



Exogenous hormone therapy to control the postpartum interval in beef cows
by Dallas Blair Foster

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE
in Animal Science

Montana State University

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Abstract:

Four studies were conducted to evaluate the effects of exogenous hormone treatments on postpartum parameters in the suckled beef cow. In all experiments treatments started about 22 days postpartum and the following three basic treatments were used: PMSG on day 9 with or without a 6 mg progesterin ear implant (SC21009, G.D, Searle Co,) from day 0 to day 9 (day 0 = start of treatments) and on day 23 PGF2 α in conjunction with the above two treatments. Treatment 3 received only PGF2 α , Ovarian activity was evaluated by rectal palpation and blood progesterone in experiments 1, 3 and 4. In the first experiment, eight cows received the progesterin implant and either 4000, 2000 or 1000 I.U. of PMSG. Six cows received only one of the three doses of PMSG and four animals received neither the implant nor PMSG. Cows in all treatment groups received 25mg PGF2 α and were bred by artificial insemination (AI) following detection of estrus, An ovarian response was evoked following PMSG with and without the progesterin implant. Estrus was observed in only one cow following PGF2 α . Experiment 2 consisted of 27 cows assigned to 3 groups (G1, G2 and G3). G1 received a combined injection of 2.5mg estradiol valerate and 1.5mg progesterin (EV-SC21009) on day 0, the progesterin implant and 1000 I.U. of PMSG. G2 received only the implant and PMSG while G3 received no EV-SC21009, implant or PMSG, All groups received 50mg PGF2 α followed by breeding (AI) for 5 days according to estrous detection and by natural service for the remainder of the 45 day breeding season. Average day of conception for the breeding season for G1, G2 and G3 was 18.2, 9.4 and 23.3, respectively with a significant difference ($P < .05$) between G2 and G3. Experiment 3 consisted of 27 cows assigned to 3 groups (G1, G2 and G3), G1 received the progesterin implant and 1000 I.U. PMSG. G2 received only the PMSG and G3 received neither the implant or PMSG, All groups were given 50mg PGF2 α followed by breeding (AI) at 72 hours with Hereford and 96 hours with Holstein semen followed by natural service to an Angus bull for the remainder of the 45 day breeding season. Serum progesterone concentrations increased from day 9 to 23 in G1 ($P < .05$), G2 ($P < .01$) and G3 ($P < .05$) with concentrations being greater at day 23 in G1. A decrease in progesterone concentrations following PGF2 α was observed between days 23 and 27 in G1 ($P < .05$), G2 ($P < .01$) and G3 ($P < .05$), Forty cows in experiment 4 were assigned to 5 groups (G1, G2, G3, G4 and G5). G1 and G3 received the progesterin implant and 1000 and 750 I.U. PMSG, respectively. G2 and G4 received only the 1000 and 750 I.U. PMSG, respectively; while G5 received neither the implant nor PMSG. All groups received 50mg PGF2 α followed by a similar breeding regime as that described in experiment 3. Serum progesterone concentrations increased from day 9 to 23 in G1, G3 and G4 ($P < .05$). A decrease in progesterone concentrations following PGF2 α was observed between day 23 and 27 in G1 and G2 ($P < .05$) and G3 and G4 ($P < .10$). These studies demonstrate induced ovarian activity and some of the treatments may be useful in shortening the calving interval.

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EXOGENOUS HORMONE THERAPY TO CONTROL
THE POSTPARTUM INTERVAL IN BEEF COWS

by

DALLAS BLAIR FOSTER

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

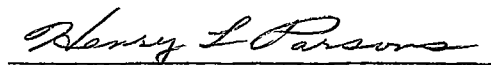
in

Animal Science

Approved:


Chairman, Graduate Committee


Head, Major Department


Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana
July, 1979

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Dr. E. L. Moody for his advice and guidance throughout my graduate program. Appreciation is also expressed to Drs. P. J. Burfening and J. A. McMillan for their assistance and cooperation as committee members.

I would also like to thank David Han for his advice and assistance throughout the course of this study and Melinda Poncelet for her laboratory assistance.

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ABSTRACT

Four studies were conducted to evaluate the effects of exogenous hormone treatments on postpartum parameters in the suckled beef cow. In all experiments treatments started about 22 days postpartum and the following three basic treatments were used: PMSG on day 9 with or without a 6 mg progestin ear implant (SC21009, G.D. Searle Co.) from day 0 to day 9 (day 0 = start of treatments) and on day 23 $\text{PGF}_2\alpha$ in conjunction with the above two treatments. Treatment 3 received only $\text{PGF}_2\alpha$. Ovarian activity was evaluated by rectal palpation and blood progesterone in experiments 1, 3 and 4. In the first experiment, eight cows received the progestin implant and either 4000, 2000 or 1000 I.U. of PMSG. Six cows received only one of the three doses of PMSG and four animals received neither the implant nor PMSG. Cows in all treatment groups received 25mg $\text{PGF}_2\alpha$ and were bred by artificial insemination (AI) following detection of estrus. An ovarian response was evoked following PMSG with and without the progestin implant. Estrus was observed in only one cow following $\text{PGF}_2\alpha$. Experiment 2 consisted of 27 cows assigned to 3 groups (G1, G2 and G3). G1 received a combined injection of 2.5mg estradiol valerate and 1.5mg progestin (EV-SC21009) on day 0, the progestin implant and 1000 I.U. of PMSG. G2 received only the implant and PMSG while G3 received no EV-SC21009, implant or PMSG. All groups received 50mg $\text{PGF}_2\alpha$ followed by breeding (AI) for 5 days according to estrous detection and by natural service for the remainder of the 45 day breeding season. Average day of conception for the breeding season for G1, G2 and G3 was 18.2, 9.4 and 23.3, respectively with a significant difference ($P < .05$) between G2 and G3. Experiment 3 consisted of 27 cows assigned to 3 groups (G1, G2 and G3). G1 received the progestin implant and 1000 I.U. PMSG. G2 received only the PMSG and G3 received neither the implant or PMSG. All groups were given 50mg $\text{PGF}_2\alpha$ followed by breeding (AI) at 72 hours with Hereford and 96 hours with Holstein semen followed by natural service to an Angus bull for the remainder of the 45 day breeding season. Serum progesterone concentrations increased from day 9 to 23 in G1 ($P < .05$), G2 ($P < .01$) and G3 ($P < .05$) with concentrations being greater at day 23 in G1. A decrease in progesterone concentrations following $\text{PGF}_2\alpha$ was observed between days 23 and 27 in G1 ($P < .05$), G2 ($P < .01$) and G3 ($P < .05$). Forty cows in experiment 4 were assigned to 5 groups (G1, G2, G3, G4 and G5). G1 and G3 received the progestin implant and 1000 and 750 I.U. PMSG, respectively. G2 and G4 received only the 1000 and 750 I.U. PMSG, respectively; while G5 received neither the implant nor PMSG. All groups received 50mg $\text{PGF}_2\alpha$ followed by a similar breeding regime as that described in experiment 3. Serum progesterone concentrations increased from day 9 to 23 in G1, G3 and G4 ($P < .05$). A decrease in progesterone concentrations following $\text{PGF}_2\alpha$ was observed between day 23 and 27 in G1 and G2 ($P < .05$) and G3 and G4 ($P < .10$). These studies demonstrate induced ovarian activity and some of the treatments may be useful in shortening the calving interval.

INTRODUCTION

The human population of the world will double its current number in about 35 years and nearly triple in 50 years if the present rates of growth continue. This increase in world population must be accompanied with a parallel increase in food production. The Food and Agriculture Organization estimate that about 450 million and possibly as many as a billion people do not receive sufficient food. To meet the food requirements of today and the future we must seek ways to provide for maximum use of the resources available to us.

Animal products are a major source of the world's diet. In the United States, livestock produce two-thirds of the protein, one-half of the fat and one-third of the energy for human consumption. Animal products in developing countries provide 10% of the calories and 20% of the protein. The United Nations Food and Agriculture Organization estimates that 20% of the population in these developing countries are undernourished and 60% are malnourished. Much of this malnutrition, especially in children, can be attributed to an insufficient protein-energy source.

An increase in beef production is one of the means for keeping pace with the world's increasing food needs. Research can lead to improvements in such areas as animal health, nutrition, genetics and reproduction.

An increase in reproductive efficiency would provide a means in which to enhance beef production. The most important causes of reproductive failure in the United States have been reported 1) the number

of cows in heat the first 21 days of the breeding season, 2) the number of cows conceiving at first service and 3) the number of cows losing calves at or near birth (Wiltbank et al. 1973). The postpartum interval and the problems associated with the early anestrous beef cow is an area in which research could lead to improvements in reproductive efficiency. The use of artificial insemination to enhance availability of quality semen and recent progress in estrus synchronization have given us a means of improving both genetic merit and reproductivity performance.

The following study was designed to determine the influence of various exogenous hormone treatments in decreasing the length of the postpartum interval in the anestrous beef cow. The objectives of this study were to investigate the basic postpartum physiology in the anestrous cow and more specifically to decrease the postpartum interval to the first fertile estrus and conception. This would lead to a reduction in the number of anestrous and low fertility cows present during the breeding season and thereby increase reproductivity efficiency.

LITERATURE REVIEW

Postpartum Interval

The term postpartum interval may be considered to be that interval beginning with parturition and ending with a designated event such as first ovulation, first estrus, completion of uterine involution, first breeding, or conception (Casida, 1968). In contrast to the more precise timing of hormonal regulation occurring during the estrous cycle, the postpartum interval in cattle is generally subject to a greater degree of variation. There are a number of factors which contribute to this variation in interval length including lactation (Wiltbank and Cook, 1958; Wagner and Hansel, 1969; Laster et al. 1973a; Bellows et al. 1974; Radford et al. 1978), plane of nutrition (Wiltbank et al. 1964; Dunn et al. 1969; Oxenreider and Wagner, 1971; Folman, 1973), age (Wiltbank and Cook, 1958; Wiltbank, 1970; Hill et al. 1972; Rosenberg et al. 1977), season (Buch et al. 1955; Rosenberg et al. 1977) disease (Morrow, 1966; Elliot, 1968; Callahan et al. 1971), and dystocia (Rasbech, 1967; Laster et al. 1973b; Foulley et al. 1976).

A better understanding is needed of the anestrous cow and the problems associated with her return to a normal cyclic hormonal pattern. This requires an examination of the endocrine physiology and its impact on the postpartum interval.

Endocrine Physiology

A review of the mechanisms involving the hypothalamus and pituitary in the control of ovarian function is necessary in order to discuss the

anestrous cow and her return to cyclic ovarian activity. Unfortunately, this system is rather complex and it is still not completely clear how the numerous feedback systems operate in order to compensate for abnormal hormonal secretion patterns. One of the disadvantages involved when approaching this problem is acquiring an in vivo model capable of giving a true representation of what is occurring under natural circumstances. The most common model for physiological studies is the rat, but the ewe is more commonly used for studies of reproductive physiology in larger mammals. There are obvious similarities in reproductive physiology of all mammals but one must be careful not to imply too much when comparing species. The following discussion will review the present understanding in the functions associated with follicular growth, estrogen secretion, ovulation, corpus luteum formation, progesterone secretion and corpus luteum regression.

Hypothalamic Function

It is now accepted that the hypothalamus controls the release of pituitary hormones via releasing and inhibiting factors (hormones) secreted by neurons in the basal hypothalamus (Gullemin, 1967; Everett, 1969; Convey, 1973; McCann et al. 1973). These hypothalamic releasing factors pass to the pituitary gland by way of a system of portal vessels capable of providing a rapid and efficient transfer of chemical messages between the brain and the pituitary (Gay, 1972). Observations have led to the prevailing theory that the release of pituitary hormones may be

controlled by varying the concentrations of the specific releasing factors in the blood which flows through the portal system to the pituitary (Gay, 1972; Convey, 1973). The primary hypothalamic releasing factors affecting reproduction are luteinizing hormone releasing factor (LH-RF) and/or follicle stimulating hormone releasing factor (FSH-RF).

There are several possible mechanisms for modification of pituitary hormone release involved in reproductive function. Convey (1973) suggests three possibilities: 1) changes in releasing and inhibiting hormone synthesis and/or release from the hypothalamus; 2) a change in pituitary responsiveness to the hypothalamic releasing factors such as changes in hormone storage, releasability or pituitary cell sensitivity; or 3) a combination of 1 and 2.

Evidence supports the concept of dual sites of target gland feedback. A principal site for feedback of gonadal steroid secretion appears to be at the hypothalamic level as indicated by the ability of hypothalamic lesions to block the stimulatory feedback (Bishop et al. 1972). Supporting evidence also shows the ability of gonadal steroids to modify the content of stored gonadotropin releasing factors and prolactin inhibiting factor in the hypothalamus (Ajika et al. 1972).

McCann et al. (1973) postulates that stimulation or inhibition of LH release may occur by actions at both the hypothalamic and pituitary levels. Evidence from various investigators suggests that steroid levels may act directly on the pituitary, thus bypassing the hypothalamus,

by sensitizing or preventing sensitization of the pituitary to LH-RF (Arimura and Schally, 1970; Hilliard et al. 1971). This theory of pituitary control thereby suggests that the influence of the hypothalamus is not cyclic and regulatory but rather the sensitivity of the pituitary is variable depending on circulating steroid levels. Increased hormone release is then a reflection of the increased sensitivity of the pituitary to the tonic secretion of releasing factors by the hypothalamus.

A different control mechanism may be responsible for the release of prolactin. Much of the evidence has led to the concept that hypothalamic control of prolactin is mediated through an inhibiting factor (PIF) rather than a releasing factor as seen for gonadotropins. This is based on the effects of hypothalamic lesions on the release of gonadotropins and prolactin (McCann and Friedman, 1960; Bishop et al. 1972). Other evidence gives support to the possibility of a prolactin-releasing factor also being involved in prolactin secretion (Mishkin et al. 1968; Bishop et al. 1972; Mitnick et al. 1973).

Pituitary-Gonadal Relationships

The concept of control of gonadal function by the anterior pituitary produces the question of what role the gonads play in controlling gonadotropin secretion rates. This leads to the discussion of feedback control mechanisms.

It is generally accepted that ovariectomy causes an increase in gonadotropin secretion rates in most species including the rat, ewe and

cow (Labhsetwar, 1969; Goding et al. 1970; Roche et al. 1970; Yamamoto et al. 1970; Howland et al. 1971; Snook et al. 1971). This indicates that the gonadal steroids estrogen and/or progesterone in the female are engaged in some type of interacting relationship with the gonadotropins.

When discussing the estrous cycle one can start at any place in time. Since ovulation is the major event associated with the estrous cycle this may be an appropriate place to begin.

It has been known for some time now that ovulation is associated with a large increase in release of luteinizing hormone (LH) from the pituitary gland. In the cow this LH surge in plasma levels is seen at estrus with the LH peak coinciding with, or following the onset of estrus by a few hours, and last for about 8 hours (Hansel and Echternkamp, 1972). Other reports (Swanson and Hafs, 1971) suggest that the LH peak may precede the onset of estrus by approximately 3 hours.

The role of this large LH increase is to bring about ovulation by causing the follicle to rupture and thereby allowing the ovum to be extruded. Ovulation in the cow occurs about 10 to 11 hours following estrus (Thibault and Levasseur, 1975). Follicular rupture is not due to increased intrafollicular pressure but rather to an increase in distensibility (strain/stress) of the follicular wall brought about by the influence of the LH surge (Rondell, 1970). As the atrum enlarges, the follicle wall becomes thinner resulting in a decrease in breaking

strength (Rondell, 1970). It is believed that this increase in distensibility is produced by the action of a collagenase-like enzyme which interferes with the intrafibrillar bonding of collagen in the follicular wall thus decreasing the breaking strength (Rondell, 1970).

It would appear that both estrogen and progesterone play an important part in bringing about the preovulatory surge in LH blood levels. Chenault et al. (1975), Howland et al. (1971) and Henricks (1971a) among others have shown an apparent positive relationship between the rise in estrogen concentration and the large increase in LH levels that occur 3 to 4 days later. Henricks (1971a) also reported that progesterone levels were highest on the fourth day prior to estrus and progressively decreased to very low levels on the day prior to estrus in the cow. This is similar to changes in progesterone activity described by others (Donaldson et al. 1970; Christenson et al. 1971; Snook et al. 1971; Sprague et al. 1971; Robertson, 1972). Henricks (1971a) also observed that plasma estrogen concentrations were not able to rise about 10 pg/ml, as opposed to the 15 to 25 pg/ml levels measured prior to the LH surge, until progesterone decreased to low levels (below 2 ng/ml). These data indicate a negative correlation between progesterone levels and the LH surge and a positive correlation between estrogen and this large preovulatory LH increase.

This raises the question as to what initiates or controls the mechanism that triggers this preovulatory LH surge? It may be the

declining progesterone concentrations, the rising estrogen levels or a combination of these two factors. Hansel and Echternkamp (1972) were able to show that no significant rise in plasma LH occurs until plasma progesterone concentrations decline to below 1 ng/ml, suggesting that the large LH release is inhibited by higher progesterone levels. This is supported by evidence that progesterone levels declined to less than 50 ng/100ml before the beginning of the LH peak (Snook et al. 1971). Henricks et al. (1971a) reported that a rise in estrogen concentrations greater than 10 pg/ml was not detected until progesterone levels were below 2 ng/ml while Fogwell et al. (1978) observed that with constant basal concentrations of LH, systemic concentrations of estradiol-17B can increase in response to withdrawal of progesterone.

Neither the inhibitory or stimulatory feedback of estrogen on LH is affected by exogenous progesterone treatment in the ovariectomized cow (Howland et al. 1971; Hausler and Malven, 1976).

This combination of high progesterone concentrations and low estrogen and LH secretions appears to inhibit follicular development and ovulation in the postpartum cow. Pituitary availability of gonadotropins does not seem to be the limiting factor since responses to exogenous GnRH administration have been seen from as early as 1 to 21 days postpartum (Cummins et al. 1975; Webb et al. 1977; Kesler et al. 1977; Fernandes et al. 1978; Schallenberger et al. 1978). This would indicate that the circulating progesterone and estrogen concentrations

may be a limiting factor in the initiation of cyclic ovarian activity in the anestrous cow.

During lactation progesterone concentrations are low (less than 1 ng/ml) until resumption of cyclic ovarian activity (Donaldson et al. 1970; Robertson, 1972; Corah et al. 1974; Prybil and Butler, 1978). It has also been seen that the first postpartum cycle is usually preceded by a transient increase in plasma progesterone concentrations 3 to 5 days before ovulation (Donaldson et al. 1970; LaVoie et al. 1979). Similar results have been observed by Corah et al. (1974), Schams et al. (1978) and Prybil and Butler (1978). Corah et al. (1974) saw a marked elevation in progesterone only in those cows which conceived suggesting that this period of progesterone elevation may be necessary for conception at first estrus. This is supported by Folman et al. (1973) who observed fertility is influenced by progesterone levels in the preceding estrous cycle. It is not known whether this transient rise in progesterone prior to the first postpartum ovulation is of adrenal or ovarian origin (Corah et al. 1974; Prybil and Butler, 1978).

A similar situation may exist in the prepuberal heifer. Gonzalez-Padilla et al. (1975a) reported no deficiency in circulating levels of pituitary or hypothalamic hormones two months prior to the onset of puberty in heifers. They also reported two distinct LH peaks shortly prior to puberty, each preceded by a period of elevated progesterone. Swanson and McCarthy (1978) were able to elicit LH response to various

doses of estradiol in prepuberal Holstein heifers at 29 ± 1 weeks of age. Donaldson et al. (1970) found that plasma progesterone concentrations at the time of puberty indicated cyclic ovarian activity before the first observed estrus.

Desjardins and Hafs (1968) determined pituitary FSH and LH in heifers from birth through puberty and determined that puberty is associated with a decrease in pituitary LH levels. They also found that plasma levels were inversely related to pituitary levels indicating an increase in circulating gonadotropin levels prior to puberty. Gonzalez-Padilla et al. (1975a) suggests that this establishment of a mature pattern of LH secretion is not a sudden but a gradual event which may be mediated by a stepwise increase in progesterone levels with each elevation serving as a primer for further maturation of the hypothalamo-pituitary-ovarian axis.

The prepuberal heifer is also similar to the anestrous cow in that the ovary is receptive to exogenous hormone therapy prior to cyclic ovarian activity and capable of follicular activity from 11 months up to puberty (Arije et al. 1969; Neville and Williams, 1973; Gonzalez-Padilla et al. 1975b).

Hormone Levels During the Bovine Estrous Cycle

With the advent of radioimmunoassays (RIA) in the latter part of the 1960's an effective and efficient method of measuring levels of blood hormones has been developed. This has given investigators the

opportunity to monitor hormone levels and develop a better understanding of the events connected with the estrous cycle.

Progesterone

Pope et al. (1969) measured plasma progesterone levels in eight normal cycling Friesian cows and found that levels near the time of ovulation were below 2 ng/ml and rose and fell during ovulation cycles as corpora lutea grew and regressed. Mean peak concentrations for 22 cycles were 9 ng/ml and occurred on an average of 13 days after ovulation. Other investigators have reported peak progesterone concentrations ranging from 3 to 12 ng/ml in dairy cows (Henricks et al. 1971a, Robertson, 1972 and Chenault et al. 1975).

Similar results have been noted in beef cows. Donaldson et al. (1970) observed progesterone concentrations were lowest at estrus (.44 ng/ml) and then increased to a maximum of 6.8 ng/ml at day 14 of the 21 day cycle. Concentrations decreased rapidly during the last 4 days before estrus. This agrees with data reported by Christensen et al. (1971) and Henricks et al. (1971b) but is different from that reported by Sprague et al. (1971) who observed maximum progesterone concentrations on days 9 and 12 followed by a decline thereafter to the next estrous.

Progesterone concentrations in both beef and dairy heifers follow a similar pattern as that seen in the mature cow. Snook et al. (1971) measured progesterone concentrations and observed low levels (<1 ng/ml) following estrus followed by a rise from day 5 to 7 of the estrous cycle.

A progesterone peak (about 4.5 ng/ml) was observed at day 15 to 17 followed by a sharp decline. These results are supported by similar data reported by Hackett and Hafs (1969) and Henricks et al. (1971a).

Pope et al. (1969) suggest that the relatively high progesterone concentrations in mid-cycle are involved in the inhibition of ovulation while the rapidly falling levels of progesterone are more closely associated with impending ovulation. This inhibitory action of progesterone on ovulation has also been reported by Chenault et al. (1975) and is supported by Hackett and Hafs (1969) who at the time of high progesterone concentrations, observed a reduction of hypothalamic LRF (lutenizing releasing factor) which is associated with release of LH and ovulation.

Estrogens

Christensen et al. (1971) measured estrogens during the estrous cycle and observed highest concentrations (176 ± 31.6 pg/ml) at about 24 hours before the LH peak associated with ovulation. They also observed elevated estrogen levels (144 ± 44.2 pg/ml) on days 5 and 6 while levels during the remaining days fluctuated between 98 and 133 pg/ml. Henricks et al. (1971a) reported plasma estradiol concentrations less than 10 pg/ml until the day prior to estrus when concentrations were 15 to 25 pg/ml. Estradiol concentrations greater than 10 pg/ml were not detected until progesterone concentrations were below 2 ng/ml. This differs from Chenault et al. (1975) who observed an increase in estradiol concentrations from 2 pg/ml at 4 days prior to ovulation to 6 pg/ml at 12 hours

before ovulation. This was followed by an abrupt increase to 7.4 pg/ml at the LH peak with a return to basal levels at 14 hours after the LH peak. Dobson (1978) reported a similar rise and decline in estradiol concentrations around the time of the preovulatory LH peak. These data support the hypothesis that estradiol or a high estradiol to progesterone ratio is responsible for triggering the preovulatory surge of LH in the cow (Chenault et al. 1975).

Gonadotropins

Snook et al. (1971) monitored LH levels in cycling heifers and observed basal levels for serum LH of 2 to 4 ng/ml. A preovulatory LH peak (7 to 50 ng/ml) was observed in every heifer with LH concentrations declining rapidly following the peak. Christensen et al. (1971) determined LH concentrations in beef cows and observed basal levels of .6 to 1.6 ng/ml with preovulatory LH peaks ranging from 18 to 86 ng/ml. These elevated levels lasted 12.4 ± 1.56 hours. Other investigators have reported LH peaks prior to ovulation ranging from 2.6 to 61 ng/ml and lasting from 6 to 10.5 hours (Sprague et al. 1971, Swanson and Hafs, 1971 and Chenault et al. 1975).

Snook et al. (1971) reported another LH peak 4 to 7 days before ovulation which was thought to be correlated with growth and atresia of anovulatory follicles. Snook et al. (1971) also found a positive correlation between serum LH and progesterone during the luteal phase (day 3 to 15) which would support the hypothesis that LH is luteotropic

in cattle.

Hackett and Hafs (1969) reported pituitary content of FSH declined from a maximum (450 ug) on day 18 of the cycle to a minimum (122 ug) at estrus and, except for a depression on day 4, increased continuously from estrus to day 18. This agrees with data by Dobson (1978) who observed a significant increase in plasma FSH and LH around the time of estrus. Dobson also reported a second FSH peak which occurred about 24 hours after the dual surge of FSH and LH.

Changes in Hormone Levels During Pregnancy, Prepartum and Postpartum Periods:

Progesterone

Pope et al. (1969) monitored plasma progesterone concentrations in dairy cows during pregnancy and the postpartum period and found concentrations during the last month of pregnancy to be in the 2.5 to 7.5 ng/ml range while dropping to .5 to 3 ng/ml at calving. Ovulatory cycles began at about 20 days postpartum with ovulations without estrus occurring more frequently in the earlier part of the postpartum period when progesterone levels were often quite low. Robertson (1972) observed in dairy cows a gradual decline in progesterone levels over a period of 35 to 70 days prior to parturition reaching a level of less than 2 ng/ml the day before parturition. Following parturition, progesterone levels remained less than .5 ng/ml until the first sign of the resumption of cyclic activity which ranged from 20 to more than 60

days. Others have also reported low concentrations of progesterone in dairy cows from parturition to first postpartum estrus or ovulation (Echternkamp and Hansel, 1973, and Stevenson and Britt, 1979).

In beef cows Donaldson et al. (1970) reported plasma progesterone concentrations during early pregnancy to be similar to luteal phase values (4 to 6 ng/ml) of the estrous cycle. These values declined during mid-pregnancy and then increased to a maximum (7 to 8 ng/ml) at about 240 days and declined 2 to 3 weeks before parturition. Schams et al. (1972) observed a similar increase in progesterone levels at the beginning of pregnancy with levels dropping slightly from day 20 to 40. There was a marked drop in concentrations within two days of parturition. Arije et al. (1974) observed progestin concentrations between 5.2 and 10 ng/ml from 21 to 14 days prior to parturition with concentrations being fairly constant at about 6 ng/ml on the day of parturition. These high progestin levels reported by Arije et al. (1974) are much higher than those reported by others at parturition (Donaldson et al. 1970, Schams et al. 1972 and Corah et al. 1974).

Postpartum progesterone concentrations remained low up to 30 to 39 days following parturition in a group of beef-dairy crossbreeds (Schams et al. 1972). These low postpartum progesterone concentrations have been reported by a number of investigators in beef cattle (Donaldson et al. 1970, Arije et al. 1974 and Corah et al. 1974).

Estrogens

Arije et al. (1974) reported estrogen concentrations the last 20 days prepartum ranging between 870 and 1300 pg/ml with a drop to 500 pg/ml at parturition. Estrogen levels decreased with the next 10 days varying from 122 to 382 pg/ml and throughout most of the postpartum period fluctuated around 200 pg/ml with occasional peaks of 300 to 400 pg/ml. Henricks et al. (1972) observed lower estrogen concentrations (510 pg/ml) 14 days prior to parturition at which time there was a continual rise to 2660 pg/ml at parturition. Stellflug et al. (1978) also noted an increase in estrogens, although not as dramatic, from -158 hours prepartum (399 pg/ml) to parturition (501 pg/ml).

Corah et al. (1974) reported estradiol concentrations peaked at parturition (62.4 ± 5.6 pg/ml), fell sharply to 10.6 ± 1.8 pg/ml one day later and fluctuated between 2 and 5 pg/ml through day 21 postpartum. This is similar to estradiol concentrations reported by Echterkamp and Hansel (1973) who also observed a parallel increase in estrone concentrations at this time.

Gonadotropins

Saiduddin et al. (1966) reported pituitary LH was very low at the time of calving, increased considerable by day 10 and thereafter increased gradually up to day 30 postpartum. Morrow et al. (1968) determined the mean interval from first to second postpartum ovulation in dairy cattle to be significantly shorter than the interval from second

to third ovulation. From this it was hypothesized that low postpartum levels of LH may be responsible for deficient maintenance and early regression of the corpus luteum resulting in a short cycle (Morrow et al. 1968).

Schams et al. (1972) reported LH levels ranged around 1 ng/ml throughout pregnancy while Ingalls et al. (1971) observed no measurable change in serum LH from 30 days before parturition until first estrus following parturition. A number of investigators have reported low LH levels ranging from .5 to 2 ng/ml during the early postpartum anestrus period (Foote, 1971, Echternkamp and Hansel, 1973 and Arije et al. 1974).

