion by PMSG-stimulated follicles. However, these workers did not measure estrogen levels in these animals. They noted that superovulation was followed by production of fully functional luteal tissue and that luteal progesterone levels after induced ovulation were maintained approximately eight days longer than in normally cycling cattle. Plasma progesterone levels were also high presumably as a result of the large mass of active luteal tissue present. The correlation between peak plasma progesterone and number of corpora lutea was 0.76. Progesterone was not detected in blood samples from a calf that failed to ovulate, leading to the conclusion that the treatment regimen did not cause

progesterone production in the absence of luteal tissue. In a separate experiment, Spilman et al. (1972) demonstrated that corpora lutea from prepuberal cattle incubated in vitro in the presence of LH increased their production of progesterone by 27 percent. One wonders if the addition of PMSG to the incubation medium would have a similar luteotrophic effect to the point that injections of PMSG early in the cycle could cause maintenance of the corpus luteum.

Superovulation of the Post-Puberal Female

Hammond (1949) reported that multiple ovulations and multiple pregnancies could be induced with single injections of PMSG although there was much variability in ovulation rates. Schilling and Holm (1963) observed a preponderence of two to three corpora lutea following a sequence of two PMSG injections, and similar results were reported by Laster et al. (1971). Several problems associated with induced superfetation in cattle have been noted including lowered conception rates (Schwartz and Shelby, 1969), retained placentas and freemartinism (Turman et al., 1971). The most difficult problem to overcome, however, has been that of trying to limit ovulations to an acceptable number. An understanding of the hormonal changes in the cow after she has been injected with PMSG might prove beneficial in this regard.

Spilman et al. (1973) noted that pituitary concentration of LH in six postpuberal heifers superovulated by PMSG was 1.1 mg LH per gram of pituitary. These results compare favorably with Desjardins et al. (1966) who found pituitary LH of sexually mature cattle to be about 2 mg per gram of fresh tissue. Spilman et al. (1973) also noted that plasma LH in sexually mature cycling heifers rose sharply after PMSG and declined

gradually thereafter to values similar to normally cycling cattle. As was the case with prepuberal calves, luteal progesterone in some of the superovulated mature heifers was maintained approximately two to three days longer than in normal cycling heifers. The level of plasma progesterone in heifers treated with PMSG on day 15 or 16 was also substantially higher than those reported in normal cows during the estrous cycle. Similarly, equine luteinizing hormone caused a significant increase in progesterone levels in heifers (Brunner et al., 1969).

Hendricks and Lamond (1972) showed that after administering PMSG plasma estrogen and LH levels were greater in cows which subsequently had multiple ovulations than in cows having one ovulation. In a more extensive trial, Hendricks et al. (1973) allotted 24 beef heifers to one of three treatment groups. The animals received either 0, 1600 or 3200 I.U. PMSG on day 16 of the estrous cycle. Plasma estrogen, LH and progesterone were quantified by RIA from blood samples collected from day 16 until 12 hours after the onset of estrous. They noted that most ovulations were associated with the longest interval between PMSG injection and estrus in the heifers treated with 1600 I.U. but not in those treated with 3200 I.U. PMSG. They also observed that five of the eight heifers receiving 3200 I.U. PMSG had large numbers of cystic-appearing follicles. Plasma progesterone began to decrease about 60 hours before estrus in the 0 and 1600 I.U. groups and about 36 hours before estrus in the 3200 I.U. group. Progesterone was less than 1 ng per ml for 24 hours, 12 hours and 6 hours before estrus in the 0, 1600 and 3200 I.U. groups, respectively. Plasma progesterone remained elevated for a longer period of time in the 3200 I.U. group but decreased at a more rapid rate before estrus. The mean rates of increase in plasma estrogen were 0.08, 1.0 and 1.2 pg per ml per

hour for the 0, 1600, and 3200 I.U. groups, respectively. Plasma estrogen was not correlated with follicle number or maximum plasma LH. In the PMSG treated groups, plasma LH began to rise about six hours prior to estrus and peaked at the time estrus was detected. In the control group, however, LH did not begin to rise until after estrus was detected and did not peak until six hours later. Since there was no correlation between number of ovulations and the LH peak, it suggests that there was enough LH for ovulation. Guthrie et al. (1974) reported a similar study in swine in which plasma estrogen, LH and progesterone were examined in mature gilts after an injection of 0, 1600 or 1200 I.U. PMSG on day 15 of the estrous cycle. Plasma estrogen for gilts receiving 1200 I.U. PMSG was significantly greater than the means for the other two groups on the day before estrus and on the first day of estrus. Plasma progesterone was also higher four and three days prior to estrus in animals receiving PMSG. The magnitude of the preovulatory LH surge, however, was not significantly affected by PMSG treatment.

In a similar study, Lamond and Gaddy (1972) treated 48 heifers with 1500 to 3000 I.U. PMSG in two separate trials. They were interested in examining the progesterone values in the heifer having multiple ovulations. Blood samples were collected at five day intervals beginning 15 days after mating. The results were variable, but generally the heifers having increased numbers of ovulations also had increased plasma progesterone levels. Regression analyses suggested that each additional corpus luteum resulted in an increase of 8.0 ± 2.14 and 9.2 ± 2.50 ng progesterone/ml for the two trials, respectively. These authors postulated that cows with the highest probability of carrying a small little to term may be those

which are able to limit blood progesterone concentrations to physiological ranges.

Response to Repeated Injections of PMSG

Willett et al. (1953) suggested that repeated injections of gonadotrophins lead to a refractory state by demonstrating that the number of corpora lutea in cattle decreased with successive superovulations. These authors suggested three possible explanations for this refractoriness: development of an antisubstance to the gonadotrophin, exhaustion of primary follicles in the ovaries or varying degrees of damage to the ovaries as a result of the treatments or surgery. They suggested further that increased responses of some cows to increased doses of hormones gave credence to the antigonadotrophin concept; while the progressive decline in the number of corpora lutea in one cow in spite of alternating species as sources of FSH indicated the contrary. When they assayed the blood serum from some of the refractory cows for antigonadotrophin using a rat bioassay described by Deutsch et al. (1950), they found that the cow serum augmented the ovarian response of rats to gonadotrophins instead of inhibiting it. Casida et al. (1943) also found only slight evidence of antihormone in serum from cows given large daily doses of sheep pituitary extract.

Hafez et al. (1964) were unable to induce multiple ovulations in cows with repeated injections of PMSG. Jainudeen et al. (1966) endeavored to determine the ovarian response to repeated doses of PMSG and to examine the serums of the cows for antigonadotrophic activity in immature female rats using the method of Cole et al. (1957). These workers gave a series of PMSG injections on day 16 of four different estrous cycles with five

to seven months between the first and second injections after which two and one estrous cycles separated subsequent injections. Their results also indicated that ovarian response was altered by repeated PMSG injections. However, in contrast to previous studies, their data showed an inhibitory influence of 2 ml of serum from cows treated with PMSG on the ovarian response of immature female rats suggesting the presence of an antigonadotrophic substance. Their work also suggests that the antigonadotrophic substance may persist from seven to eight months; while Thompson (1941) considers antigonadotrophic activity in blood serum to be usually lost one or two months after termination of injection of the hormone.

Greenwald (1970) reported a similar refractory period in the rabbit after repeated injections of human chorionic gonadotrophin (HCG). However, some rabbits that were refractory to HCG were mated and subsequently ovulated indicating that any antiserum against HCG that was present did not neutralize endogenous LH.

CHAPTER III

MATERIALS AND METHODS

Procedure

General

This study was conducted from January, 1974 to May, 1975 at the Fort Reno Livestock Research Station, El Reno, Oklahoma, and the Animal Science Physiology Laboratory on the Oklahoma State University campus in Stillwater. The experimental animals consisted of 54 straightbred Angus females including 31 three year-old lactating cows raising their first calf and 23 two year-old heifers. All cows had been treated with PMSG during the previous reproductive cycle while none of the heifers had received prior hormonal therapy. All animals exhibited at least one normal estrous cycle before being allotted to a treatment group. They were maintained on native pasture at a moderate level of winter feeding which required providing approximately 1.4 kg of a 30 percent natural protein supplement per day between January 1 and April 15.

Assay, Storage and Administration of PMSG

The pregnant mare serum gonadotrophin (PMSG) used in this study was obtained as a lyophilized powder with a stated potency of 200 I.U. PMSG per mg. Prior to use in this trial, the PMSG was assayed according to the method of Cole and Erway (1941). The assay was carried out in 21

day old immature female rats of the Texas Inbred Mice Company strain, Tex:SD (Sprague-Dawley Derived). The standard PMSG preparation used was the World Health Organization Second International Standard for Serum Gonadotrophin, Equine (Medical Research Council, Division of Biological Standard, National Institute for Medical Research, Hampstead Laboratories, Holly Hill, London NW36RB). Fifty rats received a single subcutaneous injection of 0.5 ml saline containing either 4, 6, 9, 13 or 20 I.U. of the International Standard or the PMSG used in this trial while five rats served as saline injected controls. The rats were sacrificed 48 hours later and wet ovarian weights determined. The mean ovarian weights of the five rats in each treatment group are presented in Table I. An analysis of variance utilizing a 2 x 5 factorial arrangement of treatments (Snedecor and Cochran, 1967) yielded no evidence to suggest that the test PMSG differed significantly in potency ($P > .25$) from that of the International Standard. In addition, 4 I.U. of both the test and the Standard PMSG produced significantly ($P < .05$) larger ovaries than those seen in saline treated controls. Therefore, the stated potency of 200 I.U. PMSG per mg was used in preparing hormone injections.

The lyophilized PMSG was sealed in bottles and stored at -10 C to protect against damage from heat or moisture. Near the time of injection, individual doses of PMSG were weighed, placed in 15 ml test tubes, stoppered and refrigerated in the physiology laboratory at Fort Reno. Just prior to being injected, the PMSG was reconstituted with six ml of double distilled water and injected subcutaneously in the neck or shoulder region.

TABLE I

MEAN OVARIAN WEIGHTS OF IMMATURE FEMALE RATS TREATED WITH EITHER THE SECOND INTERNATIONAL STANDARD FOR SERUM GONADOTROPHIN (STANDARD) OR THE PMSG USED IN THIS TRIAL (TEST)[a]

	I.U. PMSG				
Treatment	4	6	9	13	20
Standard	43.5±2.6[b]	42.9±1.4	58.7±1.8	61.6±3.4	68.3±5.0
Test	37.4±2.0	47.2±4.1	59.0±5.0	59.7±1.6	73.5±7.1

[a]Values are mean ± S. E. from five rats on each treatment
[b]Values are expressed in milligrams

Treatments

The treatments imposed in this study were utilized to compare the endocrine and reproductive responses of cows and heifers treated with PMSG either during the late luteal phase or both during the early and late luteal phases of the estrous cycle. Table II illustrates the treatment schedule used to pursue these objectives. Sixteen cows and 11 heifers received a single subcutaneous injection of 2000 I.U. PMSG on day 17 (day 0 = estrus) of their estrous cycle; while 15 cows and 12 heifers received 1500 I.U. PMSG on day 5 followed by 2000 I.U. PMSG on day 17. Estrus was determined by using vasectomized or penectomized bulls equipped with chinball markers.

TABLE II

TREATMENT SCHEDULE

Items	PMSG Treatment	
	Treatment 17	Treatment 5, 17
Day of Cycle		
Day 5	----------	1500 I.U. PMSG
Day 17	2000 I.U. PMSG	2000 I.U. PMSG
Number of Animals	16 cows	15 cows
	11 heifers	12 heifers

Bleeding Schedule

All animals were bled at regular intervals during the estrous cycle in which PMSG treatments were imposed. Blood samples were obtained by jugular venipuncture using a 1½ inch 16 gauge needle and a 50 cc syringe in the morning of days 1, 3, 5, 6, 7, 9, 11, 13, 15, 17, 18 of the cycle, then daily until estrus occurred, or until day 26 if estrus did not occur. Blood was transferred from the syringe to a plastic centrifuge tube containing 31.7 mg oxalic acid and centrifuged at 2700 x g for 15 minutes to remove the blood cells. The plasma was then decanted into 7-dram plastic vials and stored at -10 C until assayed for hormone concentration.

At the first estrus after the last PMSG injection, all animals were bred by natural service to Angus bulls. A final blood sample was collected approximately 12 days after breeding.

Laparotomy Technique

In order to determine the number of ovulations resulting from PMSG treatment, a high lumbar laparotomy (Laster, 1971) was performed on each animal approximately seven to 11 days after breeding. The laparotomies were performed on the animal's right side between the last rib and the external angle of the ilium. The hair was clipped and the entire operative area was scrubbed with antiseptic soap (Roccal-D). The skin and underlying tissues were anesthetized by injecting a total of 20 ml of two percent procaine in two ml quantities along either side of the incision line. Prior to surgery all instruments and the hands and forearms were scrubbed with antiseptic soap. An eight inch vertical incision was made through the skin beginning approximately four inches below the lumbar vertebrae. A slightly shorter incision was then made through the outer muscle layer. The inner muscle layer was pierced by placing the forefinger between the muscle bundles and forcing the hand through the layer. A small incision was then made in the peritoneum and the hand and forearm were forced into the body cavity. Hemorrhage was controlled by clamping hemostats on the severed blood vessels.

Once the ovaries had been located, they were grasped with the hand and observed through a laparoscope (Jacobs-Palmer Operating Peritoneoscope, Richard Wolf Medical Instruments Corp., 7046 Lyndon Ave., Rosemont, Illinois 60018) inserted into the body cavity. Ovarian size, number of corpora lutea, number of follicles and the presence of any abnormal structures such as cystic follicles were noted for each ovary. The cut tissues were closed in the reverse order that they were entered, and the operative area was covered with iodine. Following the laparotomy, each

animal received an intramuscular injection of 8 ml Pestrep. After the entire treatment regimen had been completed, all animals were placed on native grass pasture with fertile bulls for a period of 60 days after which pregnancy rates were determined by rectal palpation.

Radioimmunoassays (RIA)

A series of radioimmunoassays were conducted on each plasma sample. A detailed description of the assays used to quantify plasma progesterone, estradiol and luteinizing hormone will follow in subsequent sections.

Progesterone

Plasma progesterone was quantified by RIA similar to the method described by Kittok et al. (1973). The specificity of the antiserum (#869) used in these determinations has been reported in detail (Niswender, 1973).

To minimize background interference in this assay, all glassware was washed prior to use with detergent and rinsed in tap water, distilled water, glass distilled water and methanol. In addition, all glassware was also rinsed with 5% trimethyl chlorosilane in toluene. One ml plasma was placed in a 15 ml screw cap extraction vial, followed by the addition of 3000 dpm of ^{3}H-1,2,6,7 progesterone (New England Nuclear; 105 c/mM; repurified by column chromatography; solvent was chloroform:ethanol; 95:5, V:V; Swanson et al., 1971). The plasma and tracer were mixed gently and allowed to equilibrate for 15 min. at room temperature. Plasma and tracer were then extracted with 10 ml benzene:hexane (1:2, V:V) by vigorous inversion for 15 minutes. Samples were placed at -10 C for at least one hour to freeze the plasma. The solvent extracts were decanted

into 12 ml conical test tubes and evaporated under nitrogen to approximately 3.0 ml. A 0.5 ml aliquant was taken to estimate procedural losses, and dilution duplicates appropriate for the expected amount of progesterone present were placed in 12 x 75 mm disposable culture tubes and dried under nitrogen.