Schams et al. (1978) observed a great deal of individual variation in LH profiles with regard to onset of elevations, regularity of patterns and peak values. It was also noted that the preovulatory LH peak values tended to increase with an increase in the number of postpartum estrous cycles. The number of episodic LH peaks and average LH concentration appear to be inversely associated with the interval to first ovulation (Stevenson and Britt, 1979). That is to say, the greater the number of episodic LH peaks, the shorter the interval to first ovulation.

Foote (1971) reported FSH concentrations in early postpartum anestrus cows to follow a trend inverse to the relatively low LH activity seen at this time. This was interpreted to mean a pituitary accumulation of LH is needed in preparation for the LH surge connected with ovulation while a continual, more gradual release of FSH is required

to stimulate follicular development in preparation for ovulation.

The Anestrous Cow and Factors Effecting the Postpartum Interval

A number of factors have been shown to have a distinct effect on the postpartum interval in the cow. Because of their effects on the anestrous period, a brief discussion will be allotted to the major factors involved in lengthening or shortening this period.

Lactation

It is generally acknowledged that lactation lengthens the interval from parturition to first estrus (Graves et al. 1968; Oxenreider, 1968; Short et al. 1972; Smith and Vincent, 1972; Laster et al. 1973a; England et al. 1973, LaVoie, 1975). Graves et al. (1968) reported that the interval in the beef cows from parturition to first estrus was 35 to 54 days shorter for suckled vs nonsuckled cows. Bellows et al. (1974) reported early weaned beef heifers exhibited estrus an average of 20.5 days postpartum compared with 43.2 days for suckled dams. Other results (Radford et al. 1978) indicated that nonsuckled cows experienced regular ovarian cycles 10 to 33 days postpartum while suckled cows failed to have normal cycles until at least 14 weeks postpartum. LaVoie et al. (1979) observed an increase in the postpartum interval from 20 days in non lactating beef cows to 38 days in cows suckled ad libitum.

The amount or type of milking stimulus may also be a factor involving the postpartum interval. Wiltbank and Cook (1958) reported

the interval from calving to first estrus was about 30 days longer in nursed than in milked Shorthorn cows. Supportive evidence furnished by Wagner and Hansel (1969) showed the mean interval to the first observed postpartum estrus was 14 days in a group of milked Holstein cows while a similar nursed group had not shown estrus by 30 days postpartum. LaVoie et al. (1979) found an increase, although not significant, in the postpartum interval from 34 days in cows suckled two times daily to 38 days in cows suckled ad libitum.

The exact nature as to how lactation mediates its effect on the postpartum interval is not fully understood. Echternkamp (1978) found an increased incidence of spontaneous LH release and an increase in minimum LH concentrations at 30 days postpartum in nonsuckled ovariectomized heifers when compared to nursed groups. Short et al. (1972) observed lower LH levels in suckled cows while Wagner et al. (1969) reported follicular activity in nursed animals that did not ovulate suggesting the presence of adequate FSH with an absence of LH available for ovulation. These data would suggest that lactation suppresses gonadotropin secretion and/or synthesis in early postpartum cows (Echternkamp, 1978).

Failure of suckled beef cows to respond to exogenous estrogen at 6 weeks postpartum gives evidence of failure in positive estrogen feedback (Radford et al. 1976). Short et al. (1978) demonstrated that lactation induced anestrus in the cow may be at least partially mediated

through elevated prolactin levels as a result of suckling. Ratner and Meites (1964) indicated that in the rat suckling can deplete the hypothalamus of a prolactin inhibiting factor (PIF), thus removing hypothalamic inhibition to prolactin release. Grosvenor (1965) implied that nursing effects prolactin release in the rat by suppressing PIF release from the hypothalamus. Han and Moody (1974) found a significant difference in prolactin levels in suckled and nonsuckled beef cows suggesting reduced prolactin secretion as seen in nonsuckled cows may be necessary for the initiation of estrus. Oxenreider and Wagner (1971) have revealed a correlation between plasma glucose levels and postpartum interval and reported that lactation caused lower plasma glucose levels during the first 56 days postpartum.

Nutrition

The effect of energy level on reproductive performance has been demonstrated by a number of investigators (Wiltbank et al. 1962; Dunn et al. 1969; Lamond, 1970; Oxenreider and Wagner, 1971; Folman et al. 1973). A longer interval from calving to first estrus was seen in beef cows fed one-half the recommended level (low) before calving and the recommended level (high) after calving compared to cows fed a high level before and low level of energy after calving (Wiltbank et al. 1962). Dunn et al. (1969) reported a low energy level before or after calving lengthens the interval from parturition to first estrus.

The nutritional environment both before and after calving is seen

to influence follicular growth and ovulation (Lamond, 1970; Wagner and Oxenreider, 1971). Oxenreider and Wagner (1971) reported that energy intake had a significant effect on plasma glucose levels with low glucose levels increasing the interval to first postpartum ovulation. Dairy cows maintained on a high level of nutrition required fewer inseminations per conception, conceived earlier and had a high progesterone level 23 days earlier than cows on a standard nutrition level (Folman et al. 1973). These data would support the theory that body condition as well as energy intake are important factors affecting the postpartum interval (Wiltbank et al. 1962).

Age

Burke (1954) reported a peak in calf production in 6 and 7 year old cows with a gradual increase in calf crop percentage up to this age followed by a gradual decrease through cows 9 years and older. Wiltbank and Cook (1958) found the interval between calving and first estrus was shorter between the second through seventh lactation. Young cows nursing their first calf required 15 to 25 days longer to return to first postpartum estrus (Wiltbank 1970). Others provide supportive evidence of younger cows having longer intervals to first estrus and conception (Hill et al. 1972; Rosenberg et al. 1977).

Season

Wiltbank and Cook (1958) observed no influence of season on reproductive performance. Buch et al. (1955) indicated the interval to first

estrus was shortest in the summer and autumn while Rosenberg et al. (1977) found the conception rate lower in the summer than in the winter. The rate of uterine involution was seen to be slower in winter and most rapid during the spring and summer (Marian et al. 1968). Morrow et al. (1966) reported no significant affect of season on rate of uterine involution.

Disease-Dystocia

Uterine infection (metritis) was found to delay the first postpartum estrus in dairy cows (Callahan et al. 1971). Elliot et al. (1968) found a rapid decrease in the percent of detectable uterine infections as the interval after parturition increased. They also reported that cows failing to conceive after three or more services had more leukocytes in the endometrium than did cows conceiving at first service.

Postpartum diseases such as milk fever, dystocia and retained fetal membranes slowed ovarian activity in dairy cows (Morrow et al. 1966). Fewer cows (14.4%) experiencing dystocia were detected in estrus during a 45 day artificial insemination period resulting in a 15.6% lower conception rate (Laster et al. 1973b). Foulley et al. (1976) reports a decreased pregnancy rate after difficult calving without a caesarian section while Rasbech (1967) showed a decreased (13 to 14%) conception rate at first, second or third insemination following the birth of a dead calf.

Breed

Beef cows tend to have a longer interval from parturition to the first succeeding estrus than dairy cows. Different studies on dairy cows range from 30 to 72 days with comparable figures for beef cows being 46 to 104 days (Wis. Agr. Exp. Sta. Bull. 270, 1968). Some of these differences in postpartum interval length could be attributed to the differences in management schemes between beef and dairy cows since the degree or type of lactation stimulus, as previously reviewed, is known to effect this interval.

Uterine Involution

Several studies indicate that the involutionary state of the uterus does not exert a significant influence on the length of the postpartum periods to first estrus, ovulation and breeding (Foote et al. 1960a; Perkins and Kidder, 1963; Morrow et al. 1968). Reports on the interval from parturition to uterine involution range from 25 to 50 days (Buch et al. 1955; Morrow et al. 1966; Britt et al. 1974a). Factors affecting uterine involution included age, seasons, dystocia, parity, progestagens and GnRH (Buch et al. 1955; Morrow et al. 1966; Marion et al. 1968; Britt et al. 1974b). Factors reported not to affect uterine involution include seasons, production level, cystic follicles, suckling, anemia, ovariectomy, progestagens and estrogens (Morrow et al. 1966; Marion et al. 1968; Wagner and Hansel, 1969; Britt et al. 1974a)

Exogenous Hormone Therapy to Control the Postpartum Interval

Since the early 1960's efforts have been directed by researchers to shorten the postpartum interval by the use of various exogenous hormone treatments. Most of the treatments have been concerned with steroid, gonadotropin, hypothalamic releasing factors and various combinations of these treatments. A prostaglandin, $\text{PGF}_2\alpha$, is now recognized as a luteolytic agent in the cow (Lauderdale, 1972 and Inskeep, 1973). This has added another dimension to hormone therapy and may be of increased importance in the future.

Steroid Treatments

The effect of a single injection of progesterone (1 mg/lb body weight) at 14 days postpartum in beef cattle was shown to delay ovarian activity and subsequently the expression of first estrus after calving but did not seem to decrease the interval to uterine involution or affect conception rate (Foote et al. 1960b) Ulberg and Lindley (1960) reported that in cycling beef cows daily injections for 14 days of as little as 12.5 mg progesterone per day could inhibit both estrus and ovulation. This daily progesterone administration was also shown to have a depressing effect upon pregnancy rate of animals inseminated during the first estrus after treatment, with higher doses being even more detrimental. Marion et al. (1968) were able to show in dairy cows that higher doses (30 mg/day) of progesterone significantly increased the interval to uterine involution. Silicone rubber implants impregnated with

progesterone were implanted in the side of the neck in beef cows and left in place for 21 days (Maher et al. 1973). The implant synchronized estrus and ovulation but tended to lower conception rates following implant removal. This effect of lowered fertility following mating at first estrus seems to be predominant in a number of progesterone regimes including melengestrol acetate (Hill et al. 1971), impregnated silastic coils (Roche, 1976), progesterone implants (Chupin et al. 1975) and progesterone injections (Ulberg and Lindley, 1960).

Lamond et al. (1971) suggested that progestins such as melengestrol acetate (MGA) may cause suppression of ovarian function in the cow by altering the precise regulatory mechanism exerted by the hypothalamus on secretion of gonadotropins, resulting in an abnormal release of gonadotropins. According to this view, optimum fertility would follow a dose of progestin which causes the least alteration to the normal follicular pattern during treatment. Chupin et al. (1975) found that maximum fertility with a progestin implant (SC21009) resulted from treatments that involved a high dose (12 mg) for a short period of time (7 days).

Because of this fertility problem involving progestagens, a great deal of investigation has been directed towards the use of estrogens used in combination with progesterone to provide an effective treatment in enhancing reproductive efficiency. An injection of .5 to 10 mg estradiol benzoate following 14 daily injections of progesterone

was shown to reduce much of the variation in onset of estrus due to progesterone administration (Ulberg and Lindley, 1960). Saiduddin et al. (1968) reported effects of exogenous progesterone and estradiol at various stages postpartum in beef cows. Their results indicated that cows given estradiol alone or following progesterone treatment resumed ovarian activity earlier after parturition than did untreated or progesterone only treated cows. Treatment given earliest after parturition (5 to 15 days) caused earliest ovulations but cows treated midpostpartum (14 to 24 days) had shortest intervals to conception. It was postulated that progesterone tended to lessen the influence of estrogen in hastening estrus and ovulation, but may have increased the fertility of the estrogen treated cows since conception occurred earliest in cows given both hormones. This agrees with similar data described by Brown et al. (1972) who fed dihydroxyprogesterone acetophenide (DHAP) daily for 9 days (120 mg/day) but gave a 5 mg estradiol valerate injection on the second day of treatment. Brown's group found that animals who started treatment in the 30 to 35 day postpartum interval had the highest first service conception rate when both hormones were administered. Data provided by Hill et al. (1972) showed that progesterone administration in the form of 6-chloro Δ^6 -17 acetoxyprogesterone (CAP) fed daily from parturition for 10, 20, 30 or 40 days followed by a .5 mg estradiol-17 β injection resulted in no improvement in the interval to first estrus or conception. Britt et al. (1974a), starting MGA feedings at 21 days postpartum in

dairy cattle followed by 500 ug estradiol benzoate after 14 days of MGA, found there was no effect of treatment interval to first ovulation but the interval to first conception and services/conception were lower for treated cows. In this last particular case estradiol benzoate after MGA produced similar results as the MGA administration alone.

When reviewing steroid therapy in the anestrus cow it might also be of value to look at estrus synchronization using the cycling cow. In both the anestrus cow and the estrus synchronized cow, control of the estrus cycle is desired, thus, a treatment regime in one may give insight to an effective treatment in the other. Although normal cyclic endocrine control has not been completely reestablished in the anestrus cow, a means of providing a fertile estrus is sought for both models.

Corah et al. (1974) observed that a period of progesterone elevation prior to first estrus may be necessary for conception to occur. This is supported by Folman et al. (1973) who concluded that a high concentration of peripheral progesterone (greater than 4 ng/ml) at the peak of the luteal phase of at least one estrus cycle before insemination was positively associated with conception. Snook et al. (1971) suggested that declining progesterone levels may be one factor responsible for the pre-ovulatory LH surge. With this in mind Folman et al. (1973) postulated that this large drop in progesterone concentrations found in cows that conceived after first insemination may cause the release of greater quantities of gonadotropins affecting ovulation and estrogen production.

Exogenous progesterone may be necessary in the anestrus cow in order to acquire a progesterone "build up". An entirely different function is associated with exogenous progesterone in the synchronized cow. In this case it acts to inhibit estrus and ovulation (Hill et al. 1971a; Roche, 1974; Kinder and Ellington, 1974). The similarity exists between the two uses of progesterone therapy in the fact that a change in the pattern and secretion rate of endogenous hormones may occur (Hill et al. 1971; Henricks et al. 1973a; Hansel et al. 1975).

The stage of the estrous cycle at which treatments are administered in synchronized cows appears to be a factor in determining onset of estrus and affects on fertility (Hill et al. 1971; Roche, 1974; Humphrey et al. 1977). Roche (1974) found that estrous response was higher in animals that received progesterone implants during the luteal phase of the estrous cycle but Hill et al. (1971) observed that synchronization of estrus was better in animals in which MGA treatment was initiated on day four of the cycle. Humphrey et al. (1977) indicated that the presence of a palpable corpus luteum (CL) at initiation of treatment had no effect on the synchronized estrus but pregnancy rates were higher if a CL was present.

The difficulty in interpreting data concerning these types of hormonal treatments lies in all the variations in treatments that can arise when comparing results. This includes the type and dose of progestin administered, the way in which it is applied, the length of time the

animal is exposed to it and the stage of the cycle or postpartum period in which treatment is initiated. In addition to this, the use of supplemental hormones such as various estrogens, gonadotropins and hypothalamic releasing factors compound the number of possibilities that exist. To this one might add another very important factor and that is the time interval from the termination of the postpartum or synchronized treatment to the time of insemination.

Gonadotropic Therapy

The use of gonadotropins in stimulating ovarian activity has been demonstrated by a number of investigators (Oxenreider, 1968; Bellows et al. 1969; Chupin et al. 1975; Mulyehill and Sreenan, 1977; Archbald, 1978; Echterkamp, 1978). Pregnant mare's serum gonadotropin (PMSG), which is known to mimic FSH activity, has been shown to be an effective agent in stimulating follicular development in the cow (Lamond, 1970; Wagner et al. 1973; Henricks et al. 1973b; Gengenbach et al. 1978; Henricks and Hill, 1978). In the anestrous cow, PMSG treatments have produced varying amounts of success in effecting ovarian activity. Oxenreider (1968) maintained that the interval from parturition to first estrus and ovulation were shortened with the use of 2500 I. U. PMSG followed by 2000 I. U. of human chorionic gonadotropin (HCG) 4 days later in the early postpartum cow. Phelps et al. (1976) were able to shorten the interval to first estrus in 45 day postpartum beef cows using a 2000 I. U. dose of PMSG. No other intervals were significantly

affected although there was a slight decrease in the intervals to first ovulation and conception. Improvement in fertility was detected in postpartum beef cows with a 750 I. U. dose of PMSG following a short-term (9 days) progestagen treatment (Mulvehill and Sreenan, 1977). Similar results were obtained earlier by Chupin et al. (1975) using a 800 I. U. dose of PMSG. Brown et al. (1972) found that progesterone and estrogen therapy used in conjunction with 1000 I. U. of PMSG, followed by 750 I. U. HCG at time of insemination did not decrease intervals to estrus, ovulation or conception.

One of the problems concerning PMSG is the great amount of variation associated with dose responses. Lamond and Gaddy (1972) using PMSG doses between 1500 and 2500 I. U. in cycling beef cows showed responses varying from 1 to 28 ovulations with progesterone concentrations ranging from 1 to greater than 180 ng/ml. This agrees with similar data by Gengenbach (1978) illustrating that higher doses (2000 I. U. PMSG) resulted in greater variation in response (1 to 19 ovulations).

It has been suggested that the difficulty in using PMSG is its long half-life (5-6 days; Menzer and Schams, 1979) which might be expected to interfere with the neuroendocrine mechanisms for ovulation (Lamond, 1970). On this assumption one might interpret the higher doses of PMSG to mean a longer exposure of the ovary to the gonadotropic stimulation resulting in a greater degree of interference with the neuroendocrine control of the estrous cycle. Lamond and Gaddy (1972) contend that crowding of

CL's in an ovary as a result of higher PMSG doses, may affect local mechanisms relating to CL maintenance. They suggested that the uterus may possibly be affected by the greatly elevated progesterone concentrations, resulting in premature initiation of CL regression.

In order to create an ovulation rate and progesterone concentrations closer to physiological levels lower doses of PMSG have been tested. Henricks et al. (1973b) reported that a lower dose of PMSG (1600 I. U.) induced ovulation of as many fertile ova per heifer as the higher dose (3200 I. U.) while causing less of a disturbance in the endocrine system. Rajamabedran et al. (1976) also indicated that there was no significant difference in ovarian response between doses of 1000 and 2000 I. U. of PMSG in heifers. Ovarian responses have been reported with lower doses of 500 I. U. (Gengenbach et al. 1978), 750 I. U. (Mulvehill and Sreenan, 1977), 800 I. U. (Chupin et al. 1975) and 900 I. U. of PMSG (Lamond, 1970). Echterkamp (1978) was able to evoke stimulated estrogen secretion and subsequent LH surge in anestrous beef cows (42 days postpartum) with a 750 I. U. dose of PMSG.

Human chorionic gonadotropin (HCG) has biological activity similar to LH and appears to be responsible for maintenance of the corpus luteum of pregnancy in women (Niswender et al. 1972). Moody and Hansel (1967) have demonstrated HCG to be luteotropic in the cow. A 1000 I. U. dose of HCG in conjunction with progesterone therapy and CL removal resulted in an increase in CL size with 2000 and 4000 I. U. doses producing

additional growth responses (Veenhuizen et al. 1972). Use of a 21 day progesterone treatment followed by 6000 I. U. of HCG showed a positive affect on estrus and ovulation in mid to late (56 to 83 days) postpartum beef cows (Maher et al. 1973). Others have also shown that HCG used in conjunction with steroid therapy have resulted in improved pregnancy rates (Wagner et al. 1973; Brown et al. 1973). Wagner et al. (1973) hypothesized that HCG may increase pregnancy rate by 1) synchronizing ovulation relative to insemination and 2) increasing luteal function during the first few days following insemination. Wettemann and Hafs (1973) reported that HCG had little effect on serum LH concentrations or fertility while Kinder and Ellington (1974) showed that the addition of HCG to their progestogen treatment did not produce any marked improvement in estrous synchronization and may have had a detrimental effect.

Hypothalamic Releasing Hormones

Response to hypothalamic releasing hormones in the postpartum cow have been quite variable. In lactating Holstein cows repeated doses of gonadotropin releasing hormone (GnRH) appeared to initiate estrous cycles when administered to cows with ovarian follicular cysts (Kittok et al. 1973). The use of a GnRH implant (100 ug) placed subcutaneously in the ear of 14 day postpartum dairy cows resulted in a shorter interval to first ovulation but did not effect the interval to first estrus (Britt et al. 1974b). Webb et al. (1977) reported that treatment of early postpartum beef cows with an injection of 500 ug GnRH followed by another

injection of GnRH after the first induced luteal phase appeared to initiate normal cyclic activity. Gonadotropin releasing hormone was found to have no positive effect on pregnancy rate when administered following a progestagen treatment in beef cattle (Roche, 1976).

Reports of response to GnRH administration are also quite variable. Webb et al. (1977) reported response to luteinizing hormone-releasing hormone (LH-RH) was reduced up to 20 days postpartum while Fernandes et al. (1978) found an LH response restored between 10 and 20 days postpartum. Kesler et al. (1977) indicated pituitary responsiveness to GnRH was regained by 7 or 8 days postpartum and both Cummins et al. (1975) and Schallenberger et al. (1978) provided evidence that the immediate postpartum period in the cow is capable of giving an LH release similar to that of normal estrus. This wide range of response patterns for LH release with GnRH after parturition might be one of the reasons for the individuality in the initiation of a new estrus in postpartum cattle.

PGF₂ α

It is known that the corpus luteum plays a major role in controlling the length of the estrous cycle and ovulation through its secretory product, progesterone. The control of luteal function is thereby a means of regulating the estrous cycle. This reasoning led to the search of a uterine luteolytic factor capable of causing chemical regression of the CL.

Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) was initially tested by uterine infusion in rats (1 mg/kg/day) on days 5 and 6 of pseudopregnancy (Pharriss and Wyngarden, 1969). They saw a dramatic decrease in the progesterone content of the ovaries on day 7 and a shortening of pseudopregnancy to 7 days from the normal 14 day period. Pharriss et al. (1970) postulated that $PGF_2\alpha$ was a venoconstrictor which is released from the uterus and thereby reduces blood flow to the ovary to cause luteolysis. Pharriss et al. (1970) tested this hypothesis by cannulating the utero-ovarian vein in a group of rabbits. They found a reduced blood flow following a 1 or 2 mg dose of $PGF_2\alpha$. Other possible modes of action of $PGF_2\alpha$ includes a direct lytic effect on the steroidogenic cells (Okamura et al. 1972; Koering et al. 1973) and interference with the hormonal support of the corpus luteum (Jacobson et al. 1974; Hichens et al. 1974).

Luteolysis by $PGF_2\alpha$ has been shown in a variety of species including rats, hamsters, sheep and cattle (Pharriss and Wyngarden, 1969; Labhsetwar, 1971; Goding et al. 1972; Louis et al. 1972; Lauderdale, 1972). Exogenous $PGF_2\alpha$ is known to induce luteal regression in cattle with a functional CL during days 5 through 18 of the estrous cycle (Inskeep, 1973). Prostaglandins in cattle have been utilized primarily with estrous synchronization work and to some degree with superovulation (Archbald, 1976; Gengenbach, 1978; Henricks and Hill, 1978) and the postpartum cow (Manns and Richardson, 1976; Radford et al. 1976; Rovira et al. 1978).

The sequence of events after induction of luteolysis with $\text{PGF}_2\alpha$ seems to be a rapid follicular growth and secretion of estradiol- 17β with the resulting expression of estrus (Archbald, 1976). It appears that if estrus is to occur, follicles larger than 10 mm must be present on the ovary, even though CL regression has occurred (Archbald, 1976). This has led to the use of PMSG in association with $\text{PGF}_2\alpha$ treatments. Gengenbach et al. (1978) using Holstein heifers was able to show a tightly synchronized estrus when a low dose (500 I. U.) of PMSG was employed at the same time or 12 hours prior to $\text{PGF}_2\alpha$. Estrus tended to be later in animals receiving higher doses (1200 and 2000 I. U.) with a greater variation in onset also. They concluded that heifers returning to estrus 2 to 4 days following PMSG or $\text{PGF}_2\alpha$ tended to have lower and more uniform ovulation rates than heifers returning to estrus after 4 days. In heifers approaching puberty a 1750 I. U. dose of PMSG followed by 30 mg of $\text{PGF}_2\alpha$ 18 days later resulted in 15% of the heifers in estrus within 6 days after $\text{PGF}_2\alpha$ treatment. Henricks and Hill (1978) administered to beef heifers PMSG (1600 I. U.) and $\text{PGF}_2\alpha$ together during the mid (day 9 or 10) or late (day 16 or 17) luteal phase of the estrous cycle. They also gave to another group of heifers PMSG at mid and late luteal phase followed by $\text{PGF}_2\alpha$ two days later. They found a suppression of the effects of PMSG when $\text{PGF}_2\alpha$ was given the same day. There was no effect of treatments on pregnancy rate. Chupin and Pelot (1978), using dairy cows, administered steroid and BMSG therapy.

with and without a prostaglandin analog at the end of the treatment period. Their results showed an increased calving rate with the prostaglandin analog.

A number of exogenous hormones have been used in the postpartum cow in conjunction with prostaglandins including PMSG, FSH, HCG, GnRH, estrogens and progestins (Radford et al. 1976; Manns and Richardson, 1976; Rovira et al. 1978; Chupin and Pelot, 1978). All have had varying amounts of success with no one producing a satisfactory therapeutic model for the problems involved in the anestrous cow. The use of these hormone treatments and their ability to produce a fertile estrous cycle is the primary problem to be encountered in the postpartum cow.

MATERIALS AND METHODS

Four experiments were conducted to determine the effects of various exogenous hormone treatments on the anestrous postpartum interval in suckled beef cows. This interval is characterized by low progesterone serum levels as a result of little or no follicular activity occurring in the ovaries. The treatments were designed to stimulate ovarian activity and initiate the resumption of a normal hormonal pattern needed in order for conception to occur. In three of the four experiments ovarian activity was evaluated by rectal palpation and blood samples taken throughout the experimental period.

Experiment 1

Eighteen suckled beef cows ranging from 18 to 24 days postpartum (20.44 average) were randomly allotted to seven treatment regimes (figure 1). Cows ranged from 3 to 6 years of age and calved between March 7 and March 29, 1978. All animals were maintained on the same prepartum and postpartum nutrition scheme.

Eight cows received a 6 mg silastic progesterin implant (G. D. Searle Co.) subcutaneously in the ear on day 0 (day 0 = start of treatment). Implants were removed on day 9 followed by an injection (im) of either 4000, 2000 or 1000 I. U. of PMSG. Two cows received the 4000, 3 cows the 2000 and 3 cows the 1000 I. U. dose of PMSG at the time the implant was removed. Six cows received no progesterin implant but did receive PMSG on day 9 of the treatment schedule. This included 2 cows receiving 4000, 3 cows 2000 and 1 cow 1000 I. U. doses of PMSG. Four animals

served as controls and received neither the implant nor PMSG. All the treated animals and the controls were given (im) a 25 mg dose of $\text{PGF}_{2\alpha}$ on day 23. The cows were observed twice daily for estrus. Following the $\text{PGF}_{2\alpha}$ injection, a sterile bull equipped with a paint marking harness was also used to aid in detecting estrus. The cows were bred by artificial insemination within 8 to 22 hours following estrous detection.

Blood samples (5 to 10 ml) were taken via tail vein on days 0, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 and 41 of the treatment period. Rectal palpation, to evaluate ovarian activity, was conducted on the same days the blood samples were taken. Blood samples were stored at 4°C on the day of collection followed by centrifugation and storage of serum at -25°C the next day.

Progesterone concentrations were measured in serum samples by radioimmunoassay techniques. All serum samples for a particular cow were done in the same assay with 3 cows in each assay. Treatments were randomized throughout the assays in order to minimize assay variation between treatments. A high and low value serum progesterone standard was included at the beginning and end of each assay in order to account for intra- and inter-assay variations.

Experiment 2

This experiment was conducted at the Montana State Prison Ranch, Deer Lodge, Mt., on a group of cows that calved in the latter part of the calving period (April 16 to May 22, 1978). The cows ranged from 3

to 10 years of age and were maintained on a similar prepartum and postpartum nutrition scheme.

Twenty-seven suckled beef cows ranging from 24 to 33 days postpartum (25.56 average) were randomly assigned to 3 treatment regimes (figure 2). Ovarian activity in all cows was assessed prior to treatment by rectal palpation.

Nine cows assigned to group 1 received a 2 ml injection (im) containing 2.5 mg of estradiol valerate (EV) plus 1.5 mg SC21009 (a progestin) in sesame oil with 10% benzyl alcohol (Searle Co.). Following the EV-SC21009 injection on day 0 (day 0 = start of treatment), all cows received a silastic progestin implant (6 mg) subcutaneously in the ear. Implants were removed on day 9 followed by an injection (im) of 1000 I. U. of PMSG. Nine cows in group 2 received a similar treatment excluding the EV-SC21009 injection prior to the progestin implant. Group 3 contained 9 cows that served as controls and received no exogenous hormone treatment up through day 9 of the treatment schedule. All three groups were administered a 50 mg dose of $\text{PGF}_2\alpha$ on day 23. All cows were bred by artificial insemination (AI) according to estrous detection for a 5 day period following $\text{PGF}_2\alpha$. The AI period was followed by exposure to a bull for natural mating for an additional 40 day period. Cows were checked by rectal palpation to determine pregnancy status at 50 days following the end of the breeding period.

The resulting pregnancy information was analyzed using a chi square

analysis to test for a difference in pregnancy rate between the three treatment groups. An analysis of variance was performed across the three groups to test for a difference in the days postpartum to conception and the day of conception of the breeding season.

Experiment 3

Thirty-one suckled beef cows ranging from 16 to 33 days postpartum (21.33 average) were randomly assigned to three treatments (figure 3). The animals consisted of a group of 23 cows acquired from the Montana State Prison Ranch prior to calving. These cows calved between August 22 and September 2, 1978 and ranged from 3 to 10 years of age. They were maintained on a similar prepartum and postpartum nutrition scheme. Another group of cows were comprised of 8 animals from the Montana State University beef herd. These were 3 and 4 year old cows that calved between October 7 and November 28, 1978. Cows within this group were on a similar prepartum and postpartum nutrition scheme.

Twelve cows assigned to group 1 received a silastic progestin implant (6 mg) subcutaneously in the ear on day 0 (day 0 = start of treatment). Implants were removed on day 9 followed by an injection (im) of 1000 I. U. of PMSG. Ten cows in group 2 received no progestin implant but did receive 1000 I. U. of PMSG on day 9 of the treatment schedule. Group 3 consisted of 9 cows which received neither the implant nor PMSG. Laparotomies were performed using lidocaine as a local anesthetic, on 2 cows in each treatment on day 17 to observe ovarian activity. Cows in

all three groups were administered (im) a 50 mg dose of $\text{PGF}_2\alpha$ on day 23. Following $\text{PGF}_2\alpha$ all animals were artificially inseminated at 72 hours with Polled Hereford and 96 hours with Holstein semen. Two days after this insemination period the cows were exposed to an Angus bull for an additional 41 day natural mating period. All animals were rectally palpated to determine pregnancy status at 40 days following the AI period and again at 60 days after the conclusion of the natural mating period. Originally only the 23 Deer Lodge cows were to be used for this study. A problem with implant retention in 4 cows made it necessary to incorporate the 8 Montana State University cows into the study in order to maintain enough cows in each treatment for statistical analysis.

Ovarian activity was evaluated by rectal palpation and blood samples taken via tail vein on days 0, 9, 14, 17, 23, 27, 36, 45, 56 and 69 of the treatment schedule. Blood samples were stored at 4°C on the day of collection followed by centrifugation and storage of serum at -25°C the next day.

Serum progesterone was quantified by radioimmunoassay techniques. All serum samples for a particular cow were done in the same assay with 4 cows in each assay. Treatments were randomized throughout the assays in order to minimize assay variation between treatments. A high and low value serum progesterone standard was incorporated at the beginning and end of each assay in order to adjust for intra- and inter-assay variations.

All progesterone values were adjusted for intra- and inter-assay variation. Progesterone data were analyzed by Kruskal-Wallis non-parametric rank test to test equality of all three treatments at day 9, 14, 23 and 45 of the treatment period. A Wilcoxin signed rank test was used to test for a difference within each treatment group at days 9 vs 23, 9 vs 27, 23 vs 27 and 27 vs 36. An analysis of variance was done across all treatments at day 9, 23 and 27. Chi squared analysis was used to test for a difference at days 14 and 45 across the three treatments. A chi square test was also used to analyse treatments 1 and 2 vs 3 at day 45.

Statistical analysis was also performed on the pregnancy information acquired from the palpation data. Chi squared analysis was used to test for a difference in pregnancy rate between the three groups. An analysis of variance was performed across the three groups to test for a difference in the days postpartum to conception and the day of conception of the breeding season.

Experiment 4

Forty suckled beef cows ranging from 16 to 29 days postpartum (21.63 average) were randomly assigned to five groups (figure 4). Cows ranged from 3 to 10 years of age and calved between March 1 and April 4, 1979. All animals were maintained on a similar prepartum and postpartum treatment scheme.

The eight cows assigned to group 1 received a silastic progestin

implant (6 mg) subcutaneously in the ear on day 0 (day 0 = start of treatment). Implants were removed on day 9 followed by an injection (im) of 1000 I. U. PMSG. Eight cows in group 2 received no progestin implant but did receive 1000 I. U. PMSG on day 9 of the treatment schedule.

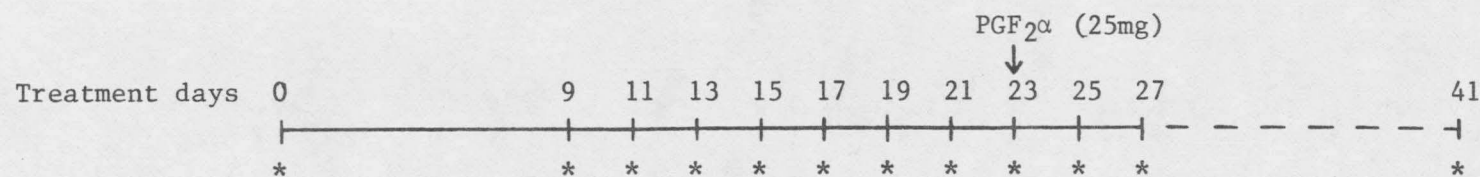
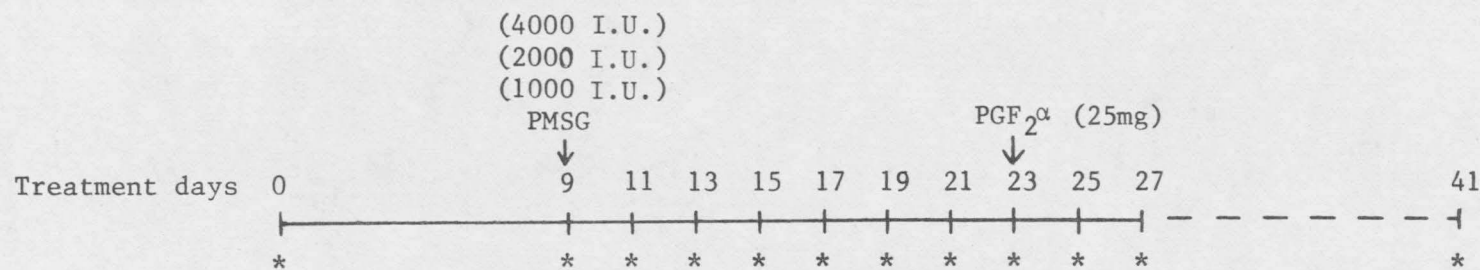
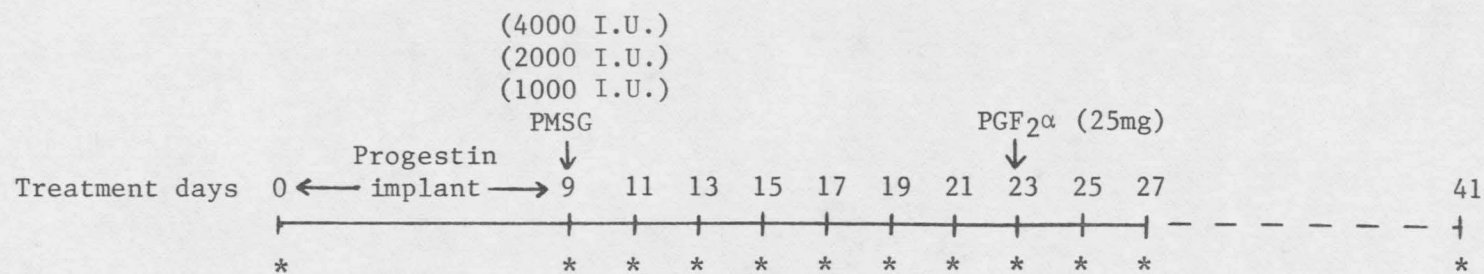
Group 3 consisted of 8 cows receiving the progestin implant on day 0 followed by implant removal and 750 I. U. of PMSG on day 9 while 8 cows assigned to group 4 received only the 750 I. U. of PMSG on day 9. Eight cows in group 5 received neither the progestin implant nor PMSG. Cows throughout all treatments were administered (im) a 50 mg dose of $\text{PGF}_2\alpha$ on day 23. Following $\text{PGF}_2\alpha$ all animals were artificially inseminated at 72 hours with Polled Hereford and 96 hours with Shorthorn semen. Two days after this insemination period the cows were exposed to an Angus bull for an additional 41 day natural mating period. Cows were rectally palpated to determine pregnancy status at 36 days following artificial insemination.

Ovarian activity was evaluated by rectal palpation and blood samples taken via tail vein on days 0, 9, 18, 23, 27 and 36 of the treatment schedule. Blood samples were stored at 4°C on the day of collection followed by centrifugation and storage of serum at -25°C the next day.

Serum progesterone was quantified by radioimmunoassay techniques. All serum samples for a particular cow were done in the same assay with 7 cows in each assay. Treatments were randomized throughout the assays in order to minimize assay variation between treatments. A high and

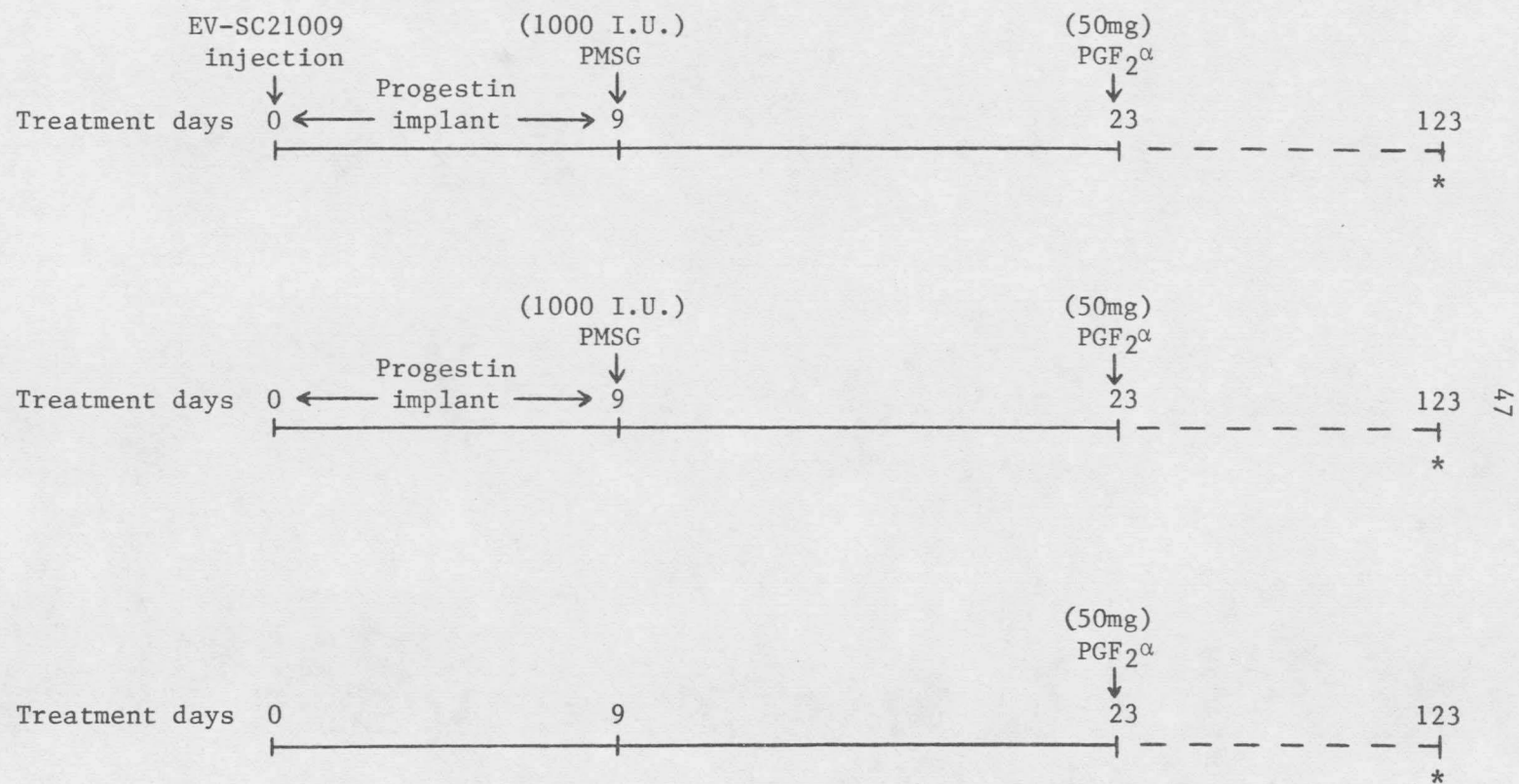
low value progesterone standard was incorporated at the beginning and end of each assay in order to adjust for intra- and inter-assay variations.

Progesterone data were analysed using a Wilcoxin signed rank test to test for a difference at days 9, 23, 27 and 36 across all treatments. This test was also used to determine if differences existed within treatment groups at days 9 vs 23, 9 vs 27, 23 vs 27, 9 vs 36, 23 vs 36 and 27 vs 36.



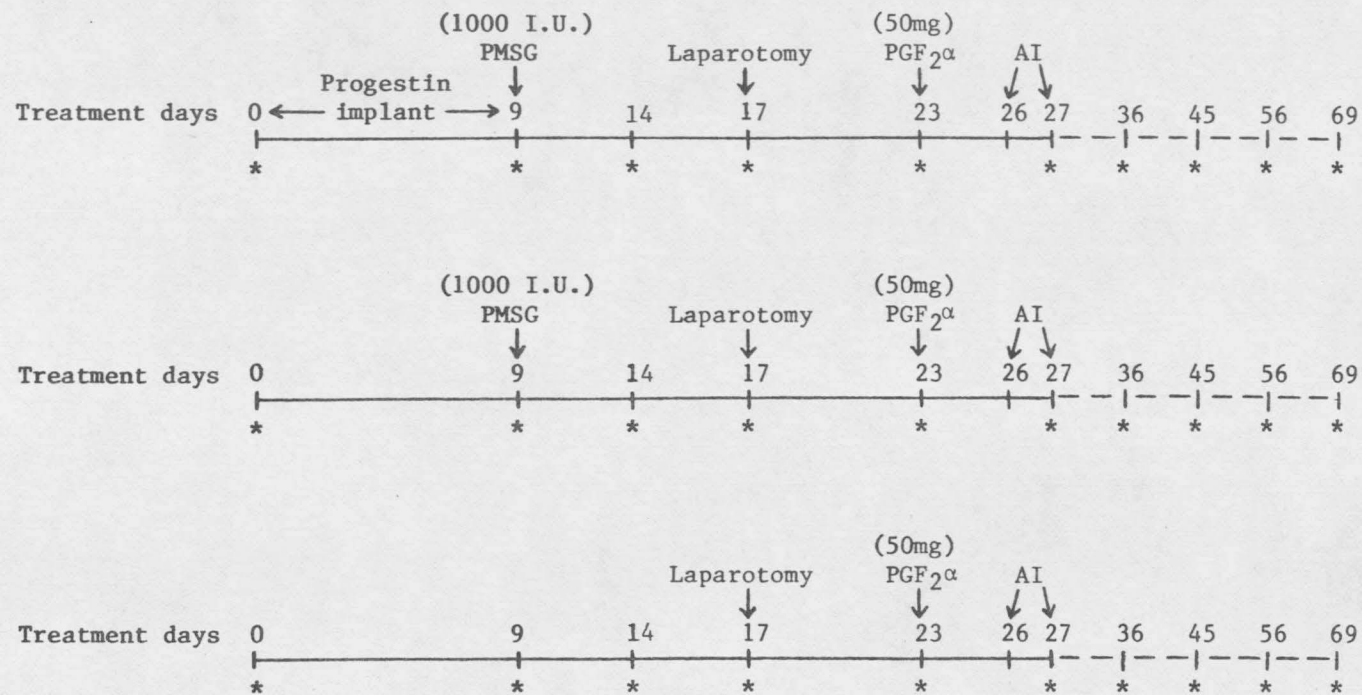
* Days cows were bled and palpated

Figure 1. Experiment 1 treatment schedule including day of progestin implant, PMSG and PGF₂ α



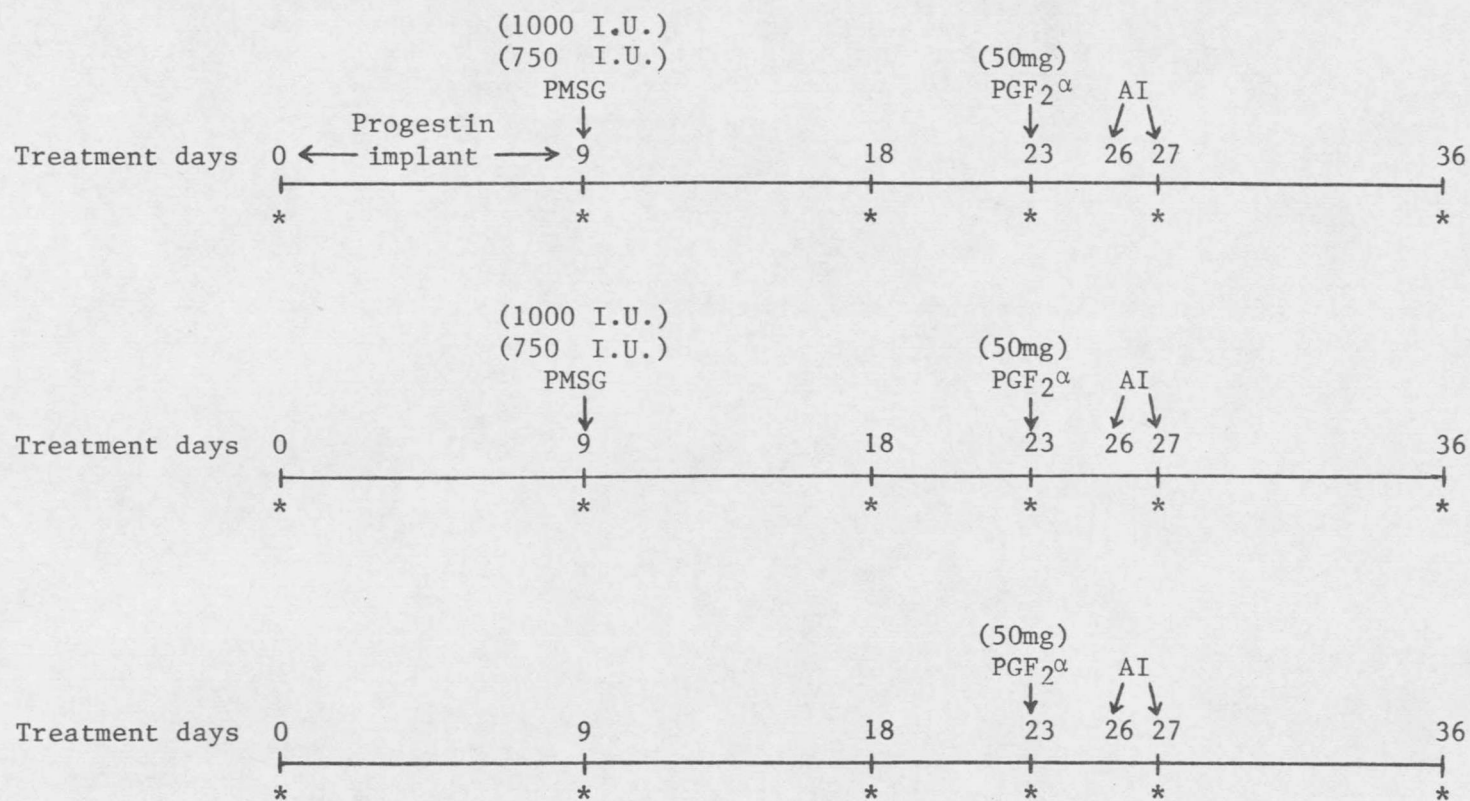
* Cows checked for pregnancy

Figure 2. Experiment 2 treatment schedule including day of EV-SC21009 injection, progestin implant, PMSG and PGF₂^α



* Days cows were bled and palpated

Figure 3. Experiment 3 treatment schedule including day of progestin implant, PMSG, laparotomy, PFG₂α and AI



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* Days cows were bled and palpated

Figure 4. Experiment 4 treatment schedule including day of progestin implant, PMSG, PGF_2^α and AI

Progesterone Assay

Progesterone concentrations were determined in serum samples using radioimmunoassay techniques.

Preparation of Reagents for Assay

Antibody Production

A progesterone antibody was kindly provided by R. B. Staigmiller from the USDA Livestock and Range Research Station, Miles City, Montana. The antibody consists of an antiserum raised in rabbits against a progesterone-11 α -BSA (bovine serum albumin) conjugate.

Buffers

The buffer used in the progesterone assay was a .1 M phosphate buffer (pH 7.0) containing 5.38 gm NaH₂PO₄ monobasic, 16.35 gm Na₂HPO₄ dibasic, 9.00 gm NaCl, 990 ml double distilled H₂O, 1.00 gm Knox Gelatin and 10 ml of 1:100 thimersol.

The buffer used for antibody dilution consisted of 18.612 gm disodium EDTA (ethylenedinitrilo tetraacetic acid, disodium salt), 1000 ml of the .1 M phosphate buffer and 10 ml of 1:100 merthiolate. The buffer was warmed and stirred in order for the EDTA to go into solution. The buffer was brought to a pH of 7.0 by slowly adding 5 N NaOH while stirring. All buffers were stored at 4°C for up to 3 months.

Radioactive Progesterone

Progesterone-1,2,6,7-³H (specific activity = 97.9 Ci/mmol) was

obtained from the New England Nuclear Corporation. Its purity was checked by the use of a $1\frac{1}{2}$ inch Sephadex LH-20 column. The LH-20 gel was soaked in benzene:methanol (9:1 v/v) for at least 4 hours prior to making the column. Tritiated progesterone was diluted in the assay buffer to give approximately 11,000 CPM/200 μ l. Another dilution of approximately 2400 CPM/100 μ l was required for the recovery purposes described in the assay procedure. The diluted ^3H -progesterone was stored at 4°C .

Progesterone Standards

Progesterone, in the form of anhydrous crystals, (Sigma Chemical Co.) was weighed on a Metler scale and dissolved and diluted with double distilled ethanol to make a stock solution. Progesterone concentrations, used to develop the standard curve, were prepared from the stock solution. The concentrations for the assay were 0 (double distilled ethanol), 10, 30, 60, 125, 250, 500, 1000 and 2000 pg/100 μ l. The progesterone standards were stored at 4°C .

Charcoal

Separation of free progesterone from antibody-bound progesterone was accomplished with a dextran-charcoal suspension. Fines were removed from the charcoal (Norit-A, Pharmacia Fine Chemicals Inc.) by repeat washing with double distilled water followed by aspiration of the charcoal fines after allowing the mixture to settle for 2 to 3 hours. The washed charcoal was dried in an oven at 100°C . The charcoal suspension for use in the assay consisted of 200 ml of the assay buffer, 1.25 gm

charcoal and .25 gm dextran (clinical grade, Sigma Chemical Co.). The suspension was shaken vigorously for 30 seconds and stored at 4°C. This preparation is stable for up to 1 month.

Scintillation Fluid

A premixed liquid scintillation fluid (READY-SOLV EP, Beckman) composed of a triton/toluene base was used for liquid scintillation counting purposes. A volume of 10 ml was used for each counting vial.

Lab Serum Standards and Serum Samples

Lab serum standards consisted of a pool of high and low value progesterone samples. The high serum value, collected from a pregnant cow, and the low serum value, collected from an ovariectomized cow, were each aliquoted into 1 ml samples and stored at -25°C. Both high and low value standards were incorporated at the beginning and end of each assay to assess intra- and inter-assay variations.

All blood samples collected for assay purposes were stored at 4°C on the day of collection. The following day samples were centrifuged for 15 minutes at 2000 rpm and the serum was pipetted off the top. All serum was stored at -25°C.

Assay Procedure

Preparation of Samples

Approximately 2400 CPM (100 ul) of ³H-progesterone, employed to assess procedural losses, were added to .5 ml aliquots of a double

distilled water sample, the high and low lab standards and each serum sample to be assayed. One hundred μ l of ^3H -progesterone was also added to three scintillation vials used to determine a value for total counts recovered (TCR). These TCR vials were capped and stored at 4°C until the end of the assay when scintillation fluid was added. After the labeled progesterone was added, the samples were vortexed at #2 speed for 2 to 3 seconds and allowed to equilibrate overnight at 4°C .

Extraction

The following day 3 ml of benzene-hexane in a 1:2 v/v mixture were added to each vial for purposes of progesterone extraction. Samples were agitated on a mechanical shaker for 15 minutes and then placed in a freezer at -25°C for one hour. The extraction vials were then removed from the freezer and the aqueous phase was poured off into corresponding vials and dried down under a hood with nitrogen gas.

Splitting the Extracted Samples

Each evaporated sample was reconstituted in 1 ml of double distilled ethanol. A 300 μ l aliquot of the redissolved extract was used to assess the procedural losses. Duplicate 300 μ l aliquots were placed in 12 x 75 mm culture tubes for quantitation of progesterone. The aliquots in the culture tubes were evaporated with nitrogen gas. The antibody and ^3H -progesterone were allowed to come to room temperature and 200 μ l of antibody followed 30 minutes later by 200 μ l of ^3H -progesterone were added to each tube. The reaction solution was vortexed at #2 speed for

2 to 3 seconds and allowed to equilibrate 14 to 18 hours at 4°C.

Separation of Free and Bound Fractions

Following the incubation period the dextran-charcoal suspension was removed from the refrigerator and placed in an ice bath on a magnetic stirrer for 20 minutes. The charcoal absorption step was done at 4°C because at higher temperatures stripping of the bound steroids will occur. The assay culture tubes were placed in refrigerated centrifuge carriers and removed from the refrigerator just prior to the addition of the charcoal suspension. Two hundred μ l of the assay buffer was added to the two total count tubes (double distilled ethanol, 0 pg progesterone/100 μ l) and 200 μ l of the charcoal solution was added to all the other tubes in order to absorb and precipitate the free steroids. The tubes were gently shaken back and forth by hand for 15 seconds and then allowed to incubate for 10 minutes in the centrifuge at 4°C.

Following the incubation period the tubes were centrifuged at 2500 rpm for 10 minutes. The aqueous phase was poured off into scintillation vials and 10 ml of scintillation fluid was added to each vial including the three total count recovery vials and an empty vial (blank) in order to get a value for the background count. The vials were capped, placed in a box and left covered for 30 minutes before being taken to the counter. The vials were counted on a Beckman liquid scintillation LS-100 system set for 5 minutes per vial at 5% error.

Recovery

Progesterone concentration in each sample was corrected for procedural losses. The experimental loss was calculated by dividing the CPM for each recovery vial into the average recovery obtained from the total count recovery vials and multiplying by 100 to give a percent recovery. The mean recovery of ^3H -progesterone from the benzene-hexane extracts of 15 assays was 78.3%.

Precision

The intra- and inter-assay variation was evaluated by the use of duplicate high and low progesterone standards that were incorporated within each assay. The coefficient of variation (CV), used to determine inter-assay precision, was estimated for both the high and low standards by the following formulas:

$$CV = \frac{S (100)}{\bar{X}}$$

$$\text{and } S^2 = \left[\frac{\sum X_i^2 - \frac{(\sum X_i)^2}{n}}{n-1} \right]$$

where S = standard deviation; $\bar{X}$ = mean of the lab standards; S^2 = variance; X_i = value of each lab standard; n = number of duplicate determinations.

Sixteen duplications were determined in eight assays for both the high and low progesterone standards. The values for the high lab standard ranged from 7.35 to 13.81 ng/ml (10.75 ng/ml average) with a

coefficient of variation of 17.72%. The high standard had an average binding capacity of 19.25% on the standard curve. The values for the low lab standard ranged from .21 to .61 mg/ml (.38 ng/ml average) with a coefficient of variation of 31.25%. The low standard had an average binding capacity of 82.88% on the standard curve.

The coefficient of variation, used to determine intra-assay precision, was estimated for both the high and low lab standards by the following formulas:

$$CV = \frac{S (100)}{\bar{X}}$$

$$SS = \left[\sum Xi^2 - \frac{(\sum Xi)^2}{N} \right]$$

and

$$s^2 = \frac{\sum SS}{n}$$

where SS = sum of squares; N = number of duplicate standards within an assay; n = number of assays.

High and low progesterone lab standards were incorporated at the beginning and end of eight assays for a total of sixteen sets of high and low standards. The intra-assay coefficient of variation for the high lab standards was 12.2% while for the low lab standards it was 3.39%

Assay Validation

Sensitivity

The standard curve was derived by plotting the percent bound against the logarithm of the dose of progesterone added (figure 5). The percent bound when no progesterone (double distilled ethanol) was added was defined as 100%. At the 95% confidence limit 30 pg was significantly different from 0 pg.

Specificity

The specificity of an assay refers to the ability to bind only the compound intended to quantify. Absolute specificity is difficult to achieve. A number of steroids were tested by cross-reaction studies to determine their ability to bind to the progesterone antibody used in this assay. The percent cross reactivity was determined by dividing the ng of progesterone required to displace 50% of the ^3H -progesterone bound to the antibody by the ng of steroid required to displace 50% of the ^3H -progesterone bound to the same antibody.