Standards were diluted in 200 proof redistilled ethanol so that 0.1 ml contained 0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, and 1.6 ng of progesterone. Two standard curves were used in each 48 tube assay, and these were also dried under nitrogen. In addition, a steer sample and a steer plus five ng progesterone were also used in each assay.

After the standard and plasma extracts were evaporated, 0.2 ml antibody (Anti-progesterone #869 generously supplied by Dr. G. D. Niswender, Department of Physiology, Colorado State University, Fort Collins) against 6B-succinyl progesterone conjugated to bovine serum albumin was added to each tube, mixed gently and allowed to equilibrate at room temperature for 15 minutes. The antibody had been diluted 1:6000 in 0.1 M phosphate buffered saline containing 0.1% gelatin (Knox Gelatin, Inc., Johnstown, N.Y.; Table VII). Following the incubation period, 0.2 ml of 0.1% gelatin in 0.1 M phosphate buffered saline containing approximately 58,000 dpm of ^{3}H-1,2,6,7-progesterone was added to each tube. The tubes were mixed gently and incubated at 4 C for 12 to 20 hours.

Following the overnight incubation, the tubes were placed in an ice water bath at 4 C for 10 minutes after which one ml dextran coated charcoal (2.5 g activated neutral norit plus 0.25 g dextran T-150 in one liter glass distilled water) was rapidly (less than two minutes) added to each tube. The contents were mixed, incubated for 10 min. at 4 C and centrifuged (1200 x g) at 4 C for 10 minutes. A 0.5 ml aliquant of the

supernatant was diluted with 10 ml of liquid scintillator (21.7 g 2, 5 diphenyl oxazole in 3.0 liter toluene) for quantification of radioactivity in a Packard Tri-Carb Liquid Scintillation Spectrometer. The mass of progesterone in unknowns was calculated by interpolation between standards and corrected for procedural losses.

Kittok et al. (1973) demonstrated the specificity of this assay by comparing it to a competitive protein binding assay and to radioimmunoassay of progesterone isolated by Sephadex LH-20 column chromatography. Since these results indicated a high degree of specificity in this assay, no further validation was carried out in our laboratory. However, 34 determinations were conducted on a pool of steer plasma and averaged .28 $\pm$ .01 ng/ml. In addition, an average of 5.03 $\pm$ .09 ng/ml (95% recovery) was obtained from 44 determinations of a steer plasma + 5 ng progesterone/ml resulting in a between assay coefficient of variation of 12.04%. Finally, the addition of 0.1 ng progesterone to the assay resulted in approximately a 30% reduction in the amount of ^{3}H-progesterone that was bound to the antibody indicating that this assay is highly sensitive at the lower hormone concentrations.

Estradiol

The procedure used to quantify estradiol was similar to that described by Wettemann et al. (1972) as modified by Hafs et al. (1974). Triplicate aliquants (1.0 ml) of plasma were placed in 13 x 100 mm disposable culture tubes which had been rinsed with redistilled methanol. Approximately 3000 dpm of ^{3}H-2,4,6,7-estradiol (111 c/mM; repurified by column chromatography) in ethanol was added to one of the triplicate tubes, vortexed gently and allowed to equilibrate for 30 minutes.

Estrogens were extracted by vortexing with 3.0 ml benzene (Nanograde) for one minute. Solvent from the tracer tube was pipetted into a scintillation vial to estimate procedural losses; while the solvent from the other two tubes were pipetted into 12 x 75 mm disposable culture tubes for radioimmunoassay. Standards were diluted in redistilled ethanol so that 0.1 ml contained 0, 1, 2, 4, 6, 10, 20, 40, 60 and 100 pg estradiol. Two standard curves were placed with each assay (48 total tubes per assay), and the standards and solvent extracts were evaporated under nitrogen. In addition, a water blank and a cow reference sample were also placed in each assay.

After evaporation 0.2 ml antibody (Antiestradiol #244 generously supplied by Dr. G. D. Niswender, Colorado State University) against 6-B succinyl estradiol conjugated to bovine serum alubmin was added to each tube, vortexed gently and incubated at room temperature for 30 minutes. The antibody had been diluted 1:100,000 in phosphate buffered saline plus 0.1% gelatin (Table VII). Approximately 30,000 dpm ^{3}H-2,4,6,7-estradiol (111 c/mM) in 0.2 ml phosphate buffered saline plus 0.1% gelatin (Table VII) was then added to each tube, vortexed gently and incubated at 4 C for three to 20 hours. Free and bound estradiol were separated, radioactivity was quantified and mass of estradiol was calculated as described for the progesterone assay except that the assay tubes were centrifuged at 1600 x g for 10 min. at 4 C after the dextran coated charcoal was added.

Previous radioimmunoassays for estradiol had been hampered by the fact that many antibodies would cross react with other estrogens requiring the separation of the estrogens by column chromatography prior to radioimmunoassay. The antibody used in this experiment was believed to

be highly specific for estradiol, but this premise had to be validated before the antiserum could be used to quantitate estradiol without isolation by chromatography.

The first step in the development of the assay was to determine the titer of the antibody that would result in the most desirable sensitivity and percent binding. The 1:100,000 dilution proved to be the most useful in that it resulted in approximately 19% of the competitor being bound to the antibody; while the addition of one pg estradiol resulted in approximately a 5% reduction in the amount of competitor that was bound. The antibody was next checked for cross reaction with various other steroids. When tested in this assay, estriol showed no crossreaction with the antibody, while 320 pg estrone was equivalent to about 10 pg estradiol. Progesterone (6.4 ng), testosterone (10 ng), cortisol (10 ng) and corticosterone (10 ng) also showed very little if any cross reactivity, while 200 pg estradiol benzoate was equivalent to about 80 pg estradiol. Figure 1 illustrates a typical standard curve for estradiol and cross reactions with estrone and estradiol benzoate as determined by RIA.

As a further validation step, estradiol was quantified in benzene extracts of 14 samples (range 1.5 to 14.9 pg/ml) and the estimates were compared to those obtained on the same samples after isolation of estradiol from Sephadex LH-20 columns (Chenault et al., 1975; solvent was benzene : methanol; 95:5; V:V). Estradiol in the extracts (3.8 ± 1.0 pg/ml) did not differ significantly ($P > .25$) from those determined after column chromatography (4.6 ± 1.0 pg/ml). In addition, a significant positive correlation coefficient was calculated between the two methods ($r = .95$; $P < .01$). Similarly, the between assay coefficient of variation as estimated from four determinations of the same sample was 7.7%.

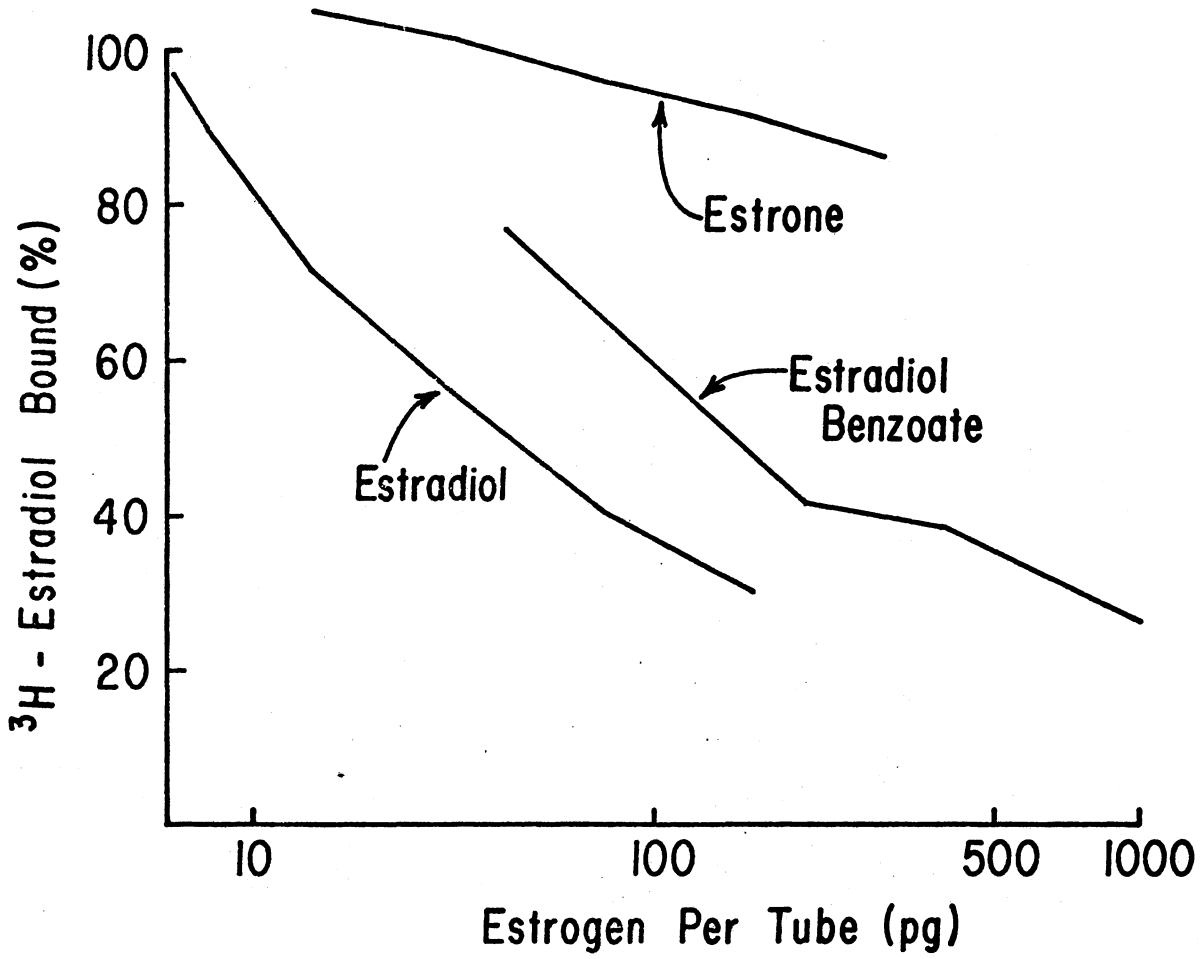

Figure 1. Standard Curve for Estradiol and Cross Reactions With Estrone and Estradiol Benzoate.

When two, four or six pg of estradiol were added to one ml plasma samples, 97 ± 7% (n = 4), 98 ± 7% (n = 4) and 106 ± 2% (n = 6), respectively, were recovered.

Luteinizing Hormone (LH)

The method used to quantify plasma LH was similar to that described by Niswender et al. (1969) as was the radioiodination procedure except ^{125}I rather than ^{131}I was used. Purified bovine LH used for iodination (LER 1072-2) was supplied by Dr. Leo Reichert (Emory University, Atlanta, Georgia). Plasma samples were assayed in dilution duplicate such that 0.2 and 0.3 ml plasma were added to 12 x 75 mm disposable culture tubes containing 0.3 and 0.2 ml phosphate buffered saline plus 0.1% gelatin, respectively (Table VII).

Bovine LH (NIH-LH-B8) was used as the standard in the assays. Standards were diluted in phosphate buffered saline plus 0.1% gelatin (Table VII) so that 0.5 ml contained 0, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, and 25.6 ng LH. Three sets of standards were included in each assay of 300 to 400 tubes. Two hundred ul of an antibody (Anti-LH-B225 generously supplied by Dr. G. D. Niswender, Colorado State University, Fort Collins, Colorado) against bovine LH was added to each tube, vortexed gently and incubated at 4 C for 24 hours. The antibody was diluted 1:80,000 in 1:400 normal rabbit serum which had been suspended in 0.05M phosphate buffered saline plus EDTA (Table VII). On the following day, 0.1 ml of approximately 20,000 cpm ^{125}I-LH in phosphate buffered saline plus 0.1% gelatin (Table VII) was added to each tube vortexed gently and incubated for another 24 hours at 4 C. Two hundred ul of an appropriate dilution of a second antibody (sheep anti-rabbit gamma globulin) against

rabbit gamma globulin fraction II (Nutritional Biochemical Corp., Cleveland, Ohio) was then added to each tube to insure that the LH-anti-LH complexes would be precipitable. The second antibody was usually diluted 1:6 in phosphate buffered saline plus EDTA (Table VII). Each tube was vortexed gently a final time and then incubated at 4C for 72 hours. After the final incubation period, three ml of cold phosphate buffered saline was added to each tube, and the free and bound LH were separated by centrifugation at 2300 x g for 30 minutes at 4 C. The supernatant was carefully decanted and the precipitate counted in a Packard Tri-Carb Liquid Scintillation Spectrometer with a tandem gamma unit.