Cross-reactivity was tested for the following steroids with their concentrations expressed in pg/ml:

Pregnenolone (2000, 1000, 500, 250), Cholesterol (4000, 2000, 1000), 20 β -Dihydroxyprogesterone (2000, 1000, 500, 250), Testosterone (2560, 1280, 640, 160), Hydrocortisone (2000, 1000, 500, 250), Estrone (2000, 1000, 500, 250), 17 β -Estradiol (2000, 1000, 500, 250) and Estriol (2000, 1000, 500, 250).

Two steroids showed some cross-reactivity, namely, pregnenolone

(35%) and 20 β -dihydroxyprogesterone (15.9%). Cross-reactivity was not detected with the other steroids tested.

Quantitative Recovery

The accuracy of the assay was tested by recovery experiments. A known amount of progesterone was added to serum containing a low level (200 pg/ml) of progesterone. The serum was from a pool obtained for the low standard used in the assay. The progesterone concentration of the serum was decreased by adding charcoal (Norit-A) to absorb free steroids. The charcoal-serum suspension was centrifuged and the serum pipetted off the top. Quantities of 0, 250, 500, 1000, 2000, 4000 and 8000 pg progesterone/ml were added to .5 ml aliquots of the serum. The quantity of progesterone estimated differed by 22.7% at the 4000 pg/ml concentration but was less than 15% at remaining concentrations (table 1).

Parallelism

A demonstration of parallelism between the standard curve and various dilutions of a medium containing progesterone was performed. Varying quantities (500, 250, 100, 50 μ l) of the high progesterone lab standard were assayed and plotted against the standard curve (figure 6). The results indicated the activity of the high progesterone lab standard to be parallel to that of the standard curve.

TABLE 1. QUANTITATIVE RECOVERY FOR PROGESTERONE ASSAY

Progesterone (pg) added to SS ^a	Expected recovery (pg/ml)	Quantity recovered (pg/ml)	% deviation from expected recovery
250	700	770	11.3
500	1200	1379	14.9
1000	2200	2214	.6
2000	4200	4222	.5
4000	8200	10063	22.7
8000	16200	16817	3.8

^a Progesterone added to .5 ml stripped serum (SS) containing 200 pg progesterone

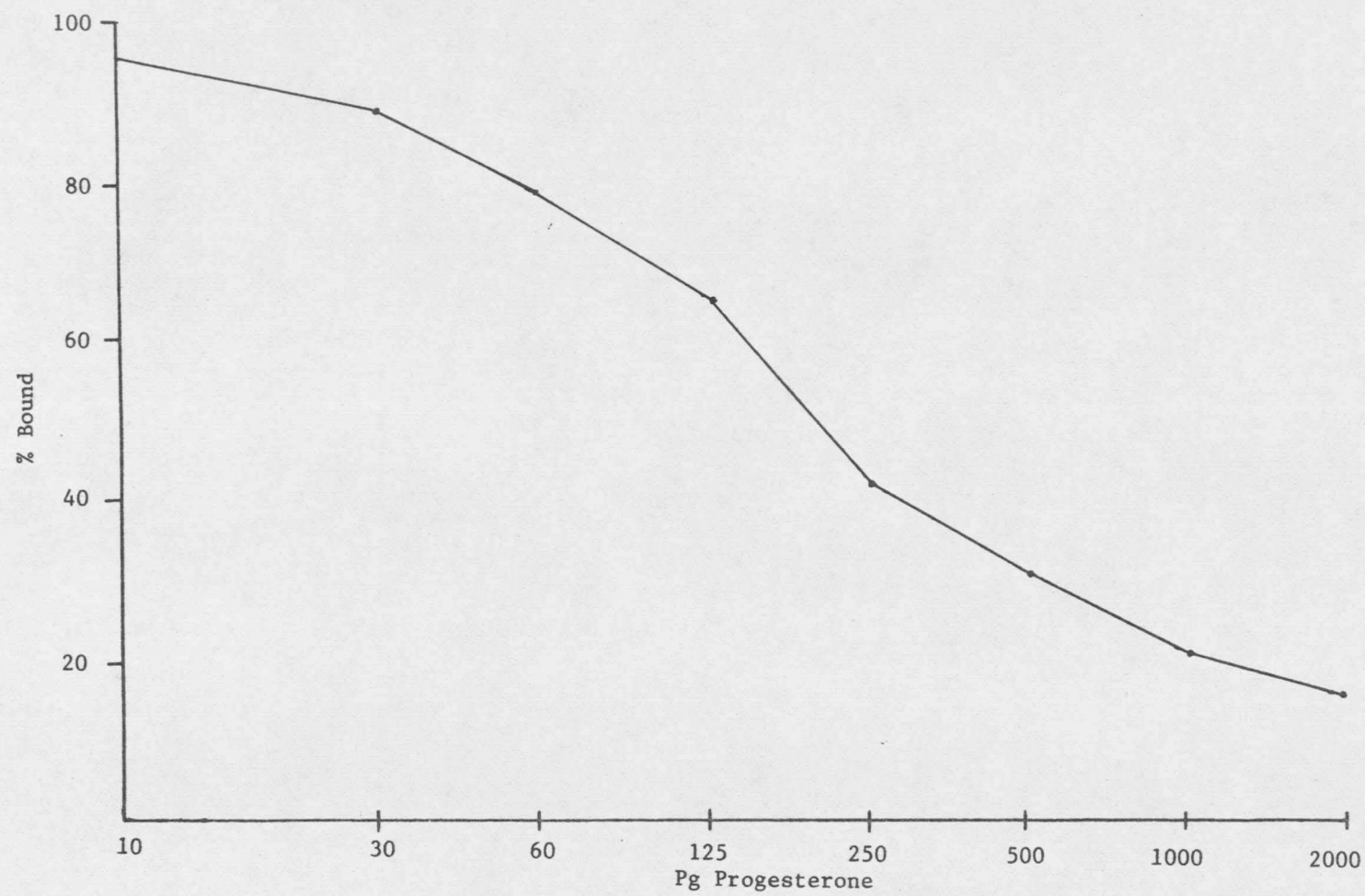


Figure 3. Standard curve with antibody dilution 1/2000

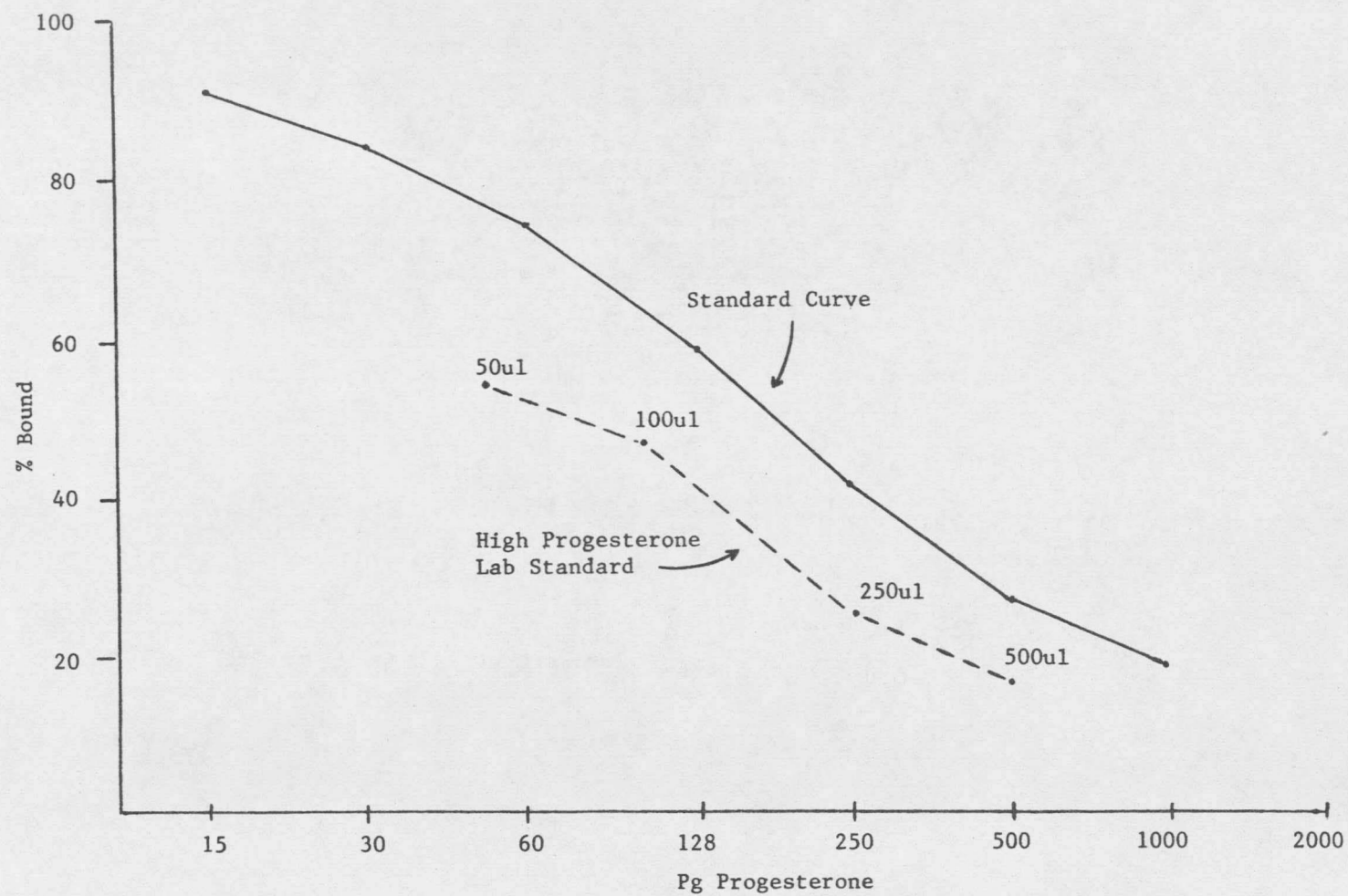


Figure 6. Demonstration of parallelism between standard curve and varying quantities of the high progesterone lab standard.

RESULTS

Experiment 1

Ovarian activity, as determined by serum progesterone concentrations ≥ 1.5 ng/ml, indicated that seven of the eighteen cows in the study were secreting progesterone prior to the time of PMSG administration. This included one cow in the group receiving 4000 I. U. PMSG, two receiving 2000 I. U. PMSG, one receiving the implant plus 1000 I. U. PMSG, one receiving 1000 I. U. PMSG and two in the control group receiving no PMSG.

Ovarian activity, as evaluated by rectal palpation, showed that in only two of the seven cows with serum progesterone values ≥ 1.5 ng/ml was there a detectable corpus luteum present.

Treatment with PMSG caused an increase in serum progesterone concentrations to >5.5 ng/ml in all treated cows following the 1000, 2000, or 4000 I. U. injections, with or without implants. This increase in serum progesterone concentrations was observed within 4 to 6 days after the PMSG injection in 12 of the 14 treated animals while an increase was seen in the remaining two cows by 8 days (figures 7-12). These latter two cows, numbers 210 and 546, had serum progesterone levels of 3.8 and 5.9 ng/ml, respectively, on the day of PMSG administration, indicating that some type of luteal activity was present at that time (figures 8 and 10).

Serum progesterone concentrations continued to increase up to day 23 of the treatment period (day of $\text{PGF}_2\alpha$ injection) in 9 of the 14 PMSG treated cows (Table 2). Three cows had peak progesterone values at day

21, one at day 19 and one cow at day 17 of the treatment period. The cow with the progesterone peak at day 17 showed a dramatic fall to levels <0.5 ng/ml on days 19 and 21 followed by an increase to 6.5 ng/ml on day 23 (Figure 9).

Maximum serum progesterone levels following PMSG stimulation varied considerably among cows not only between treatments but also within treatment groups (Table 2). The highest progesterone peak value (40.7 ng/ml) was observed in a cow receiving the 2000 I. U. of PMSG while the lowest individual peak value (5.0 ng/ml) was seen in a cow receiving the progestin implant +1000 I. U. PMSG. It is interesting to note that the two cows in the 4000 I. U. PMSG treatment had progesterone levels nearly twice that of the two cows receiving the implant plus 4000 I. U. PMSG. One of the non-stimulated control animals appeared to be cycling as evidenced by her progesterone profile (Figure 13). She showed a gradual increase in serum progesterone from day 11 (0.3 ng/ml) up to day 19 (11.1 ng/ml) of the treatment period followed by a leveling off at 9.4 and 9.0 ng/ml on days 21 and 23, respectively. One other control cow had small progesterone peaks at day 0 (1.9 ng/ml), day 11 (2.1 ng/ml) and day 23 (1.6 ng/ml). The serum progesterone concentrations of the two other control animals remained <1.5 ng/ml throughout the treatment period.

The 25 mg dose of $\text{PGF}_2\alpha$ injected on day 23 decreased progesterone values to <1.5 ng/ml within 2 days of injection in 6 of the 14 PMSG

treated cows and in both of the controls that possessed an elevated (≥ 1.5 ng/ml) progesterone level (Table 2). The two cows in the 4000 I. U. PMSG treatment showed only partial luteal regression as evidenced by the high (11.4 and 14.3 ng/ml) progesterone values that occurred 2 days following the $\text{PGF}_2\alpha$ injection. Incomplete luteal regression by 2 days post- $\text{PGF}_2\alpha$ was also seen in one cow in the implant +1000 I. U. PMSG treatment (6.3 ng/ml). One cow in the implant +2000 I. U. PMSG group and another in the implant +1000 I. U. PMSG group showed a slight degree of luteal activity 2 days post- $\text{PGF}_2\alpha$ (1.6 and 1.8 ng/ml, respectively). An animal in the 2000 I. U. PMSG treatment had similar progesterone levels following $\text{PGF}_2\alpha$ (39.9 ng/ml) as before (40.7 ng/ml), while two other cows, one in the implant +2000 I. U. PMSG group and another in the 2000 I. U. PMSG group showed increased progesterone levels to 14.0 and 9.1 ng/ml, respectively 2 days post- $\text{PGF}_2\alpha$ (Table 2).

Estrus was detected following $\text{PGF}_2\alpha$ in one cow (318) out of the fourteen PMSG treated animals and none of the control cows. This cow, from the implant +4000 I. U. PMSG group, was detected in estrus 3 days following $\text{PGF}_2\alpha$ administration and was artificially inseminated the following morning. She did not conceive from that insemination period.

Serum progesterone concentrations at 18 days following the $\text{PGF}_2\alpha$ injection indicated that 10 of the 14 PMSG treated cows showed luteal activity (≥ 1.5 ng/ml) while none of the controls were seen to have progesterone levels ≥ 1.5 ng/ml (Table 2). All of the cows in the 1000

I. U. PMSG, implant +1000 I. U. PMSG, and 2000 I. U. PMSG treatments had progesterone values ≥ 1.5 ng/ml with the cows in the implant +1000 and the 1000 I. U. PMSG groups having the highest progesterone values. One cow in each of the three remaining treatments had progesterone values ≥ 1.5 ng/ml (Table 2).

TABLE 2. EXPERIMENT 1 SERUM PROGESTERONE CONCENTRATIONS (ng/ml) DURING TREATMENT PERIOD

Treatment	Cow No.	Day of maximum progesterone concentration prior to $\text{PGF}_2\alpha$	Maximum concentration prior to $\text{PGF}_2\alpha$	2 days post- $\text{PGF}_2\alpha$	18 days post- $\text{PGF}_2\alpha$
Implant					
+4000 I.U.	431	23	13.4	1.0	0.4
PMSG	318	21	11.6	1.0	8.3
4000 I.U.	546	21	26.9	11.4	0.8
PMSG	410	23	21.3	14.3	3.3
Implant	515	23	21.3	1.1	0.5
+2000 I.U.	436	17	8.7	14.8	1.8
PMSG	551	23	15.5	1.6	1.1
2000 I.U.	528	23	9.3	1.1	6.4
PMSG	210	23	8.0	9.1	1.6
	534	23	40.7	39.9	1.7
Implant	322	23	13.6	6.3	5.6
+1000 I.U.	503	21	5.8	0.6	5.1
PMSG	228	19	24.0	1.8	11.1
1000 I.U.					
PMSG	553	23	12.5	0.8	5.7
Control	512	21	0.4	0.5	0.4
	253	21	11.1	0.8	0.5
	516	11	2.1	0.3	0.4
	556	9	1.2	0.6	0.4

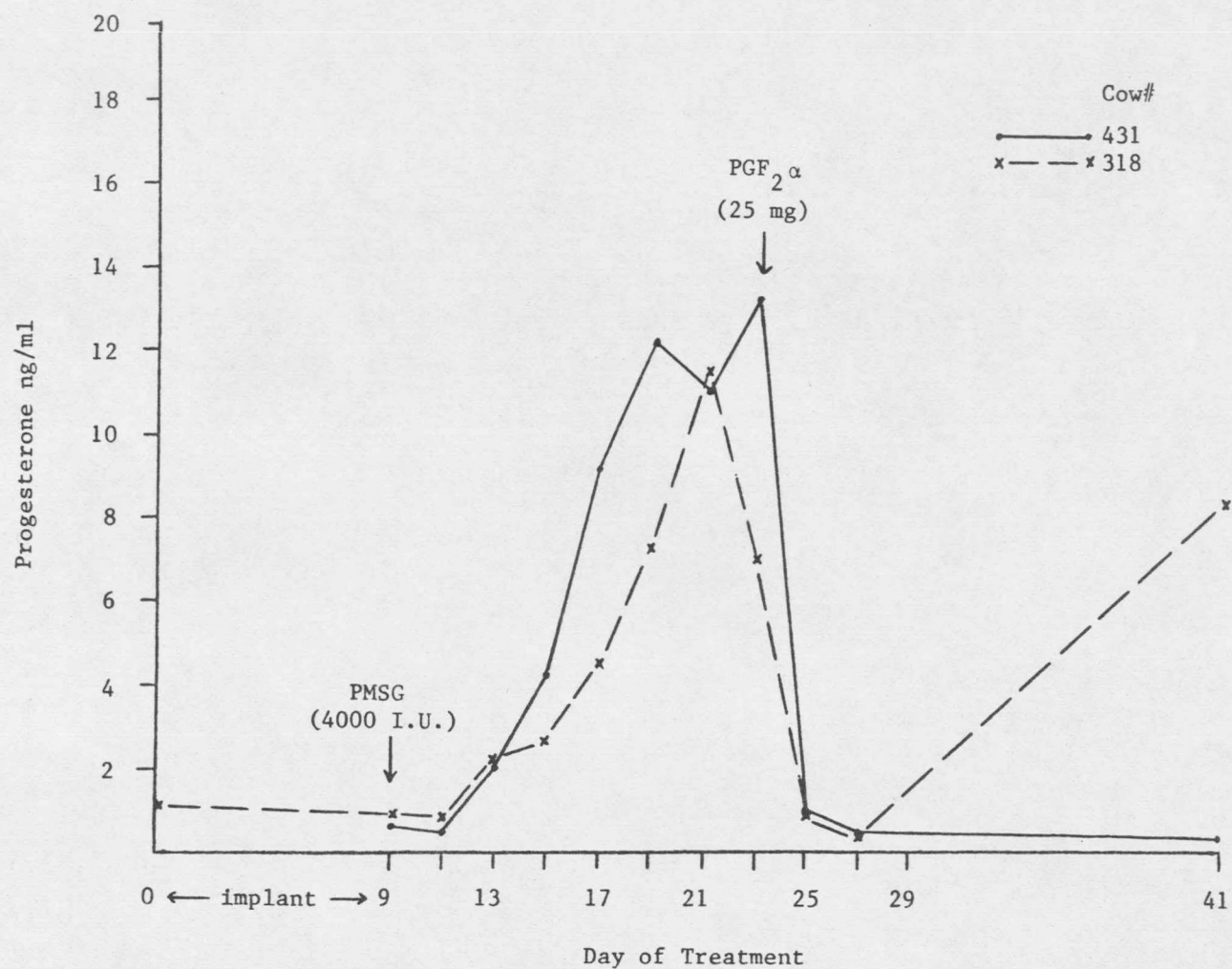


Figure 7. Serum progesterone concentrations for cows treated with progestin implant, 4000 I.U. PMSG and PGF₂α

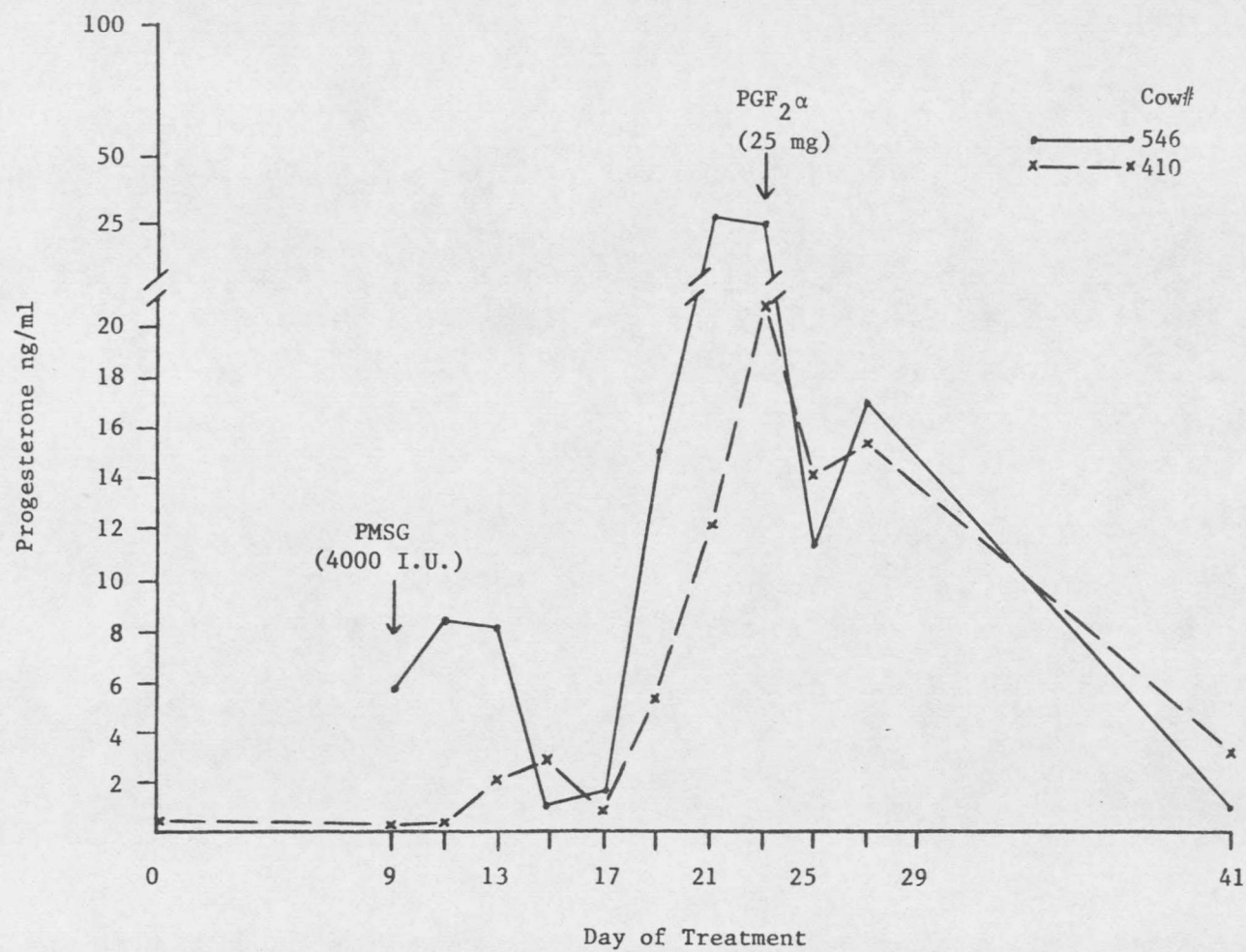


Figure 8. Serum progesterone concentrations for cows treated with 4000 I.U. PMSG and PGF₂ α

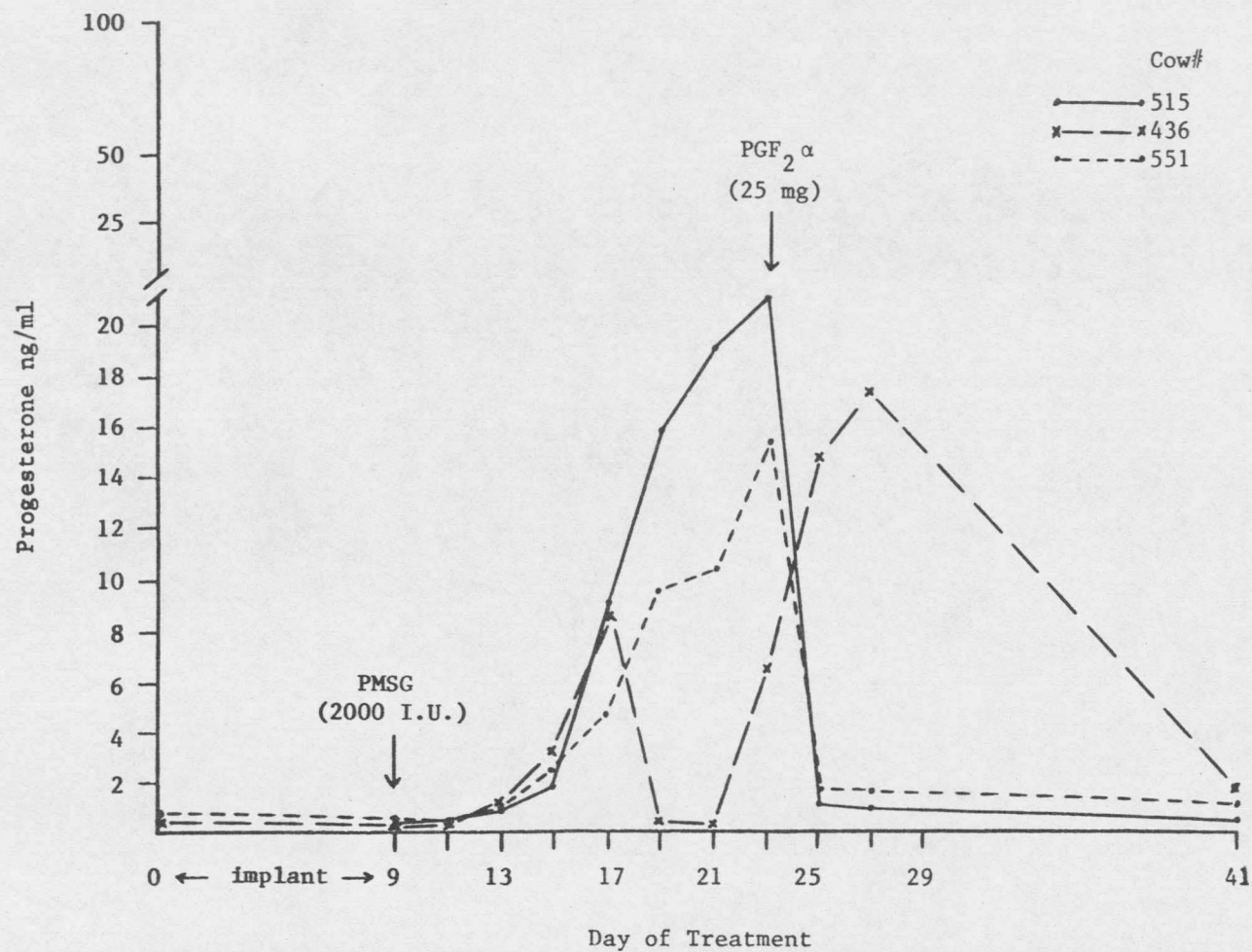


Figure 9. Serum progesterone concentrations for cows treated with progestin implant, 2000 I.U. PMSG and PGF₂α

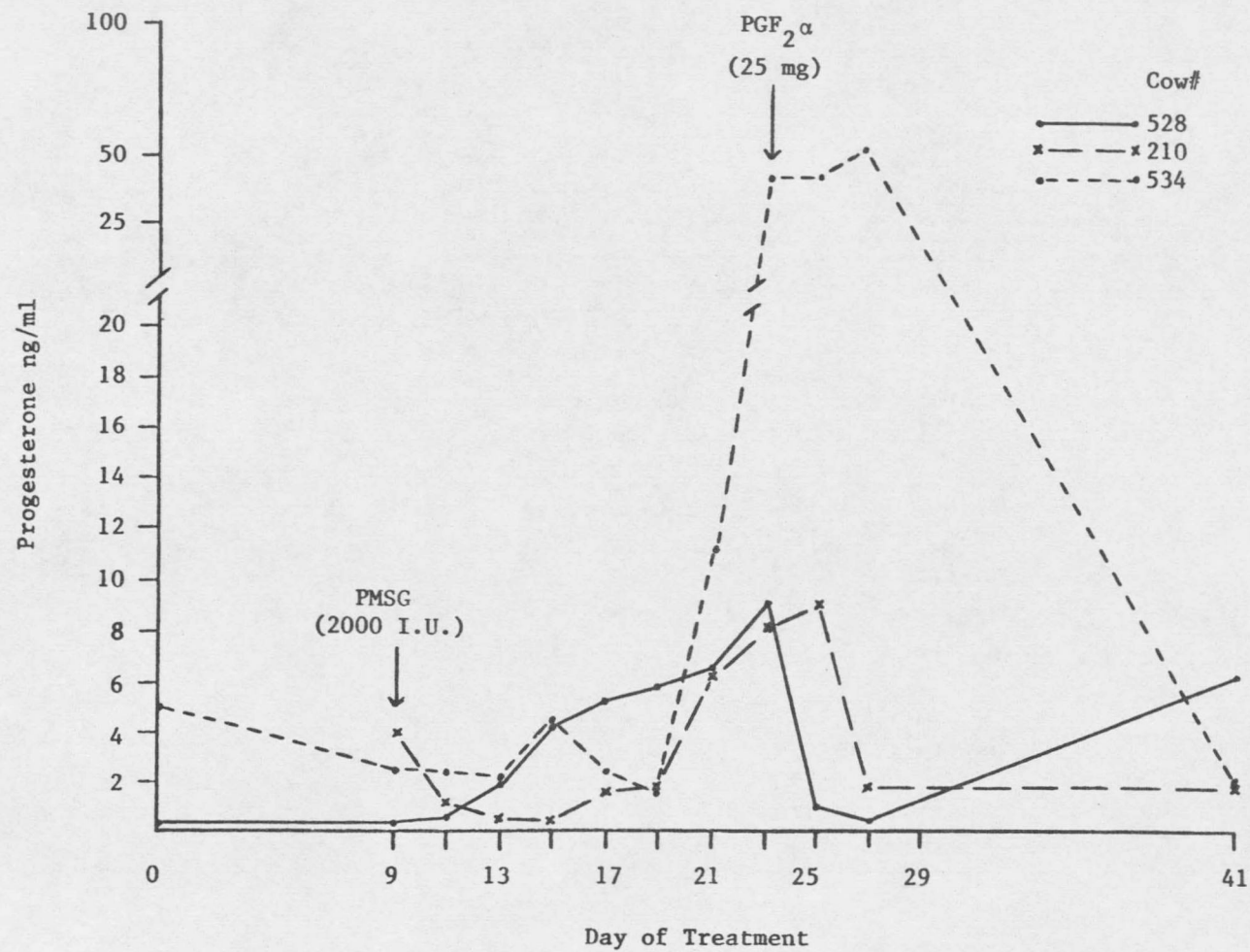


Figure 10. Serum progesterone concentrations for cows treated with 2000 I.U. PMSG and PGF₂α

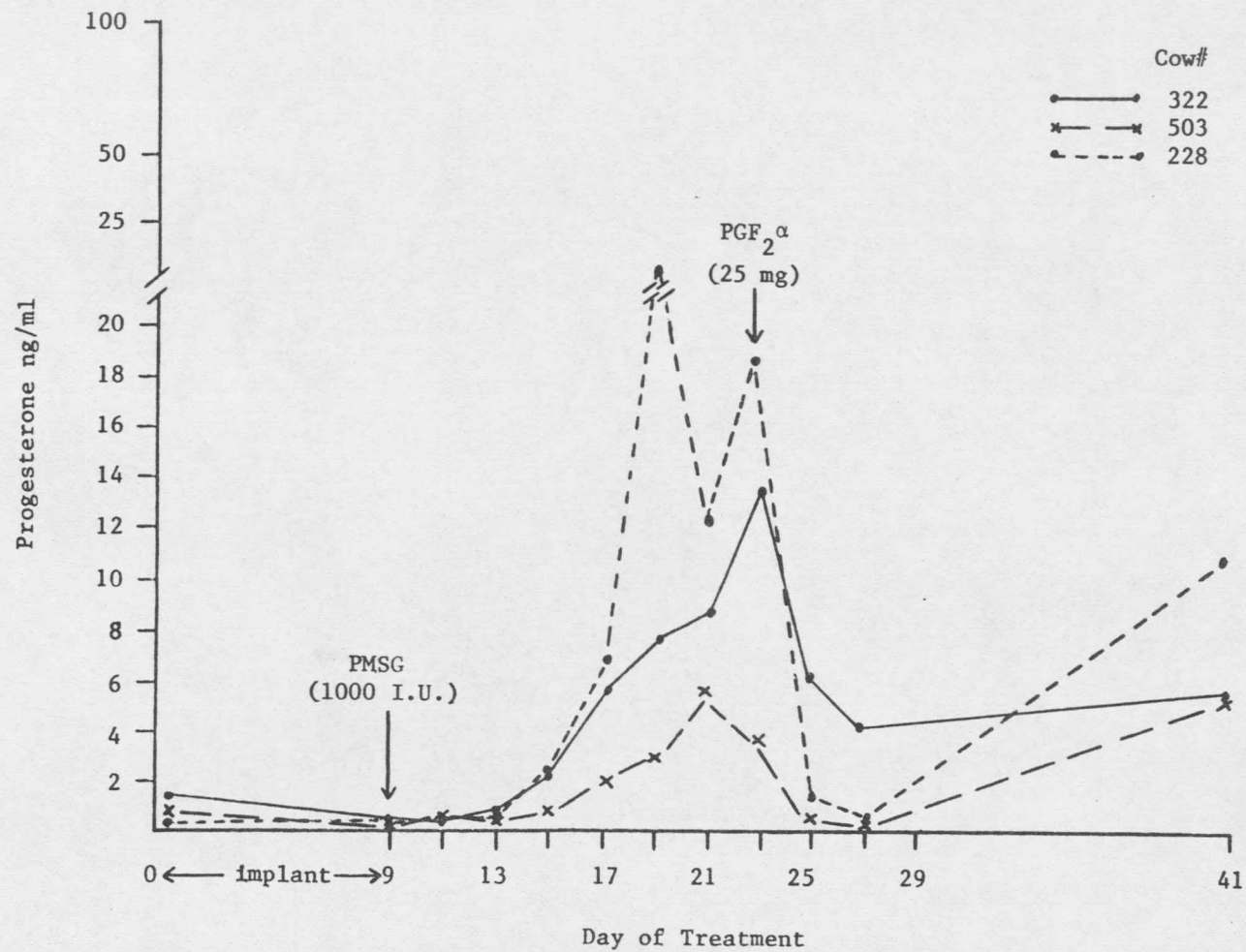


Figure 11. Serum progesterone concentrations for cows treated with progestin implant, 1000 I.U. PMSG and PGF₂^α

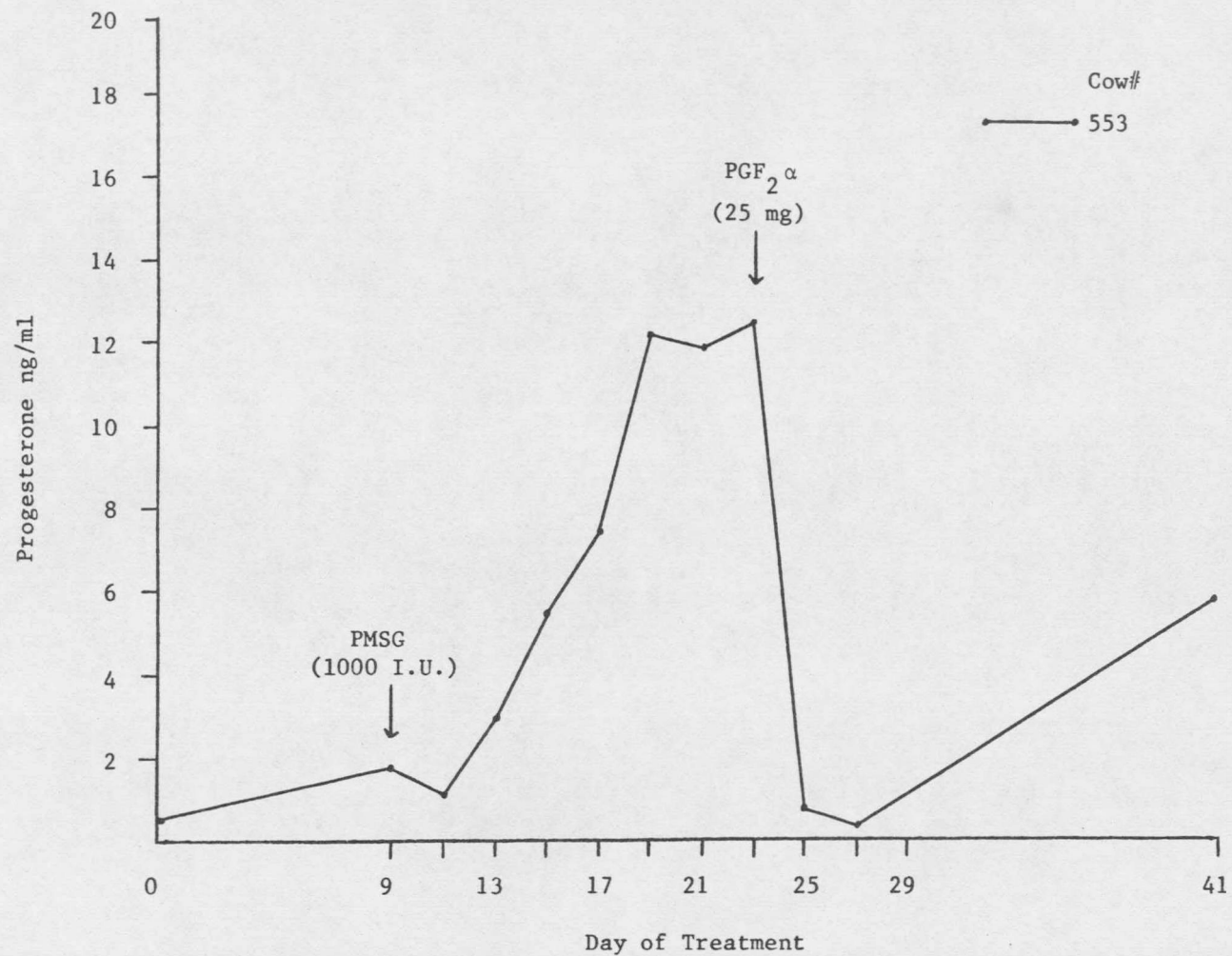


Figure 12. Serum progesterone concentrations for cows treated with 1000 I.U. PMSG and $\text{PGF}_2\alpha$

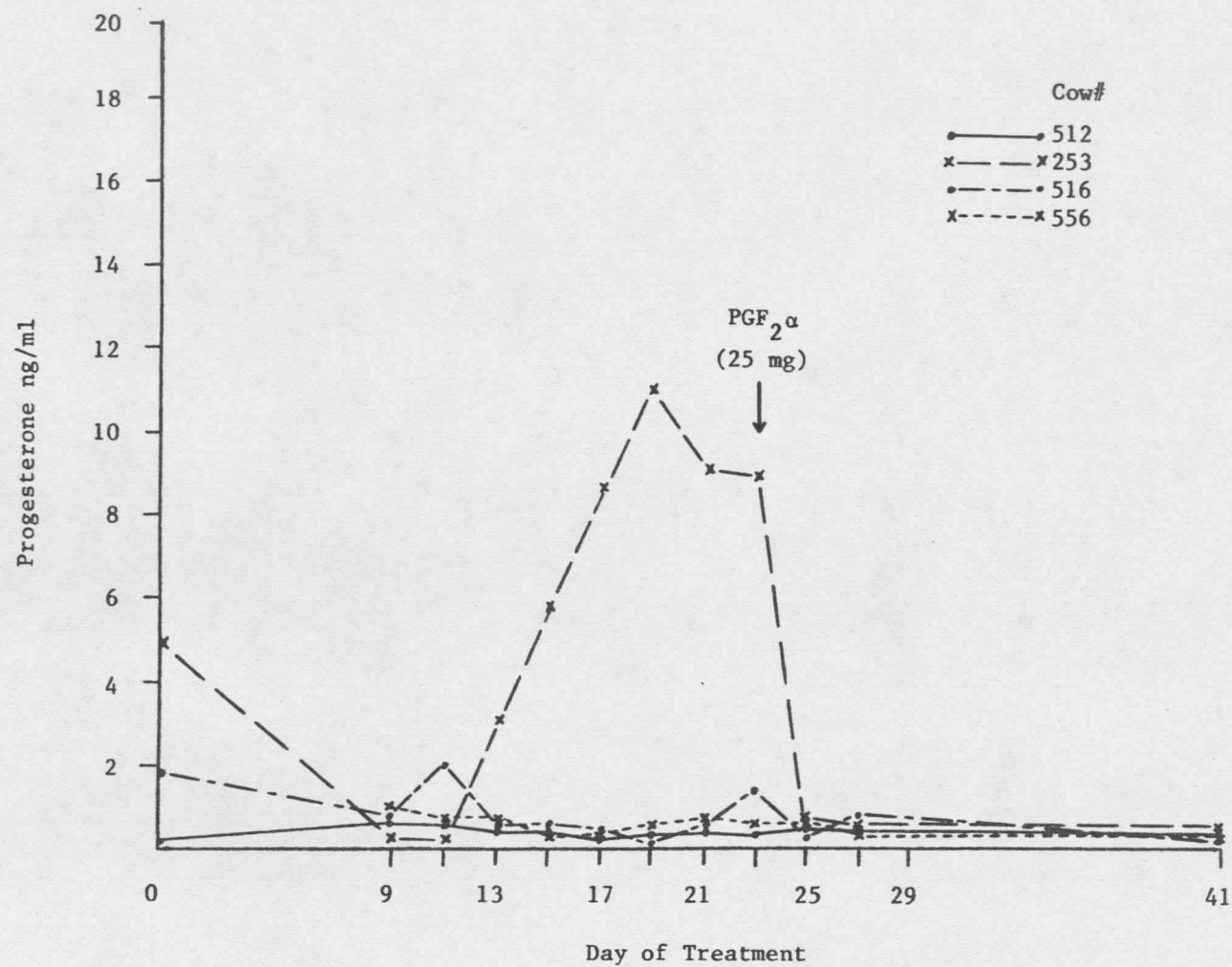


Figure 13. Serum progesterone concentrations for non-PMSG treated cows receiving PGF₂α

Experiment 2

Palpation of ovaries indicated no follicle >10 mm or CL development present prior to the treatment period in any of the 27 animals in the study.

Calving information subsequent to postpartum treatment and breeding was not available for two cows in group 3 (735 and 3823) so conception data was based on information obtained by rectal palpation used to determine pregnancy status. Data for all the other animals were based on actual calving information.

The percentage of cows bred during the five day AI period was 0.0, 33.3 and 33.3% for groups 1, 2 and 3, respectively with no cows conceiving from that insemination period (Table 3). The cows conceiving by natural service and thus the total conception rate for each group was 66.7 (group 1), 100.0 (group 2) and 88.9% (group 3). Statistical analysis revealed no significant difference in conception rate between treatment groups.

The average days postpartum to conception for groups 1, 2 and 3 were 67.0, 58.1, and 72.0, respectively (Table 3). No significant difference in days postpartum to conception was established between groups. The average days to conception following the 50 mg dose of $\text{PGF}_2\alpha$ was 19.2, 10.4 and 24.3 days for group 1, 2 and 3, respectively. The average day of conception of the breeding season which started the day following $\text{PGF}_2\alpha$ was 18.2, 9.4, 23.3 for groups 1, 2 and 3,

respectively. A significant difference ($P < .05$) was seen between groups 2 and 3 for the day of conception of the breeding season.

The range within treatment groups for days to conception post-PGF_{2α} was considerably shorter in group 2 than either of the other groups. The range for group 2 was 6 to 25 days while group 1 was 6 to 43 days and group 3, 6 to 45 days (Table 4).

TABLE 3. EXPERIMENT 2 POSTPARTUM, CONCEPTION AND PREGNANCY DATA

	(Group 1) EV-SC21009 injection + progestin implant + 1000 I. U. PMSG	(Group 2) Progestin implant + 1000 I. U. PMSG	Group 3) Control	Total
No. cow	9	9	9	27
No. cows AI'd by estrus detection (%)	0 (0.0)	3 (33.3)	3 (33.3)	6 (21.4)
No. cows conceiving by AI (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. cows conceiving by natural service (%)	6 (66.7)	9 (100.00)	8 (88.9)	23 (85.2)
Total no. cows conceiving (%)	6 (66.7)	9 (100.00)	8 (88.9)	23 (85.2)
No. cows open (%) (non-pregnant)	3 (33.3)	0 (0.0)	1 (11.1)	4 (14.8)
Ave. days postpartum at start of treatment	26.3	26.7	26.6	26.5
Ave. days postpartum to conception	67.0	58.1	72.0	65.3
Ave. days to conception post-PGF ₂ α	19.2	10.4 ^a	24.3 ^b	17.5
Ave. days conception of breeding season	18.2	9.4 ^a	23.3 ^b	16.5

a,b
p<.05

TABLE 4. EXPERIMENT 2 POSTPARTUM, CONCEPTION AND PREGNANCY DATA FOR INDIVIDUAL COWS

Treatment	Cow no.	Days postpartum at start of treatment	Days postpartum to conception	Days to conception post-PGF ₂ ^a	Day conception of breeding season
EV-SC21009 injection + progestin implant + 1000 I. U. PMSG (group 1)	5072	24	open	open	open
	3468	24	open	open	open
	5133	23	64	19	18
	3475	26	72	25	24
	3638	26	52	6	5
	5032	27	open	open	open
	3494	27	91	43	42
	4145	28	57	8	7
	5800	31	66	14	13
Progestin implant + 1000 I. U. PMSG (group 2)	3096	24	51	6	5
	8050	24	51	6	5
	4152	24	55	10	9
	5165	26	72	25	24
	5236	26	53	6	5
	4050	27	54	6	5
	3688	28	69	20	19
	2011	28	58	9	8
	767	33	60	6	5
Control (group 3)	3506	24	51	6	5
	3586	24	64	19	18
	3812	26	open	open	open
	5906	27	57	9	8
	2016	27	95	46	45
	0093	28	74	25	24
	3614	28	55	6	5
	735 ^a	24	82	37	36
	3823 ^a	31	98	46	45

^aConception data based on rectal palpation used to determine pregnancy status

Experiment 3

Four cows were rejected from the study due to problems encountered with retention of the progestin implant. The implant was missing in two cows at the time of implant removal while in the other two cows only part of the implant was found at the time of removal. It is from the remaining 27 of 31 cows in the study that the following data was analyzed.

Ovarian activity, as determined by serum progesterone concentrations >1.5 ng/ml on day 9, indicated that 11 of the 27 cows were secreting progesterone above basal levels (<1.5 ng/ml) prior to the time of PMSG administration. This included four cows in each of the first two groups and three cows in group 3 (figures 14-20). A corpus luteum or follicle >10 mm was palpable in 2 of the 11 cows, with the elevated progesterone levels.

Serum progesterone concentrations increased from day 9 to 23 in group 1 ($P<.05$) and group 2 ($P<.01$) following PMSG on day 9 and in group 3 ($P<.05$) without the PMSG injection (Table 5). Seven of the eight cows in group 1 exhibited peak progesterone concentrations on day 23 of the treatment period while the remaining cow reached a peak level by day 17 (Figure 14 and 15). Seven of the 10 animals in group 2 reached peak progesterone levels on day 23 with the other three cows displaying peak values by day 17 (Figures 16 and 17). Group 3 which received no PMSG, also exhibited an increase in serum progesterone concentrations during days 9 through 23 of the treatment schedule (Figures 18, 19, 20). Three

animals in group 3 reached peak progesterone levels by day 14, two by day 17, and four by day 23. Average serum progesterone concentrations were higher ($P < .001$) at day 23 in group 1 (33.5 ng/ml) than in group 2 (11.17 ng/ml) or group 3 (6.22 ng/ml; Table 4). Maximum progesterone levels on day 23 (prior to $\text{PGF}_2\alpha$) varied considerably among cows not only between treatments but also within treatment groups. Concentration ranges were 4.5-101.1, 3.9-21.2, and .62-13.4 ng/ml for groups 1, 2, and 3, respectively (Table 5).

One or more CL's were visible at the time of laparotomy on day 17 in two cows from each of the first two groups. No follicles >10 mm or CL's were present in one of the group 3 animals laparotomized yet a serum progesterone value of 5.6 ng/ml was recorded for that day. The other cow in group 3 laparotomized had one CL present with a progesterone value of 9.4 ng/ml.

A decrease in progesterone levels following the 50 mg dose of $\text{PGF}_2\alpha$ was observed between days 23 and 27 in group 1 ($P < .05$), group 2 ($P < .01$), and group 3 ($P < .05$). Progesterone concentrations decreased to <1.5 ng/ml by day 27 in 6 of 8 cows in group 1, 9 of 10 cows in group 2 and 7 of 9 cows in group 3. Two of the four cows in group 1 exhibiting high (>20.0 ng/ml) progesterone concentrations on day 23 showed declines from 56.4 and 39.7 to 4.6 and 9.7 ng/ml, respectively on day 27. All other animals in the three groups exhibiting progesterone levels >1.5 ng/ml on day 27 were in the 1.5 to 2.4 ng/ml range following $\text{PGF}_2\alpha$ on day 23 (Figures

14-20).

A significant increase in serum progesterone concentrations was not seen between days 27 and 36 in group 1 but was observed in group 2 ($P < .10$) and group 3 ($P < .05$). Average progesterone concentrations on day 36 were 1.6, 3.3, and 5.7 ng/ml for groups 1, 2 and 3, respectively (Table 5). Ranges for the previously mentioned groups were .57-2.5, .52-5.3 and .49-11.1 ng/ml.

All conception information is based on rectal palpation used to determine pregnancy status. Results showed 37.5, 0.0, and 33.3% cows conceived by AI for groups 1, 2, and 3, respectively; while 37.5, 70.0 and 55.6% conceived by natural service (Table 6). This gives a total conception rate of 75.0, 70.0 and 88.9% for groups 1, 2 and 3, respectively. No significant difference in conception rate was seen between treatment groups.

The average days postpartum to conception for the groups 1, 2, and 3 were 57.2, 71.5 and 59.5 days, respectively (Table 6). The average days to conception following the $\text{PGF}_{2\alpha}$ injection for groups 1, 2, and 3 were 11.7, 25.0 and 14.9 days, respectively; while the average day of conception of the breeding season was 8.7, 22.0, and 11.9 for the three groups. A significant difference between groups was not seen for days postpartum to conception or day of conception of the breeding season.

TABLE 5. EXPERIMENT 3 SERUM PROGESTERONE CONCENTRATIONS (ng/ml) DURING TREATMENT PERIOD

	(Group 1) Progestin implant +1000 I.U. PMSG	(Group 2) 1000 I.U. PMSG	(Group 3) Control
No. cows	8	10	9
Ave. progesterone concentration day 9 (range)	2.55 ^a (.13-9.0) ±3.66*	1.52 ^c (.31-5.5) ±1.56	1.44 ^a (.42-4.8) ±1.41
Ave. progesterone concentration day 23 (range)	33.52 ^b (4.5-101.1) ±32.19	11.17 ^d (3.9-21.2) ±5.78	6.22 ^b (.62-13.4) ±4.60
Ave. progesterone concentration day 27 (range)	2.30 ^a (.14-9.7) ±3.33	1.01 ^c (.39-2.4) ±.57	1.02 ^a (.39-2.4) ±.65
Ave. progesterone concentration day 36 (range)	1.55 (.57-2.5) ±.72	3.33 (.52-5.3) ±1.92	5.74 (.49-11.1) ±3.40

*Standard deviation

a,b $P < .05$ c,d $P < .01$

TABLE 6. EXPERIMENT 3 POSTPARTUM, CONCEPTION AND PREGNANCY DATA

	(Group 1) Progestin implant +1000 I.U. PMSG	(Group 2) 1000 I.U. PMSG	(Group 3) Control	Total
No. cows	8	10	9	27
No. cows conceiving by AI (%)	3 (37.5)	0 (0.0)	3 (33.3)	6 (22.2)
No. cows conceiving by natural service (%)	3 (37.5)	7 (70.0)	5 (55.6)	15 (55.5)
Total no. cows conceiving (%)	6 (75.0)	7 (70.0)	8 (88.9)	21 (77.8)
No. cows open (%) (non-pregnant)	2 (25.0)	3 (30.0)	1 (11.1)	6 (22.2)
Ave. days postpartum at start of treatment	22.4	21.3	21.9	21.8
Ave. days postpartum to conception	57.2	71.5	59.5	62.4
Ave. days to conception post-PGF ₂ α	11.7	25.0	14.9	17.4
Ave. days conception of breeding season	8.7	22.0	11.9	14.4

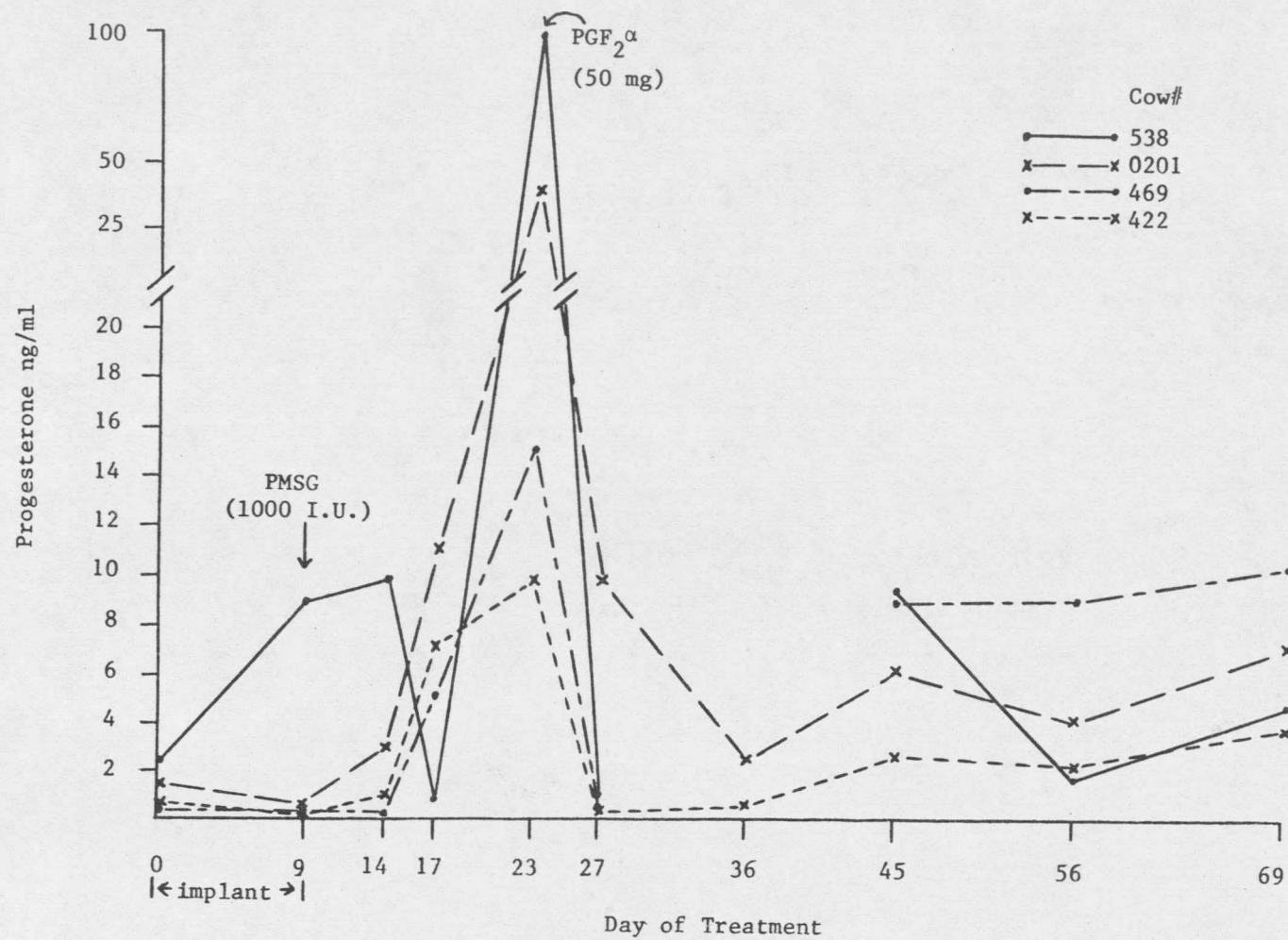


Figure 14. Serum progesterone concentrations for cows treated with progestin implant, 1000 I.U. PMSG and PGF₂α (group 1)