As was the case in the estradiol assay, the specificity of the antibody used in this experiment for bovine LH had not been demonstrated. Validation of this antibody was, therefore, imperative before assay of the unknowns could be accomplished. Several concentrations of the antibody were tested with the 1:80,000 dilution yielding between 10 and 20% bound ^{125}I-LH as well as adequate depression at the 0.1 ng LH level (approximately 10% reduction). In addition, dose response curves for plasma from steers, cows, and anterior pituitary homogenates were parallel to the LH standard curve (Figure 2). Figure 3 illustrates the cross reaction of the antiserum with other pituitary gonadotrophins. When 100 ng of growth hormone (NIH-GH-B_{17}) and 200 ng of prolactin (NIH-P-B_3) were tested in this assay, each had less than one ng equivalent LH; and one ug of follicle stimulating hormone (NIH-FSH-S_9) was equivalent to about two ng LH. Ten ng thyroid stimulating hormone (NIH-TSH-B6) was, however, equivalent to about three ng LH. Selenkow et al. (1966) and Guillemin (1967) have suggested that this cross reaction with TSH may be due to

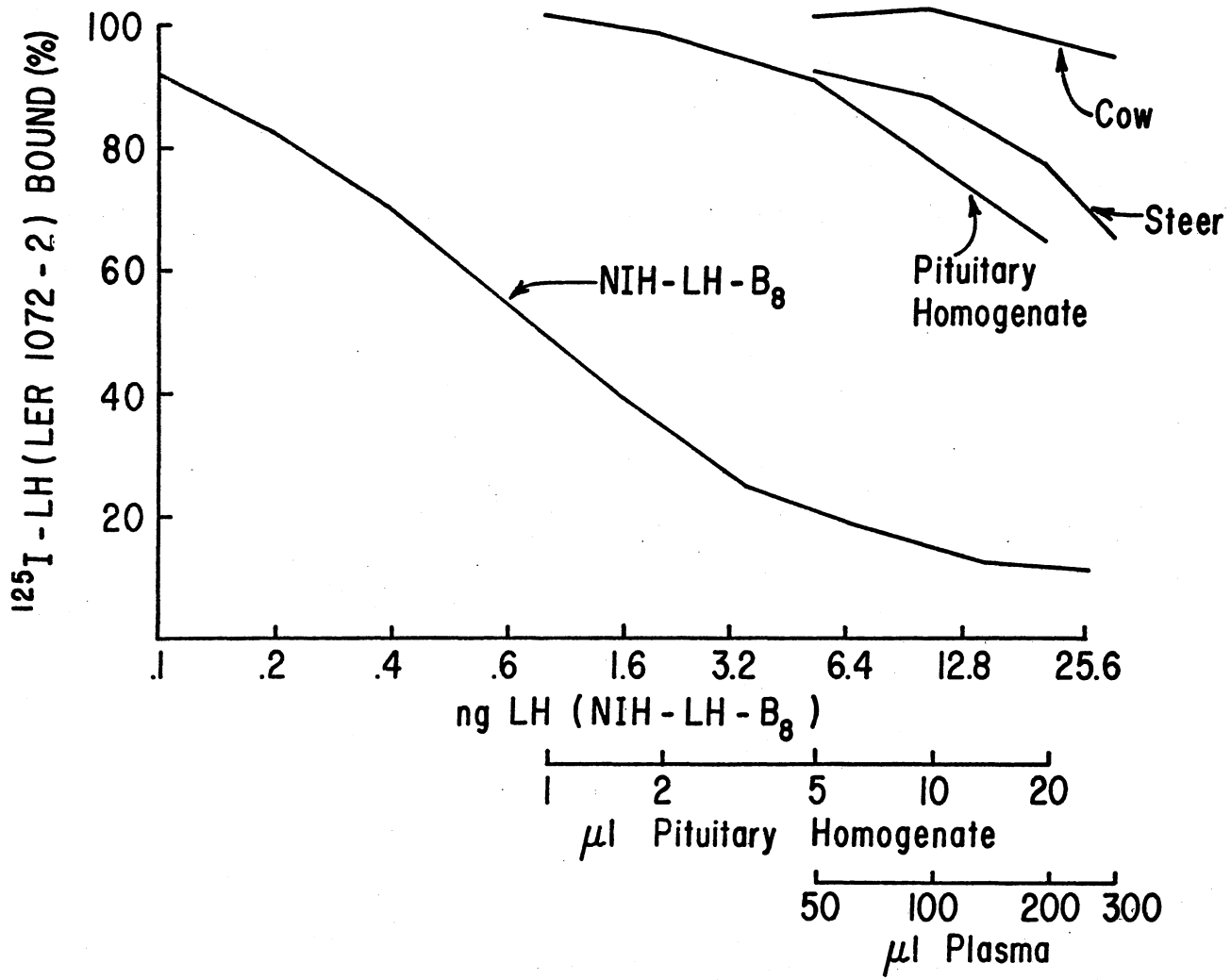

Figure 2. Standard Curve for LH and Dose Response Curves for Plasma and Anterior Pituitary Homogenate.

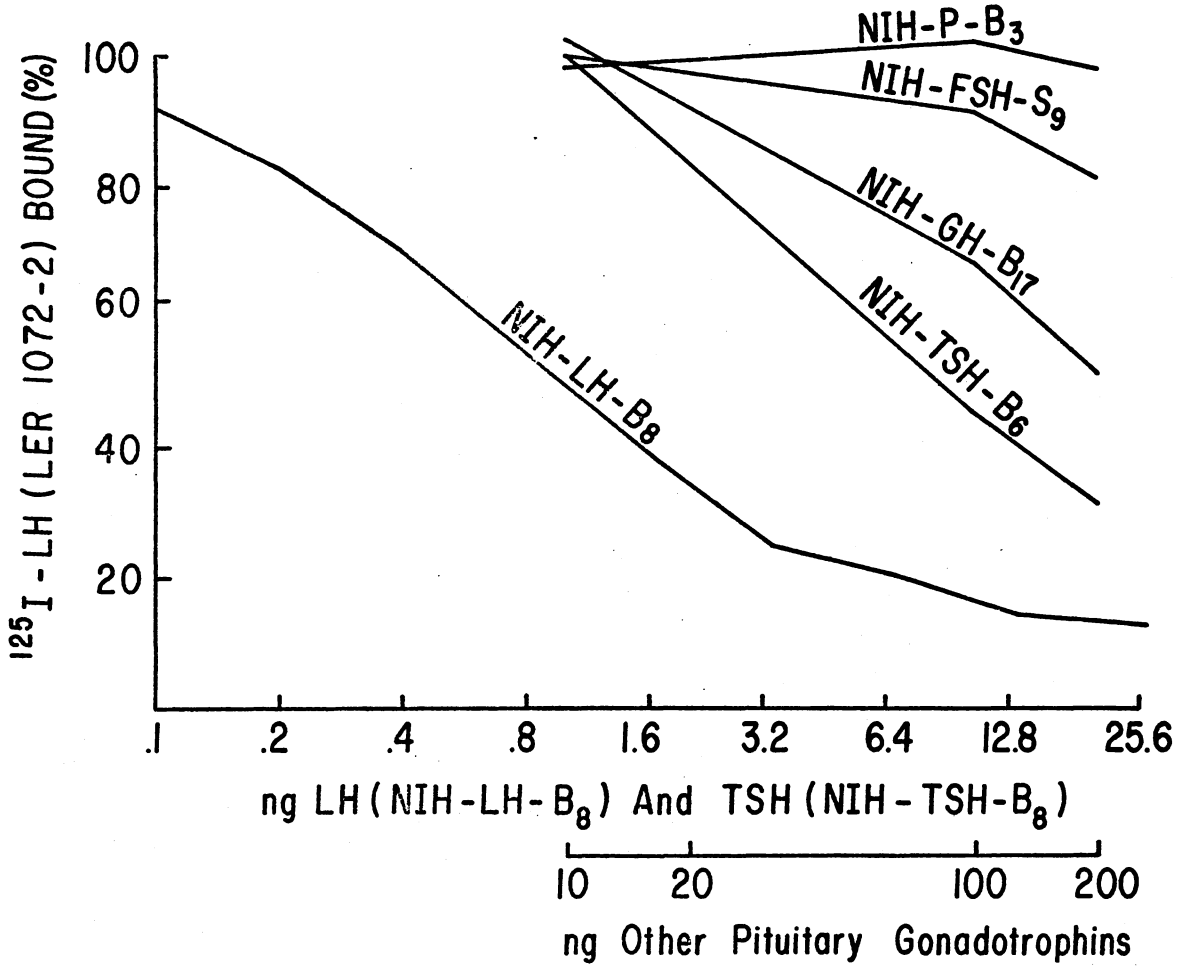

Figure 3. Standard Curve for LH and Cross Reactions with Various Other Pituitary Gonadotrophins.

contamination of the TSH with immunologically active LH since the two hormones appear to be related immunologically.

To estimate the precision of the LH assay, a sample from a pool of steer plasma was included in each assay resulting in an average of 2.1 $\pm$ .3 ng/ml with a between assay coefficient of variation of 30.3%. Similarly a series of determinations on the same sample were conducted in a single assay yielding an average of 0.7 $\pm$ 0.03 ng/ml with a within assay coefficient of variation of 10.6%. When two, four or six ng LH were added to bovine plasma, 70.6 $\pm$ 2.5% (n = 5), 70.7 $\pm$ 5.8% (n = 5) and 58.3 $\pm$ 3.2% (n = 5), respectively, were recovered.

Statistical Analysis

Several analyses were conducted to adequately evaluate the data. Standard procedures described by Snedecor and Cochran (1967) were employed to examine some variables. Estrous cycle length and ovulation rate were analyzed by analyses of variance using completely randomized designs. Chi squares techniques were employed to test for significant differences in conception rate and percent of animals having various numbers of ovulations. Simple correlation and regression coefficients were also utilized to describe the relationship between the various endocrine parameters and number of corpora lutea.

Endocrine data were subjected to split plot analyses of variance as described by Gill and Hafs (1971) for repeated measurements on animals. Table III illustrates the analysis used to determine the influence of PMSG treatment on plasma progesterone in the cow using two between block treatments (PMSG) and one within block treatment (Bleeding period). When a significant treatment x bleeding period interaction was detected, an

analysis of variance was utilized to evaluate treatment differences within a bleeding period (Snedecor and Cochran, 1967).

TABLE III

SPLIT PLOT ANALYSIS OF PLASMA PROGESTERONE IN COWS AFTER TREATMENT WITH PMSG

Source of Variation	df
Total	511
Between Animals	30
Treatments	1
Animals Within Treatments	29
Within Animals	481
Bleeding Period	19
Treatment x Period	19
Animal x Period Within Treatment	443

CHAPTER IV

RESULTS AND DISCUSSION

Reproductive Response

Overall reproductive response from the animals in this experiment was estimated by examining several variables as shown in Table IV. Approximately the same percentage of the cows in both treatment groups and heifers in Trt 17 exhibited estrus after PMSG injection, while all heifers receiving two injections (Trt 5, 17) were observed in estrus. Cows treated with two injections of PMSG showed a significantly longer estrous cycle than those treated only on day 17 (23.9 $\pm$ .5 vs 21.9 $\pm$ .7 days, respectively; $P < .05$). A similar trend was evident in the heifers with animals given two injections cycling in 23.6 $\pm$ .5 days while heifers treated only on day 17 cycled in 21.6 $\pm$ 1.1 days ($P < .10$). Cows treated twice returned to estrus in 6.9 $\pm$ .5 days after the last injection, while those given a single injection were in heat 4.9 $\pm$ .7 days later ($P < .05$). Similarly, heifers given two injections returned to estrus in 6.6 $\pm$ .5 days, while those receiving a single injection returned in 4.6 $\pm$ 1.1 days ($P < .10$).

Animals conceiving to the first post-PMSG estrus as determined by rectal palpation 60 days after breeding or by subsequent calving dates are also shown in Table IV. The conception rates determined by means of rectal palpation for both groups of cows and for heifers on Treatment 5,

TABLE IV

ESTROUS CYCLE LENGTHS, PERCENT OF ANIMALS SHOWING ESTRUS AND CONCEPTION RATES AFTER TREATMENT WITH PMSG

	Cows		Heifers	
Item	Trt 17	Trt 5, 17	Trt 17	Trt 5, 17
Animals	16	15	11	12
Exhibiting Estrus (%)	75.0	66.7	63.6	100.0
Cycle Length (days)[a]	21.9 ± .7[b]	23.9 ± .5	21.6 ± 1.1[c]	23.6 ± .5
Pregnant at 60 days (%)	62.5	60.0	45.4	75.0
Pregnant to Trt at Term (%)	56.2	40.0	18.2	33.3

[a]Mean ± S. E.
[b]Means Differ Significantly ($P < .05$)
[c]Difference Approaches Significance ($P < .10$)

17 are similar to those reported by Turman et al. (1971) and Laster et al. (1971) for superovulated cows. Heifers of Treatment 17 are somewhat lower, but differences were not significant. Although no untreated control animals were available for comparison, the post-PMSG conception rates did not appear to be adversely affected which substantiates the findings of Hammond (1949) and Hafez et al. (1964). However, the pregnancy rates to the post-PMSG estrus as determined by the time of parturition were somewhat lower than those determined by palpation 60 days post-breeding. This discrepancy was attributed to an inability to determine the date of conception accurately by rectal palpation. In addition, only 33% of those animals having multiple ovulations and conceiving to the first post-PMSG estrus gave birth to twins. These data would tend to support the results of Gordon et al. (1962) and Schwartz and Shelby (1969) that both rectal palpation and laparotomy may be involved in embryonic mortality and reduced conception rates.

Ovarian Response

Ovarian response was determined by laparotomy seven to 11 days after the first post-PMSG estrus. The various ovarian measurements taken are shown in Table V. No significant differences were detected in total ovarian diameter in either the cow or heifer groups. In a like manner, all treatment groups exhibited a similar number of follicles less than 10 mm in diameter, while cows given one injection of PMSG tended to have more follicles greater than 10 mm in diameter than those receiving two injections ($2.8 \pm .5$ vs $1.5 \pm .4$, respectively; $P < .10$). Hendricks et al. (1973) reported increased ovarian weights and follicle numbers in

cow receiving PMSG compared to controls, but untreated animals were not available for comparison in our study.

TABLE V

OVARIAN[a] RESPONSE OF ANIMALS TREATED WITH PMSG

Item	Trt 17	Trt 5, 17	Trt 17	Trt 5, 17
Ovarian Diameter (mm)[b]	64.6 ± 6.7	68.6 ± 7.0	71.6 ± 8.0	69.1 ± 5.2
No. Follicles 10 mm	2.7 ± 1.0	3.2 ± 1.1	1.4 ± .4	2.1 ± .6
No. Follicles 10 mm	2.8 ± .5[c]	1.5 ± .4	3.7 ± 1.4	1.8 ± .7
Ovulation Rate	2.2 ± .4[d]	1.3 ± .3	3.1 ± 1.2	4.3 ± 1.1
Ovulations (%)				
0	6.2	20.0	18.2	0
1	37.5	60.0	27.3	25.0
2 to 4	50.0	13.3	36.4	50.0
> 4	6.2	6.7	18.2	25.0

[a]Visual Estimates Determined at Laparotomy
[b]Mean ± S. E.
[c,d]Difference Approaches Significance ($P < .10$)

In addition, 12.5% of the cows and 27.3% of the heifers receiving a single injection of PMSG had follicles that appeared to be cystic or luteinized, while 20% of the cows and 16.7% of the heifers receiving two injections had similar abnormalities. Laster et al. (1971) noted similar occurences in one of their trials as did Hendricks et al. (1973).

Cows treated with a single injection of PMSG had a higher ovulation rate than those treated on days 5 and 17 (2.2 $\pm$ .5 vs 1.3 $\pm$ .3, respectively; $P < .10$). Although the difference in ovulation rate in heifers was not significant ($P > .25$), animals treated on days 5 and 17 tended to have more ovulations than those treated only on day 17 (4.3 $\pm$ 1.1 vs 3.1 $\pm$ 1.2, respectively). These results would suggest that animals treated during previous years with PMSG might respond best to a single injection; while animals having never received PMSG such as the heifers may respond adequately to either one or two injections. Laster et al. (1971) reported similar ovulation rates for cows receiving HCG after a series of two PMSG treatments, while their ovulation rates from heifers receiving PMSG and HCG were slightly lower than those seen in this trial.

Percent of animals having various numbers of ovulations in also shown in Table V. With the exception of heifers given two injections of PMSG, one or more animals in each of the other treatment groups failed to ovulate. In the majority of cases, the animals that did not ovulate were those not showing signs of estrus. No significant differences were evident between treatments within age groups for animals having only one corpus luteum, although 25% of the heifers compared to 60% of the cows receiving two injections responded with a single ovulation. Since all cows had been subjected to PMSG treatment in years past, the fact that 43.8% of the cows given a single injection and 80% of the cows given two

injections either failed to ovulate or shed only one ova would seem to lend credence to the thesis of Willett et al. (1953) that animals may become refractory to PMSG.

Fifty percent of the cows receiving a single injection and 13% of those receiving two injections had two, three or four ovulations. This trend was, however, reversed in the heifer group with 50% of the double treated heifers and 36% of the single treated having two to four egg ovulations. Laster et al. (1971) reported similar responses when cows and heifers were given the sequence of two PMSG injections plus HCG on the day of estrus in the cows or HCG three days after the last PMSG injection in the heifers. Turman et al. (1971) and Johnson et al. (1975) reported similar findings when over 50% of the animals conceiving to the first post-PMSG estrus gave birth to twins, triplets or quadruplets. It is encouraging that generally less than 20% of all animals treated showed more than four ovulations, since uncontrolled ovulations has been one of the major difficulties arising from multiple birth studies.

Hammond (1949), Brock and Rowson (1952) and Scanlon et al. (1968) have reported that a more desirable and restricted level of superovulation can be obtained when the interval between the last PMSG or FSH injections and subsequent estrus is relatively short. Similarly Johnson et al. (1975) indicated that the majority of multiple births resulting from PMSG injection occurred in cows that were in estrus two, three, or four days after estrus, while Gordon et al. (1962) and Turman et al. (1971) suggested an interval of at least three days as being most desirable. In the present study, the within age within treatment correlations between interval after PMSG to estrus and number of corpora lutea were nonsignificant ($P > .05$) and ranged from .41 to .32 suggesting that the

relationship between these two variables may be minimal. However, since cows receiving two injections showed a very low superovulatory response while also having a mean treatment to estrus interval of 6.9 ± .5 days, there is the possibility that there is a real relationship between length of the interval from PMSG to estrus and superovulatory response.

Endocrine Response

Limited data are available concerning the endocrine response of the bovine female following the injection of superovulatory levels of PMSG. Such information could yeild useful clues to controlling the many variables associated with the artificial induction of superovulation in cows. This study was primarily aimed at investigating this response, and subsequent sections will deal with the influence of PMSG on plasma progesterone, estradiol and LH in both the cow and heifer.

Progesterone

Plasma progesterone was determined by radioimmunoassay of jugular blood collected at regular intervals during the estrous cycle in which PMSG was administered as described previously. Progesterone in cows treated with PMSG and plotted by days after estrus (day 0 = estrus) is presented in Table IX and Figure 4. Until day 17, the cows given a single injection of PMSG were considered as controls to the cows given two injections, and exhibited a near normal progesterone curve as evidenced by peak values of 8.7 ± .5 ng/ml on day 15. Similar results have been reported by many workers including Plotka et al. (1967), Stabenfeldt et al. (1969), Sprague et al. (1971), Kazama and Hansel (1970), Wettemann et al. (1972), and Glencross et al. (1973). On day 5, cows receiving a

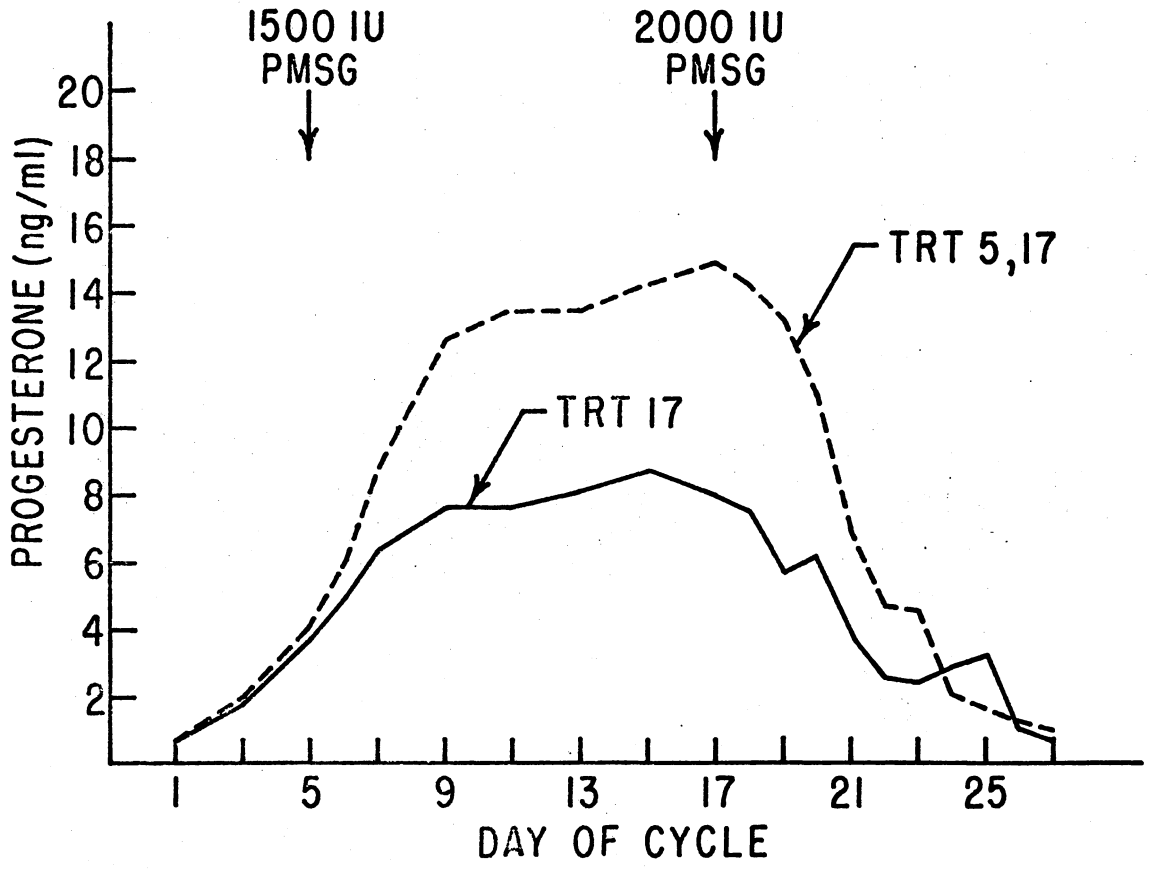

Figure 4. Plasma Progesterone in Cows Treated With PMSG.

single injection of PMSG on day 17 (Trt 17) had similar progesterone to those receiving two (Trt 5, 17) injections (3.8 $\pm$ .3 vs 4.1 $\pm$ .3 ng/ml, respectively; $P > .25$). By 48 hours after Trt 5, 17 received the initial day 5 injection, significantly ($P < .025$) greater progesterone concentrations were detected in their peripheral circulation than in those cows on Trt 17 (12.5 $\pm$ .8 vs 7.6 $\pm$ .6 ng/ ml, respectively). Progesterone continued to increase to a peak value of 14.9 $\pm$ 2.0 ng/ml on day 17 in Trt 5, 17 at which time the concentration for Trt 17 was 7.9 $\pm$ .6 ng/ml ($P < .005$). Several explanations could be postulated concerning this progesterone increase after PMSG injection early in the estrous cycle. Hoffman et al. (1974) have suggested that LH is luteotrophic in the cow. Perhaps the PMSG used in this trial served a similar function on the developing corpus luteum from the previous ovulation. Another possibility could be that PMSG may cause an endogenous LH release which subsequently has a luteotrophic effect. Results reported earlier in this thesis noted a number of luteinized follicles after PMSG which was also observed by Laster et al. (1971) and Hendricks et al. (1973). The possiblity would, therefore, exist that other ovarian tissue stimulated by PMSG might have added to the progesterone pool.

Regardless of the source, progesterone remained elevated for several days after the peak in Trt 5, 17 and did not reach a low level in all animals until day 25 (1.6 $\pm$ .6 ng/ml). After the PMSG injection on day 17, animals on Trt 17 were not stimulated to produce massive amounts of progesterone; however, plasma progesterone did not decrease to low quantities as rapidly as might be expected in a typical nontreated cow.

Plasma progesterone in only the cows showing estrus after PMSG is treated in Figure 5 and Table X and is plotted by days after the estrus

from which treatments were initiated and by days prior to the subsequent estrus. The values up to and including day 17 are similar to those reported for all cows (Figure 4). It should be noted, however, that the significant increase observed in Trt 5, 17 after the day 5 injection was evident one day earlier in the cows showing estrus than in all cows combined. Cows in Trt 5, 17 had elevated progesterone compared to Trt 17 from one day after the initial treatment until four days prior to the next estrus. By day -3 both groups had similar concentrations and continued to decrease to basal levels on the day of estrus ($0.8 \pm .1$ vs $1.4 \pm .5$ ng/ml for Trt 17 and Trt 5, 17, respectively; $P > .10$).

Figure 6 and Table XI illustrate plasma progesterone in heifers after treatment with PMSG. The results depicted are similar to those seen in the cows. The two treatment groups had similar values to day 5; after which, a highly significant ($P < .005$) increase to 19.9 ng/ml on day 13 was seen in the heifers given two injections. After the second injection, animals on Trt 5, 17 showed another slight increase on day 18 (18.3 ± 1.6 ng/ml); but this could have been due to sampling time. Progesterone in the single treatment groups increased normally to $10.0 \pm .9$ ng/ml on day 15; but rather than fall to basal levels, it reamined at 10.0 ng/ml and then increased to 14.5 ± 3.2 ng/ml four days after treatment. These data contradict that reported for cows in that the older animals on Trt 17 did not show a progesterone increase after the day 17 treatment. Spillman et al. (1973) reported that there was no precise time after superovulation at which luteal regression occurs in calves. Perhaps luteal regression had not occurred in the heifers on day 17; and the PMSG was, therefore, able to exert some type of luteotrophic effect; while regression had or was occuring in the cows and further PMSG was ineffective.

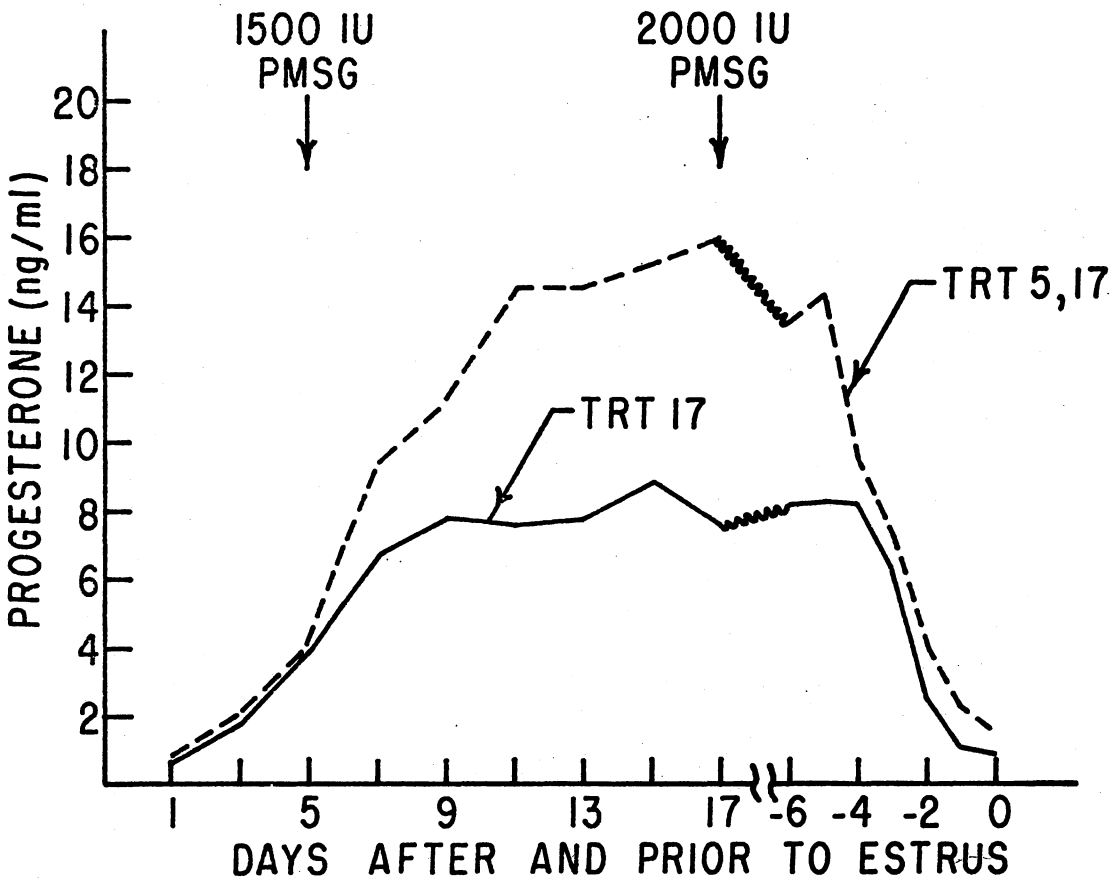

Figure 5. Plasma Progesterone in Cows Showing Estrus After Treatment With PMSG.

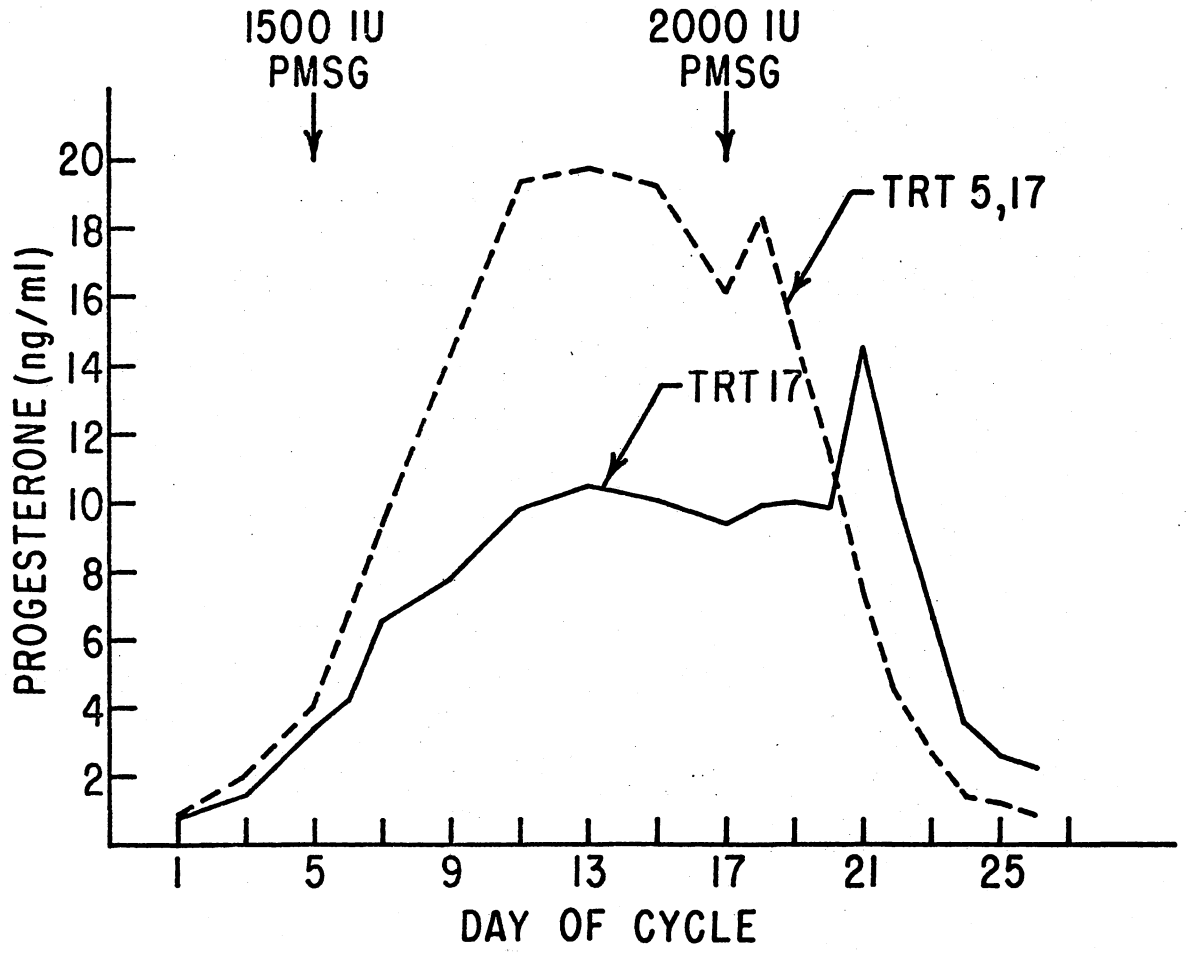

Figure 6. Plasma Progesterone in Heifers Treated with PMSG.