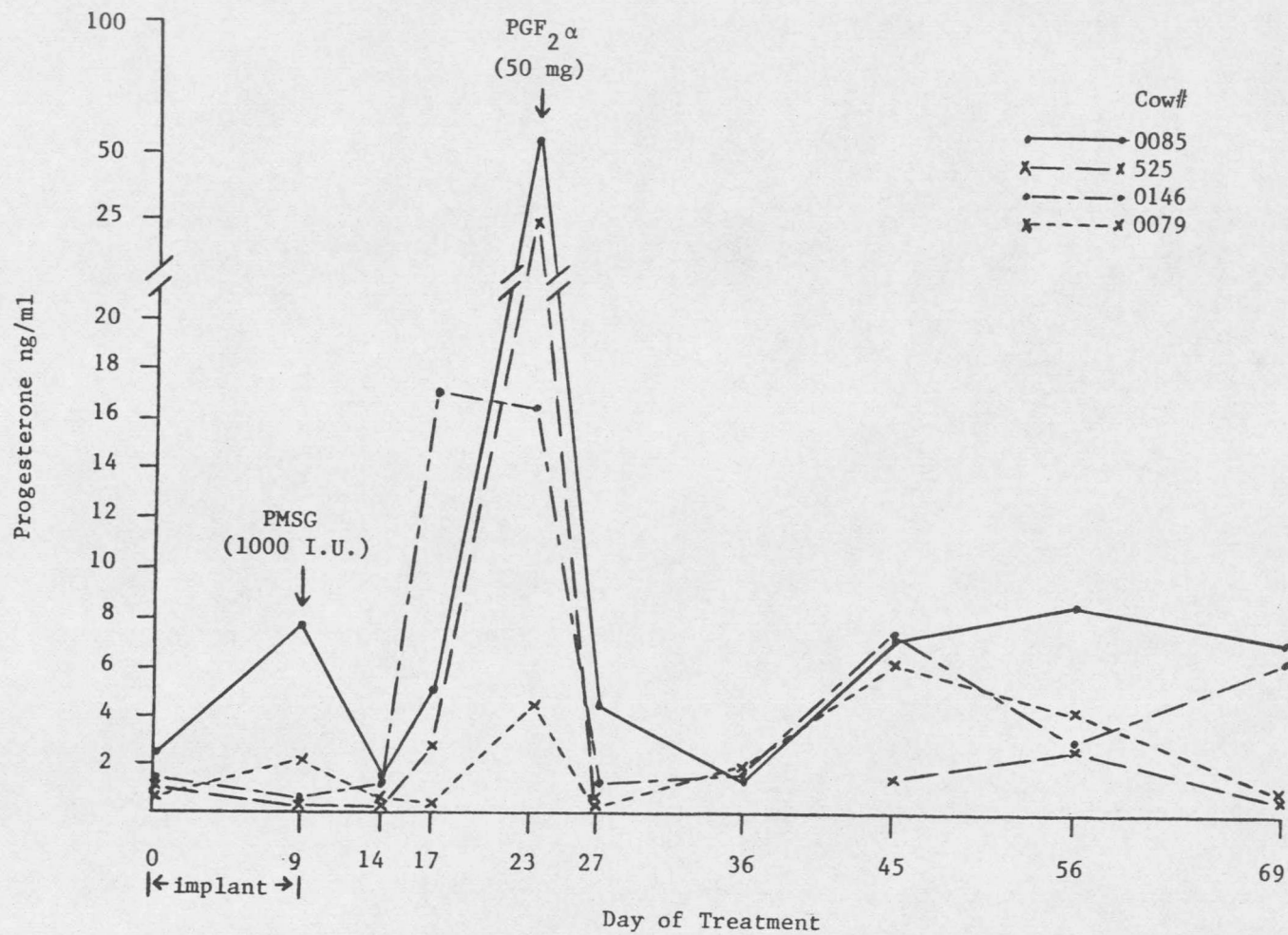


Figure 15. Serum progesterone concentrations for cows treated with progestin implant, 1000 I.U. PMSG and PGF₂α (group 1)

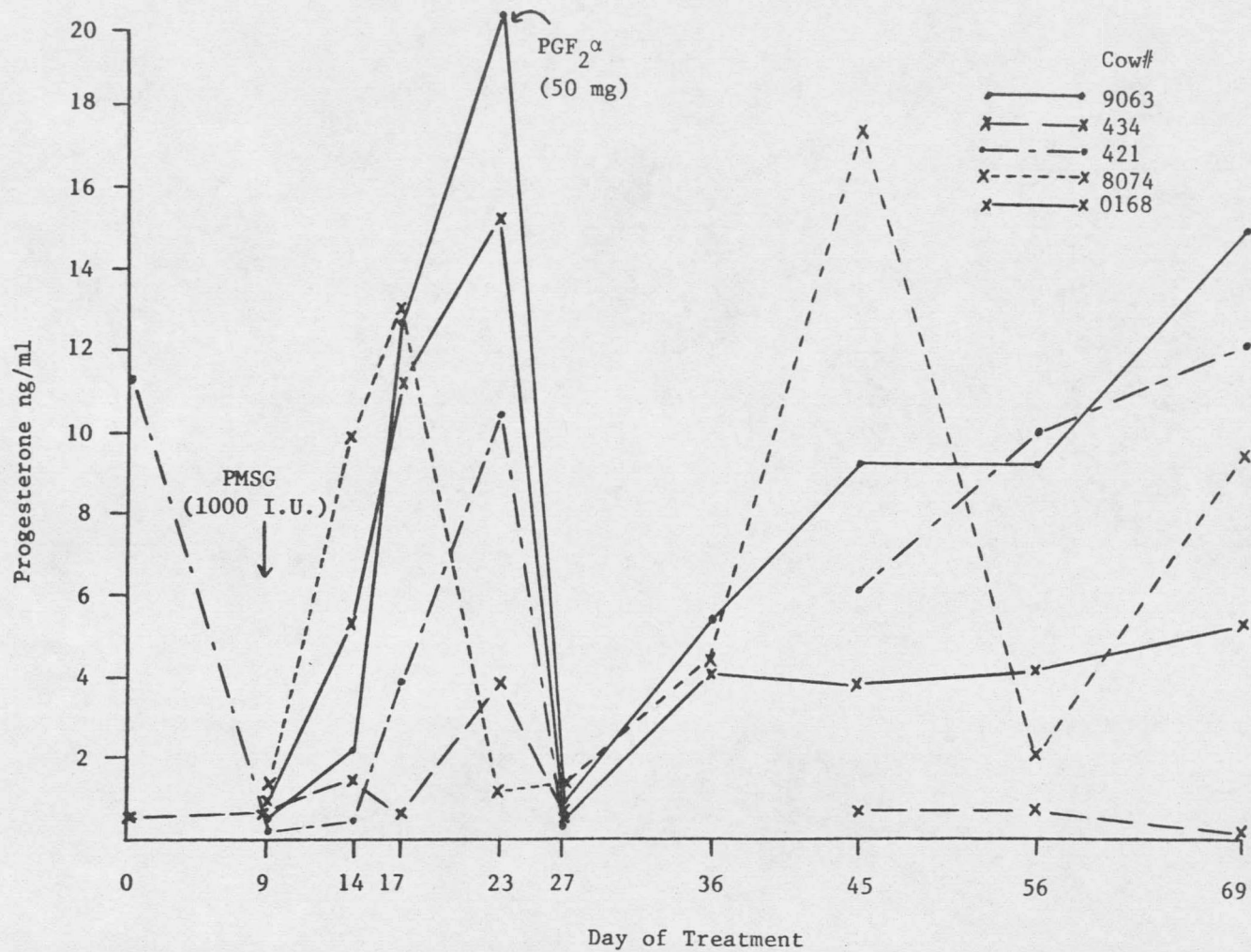


Figure 16. Serum progesterone concentrations for cows treated with 1000 I.U. PMSG and $\text{PGF}_2\alpha$ (group 2)

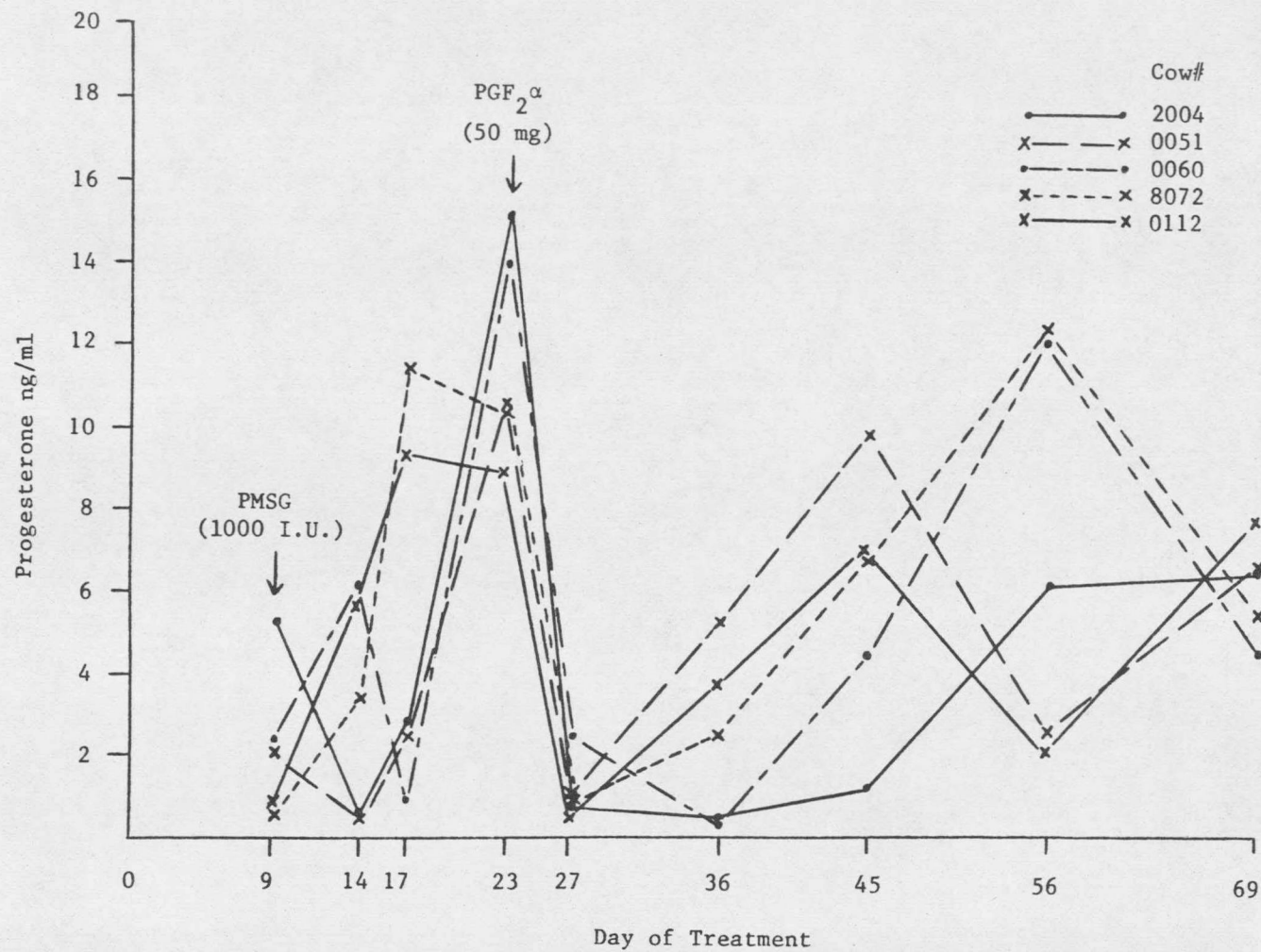


Figure 17. Serum progesterone concentrations for cows treated with 1000 I.U. PMSG and PGF₂ α (group 2)

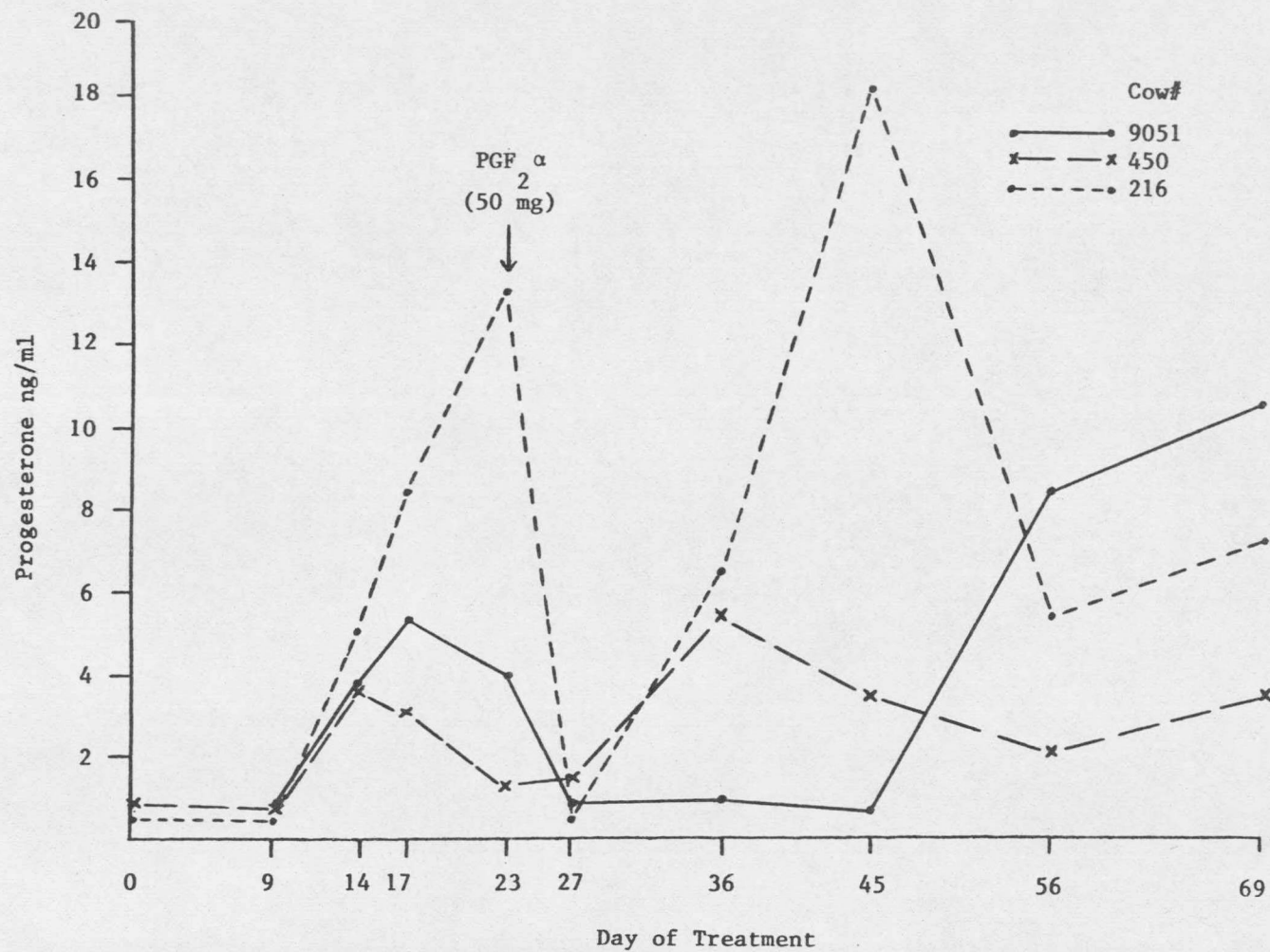


Figure 18. Serum progesterone concentrations for non-PMSG treated cows receiving PGF $_2\alpha$ (group 3)

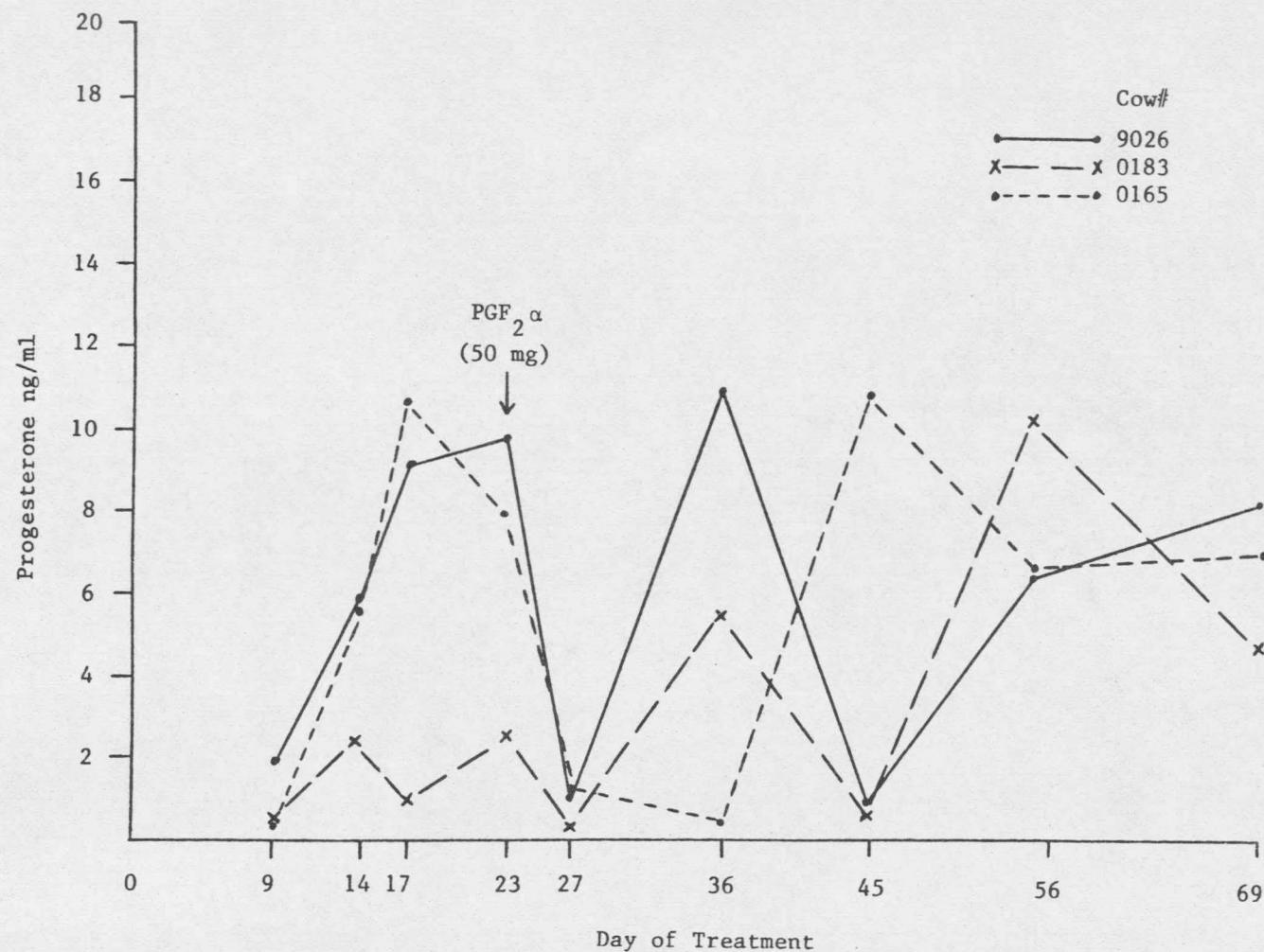


Figure 19. Serum progesterone concentrations for non-PMSG treated cows receiving $\text{PGF}_2\alpha$ (group 3)

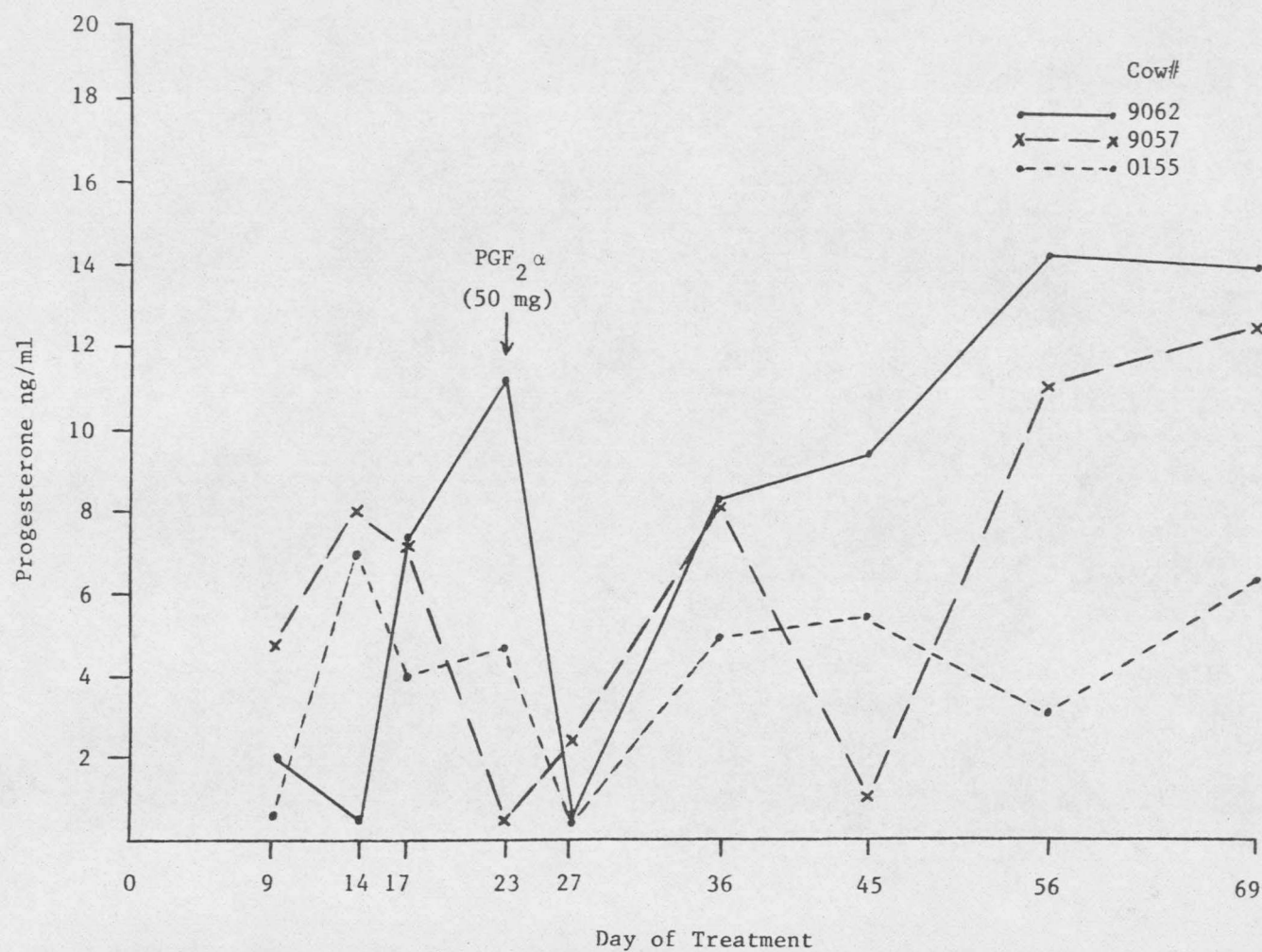


Figure 20. Serum progesterone concentrations for non-PMSG treated cows receiving PGF₂ α (group 3)

Experiment 4

Ovarian activity, as determined by serum progesterone concentrations ≥ 1.5 ng/ml on day 9, indicated that 13 of the 40 cows were secreting progesterone above basal levels (< 1.5 ng/ml) prior to the time of PMSG administration. This included three cows in group 1, four in group 2, one in group 3, two in group 4, and three in group 5. A corpus luteum or follicle > 10 mm was palpable in 1 of the 13 cows with the elevated progesterone levels.

Serum progesterone concentrations increased from day 9 to 23 in groups 1 ($P < .05$), 3 ($P < .05$) and 4 ($P < .05$) following PMSG on day 9. No significant increase was seen in group 2 following PMSG or in group 5 which received no PMSG.

Seven of the eight cows in group 1 exhibited peak progesterone concentrations on day 23 while the remaining cow reached a peak by day 18 of the treatment period (Figures 21 and 22). Four of the eight animals in group 2 reached peak progesterone levels by day 23 and two by day 18 while the two other animals did not show a response following PMSG (Figures 23 and 24). Group 3 had 6 of 8 cows reaching a peak at day 23, one at day 18, and one (436) having similar levels at day 18 (4.51 ng/ml) and day 23 (4.44 ng/ml; Figures 25 and 26). Group 4 consisted of four cows displaying progesterone peaks at day 23 and four at day 18 (Figures 27 and 28). Group 5, which received no PMSG, had 6 of 8 cows exhibiting elevated (≥ 1.5 ng/ml) progesterone levels by day 23 (Figures 29 and 30).

Average serum progesterone concentrations on day 23 for groups 1 through 5 were 33.03, 8.49, 14.27, 7.90, and 3.51 ng/ml, respectively (Table 7). Maximum progesterone levels on day 23 (prior to $\text{PGF}_2\alpha$) varied considerably among cows not only between, but also within treatment groups. Concentration ranges were 2.52-156.90, .24-29.01, 1.70-30.59, .55-22.80 and .53-8.69 ng/ml for groups 1 through 5, respectively (Table 7).

A decrease in serum progesterone levels following the 50 mg dose of $\text{PGF}_2\alpha$ was observed between days 23 and 27 in groups 1 ($P<.05$), 2 ($P<.05$), 3 ($P<.10$) and 4 ($P<.10$) while no significant difference was seen in group 5. A decrease in progesterone levels below 1.5 ng/ml following $\text{PGF}_2\alpha$ was seen in 6 (75%), 8 (100%), 6 (75%), 4 (50%), and 2 (25%) of the cows in groups 1 through 5, respectively (Figures 21-30). An increase in progesterone concentrations following $\text{PGF}_2\alpha$ was observed in one cow in group 3, one in group 4, and five in group 5 (Figures 25, 28, 29 and 30).

An increase in progesterone values from day 27 to 36 was seen in groups 1 ($P<.05$), 2 ($P<.05$), 4 ($P<.10$), and 5 ($P<.10$) but not in group 3. No significant difference between day 9 and 27 was seen in any of the groups except group 3 ($P<.05$). A significant difference was observed for progesterone values between day 9 and 36 in groups 2 ($P<.05$), 3 ($P<.05$), and 4 ($P<.05$), but not in group 1 or 5.

Analyses across all treatments indicated significant differences

at day 9 vs. 27 ($P<.001$), 23 vs. 27 ($P<.001$), 27 vs. 36 ($P<.001$), and 9 vs. 36 ($P<.001$), but not at day 9 vs. 27.

Pregnancy data is incomplete at this time but all animals were palpated and fetuses aged in order to determine if conception occurred on the days of artificial insemination. Results indicated that four cows conceived at the time of AI in group 1, two in group 2, one in group 3, and two in group 4. No cows conceived by AI in group 5.