Progesterone in heifers showing estrus after PMSG is plotted in Figure 7 and Table XII. The vast differences in concentrations were also evident as early as one day after treatment in those animals that subsequently cycled (4.3 $\pm$ .7 vs 6.7 $\pm$ .8 ng/ml for Trt 17 and Trt 5, 17, respectively; $P < .10$). These differences remained apparent until day -5; after which, both groups decreased to about 1.0 ng/ml by the day of estrus.

Spilman et al. (1973) also reported that luteal and plasma progesterone in superovulated mature heifers was maintained approximately two to three days longer than in normal cycling heifers. Similarly, Hendricks et al. (1973) reported that progesterone remained high for a longer period of time in heifers treated with 3200 I.U. PMSG on day 16 than in those treated with 0 or 1600 I.U. PMSG. Guthrie et al. (1974) observed that progesterone in mature gilts treated with PMSG was higher four and three days prior to estrus than in untreated animals. These suggestions lead to speculation concerning the role of elevated progesterone in estrous cycle length. Considering only the cows that exhibited estrus after PMSG, the within age within treatment correlations for interval from the last PMSG to estrus and peak progesterone concentration were .76 ($P < .01$, $n = 12$) and .36 ($P > .05$, $n = 10$) for Trt 17 and Trt 5, 17, respectively. Similar correlations for the heifer groups were .97 ($P < .01$, $n = 7$) and .83 ($P < .01$, $n = 12$) for Trt 17 and Trt 5, 17, respectively, suggesting that heifers with a longer than average interval from PMSG to estrus tend to have a higher than average peak progesterone. Although numbers are inadequate to develop substantial cause-effect relationships, further studies in this area could develop procedures whereby induced luteolysis after PMSG might prove beneficial in controlling

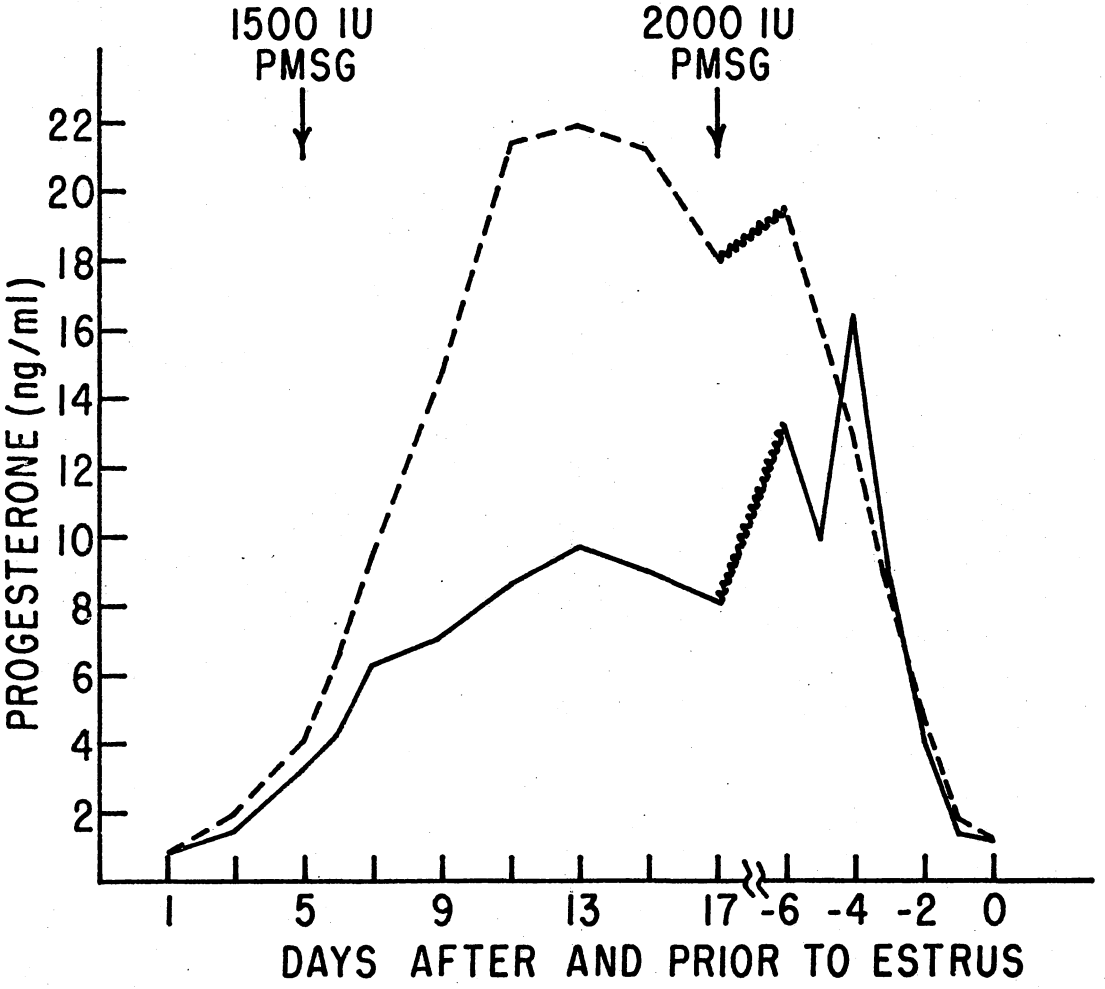

Figure 7. Plasma Progesterone in Heifers Showing Estrus After Treatment With PMSG.

problems related to the estrous cycle. Bellows et al. (1974) have reported reduced fertility in cows after progestogen treatment. One wonders if the increased progesterone seen after PMSG might serve as a pharmacologic dose sufficient to not only alter the estrous cycle, but also conception rates.

Spilman et al. (1973) observed a correlation of .76 between peak plasma progesterone and number of corpora lutea in superovulated prepuberal heifers. Lamond and Gaddy (1972) observed a similar tendency when they treated mature heifers with PMSG in two separate trials. These workers reported regression coefficients for corpora lutea number and plasma progesterone for the two trials of 8.0 $\pm$ 2.14 ng and 9.2 $\pm$ 2.50 ng. The results obtained in this study closely paralleled these reports in that a correlation was observed for all animals treated between number of corpora lutea and plasma progesterone of .75 ($P < .01$) with a regression coefficient of 9.65 $\pm$ 1.20 ng/ml (Figure 8). These values were determined from a blood sample taken 12 days after estrus following the final PMSG injection. In addition, Spilman et al. (1973) reported no detectable progesterone in blood samples from a calf that failed to ovulate suggesting that PMSG does not induce progesterone production in the absence of luteal tissue. Our data were in agreement with this observation in that progesterone in animals failing to ovulate was generally less than one ng 12 days after estrus. These data also indicated no apparent relationship between peak progesterone after the final PMSG treatment but prior to estrus and subsequent ovulation rate.

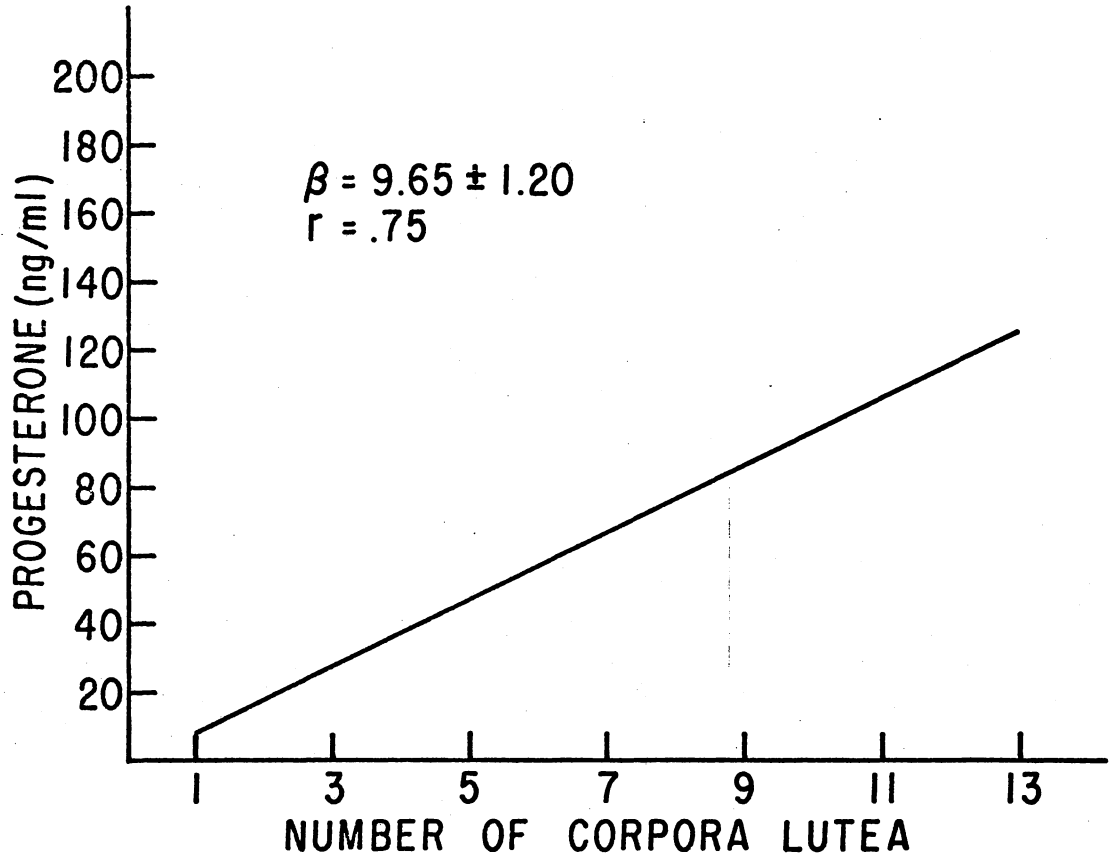

Figure 8. Influence of CL Number on Progesterone in the Bovine at 12 Days After Ovulation.

Estradiol

Plasma estradiol was quantified from all blood samples by radioimmunoassay as described earlier. Figures 9 and 10 and Tables XIII and XIV depict plasma estradiol in cows and heifers, respectively, after treatment with PMSG. Wettemann et al. (1972) reported that estradiol remains at low levels until approximately day -3 when a slight increase was noted followed by a continual rise to 9.7 pg/ml 0.5 days before estrus. Echternkamp and Hansel (1973) reported similar findings. The data obtained in this study for Trt 17 cows and heifers support these findings in that estradiol generally remained below 5 pg/ml from day 1 through day 17. Animals receiving a PMSG injection on day 5, however, showed dramatic increases in estradiol by two days after treatment in the cow ($P < .005$) and one day after treatment ($P < .025$) in the heifer groups. On day 7, estradiol in cows on Trt 5, 17 was 5.4 ± 1.2 pg/ml compared to $2.4 \pm .5$ pg/ml in cows on Trt 17 ($P < .025$). Similar results were evident on day 7 in the heifer groups except the magnitude of difference was much greater (15.1 ± 1.7 vs 4.1 ± 4 pg/ml for Trt 5, 17 and Trt 17, respectively; $P < .005$). The assumption was made that this estradiol increase resulted from follicular growth stimulated by PMSG although this was not verified since laparotomies were not performed early in the cycle. No animals were detected in estrus after day 5 PMSG treatment suggesting that even though estrogen was increased, the concurrent increase in progesterone was still the dominant endocrine factor regulating the estrous cycle.

Estradiol increases were also evident in each treatment group after all animals were treated on day 17. Care must be taken in evaluating

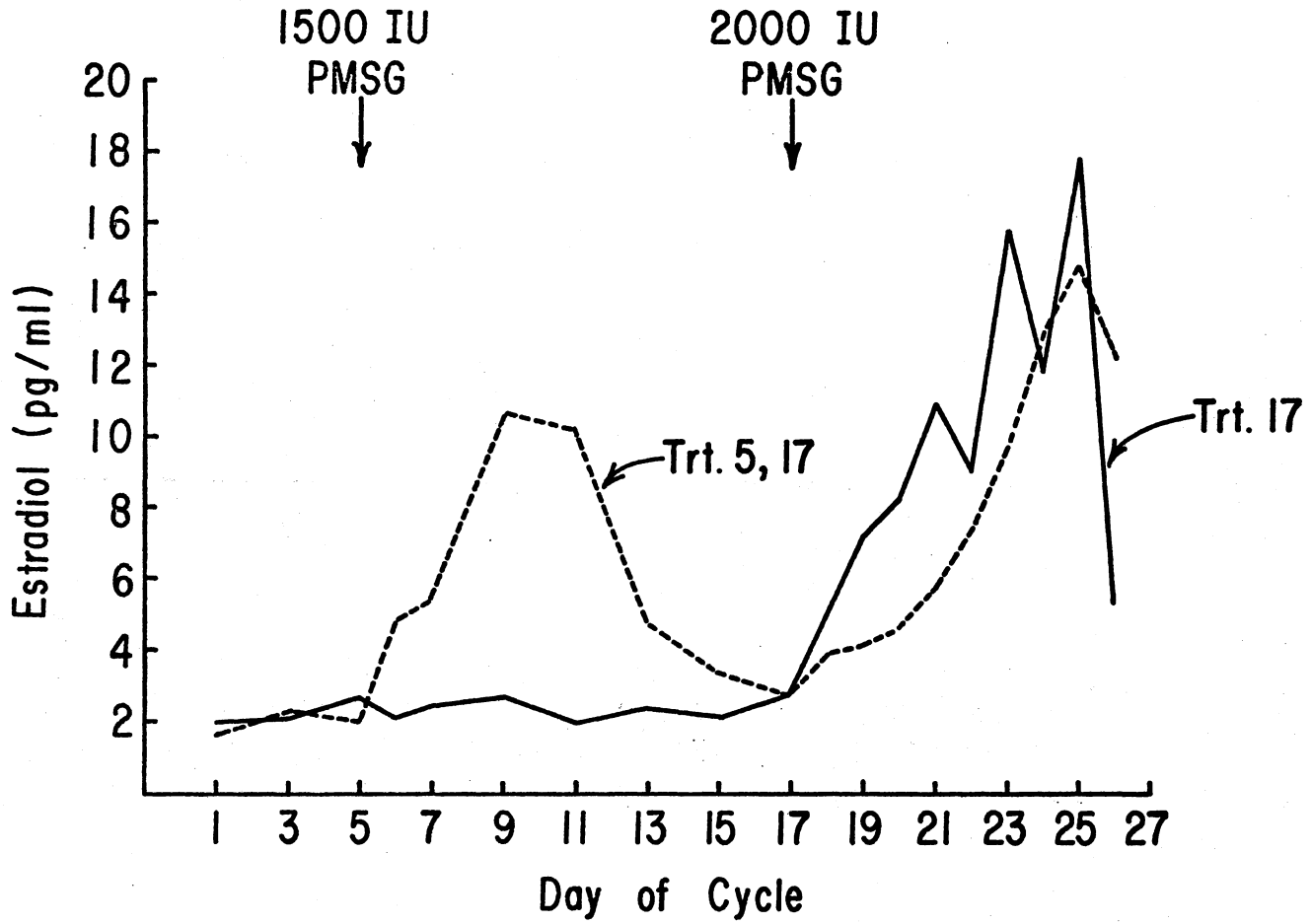

Figure 9. Plasma Estradiol in Cows Treated with PMSG.

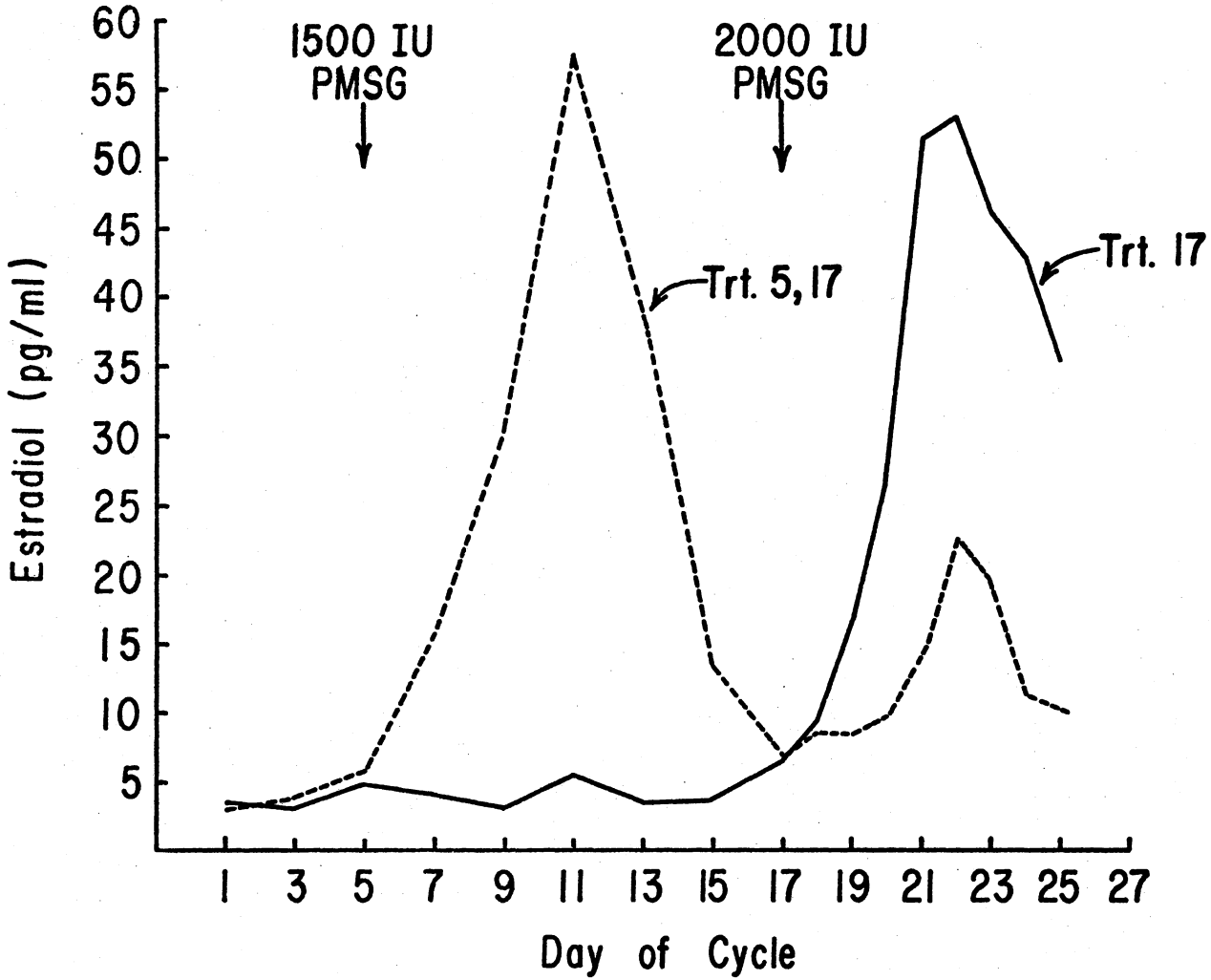

Figure 10. Plasma Estradiol in Heifers Treated with PMSG.

these data, however, since the normal pre-estrus surge of estradiol could be confounded with an estradiol rise resulting from PMSG. Estradiol values for cows on day 17 were 2.8 $\pm$ .4 in Trt 17 compared to 2.8 $\pm$ .8 in Trt 5, 17 ($P > .25$). By two days after the day 17 treatment, cows on Trt 17 had significantly ($P < .025$) more estradiol than did those on Trt 5, 17 (7.2 $\pm$ .9 vs 4.2 $\pm$ .5 pg/ml, respectively). Similarly, in the heifer groups animals on Trt 17 had almost twice as much estradiol as did those on Trt 5, 17 on day 19 (17.2 $\pm$ 3.2 vs 8.3 $\pm$ 2.3 pg/ml, respectively; $P < .05$). These differences remained apparent until approximately day -5 for cows and day -2 for heifers (Figures 11 and 12 and Table XV and XVI) at which time no significant differences in estradiol concentrations between treatment groups were apparent. On the day of estrus, all treatment groups showed 10-15 pg/ml estradiol. These data indicate that follicular growth in both cows and heifers can be stimulated by PMSG given either early or late in the estrous cycle. This study was not designed to permit determining whether the follicles stimulated by the day 5 injection were the same as those that were subsequently ovulated as suggested by Schilling and Holm (1963). Laster et al. (1971) indicated that these may be two different sets of follicles. Similar estrogen increases after PMSG late in the cycle were reported for cattle (Hendricks et al., 1973) and swine (Guthrie et al., 1974).

Hendricks et al. (1973) reported that plasma estrogen was not correlated with follicle number. The data from this study support this suggestion since no relationship was apparent between peak estradiol after PMSG on day 5 or 17 and corpora lutea number. Some evidence was observed in cows on Trt 17, however, concerning a slight relationship between peak estradiol and number of corpora lutea plus number of

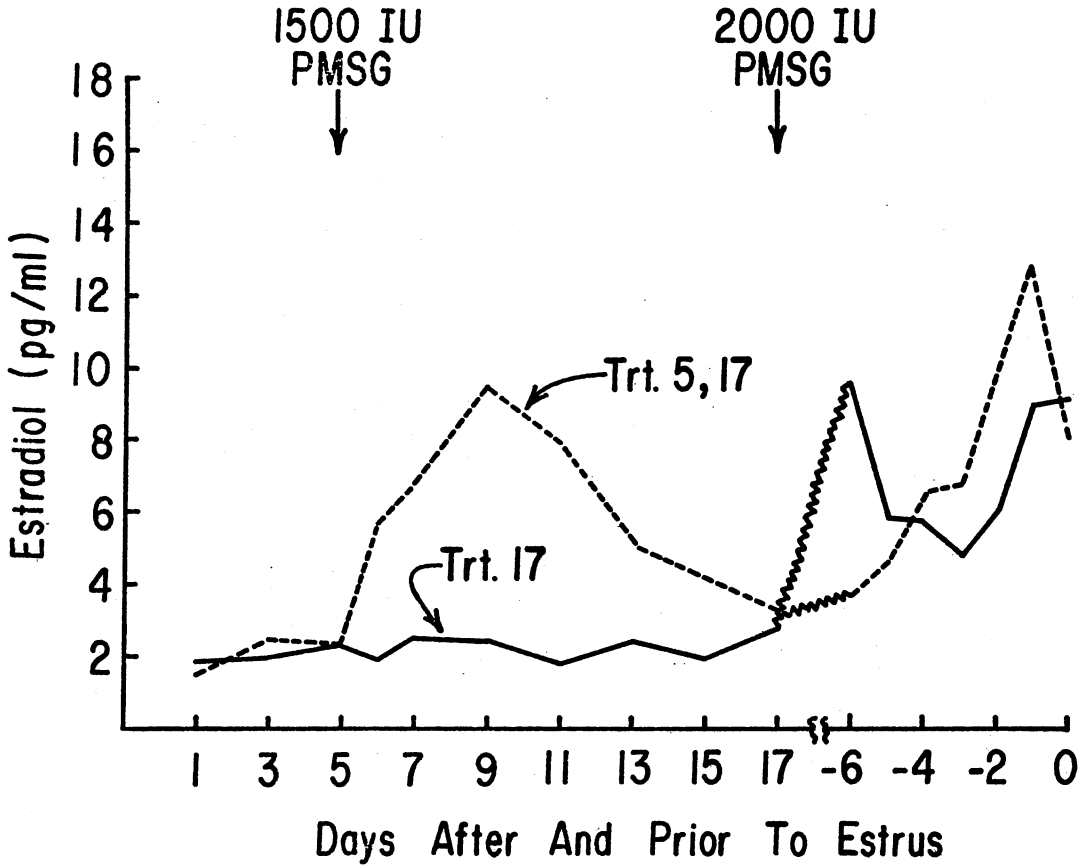

Figure 11. Plasma Estradiol in Cows Showing Estrus After Treatment with PMSG.

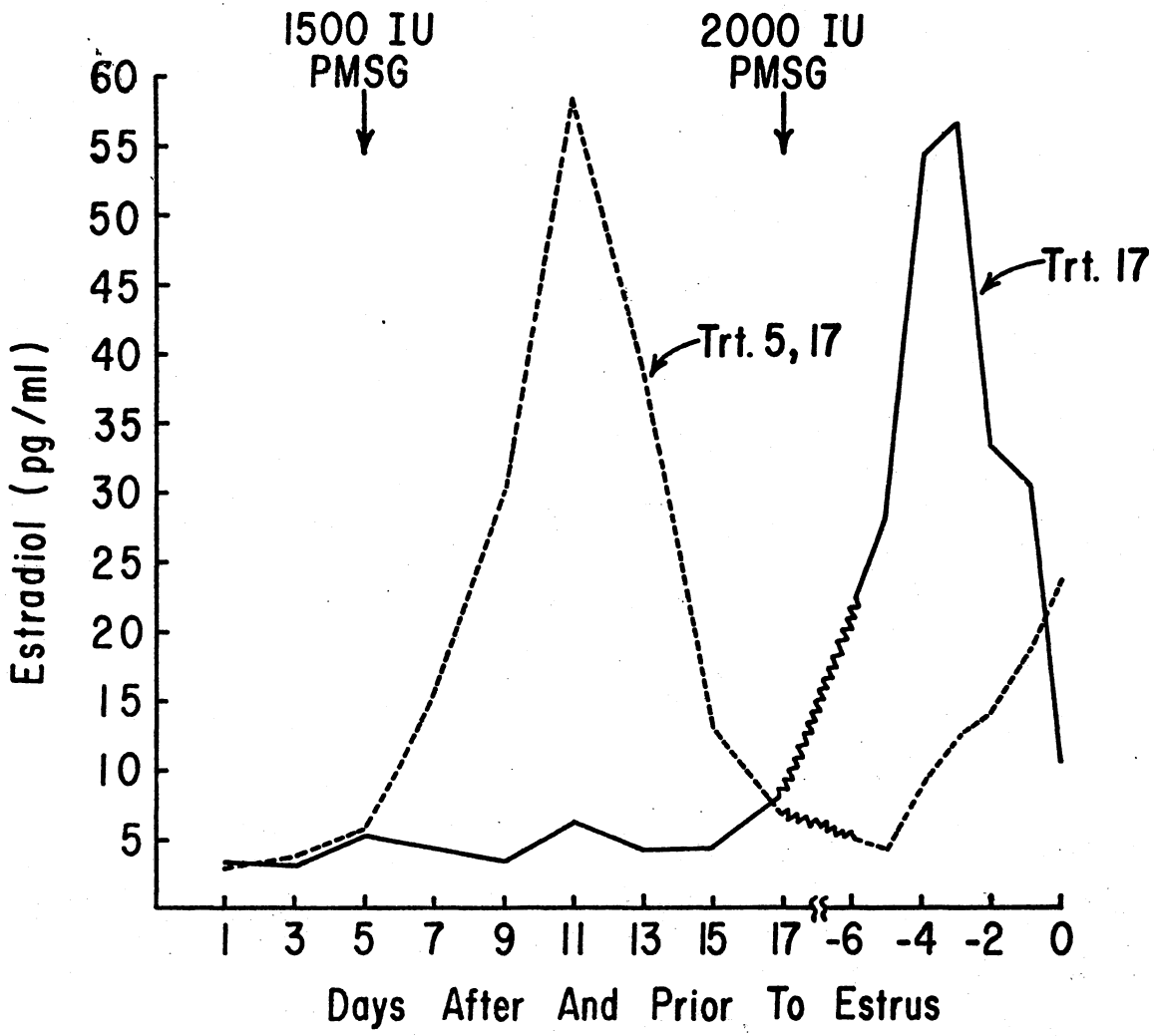

Figure 12. Plasma Estradiol in Heifers Showing Estrus After Treatment with PMSG.

follicles greater than 10 mm in diameter present at laparotomy ($r = .58$; $n = 16$; $P < .05$). A similar relationship in heifers on Trt 17 only approached significance. Similar correlations for peak estradiol after the day 5 injection in all animals on Trt 5, 17 were low and nonsignificant suggesting that follicular growth stimulated early in the cycle may not be related to the growth of follicles that ovulated. This supposition is clouded, however, by the fact that the follicles observed at laparotomy 12 days after estrus may not have been the same as those stimulated during the previous cycle with PMSG.

Several workers cited earlier have suggested that animals may develop a refractory condition to repeated injections of PMSG. This study yielded inconclusive evidence concerning this question. All cows had been treated in previous years with PMSG. The superovulatory response of cows on Trt 17 was low but acceptable; while the response of cows on Trt 5, 17 was low and unacceptable with 80% of these animals having no or one egg ovulations. Similarly, an increase in plasma estradiol after PMSG was evident but not nearly the magnitude of that seen in the heifers. In addition, heifers on Trt 5, 17 did not show as great an increase in estradiol after the second injection as that seen after the first injection; but the superovulatory response of Trt 5, 17 heifers was at least equal to that seen in Trt 17 heifers. These observations would, therefore, possibly suggest that repeated injections of PMSG over several breeding seasons may lead to a refractory state; but the full effect of this condition may require at least two injections in those animals previously immunized. These data would not, however, support the contention that two injections during the same estrous cycle of an animal produces refractoriness if that animal had not been treated previously. Studies

in which animals are laparotomized and follicles observed after a series of injections might yeild useful information on this subject.