TABLE 7. EXPERIMENT 4 SERUM PROGESTERONE CONCENTRATIONS (ng/ml) DURING TREATMENT PERIOD

Treatment	No. cows	Serum progesterone concentrations (ng/ml)			
		Average concentration day 9	Average concentration day 23	Average concentration day 27	Average concentration day 36
Implant + 1000 I. U. PMSG (range)	8	1.62 ^a (.31-4.22) ±1.26*	33.03 ^b (2.52-156.90) ±51.64	1.47 ^c (.48-3.99) ±1.14	3.71 ^e (.44-12.67) ±4.04
1000 I. U. PMSG (range)	8	1.54 (.01-2.99) ±1.10	8.49 ^b (.24-29.01) ±9.95	.77 ^c (.13-1.47) ±.47	3.58 ^e (1.43-6.53) ±1.74
Implant = 750 I. U. PMSG (range)	8	.67 ^a (.14-1.96) ±.60	14.27 ^b (1.70-30.59) ±10.30	3.85 ^d (.12-23.36) ±7.92	5.41 (.23-15.83) ±4.93
750 I. U. PMSG (range)	8	1.70 ^a (0.01-6.99) ±2.22	7.90 ^b (.55-22.80) ±8.08	2.69 ^d (.14-7.66) ±2.73	6.87 ^f (.27-27.77) ±9.12
Control (range)	8	2.48 (.54-12.85) ±4.21	3.51 (.53-8.69) ±2.95	4.86 ^c (.45-15.45) ±4.73	7.28 ^e (.54-16.10) ±5.39

*Standard deviation

a,b; b,c, and c,e_{p<.05}b,d and d,f_{p<.10}

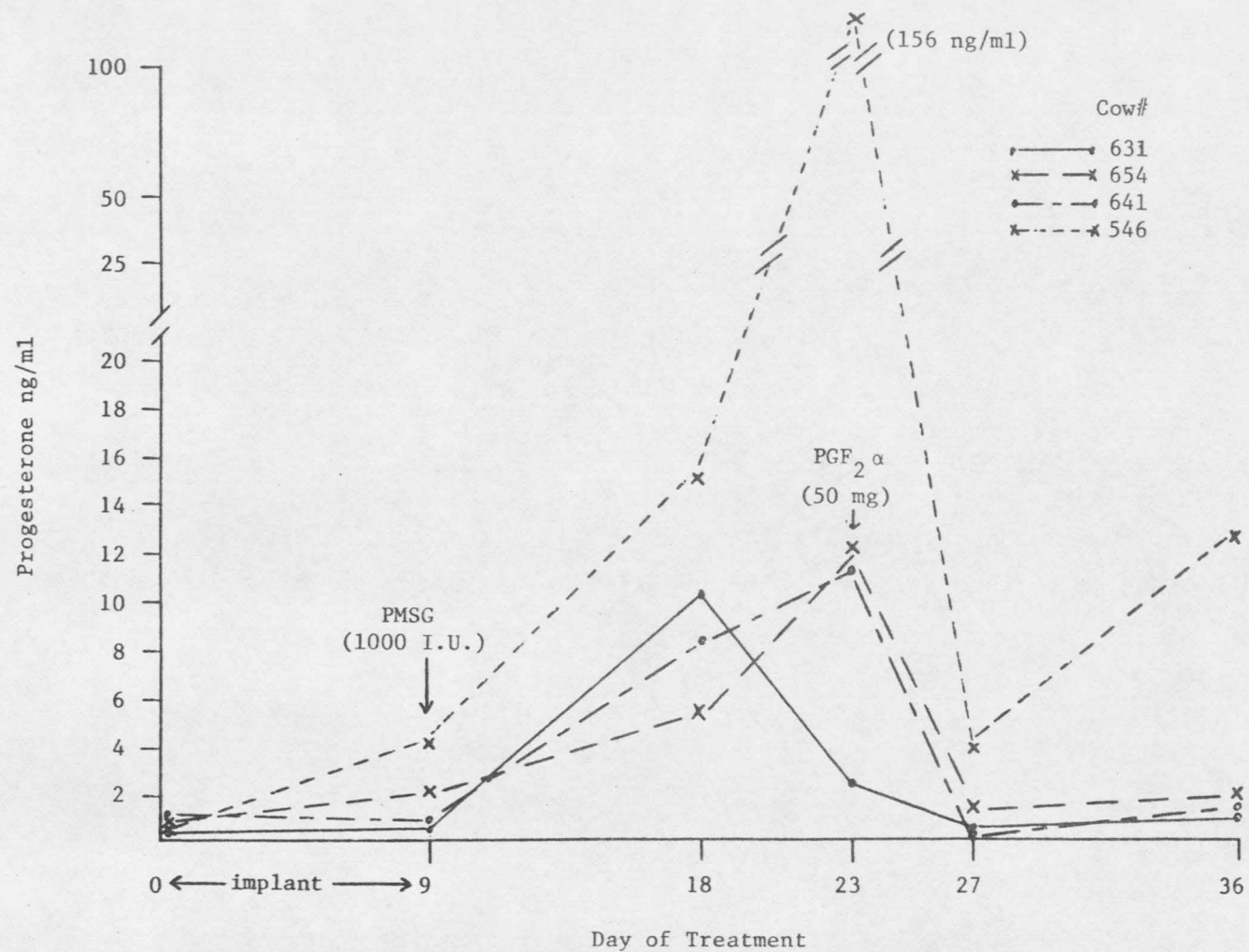


Figure 21. Serum progesterone concentrations for cows treated with progestin implant, 1000 I.U. PMSG and PGF₂α (group 1)

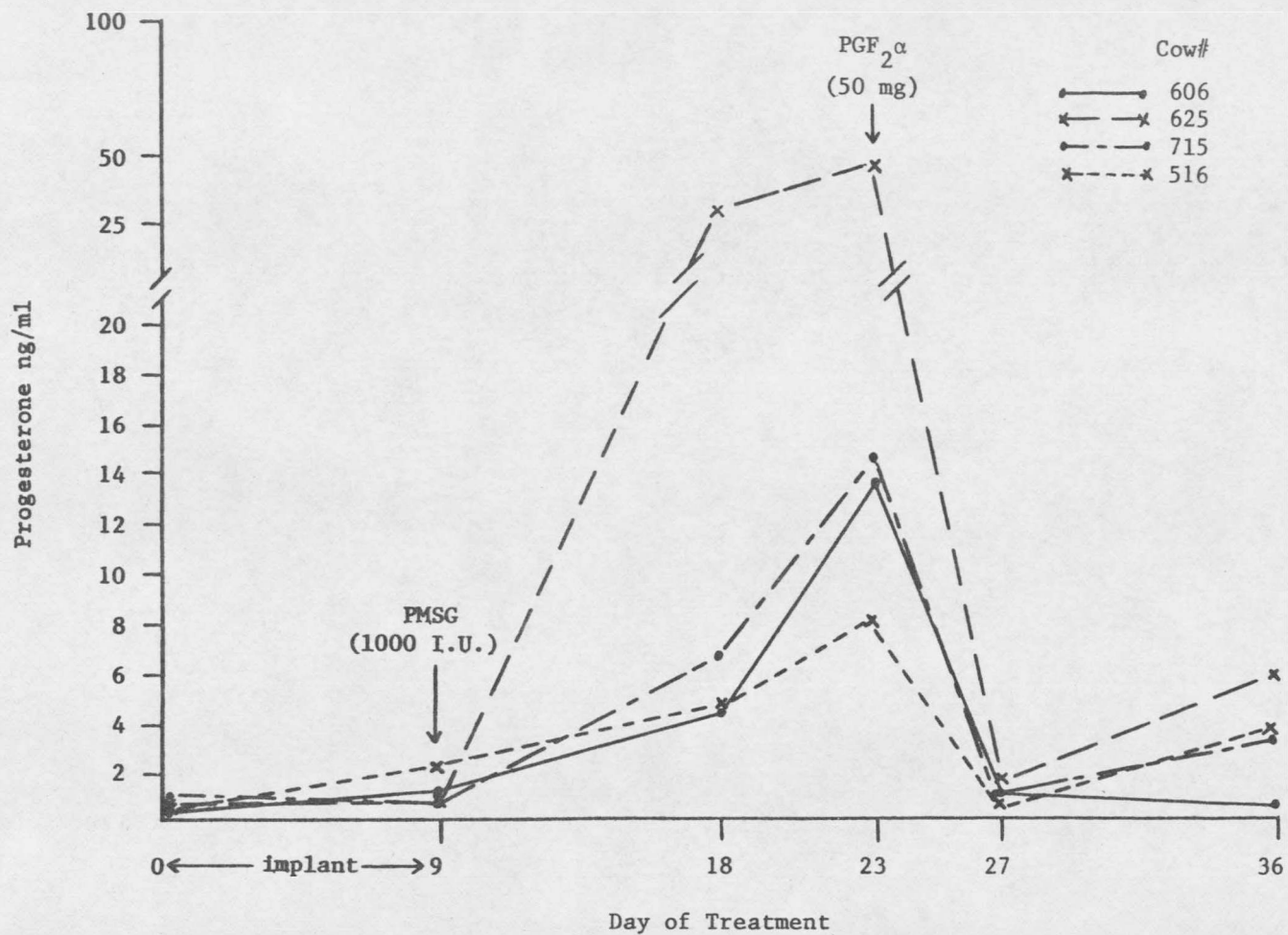


Figure 22. Serum progesterone concentrations for cows treated with progestin implant, 1000 I.U. PMSG and PGF₂α (group 1)

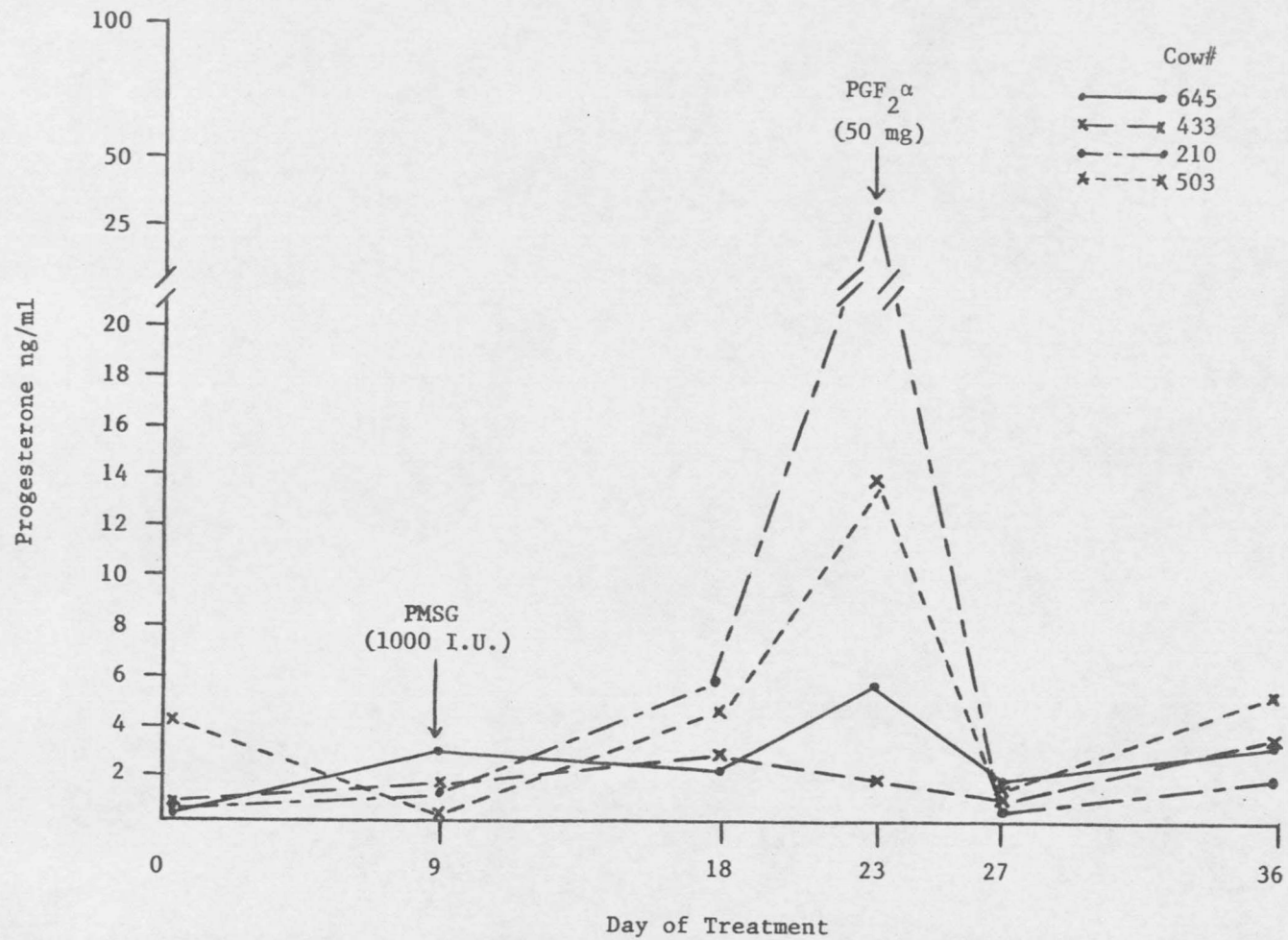


Figure 23. Serum progesterone concentrations for cows treated with 1000 I.U. PMSG and PGF₂α (group 2).

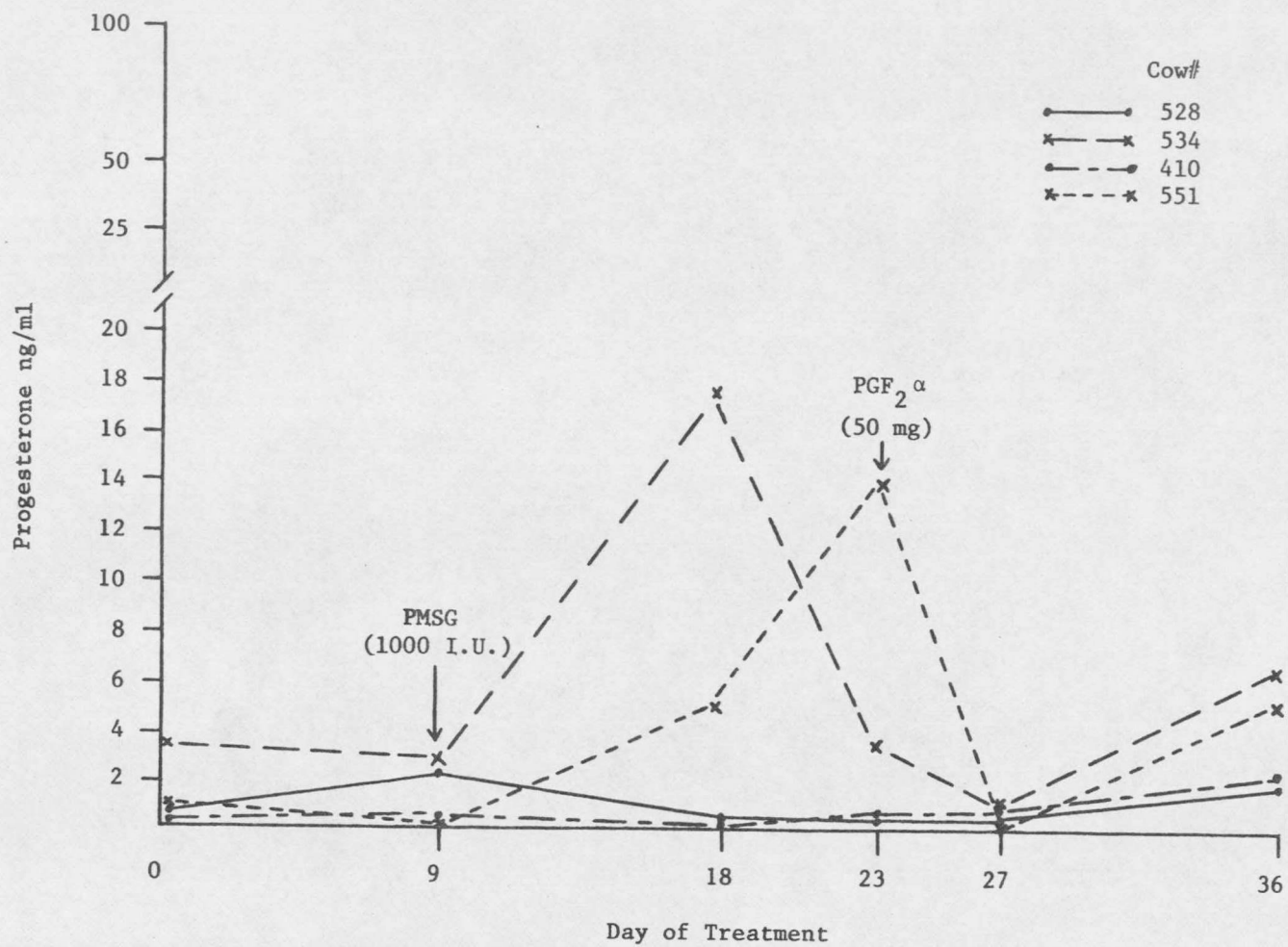


Figure 24. Serum progesterone concentrations for cows treated with 1000 I.U. PMSG and $\text{PGF}_2\alpha$ (group 2)

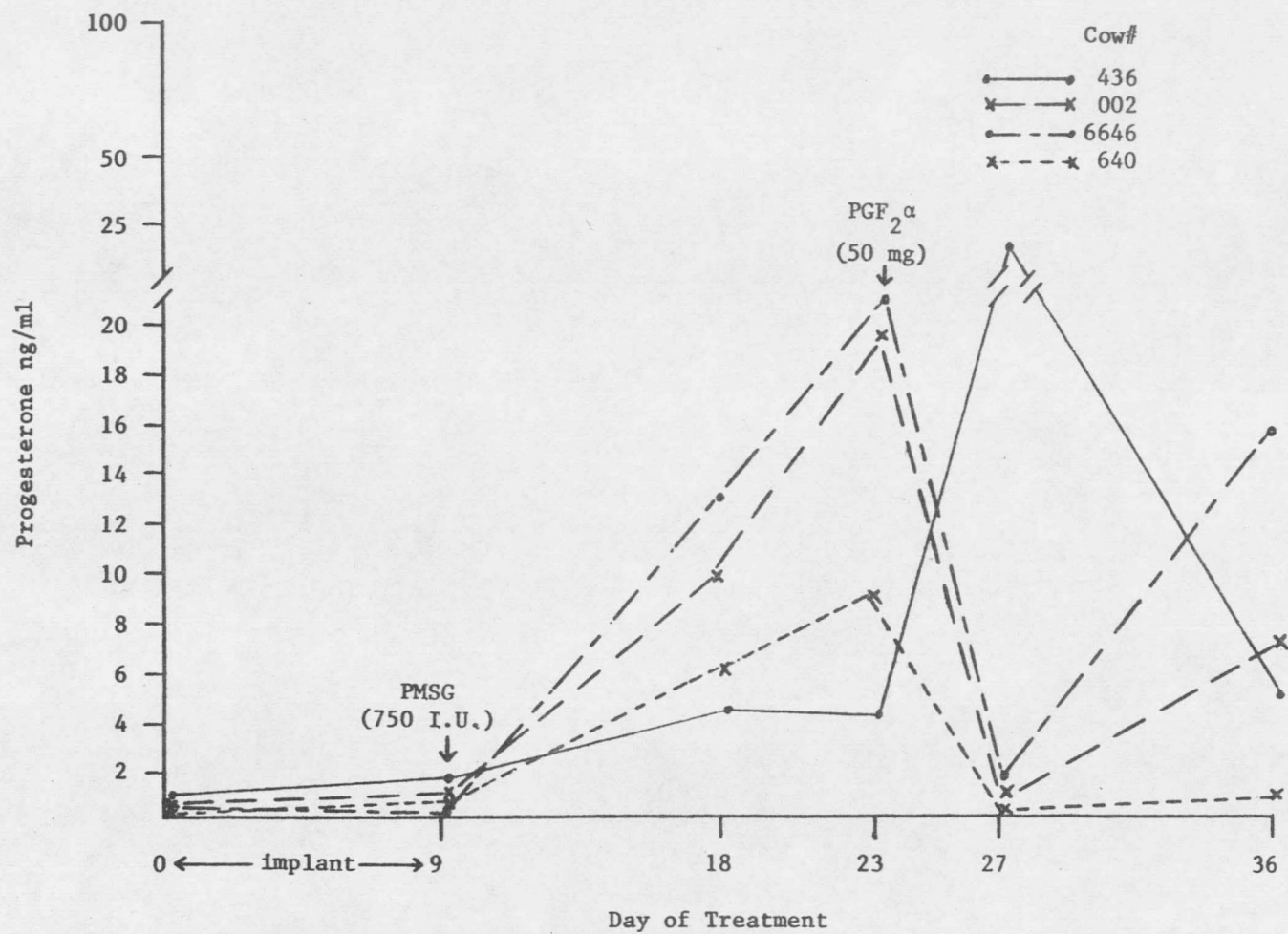


Figure 25. Serum progesterone concentrations for cows treated with progestin implant, 750 I.U. PMSG and PGF₂^α (group 3)

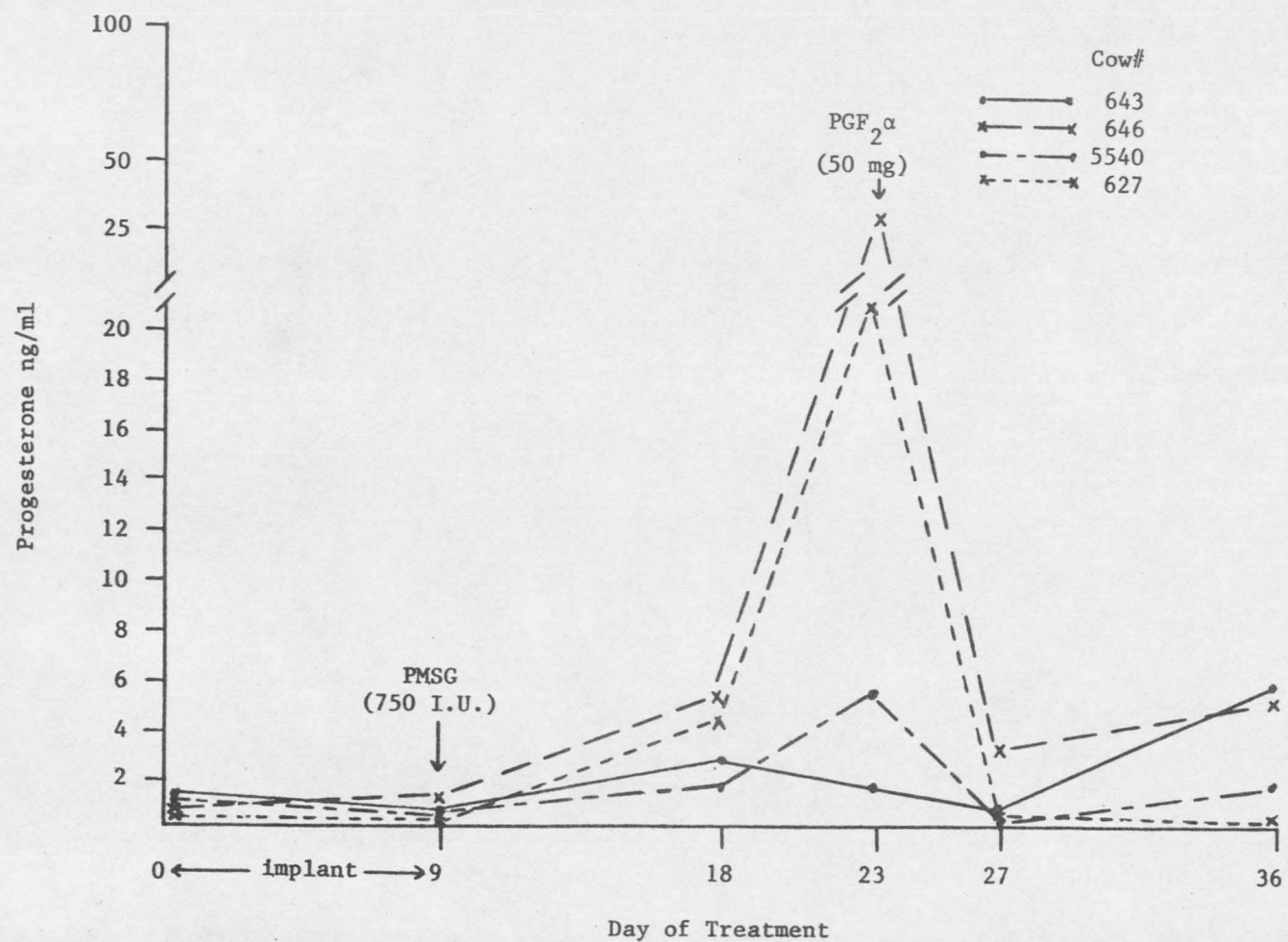


Figure 26. Serum progesterone concentrations for cows treated with progestin implant, 750 I.U. PMSG and PGF₂α (group 3)

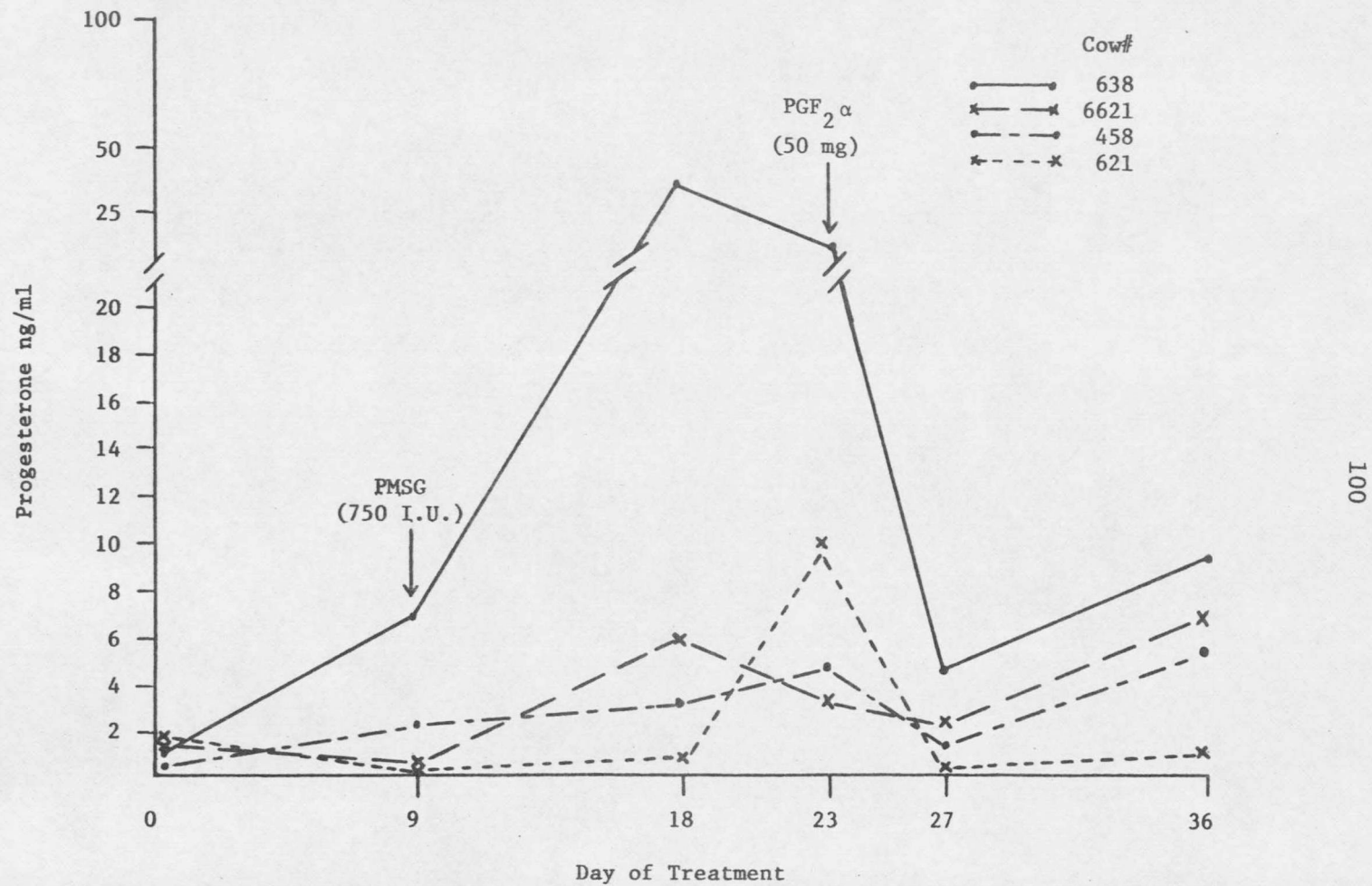


Figure 27: Serum progesterone concentrations for cows treated with 750 I.U. PMSG and PGF₂α (group 4)

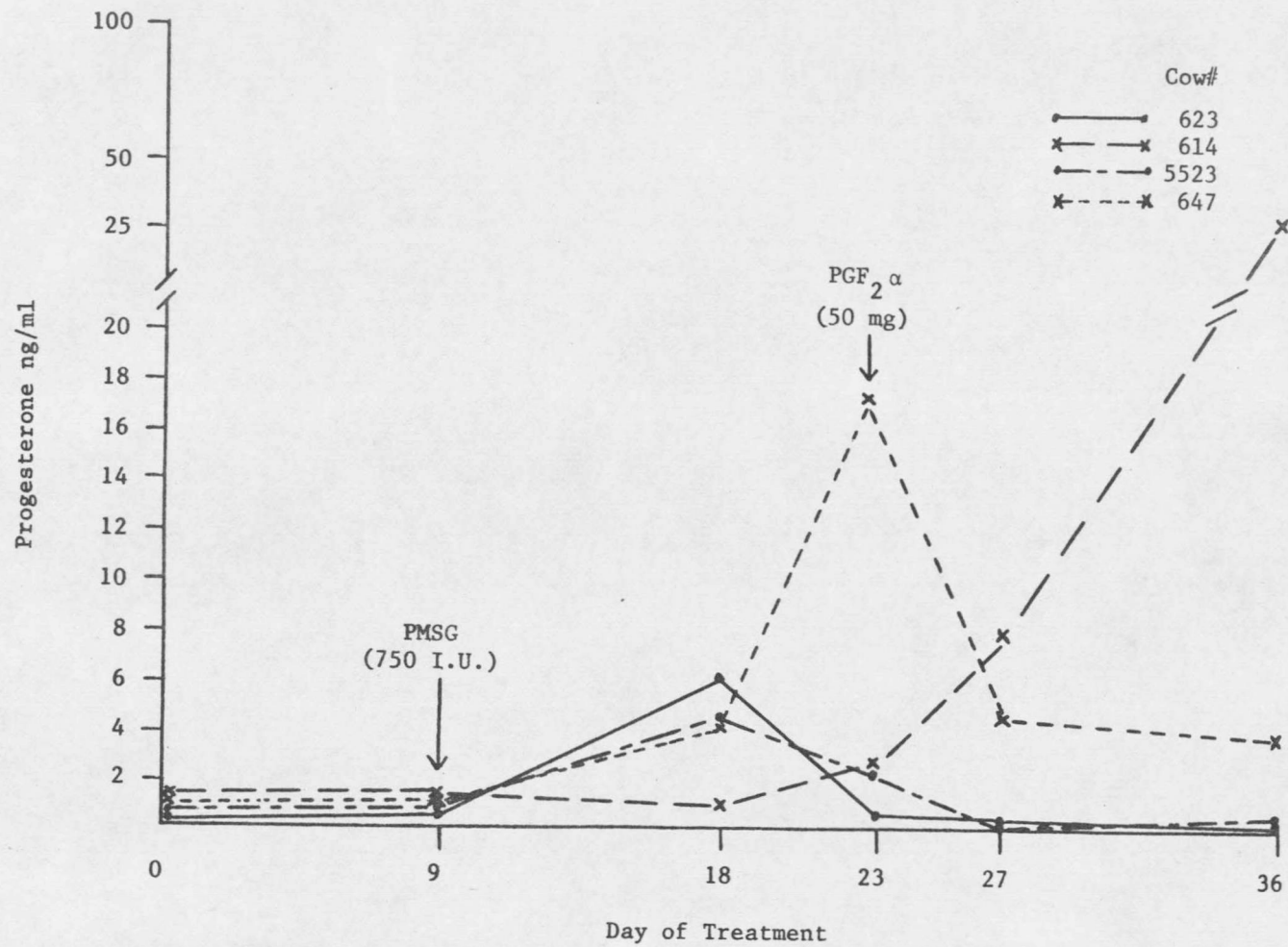


Figure 28. Serum progesterone concentrations for cows treated with 750 I.U. PMSG and PGF₂α (group 4)