Luteinizing Hormone

Plasma LH was determined from the once-daily bleedings as described earlier. Swanson and Hafs (1971) demonstrated that LH began a gradual but nonsignificant rise five days before estrus which was followed by a highly significant peak three hours before estrus. This peak was followed by a rapid decline to basal levels four to six hours later in normal cycling animals. Spilman et al. (1973) demonstrated that plasma LH rose sharply on the day after PMSG injection and declined thereafter in prepuberal and mature heifers. Estrogen secretion may regulate LH release in cattle (Wetteman et al., 1972). Spilman et al. (1972) reasoned, therefore, that the LH increase seen after PMSG resulted from endogenous estrogen secretion from PMSG-stimulated follicles. Hendricks et al. (1973) reported an LH rise earlier in PMSG treated heifers than in controls prior to estrus. These previous results would indicate that plasma LH may be affected by PMSG treatment.

LH data from the present study were variable due most likely to the fact that samples were collected on a daily basis. Figures 13 and 14 and Table XVII and XVIII show plasma LH in cows and heifers treated with PMSG. LH was not influenced by treatment within age groups and values throughout the estrous cycle remained approximately 1.0 ng/ml. In addition, no elevations in LH above two ng/ml were evident in any of the treatment groups between days 5 and 7 of the estrous cycle. However, 55% and 67% of the heifers on Trt 17 and Trt 5, 17, respectively, had at least one sample with greater than two ng/ml LH between day 17 and estrus.

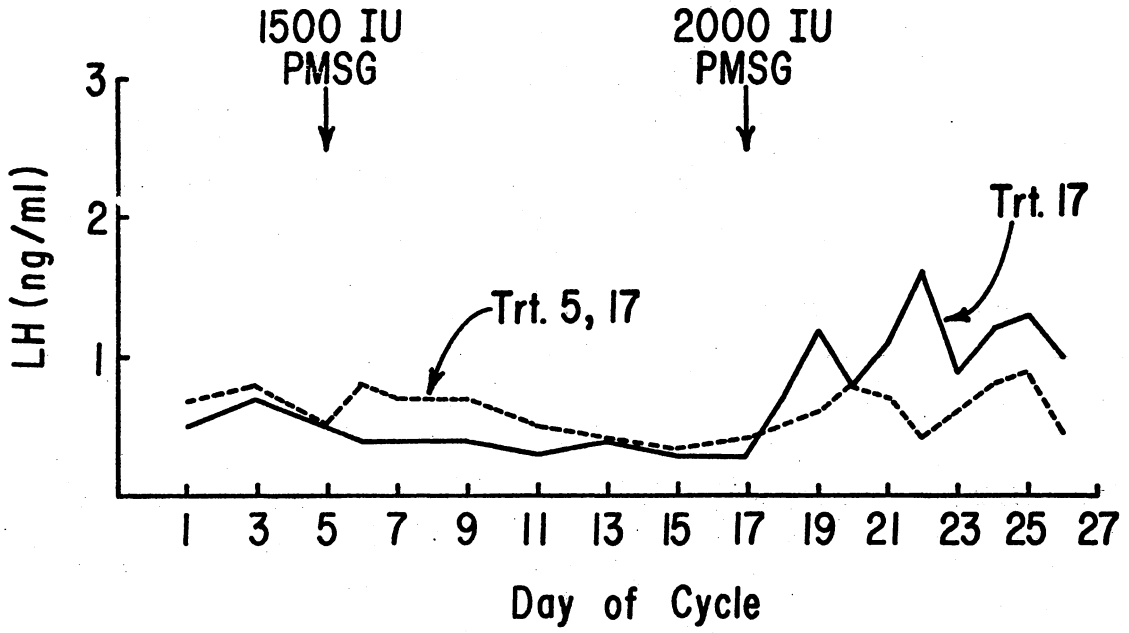

Figure 13. Plasma LH in Cows Treated With PMSG.

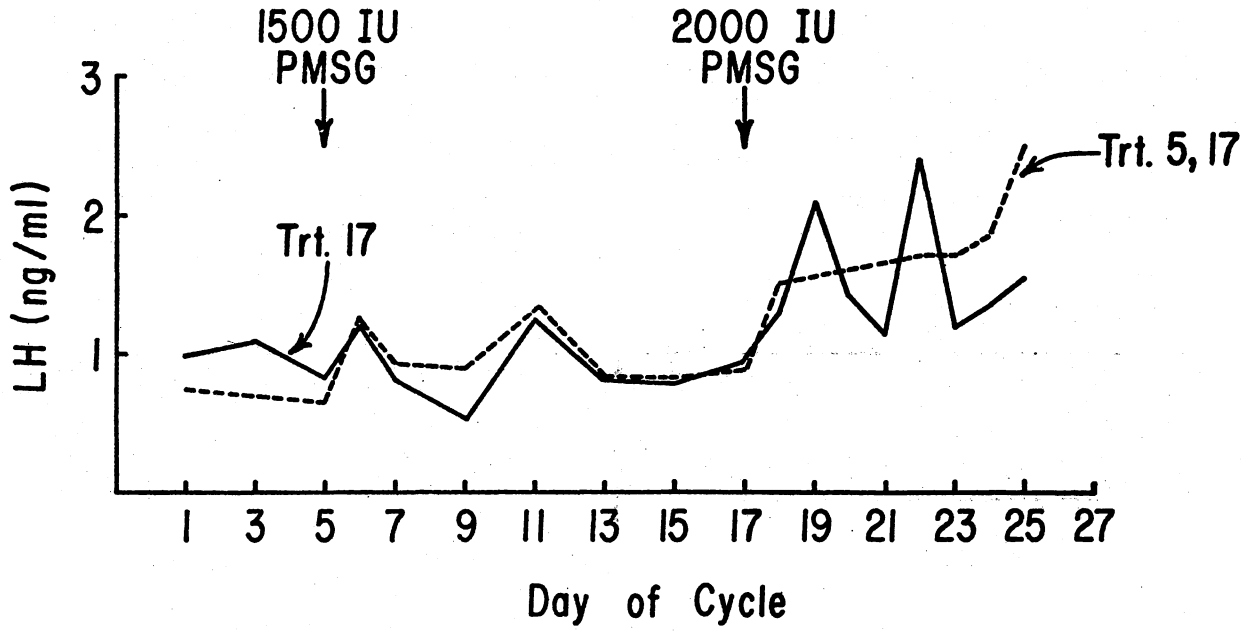

Figure 14. Plasma LH in Heifers Treated with PMSG.

Fifty-six percent of the cows on Trt 17 had similar elevations; while only 13% of the cows on Trt 5, 17 showed increased LH near the expected time of estrus. This failure of the cows on Trt 5, 17 to show elevated LH may at least partially explain the fact that 20% of these animals failed to ovulate after PMSG treatment.

No significant relationship was detected between plasma estrogen and plasma LH or between plasma LH and ovulation rate. Similarly, Hendricks et al. (1973) failed to detect a relationship between plasma LH and reproductive criteria after PMSG treatment.

CHAPTER V

SUMMARY

This study was designed to examine the endocrine and reproductive response of the bovine female to PMSG. Treatments were employed that would allow these responses to be compared in animals treated only during the late luteal phase of the estrous cycle to the response when animals were treated both during the early and the late luteal phases of the cycle. Fifty-four straightbred Angus females including 31 multiparous lactating cows and 23 two year-old heifers were used in this trial. Each age group of animals were randomly allotted to one of two treatment groups after each animal had exhibited at least one normal estrous cycle.

Sixteen cows and 11 heifers received a single subcutaneous injection of 2000 I.U. PMSG on day 17 (day 0 = estrous) of their estrous cycle; while 15 cows and 12 heifers received 1500 I.U. PMSG on day 5 followed by 2000 I.U. PMSG on day 17. All animals were bled by jugular venipuncture at regular intervals during the estrous cycle in which PMSG treatments were imposed, and plasma progesterone, estradiol and luteinizing hormone were subsequently determined by radioimmunoassay. At the first estrus after the last PMSG injection, the animals were bred by natural service to Angus bulls. A high lumbar laparotomy was performed on each animal seven to 11 days after breeding, and a final blood sample was collected 12 days after breeding.

Seventy-five percent of the cows and 63.6% of the heifers on Trt 17 exhibited estrus after PMSG; while 66.7 and 100% of the cows and heifers, respectively, on Trt 5, 17 showed estrus after PMSG. Estrous cycle length was significantly ($P < .05$) increased in cows by giving PMSG both during the early and late luteal phases of the cycle compared to injection only during the late luteal phase ($23.9 \pm .5$ vs $21.9 \pm .7$ days, respectively). Similar results were obtained in the heifer groups ($23.6 \pm .5$ vs 21.6 ± 1.1 days for Trt 5, 17 and Trt 17, respectively; $P < .10$). Conception rates at the first estrus after PMSG as determined by rectal palpation were 62.5% and 45.4% for cows and heifers, respectively, on Trt 17 and 60% and 75% for cows and heifers, respectively, on Trt 5, 17. Similar pregnancy rates determined by examining breeding dates after parturition were 56.2% and 18.2% for Trt 17 and 40% and 33.3% for Trt 5, 17.

Cows treated with a single injection of PMSG had a higher ovulation rate than those treated on days 5 and 17 ($2.2 \pm .4$ vs $1.3 \pm .3$, respectively; $P < .10$). Although the difference in ovulation rate in heifers was not significant ($P > .25$), animals treated on days 5 and 17 tended to have more ovulations than those treated only on day 17 (4.3 ± 1.1 vs 3.1 ± 1.2; respectively). Several animals in each treatment group failed to ovulate with the exception of heifers given two injections of PMSG. Similarly, no treatment differences were evident for animals showing a single ovulation. Fifty percent of the cows and 36% of the heifers on Trt 17 had two, three or four ovulations; while 13% of the cows and 50% of the heifers on Trt 5, 17 had two to four egg ovulations. Approximately six percent of the cows and 20% of the heifers had more than four ovulations. No significant differences in total ovarian diameter or number of follicles less than 10 mm in diameter were detected in any of the treatment groups;

while cows given one injection of PMSG tended to have more follicles greater than 10 mm in diameter than those receiving two injections (2.8 $\pm$ .5 vs 1.5 $\pm$ .4, respectively; $P < .10$).

Cows on Trt 17 showed a near normal progesterone curve with a peak of 8.7 $\pm$ .5 ng/ml on day 15. A slight but nonsignificant increase was seen in these animals on day 20, after which the progesterone values decreased to basal levels. Progesterone did not seem to be adversely affected in cows when PMSG was given late in the luteal phase of the estrous cycle. Cows treated with two injections of PMSG had similar values to day 5 after which a sharp increase was evident to a peak of 14.9 $\pm$ 2.0 ng/ml on day 17 ($P < .005$). A further progesterone increase was not apparent in these animals after the day 17 injection although the initial progesterone surge was maintained for a longer period of time than would be expected in a normal cycling animal.

A similar response to early luteal PMSG was noted in heifers. By four days after treatment, heifers on Trt 5, 17 had a progesterone value of 14.8 $\pm$ 1.2 ng/ml compared to 7.8 $\pm$ .7 ng/ml at the same time for Trt 17. These high values were maintained in Trt 5, 17 until approximately three days after the second injection after which they fell to basal levels. Progesterone in the single treated heifers increased normally to 10.5 $\pm$ 1.1 ng/ml on day 13 and remained at that level until day 20 then increased to 14.5 $\pm$ 3.2 ng/ml four days after treatment. This increase was followed by a rapid decline to basal levels at the time of estrus. These data indicate that a progesterone response can be elicited in heifers by giving PMSG either early or late in the luteal phase of the estrous cycle.

Some degree of relationship was noted between plasma progesterone after day 17 and the interval from PMSG to estrus. In addition, plasma progesterone 12 days post breeding was significantly related to number of corpora lutea ($r = .75$, $P < .01$). No apparent realtionship between peak progesterone after PMSG and ovulation rate was detected.

Plasma estradiol in cows and heifers that were not treated during the early luteal phase of the estrous cycle generally remained below 5 pg/ml from day 1 through day 17. Animals receiving PMSG on day 5, however, showed dramatic increases in estradiol by 2 days after treatment in the cow ($P < .005$) and one day after treatment ($P < .025$) in the heifer groups suggesting increased follicular growth after PMSG. None of the animals that were treated on day 5 exhibited estrus as a result of increased estradiol which was due most probably to the concurrent rise in progesterone at the same time.

Estradiol increases were also evident after all animals were treated on day 17. By two days after the day 17 treatment, cows on Trt 17 had significantly more estradiol ($P < .025$) than did those on Trt 5, 17 ($7.2 \pm .9$ vs $4.2 \pm .5$ pg/ml, respectively). Heifers on Trt 17 had almost twice as much estradiol on day 19 as did those on Trt 5, 17 (17.2 ± 3.2 vs 8.3 ± 2.3 pg/ml, respectively). These differences remained apparent until day -5 for cows and day -2 for heifers at which time no treatment differences were detected. These data indicate that follicular growth can be stimulated in both cows and heifers by PMSG given during either the early or late luteal phases of the estrus cycle. Considering that all the cows and none of the heifers had been treated in previous years with PMSG, one might speculate by examining the ovulation and estradiol data that animals treated over a several year period may develop some

degree of refractoriness to the second of two PMSG injections given in the same cycle.

No relationship was apparent between peak estradiol concentration after PMSG on day 5 or 17 and corpora lutea number. A slight relationship between animals treated on day 17 was evident between peak estradiol and number of corpora lutea considered in conjunction with follicles greater than 10 mm in diameter. No such relationship after the day 5 injection was seen in the animals on Trt 5, 17.

Plasma LH concentrations were highly variable throughout the estrous cycle in which PMSG injections were imposed and generally remained below 1.0 ng/ml. No significant differences in plasma LH were detected in any of the treatment groups suggesting that PMSG does no influence plasma LH values. However, LH concentration seems to be highly variable and rapidly metabolized suggesting that once daily bleedings may not be sufficient to detect changes in LH associated with PMSG treatment.

The data accumulated in this study suggest that PMSG has profound effects on both the ovarian and endocrine response of the bovine female. Research efforts aimed at controlling the progesterone and estradiol concentrations around the time of the post-PMSG estrus may answer some of the questions associated with inducing multiple pregnancies in cattle.

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APPENDIX

TABLES

TABLE VI

STOCK BUFFER SOLUTIONS USED IN IMMUNOASSAY PROCEDURES

A. Monobasic Sodium Phosphate Buffer (0.5 M)

Weigh 69.0 g $NaH_2PO_4 \cdot H_2O$ (monobasic) and dilute to 1,000 ml with glass distilled water. Store at 5 C.

B. Dibasic Sodium Phosphate Buffer (0.5 M)

Weigh 71.0 g andydrous or 134 g heptahydrate Na_2HPO_4 (dibasic) and dilute to 1,000 ml with glass distilled water. Store at 5 C.

C. Stock Phosphate Buffered Saline (0.05 M)

1. 120 ml of Monobasic Buffer (A)
2. 240 ml of Dibasic Buffer (B)
3. 143 g sodium chloride, NaCL
4. 1.75 g Thimerosol (Merthiolate)
5. Add glass distilled water to a final volume of 3500 ml
6. Check pH 7.0; adjust if necessary using sodium hydroxide or phosphoric acid and store at 5 C

TABLE VII

WORKING BUFFER SOLUTIONS USED IN IMMUNOASSAY PROCEDURES

A. Phosphate Buffered Saline Working Solution (PBS)

Dilute one part PBS Stock with four parts glass distilled water.

B. Phosphate Buffered Saline Plus 0.1% Gelation (PBS + Gel)

Weigh one gram Knox Gelatin and dilute to 1,000 ml with PBS Working Solution.

C. Phosphate Buffered Saline Plus Ethylenedinitrilotetra-acetic acid-Disodium Salt (PBS + EDTA) (0.05 M)

1. Weigh 18.61 g disodium EDTA
2. Add about 800 ml PBS, warm and stir until dissolved
3. Adjust pH to 7.0 by adding 5 N NaOH while stirring
4. Adjust volume to 1,000 ml with PBS and store at 5 C

TABLE VIII

PREPARATION OF LIQUID SCINTILLATION FLUIDS

A. Steroid Counting Fluid

Toluene (Scintillation Grade)	3800 ml
PPO	15 g
POPOP	0.15 g

Mix until dissolved

B. Protein Binding Counting Fluid

Toluene (Scintillation Grade)	3000 ml
PPO	21.7 g

Mix until dissolved

TABLE IX

PLASMA PROGESTERONE IN COWS TREATED WITH PMSG

Day of Cycle	Trt 17 (ng/ml)	Trt 5, 17 (ng/ml)	Probability (Type I)
1	0.7 ± .1[a]	0.6 ± .1	> .25
3	1.8 ± .2	1.9 ± .3	> .25
5	3.8 ± .3	4.1 ± .3	> .25
6	5.1 ± .4	6.1 ± .5	> .10
7	6.5 ± .3	8.7 ± .7	< .025
9	7.6 ± .6	12.5 ± .8	< .005
11	7.6 ± .5	13.6 ± 1.1	< .005
13	8.1 ± .7	13.5 ± 1.0	< .005
15	8.7 ± .5	14.3 ± 1.7	< .005
17	7.9 ± .6	14.9 ± 2.0	< .005
18	7.5 ± 1.0	14.3 ± 1.9	< .005
19	5.6 ± 1.1	13.1 ± 2.4	< .05
20	6.1 ± 1.6	10.9 ± 1.9	< .10
21	3.6 ± 1.0	6.9 ± 1.6	> .10
22	2.4 ± 1.3	4.7 ± 1.3	> .25
23	2.3 ± 1.1	4.6 ± 1.7	> .25
24	2.8 ± 1.8	2.0 ± .8	> .25
25	3.2 ± 2.1	1.6 ± .6	> .25

[a]Mean ± S. E.

TABLE X

PLASMA PROGESTERONE IN COWS SHOWING ESTRUS AFTER TREATMENT WITH PMSG

Day of Cycle	Trt 17 (ng/ml)	Trt 5, 17 (ng/ml)	Probability (Type I)
1	0.6 ± .1[a]	0.6 ± .1	> .25
3	1.8 ± .2	2.0 ± .4	> .25
5	4.0 ± .3	4.2 ± .3	> .25
6	5.4 ± .5	7.0 ± .5	< .05
7	6.7 ± .4	9.4 ± .9	< .025
9	7.8 ± .7	13.2 ± .9	< .005
11	7.6 ± .6	14.5 ± 1.2	< .005
13	7.8 ± .8	14.5 ± 1.0	< .005
15	8.8 ± .6	15.2 ± 2.3	< .025
17	7.6 ± .7	16.0 ± 2.1	< .005
-6	8.2 ± 1.2	13.5 ± 2.6	< .05
-5	8.2 ± 1.2	14.4 ± 2.1	< .025
-4	8.2 ± 1.4	9.6 ± 1.0	< .025
-3	6.3 ± 1.0	7.3 ± 2.0	> .10
-2	2.4 ± .5	4.1 ± 1.4	> .10
-1	1.0 ± .1	2.2 ± 1.1	> .10
0	0.8 ± .1	1.4 ± .5	> .10

[a]Mean ± S. E.

TABLE XI

PLASMA PROGESTERONE IN HEIFERS TREATED WITH PMSG

Day of Cycle	Trt 17 (ng/ml)	Trt 5, 17 (ng/ml)	Probability (Type I)
1	0.9 ± .1[a]	0.9 ± .2	> .25
3	1.5 ± .2	2.0 ± .4	> .25
5	3.5 ± .4	4.1 ± .5	> .25
6	4.3 ± .5	6.7 ± .8	< .05
7	6.6 ± .7	9.6 ± 1.1	< .05
9	7.8 ± .7	14.8 ± 1.2	< .005
11	9.8 ± .9	19.4 ± 1.2	< .005
13	10.5 ± 1.1	19.9 ± 1.0	< .005
15	10.0 ± .9	19.2 ± 1.8	< .005
17	9.3 ± 1.2	16.1 ± 1.3	< .005
18	9.9 ± 1.9	18.3 ± 1.6	< .005
19	10.0 ± 2.1	14.8 ± 1.8	> .10
20	9.8 ± 2.0	11.4 ± 2.2	> .25
21	14.5 ± 3.2	7.4 ± 2.2	> .10
22	10.0 ± 2.4	4.2 _ 1.3	< .05
23	6.6 _ 2.7	2.6 _ .7	> .10
24	3.5 ± 1.4	1.4 ± .3	> .10
25	2.6 ± .5	1.2 ± .2	< .05

[a]Mean ± S. E.

TABLE XII

PLASMA PROGESTERONE IN HEIFERS SHOWING ESTRUS AFTER TREATMENT WITH PMSG

Day of Cycle	Trt 17 (ng/ml)	Trt 5, 17 (ng/ml)	Probability (Type I)
1	1.0 ± .1[a]	0.9 ± .2	> .25
3	1.5 _ .3	2.0 ± .4	> .25
5	3.3 ± .5	4.1 ± .5	> .25
6	4.3 ± .7	6.7 ± .8	< .10
7	6.3 _ 1.0	9.6 ± 1.1	= .05
9	7.1 ± 1.0	14.8 ± 1.2	< .005
11	8.7 ± 1.1	19.4 ± 1.2	< .005
13	9.7 _ 1.6	19.9 ± 1.0	< .005
15	9.0 ± 1.3	19.2 ± 1.8	< .005
17	8.1 ± 1.7	16.1 ± 1.3	< .005
-6	13.2 ± 1.8	17.4 ± 2.0	> .10
-5	9.9 ± 1.6	15.3 ± 1.7	< .025
-4	15.8 ± 2.3	13.0 ± 1.8	> .25
-3	8.9 ± 1.9	8.3 ± 1.0	> .25
-2	4.0 ± 1.1	4.6 ± 1.0	> .25
-1	1.4 ± .2	1.7 ± .2	> .25
0	1.1 ± .2	1.1 ± .1	> .25

[a]Mean ± S. E.

TABLE XIII

PLASMA ESTRADIOL IN COWS TREATED WITH PMSG

Day of Cycle	Trt 17 (pg/ml)	Trt 5, 17 (pg/ml)	Probability (Type I)
1	2.0 ± .3[a]	1.6 ± .2	> .10
3	2.1 ± .2	2.2 ± .4	> .25
5	2.7 ± .4	2.1 ± .3	> .10
6	2.1 ± .4	4.9 ± .6	< .005
7	2.4 ± .5	5.4 ± 1.2	< .025
9	2.7 ± .4	10.6 ± 1.6	< .005
11	2.0 ± .2	9.9 ± 2.6	< .005
13	2.4 ± .3	4.8 ± 1.4	> .10
15	2.2 ± .2	3.4 ± 1.0	> .10
17	2.8 ± .4	2.8 ± .8	> .25
18	5.0 ± .5	4.0 ± 1.3	> .25
19	7.2 ± .9	4.2 ± .5	< .025
20	8.2 ± 1.0	4.6 ± .6	< .025
21	11.0 ± 2.7	5.7 ± 1.1	< .10
22	8.9 ± 1.3	7.4 ± 1.7	> .25
23	15.8 ± 4.2	9.6 ± 2.1	> .10
24	11.9 ± 7.7	12.7 ± 3.4	> .25
25	17.8 ± 14.8	14.8 ± 4.7	> .25

[a]Mean ± S. E.

TABLE XIV

PLASMA ESTRADIOL IN HEIFERS TREATED WITH PMSG

Day of Cycle	Trt 17 (pg/ml)	Trt 5, 17 (pg/ml)	Probability (Type I)
1	3.6 ± .5[a]	2.9 ± .4	> .25
3	3.2 ± .4	3.8 ± .3	= .25
5	5.0 ± .4	5.6 ± .7	> .25
6	4.6 ± .5	10.0 ± 1.8	< .025
7	4.1 ± .4	15.1 ± 1.7	< .005
9	3.0 ± .4	30.0 ± 4.9	< .005
11	5.4 ± .8	57.6 ± 14.4	< .005
13	3.5 ± .7	38.8 ± 13.7	< .05
15	3.9 ± .9	13.3 ± 7.3	= .25
17	6.5 ± 1.8	6.7 ± 2.0	> .25
18	9.6 ± 1.4	8.3 ± 2.8	> .25
19	17.2 ± 3.2	8.3 ± 2.3	< .05
20	27.0 ± 7.7	9.8 ± 2.2	< .05
21	51.5 ± 18.9	14.3 ± 3.7	< .05
22	53.1 ± 18.9	22.5 ± 6.7	= .10
23	46.1 ± 18.8	19.0 ± 8.6	> .10
24	42.9 ± 17.5	11.2 ± 3.8	> .10
25	35.3 ± 32.2	10.1 ± 3.4	> .25

[a]Mean ± S. E.

TABLE XV

PLASMA ESTRADIOL IN COWS SHOWING ESTRUS AFTER TREATMENT WITH PMSG

Day of Cycle	Trt 17 (pg/ml)	Trt 5, 17 (pg/ml)	Probability (Type I)
1	1.9 ± .3[a]	1.5 ± .2	= .25
3	2.0 ± .2	2.5 ± .4	> .10
5	2.3 ± .4	2.3 ± .4	> .25
6	1.9 ± .4	5.7 ± .6	< .005
7	2.5 ± .7	6.7 ± 1.6	< .025
9	2.4 ± .5	9.6 ± 1.8	< .005
11	1.8 ± .2	7.9 ± 2.0	< .005
13	2.4 ± .3	5.1 ± 2.0	> .10
15	1.9 ± .2	4.1 ± 1.4	> .10
17	2.7 ± .6	3.3 ± 1.1	> .25
-6	9.6 ± 1.5	3.7 ± .5	< .005
-5	5.8 ± .9	4.6 ± 1.4	> .25
-4	5.7 ± 1.7	6.5 ± 2.3	> .25
-3	4.8 ± 1.0	6.7 ± 1.9	> .25
-2	6.0 ± 1.1	9.9 ± 2.9	> .10
-1	8.9 ± 1.3	12.9 ± 4.0	> .25
0	9.1 ± 2.2	8.0 ± 2.5	> .25

[a]Mean ± S. E.

TABLE XVI

PLASMA ESTRADIOL IN HEIFERS SHOWING ESTRUS AFTER TREATMENT WITH PMSG

Day of Cycle	Trt 17 (pg/ml)	Trt 5, 17 (pg/ml)	Probability (Type I)
1	3.3 ± .5[a]	2.9 ± .4	> .25
3	3.1 ± .5	3.8 ± .3	> .10
5	5.1 ± .3	5.6 ± .7	> .25
6	4.8 ± .8	10.0 ± 1.8	= .05
7	4.3 ± .4	15.1 ± 1.7	< .005
9	3.3 ± .6	30.0 ± 4.9	< .005
11	6.7 ± 1.1	57.6 ± 14.4	< .025
13	4.1 ± .9	38.8 ± 13.7	< .10
15	4.2 ± 1.4	13.3 ± 7.3	> .25
17	8.1 ± 2.7	6.7 ± 2.0	> .25
-6	22.0 ± 9.9	5.0 ± .7	< .05
-5	28.6 ± 15.2	4.2 ± .4	< .025
-4	54.2 ± 36.3	8.9 ± 3.0	= .025
-3	56.5 ± 36.0	12.1 ± 2.5	< .025
-2	33.2 ± 20.1	14.1 ± 3.5	> .10
-1	30.6 ± 10.9	18.8 ± 5.0	> .25
0	10.6 ± 2.2	23.5 ± 6.7	> .10

[a]Mean ± S. E.

TABLE XVII

PLASMA LH IN COWS TREATED WITH PMSG[a]

Day of Cycle	Trt 17 (ng/ml)	Trt 5, 17 (ng/ml)
1	0.5 ± .1[b]	0.7 ± .1
3	0.7 ± .1	0.8 ± .1
5	0.5 ± .1	0.5 ± .1
6	0.4 ± .1	0.8 ± .2
7	0.4 ± .1	0.7 ± .1
9	0.4 ± .1	0.7 ± .1
11	0.3 ± .1	0.5 ± .1
13	0.4 ± .1	0.4 ± .1
15	0.3 ± .1	0.3 ± .1
17	0.3 ± .1	0.4 ± .1
18	0.7 ± .2	0.5 ± .1
19	1.2 ± .6	0.6 ± .1
20	0.8 ± .2	0.8 ± .2
21	1.1 ± .2	0.7 ± .2
22	1.6 ± 1.0	0.4 ± .1
23	0.9 ± .4	0.6 ± .2
24	1.2 ± .7	0.8 ± .2
25	1.3 ± .5	0.9 ± .4

[a]No Significant Treatment Differences
[b]Mean ± S. E.

TABLE XVIII

PLASMA LH IN HEIFERS TREATED WITH PMSG[a]

Day of Cycle	Trt 17 (ng/ml)	Trt 5, 17 (ng/ml)
1	1.0 ± .3[b]	0.8 ± .2
3	1.1 ± .2	0.7 ± .1
5	0.8 ± .3	0.6 ± .2
6	1.2 ± .4	1.2 ± .1
7	0.8 ± .2	0.9 ± .2
9	0.5 ± .2	0.9 ± .1
11	1.2 ± .6	1.3 ± .1
13	0.8 ± .2	0.8 ± .1
15	0.8 ± .2	0.8 ± .1
17	1.0 ± .2	0.9 ± .1
18	1.3 ± .2	1.5 ± .2
19	2.1 ± .7	1.5 ± .1
20	1.4 ± .5	1.6 ± .2
21	1.2 ± .4	1.6 ± .2
22	2.4 ± 1.0	1.7 ± .2
23	1.2 ± .3	1.7 ± .2
24	1.3 ± .3	1.8 ± .2
25	1.6 ± .4	2.5 ± 1.3

[a]No Significant Treatment Differences
[b]Mean ± S. E.

VITA

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