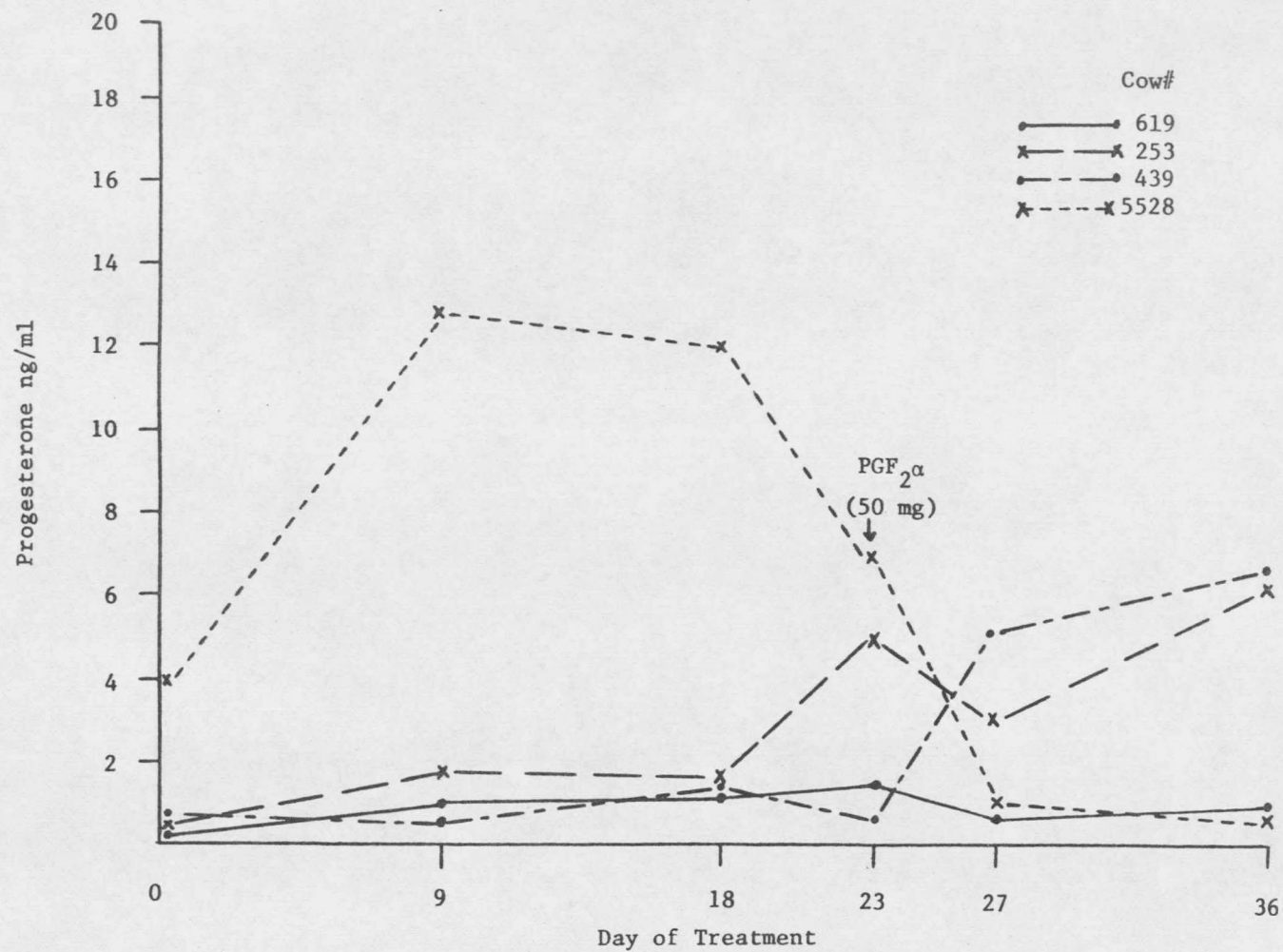


Figure 29. Serum progesterone concentrations for non-PMSG treated cows receiving $\text{PGF}_2\alpha$ (group 5)

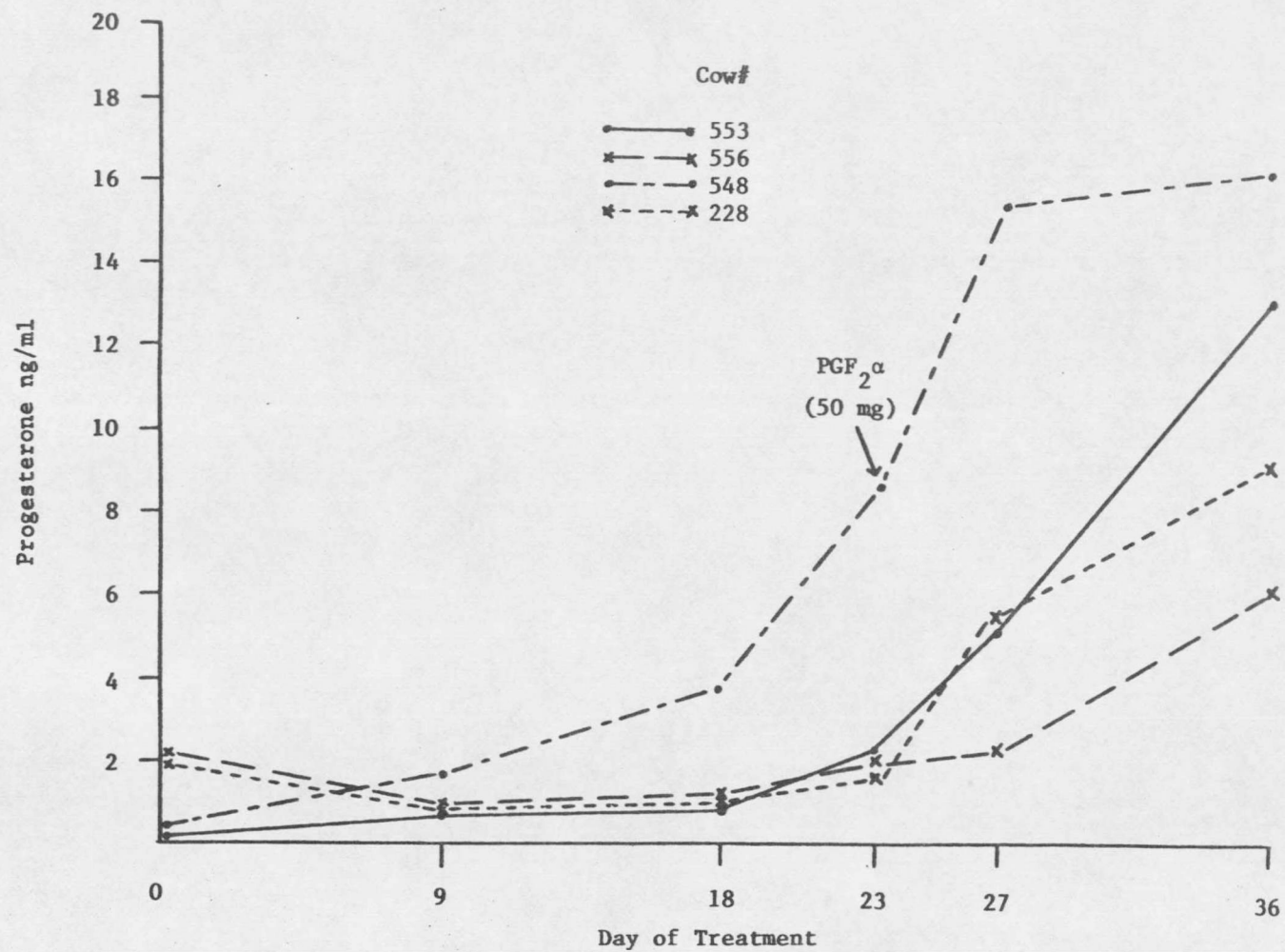


Figure 30. Serum progesterone concentrations for non-PMSG treated cows receiving PGF₂^α (group 5)

DISCUSSION

Ovarian activity, as evaluated by rectal palpation on day 9 showed that in 5 of 31 cows with serum progesterone values >1.5 ng/ml from experiments 1, 3, and 4 there was a detectable corpus luteum or follicle >10 mm present. This would indicate several possibilities that could account for the discrepancy between elevated serum progesterone levels and a lack of a palpable corpus luteum.

Two possibilities concern the source of the progesterone which could be derived from either adrenal or ovarian origin. Wagner et al. (1969) have reported a substantial increase in adrenal progesterone levels 7-30 days after calving. It seems unlikely in this instance that the adrenals could totally account for the elevated levels, >3 ng/ml, seen in many of the animals. Gonzalez-Padilla et al. (1975a) postulated that the first progesterone elevation prior to puberty in heifers could be at least partially produced by the adrenals to cause a priming LH peak which could induce luteinization of follicles or form a small CL. These luteinized follicles or the CL would account for a second progesterone rise originating from the ovary which would be related to the pubertal LH peak. A similar mechanism may exist in the postpartum cow whereby adrenal progesterone would cause the priming LH peak to induce luteinization of follicles or a small CL which in turn may activate the ovary to produce its own progesterone. The slightly elevated progesterone levels observed may be of adrenal origin while the high levels (>3 ng/ml) may be from luteinized follicles or a small CL.

Other evidence supports the hypothesis that the first progesterone elevation postpartum is primarily of ovarian origin. Castenson et al. (1976) reported the initial progesterone rise as a result of CL formation in 7 of 8 animals and a luteinized follicle in 1 of 8. Erb et al. (1971) postulated that a morphological alteration of follicles may occur to alter steroid synthesis to favor progestins instead of estrogen. In either case this would account for the lack of a palpable follicle or CL in the presence of elevated progesterone levels. This would also account for the absence of a visual CL at the time of laparotomy for the cow in experiment 3 having a progesterone value of 5.6 ng/ml.

An increase in serum progesterone concentrations was not observed from day 0 to 9 of the treatment period following removal of the progestin implant at day 9. The purpose of the implant was to cause a progesterone "build up" which would mimic the increase reported to occur naturally prior to the first postpartum estrus. This does not necessarily mean that the implant had no effect in this instance. The SC21009 progestin implant is impregnated with a synthetic progesterone. This could imply that it may still produce its effect by elevating endogenous progestin levels but these levels may not be detected by the radioimmunoassay (RIA) used to measure progesterone. This assay is based on an immune response to the natural occurring progesterone. Since the synthetic progestin and the naturally occurring progesterone have slightly different structures, the progesterone antibody may not be able to react

with the synthetic progestin in order to quantitate circulating levels in the blood.

Experiment 1

Ovarian response, as determined by serum progesterone concentrations, was evoked following PMSG administration with and without the nine day progestin pre-treatment. The cows in experiment 1 received PMSG on the average of 29.4 days postpartum. These cows were stimulated with PMSG at an earlier stage postpartum than many of the previous reports (Brown *et al.* 1972; Chupin *et al.* 1975; Phelps *et al.* 1976; and Echternkamp, 1978). Because these cows were at an earlier stage postpartum it was decided to use larger doses of PMSG on the assumption that they may be more refractory to the PMSG stimulation. A dose titration of PMSG was therefore incorporated into the treatment regime using 4000, 2000, or 1000 I.U. of PMSG on day 9 of the treatment period. The results showed a definite ovarian response at all three doses.

Statistical analysis was not applied to the progesterone data due to the small number of cows available for each treatment. The responses following the higher doses (4000 and 2000 I. U.) varied greatly within treatments (Table 2). This agrees with data observed with superovulation treatments in which a great amount of variation is associated with higher PMSG doses (Lamond and Gaddy, 1972 and Gengenbach, 1978). It may be interesting to note that the cow (534) with the highest progesterone value (40.7 ng/ml) prior to $\text{PGF}_2\alpha$ also had declining levels (4.9-2.6 ng/ml).

from day 0 to 9 (Figure 10). This would indicate that the cow was cycling and at the time of PMSG stimulation was nearing the end of her luteal phase. The effect of the PMSG may have coincided with the endogenous FSH present four to six days later to produce excessive follicular development resulting in the high progesterone levels.

Ovarian size, as determined by rectal palpation, was not always indicative of the serum progesterone concentrations. All the cows stimulated with 4000 I. U. PMSG had ovaries 2 to 3 times (5.5-7.5 cm) the size of a normal luteal phase ovary (2.5-3.5 cm), yet the animals receiving the progestin implant prior to the 4000 I. U. of PMSG had progesterone values half that of those not receiving the implant. Stimulation with the 2000 I. U. of PMSG resulted in ovaries ranging from 4.0 to 6.0 cm with the cow (534) having the 40.7 ng/ml progesterone peak also having the largest (6.0 cm) ovary. Ovaries from the 1000 I. U. dose of PMSG ranged from 3.5 to 4.5 cm. Both ovaries were stimulated in all the PMSG treated cows except for one cow (503) in the group receiving the implant and 1000 I. U. PMSG in which only one ovary had a detectable change in size following PMSG.

The day of maximum progesterone concentration was day 21 or 23 in all PMSG treated cows except for one animal (228) which peaked at day 19 and another (436) at day 17. This last cow's progesterone profile followed a pattern throughout the treatment period completely different from any of the other PMSG stimulated cows (Figure 9). This irregular

response may be a result of some type of neuroendocrine interference caused by the high (2000 I. U.) dose of PMSG.

One of the problems encountered with using PMSG is its long half-life. Menzer and Schams (1979), using a radioimmunoassay for PMSG, found concentrations highest following a 1500 or 3000 I. U. dose in cows at about 12 to 24 hours after a single injection. They also found measurable quantities of PMSG up to 10 days following a PMSG injection and thereby indicated a half-life similar to that seen in the horse (5-6 days). The long half-life could have a detrimental effect, especially at the higher dose levels of 4000 and 2000 I. U., by increasing the length of time the ovary is exposed to stimulation. This continuing stimulation may be producing a constant luteinization of new follicles in the presence of already high progesterone levels. The result would lead to a hormonal imbalance that may have a disturbing effect on the neuroendocrine mechanisms needed for resumption of cyclic activity.

The continual PMSG stimulation may also be one of the causes for the incomplete luteal regression or increased luteal activity following the $\text{PGF}_2\alpha$ given on day 23. This was evidenced by a continued luteinization as seen on day 27 in some of the animals in the 4000 and 2000 I. U. groups. The other possibility involved here may be the dose of $\text{PGF}_2\alpha$ administered. The 25 mg dose used may have been inadequate to cause complete luteal regression in the presence of abnormally high (>20 ng/ml) serum progesterone concentrations.

The elevated (≥ 1.5 ng/ml) progesterone levels seen in 10 of the 14 PMSG stimulated cows at day 18 post-PGF₂ α would indicate that these animals had initiated ovarian activity following PGF₂ α without any further exogenous stimulation. The fact that only one cow exhibited estrus during the breeding period and did not conceive following insemination would also suggest that the other PMSG treated animals in the study had not developed a hormonal pattern indicative of that required for estrus to occur.

Carrick and Shelton (1969), using ovariectomized heifers observed that physiological quantities of estrogen would readily induce estrus, but if the animals were pretreated with large doses of estrogen a state of refractoriness to the physiological dose of estrogen was produced and no estrus was observed. A similar mechanism may be occurring in these PMSG treated cows in which large quantities of estrogens were being secreted during follicular development. This would indicate that a refractory state may be negating the effects resulting from the cow's endogenous estrogens produced following PGF₂ α and estrus was not being exhibited.

It would appear that adequate ovarian stimulation was provided by the 1000 I. U. dose of PMSG and there would be no value and possibly some detrimental effects in using the higher doses of 4000 and 2000 I. U. The effects of the progestin implant could not be ascertained from this study.

Experiment 2

Results from the field trial conducted on the group of late calving cows in experiment 2 indicated a positive effect was produced by the exogenous hormone treatment in group 2 as seen by the conception data comparing group 2 vs. group 3. A significant difference ($P < .05$) was seen between groups 2 and 3 in the day of conception of the breeding season (Table 3). No significant difference between average days postpartum to conception was established between any of the three groups but this data may be misleading due to the small number of animals available for analysis. The estradiol valerate injection in group 1 would appear to have a detrimental effect on cows showing estrus as seen by the percent of cows (0.0%) AI'd by estrous detection. Due to a difference in treatment regimes it is difficult to compare these results with other investigations but Radford et al. (1976) also saw a lack of estrus exhibited following estrogen treatment in suckled postpartum cows. Other reports indicate an enhanced estrus response following estrogens used alone or in conjunction with progesterone treatments (Foote and Hunter, 1964; Saiduddin et al. 1968; Brown et al. 1972; and Chupin et al. 1975).

It could be postulated that the failure of cows in group 1 to show estrus may be a result of the effects of the exogenous estrogen on the hypothalamo-hypophyseal axis. The short progesterone rise seen prior to the first postpartum estrus (Donaldson et al. 1970; Corah et al. 1974; Prybil and Butler, 1978; and LaVoie et al. 1979) may act to change the

sensitivity of this axis. If exogenous estrogen is given prior to this change in sensitivity, the hypothalamo-hypophyseal axis may be very susceptible to high levels of estrogen at this anestrus period and thereby interfere with neuroendocrine events for a period of time up to, including, and shortly after the PMSG and $\text{PGF}_2\alpha$ administration. This may mean that exogenous ovarian stimulation by PMSG and luteolysis by $\text{PGF}_2\alpha$ could have occurred but the neural events connected with behavioral estrus were not able to be manifested.

This theory may also be used to explain the differences in conception rate between groups 1 (66.7%) and 2 (100.0%). The luteal activity following the PMSG induced follicular development would provide progesterone levels capable of changing the sensitivity of the hypothalamo-hypophyseal axis and thereby allow the endocrine events associated with a cyclic hormonal pattern to occur. This would mean the elevated progesterone values provided exogenously from day 0 to 9 of the treatment period in group 2 would not be occurring in group 1 until possibly day 12 to 23, a shift of 13 days. The 13 day difference would shorten the breeding period considerably for group 1 and could thereby account for the lower pregnancy rate. This 13 day shift could also account for the difference in the average day of conception of the breeding season for group 1 (18.2) and group 2 (9.4).

The effect of the progestin implant in group 2 may have been to provide the progesterone "priming" needed to initiate a change in

neuroendocrine events. The removal of the implant followed by PMSG stimulated follicular development, luteinization and $\text{PGF}_2\alpha$ induced luteolysis would provide a similar pattern to the naturally occurring events of declining progesterone levels followed by follicular development, luteinization and luteolysis. This would provide the animals in group 2 with an additional exogenously produced estrous cycle and thereby enhance the chances of conception the following cycle. Thatcher and Wilcox (1973) have suggested that as the number of estrous cycles increase the services per conception decrease. This would indicate an increased possibility of conception occurring in group 2 vs group 3 at this postpartum stage. This is supported by data for average days conception of breeding season, 9.4 and 23.3 and average days postpartum to conception, 58.1 and 72.0 for groups 2 and 3 respectively.

Experiment 3

An increase in serum progesterone from day 0 to 23 was observed in all three treatment groups. This increase may have been expected following PMSG stimulation in groups 1 and 2 but the rise in progesterone levels in all of the non-PMSG stimulated animals in group 3 was somewhat unexpected. The animals were randomly grouped in pens so the animals from all treatments were in close physical contact with one another. Many of the cows were also exhibiting estrous behavior following PMSG stimulation. This would indicate that estrogen levels were high enough to cause estrus and may have been sufficient to effect ovarian activity

by some type of pheromone action. Ovarian synchrony has been demonstrated in rats (McClintock, 1978) and women (McClintock, 1971) after a period of 3 to 4 cycles of continual exposure to fellow living companions via air-born chemical messages. The fact that all animals in group 3 exhibited a rise in progesterone levels would indicate that some similar type of neural response may be responsible for initiating ovarian activity.

There was variation in all treatment groups as to the day when maximum progesterone concentrations were observed. This individual variation among cows may be a result of individual variation in gonadotropin levels. Schallenberger *et al.* (1978) observed a wide range in LH response patterns following postpartum GnRH treatment. This variation in LH levels could account for high or low levels of progesterone depending on the amount of LH available to cause luteinization of the stimulated follicles. This would also account for the early decline in progesterone concentrations prior to PGF₂ α administration. Since LH is luteotropic in the cow, a short luteal period could result from inadequate LH levels needed to maintain luteal function.

The higher progesterone concentrations seen on day 23 in groups 1 (33.5 ng/ml) and 2 (11.17 ng/ml) vs. group 3 (6.22 ng/ml) would indicate that 1000 I. U. of PMSG is sufficient to stimulate ovarian activity. The augmented response to PMSG in the animals previously exposed to the progestin implant (group 1) may be a result of a change in sensitivity of the hypothalamo-hypophyseal axis. This could allow the increasing

estrogen levels, as a result of PMSG stimulated follicular development, to exert its positive feedback role on LH to cause an LH spike large enough to ovulate the follicles present at this time. The result would be a large number of follicles ovulating and available for luteinization to occur. This positive estrogen feedback mechanism may not be functioning if the neuroendocrine mechanism has not been previously sensitized with the progestin. The effect would be either an insufficient LH spike for ovulation or insufficient LH for CL maintenance if ovulation did occur.

The incomplete luteal regression seen in some animals following $\text{PGF}_2\alpha$ administration may primarily be from an inadequate dose of $\text{PGF}_2\alpha$ needed to cause complete regression in cows having excessively high (>20 ng/ml) progesterone concentrations.

A significant increase in progesterone levels from day 27 to 36 was observed in groups 2 and 3 but not in group 1. The elevated progesterone levels on day 36 would be indicative of ovarian activity in response to endogenous hormone levels. This may not be a totally accurate representation of ovarian activity due to the fact that some animals may have been at a stage in which elevated progesterone levels were not present.

The average day of conception of the breeding season was 8.7, 22.0, and 11.9 for groups 1, 2, and 3, respectively. This would appear to be somewhat contradictory to the progesterone data on day 36. The discrepancy may be a result of 1) a few days error in the day of conception which was based on palpation information, and/or 2) the rise in serum

progesterone concentrations may have occurred shortly after the 36 day blood sample indicating ovarian activity may have been present but had not reached the stage where elevated progesterone levels would be detected.

There seemed to be no pattern associated with earlier conception and elevated (≥ 1.5 ng/ml) progesterone levels as seen prior to the treatment period in any of the groups. There also appeared to be no pattern established between either progesterone levels following PMSG or $\text{PGF}_2\alpha$ and early conception.

Experiment 4

An increase in serum progesterone concentrations from day 9 to 23 in groups 1, 3 and 4 would indicate that 750 I. U. of PMSG was sufficient to stimulate ovarian activity. The extreme range in progesterone values on day 23 was considerably reduced by the 750 I. U. of PMSG as seen in group 3 (1.70-30.59 ng/ml) vs. 1 (2.52-156.90 ng/ml). The progestin implant appeared to cause an augmented response to the PMSG doses at day 23 as seen by comparing groups 1 (33.03 ng/ml) and 3 (14.27 ng/ml) vs. groups 2 (8.49 ng/ml) and 4 (7.90 ng/ml). This augmented response produced by the progestin implant could be a result of the change in sensitivity of the hypothalamo-hypophyseal axis as previously discussed in experiment 3.

A significant increase in progesterone levels between day 9 and 23 was not observed in groups 2 and 5. The fact that a significant increase

was not established in group 2 may be due to the lack of a sufficient number of animals showing elevated levels following PMSG (6 or 8), thus a significant increase might be seen if a greater number of animals were studied. Group 5 had an increase in average serum progesterone concentrations from day 9 to 23 of 2.48 to 3.51 ng/ml. These figures may be somewhat deceiving due to a cow (5528) which appeared to be cycling and had progesterone levels which declined from day 9 (12.85 ng/ml) to 23 (6.81 ng/ml). Five of the eight cows in the non-PMSG stimulated group had increasing progesterone levels following PMSG stimulation of the cows in the other groups (Figures 29 and 30). This trend is similar to that seen in the non-PMSG stimulated animals in experiment 3, although levels did not increase as dramatically. This increase in progesterone levels may be mediated through pheromone action as previously discussed in experiment 2.

The significant decrease in average progesterone levels in groups 1, 2, 3 and 4 between days 23 and 27 indicates that $\text{PGF}_2\alpha$ was effective in causing luteal regression in the PMSG stimulated animals. Incomplete luteal regression as seen by progesterone values ≥ 1.5 ng/ml was observed in 2, 1 and 3 cows in groups 1, 3 and 4, respectively and may have been a result of an inadequate dose of $\text{PGF}_2\alpha$ needed for the higher levels of progesterone present at the time of injection. One cow in both groups 3 and 4 and five cows in group 5 had an increase in progesterone following $\text{PGF}_2\alpha$. The reaction to the $\text{PGF}_2\alpha$ in group 5 was quite consistent when

looking at the type of individual cows involved. One animal (619) had basal levels (<1.5 ng/ml) throughout the treatment period, thus $\text{PGF}_2\alpha$ would have no effect. Another animal (5528) appeared to be cycling as seen by her increasing and decreasing progesterone levels from day 0 to 23 and was responsive to the $\text{PGF}_2\alpha$. Of the remaining six animals, one cow (253) had a small decrease from day 23 (4.85 ng/ml) to day 27 (3.47) while the remaining 5 cows had an increase in progesterone values from day 23 to 27. This would indicate that the $\text{PGF}_2\alpha$ had little or no effect in causing luteal regression in these last six cows.

The results following $\text{PGF}_2\alpha$ from these cows in group 5 could be interpreted in two ways. First, the continuing rise in progesterone levels could mean that the $\text{PGF}_2\alpha$ had no effect and these increasing levels may have occurred without $\text{PGF}_2\alpha$. This would indicate that the ovaries were in a refractory state and unresponsive to the $\text{PGF}_2\alpha$ as seen in the normal cycling cow at days 1 to 5 of the estrous cycle. The second possibility is the increase in progesterone levels may be a result of the $\text{PGF}_2\alpha$ injection. This would suggest that the luteinized tissue present in these animals may not be the same as that seen in normal cycling cows, thus regression does not occur. This would imply that the progesterone may be derived from a luteinized follicle rather than a corpus luteum and that $\text{PGF}_2\alpha$ either has no effect or possibly a stimulatory effect on the luteinized follicle. Reports of $\text{PGF}_2\alpha$ effects on luteinized follicles seem to be missing in the literature and is an area in which further

work may provide some insight into this problem.

The significant increase between days 27 and 36 in groups 1, 2, 4 and 5 would indicate that these animals had initiated ovarian activity following $\text{PGF}_2\alpha$ without any further exogenous stimulation.

Pregnancy data is incomplete at this time except for the AI pregnancy data. It would be premature to speculate on the effect of the treatments on conception until calving data is available.

SUMMARY AND CONCLUSIONS

Four experiments were conducted to determine the effects of exogenous hormone therapy on the postpartum interval to conception in anestrous, suckled beef cows. Blood samples and ovarian palpation were used in experiments 1, 3, and 4 to monitor serum progesterone concentrations and ovarian structures while experiment 2 was conducted as a field trial. Animals throughout all experiments ranged from 16 to 33 days postpartum at the start of the treatment period.

Experiment 1 contained 18 cows randomly allotted to seven treatment regimes. Three treatments consisted of a progestin implant (6 mg) subcutaneously inserted in the ear on day 0 (day 0 = start of treatment) followed by removal of the implant on day 9 and injections of either 4000, 2000, or 1000 I. U. of PMSG. Three other treatments received only the PMSG on day 9 while the last treatment received neither the progestin implant or PMSG. All animals received $\text{PGF}_2\alpha$ (25 mg) on day 23 of the treatment period and were bred by estrous detection thereafter. An ovarian response, as determined by serum progesterone concentrations, was evoked following PMSG stimulation with and without the progestin implant. There was a greater degree of variation in response to the PMSG in the higher doses (4000 and 2000 I. U.). Adequate ovarian stimulation was provided by the 1000 I. U. dose of PMSG and there appeared to be no value and possibly some detrimental effects in using the higher doses. Luteal regression following $\text{PGF}_2\alpha$ was not always complete, possibly due to an inadequate dose or altered response to the

PGF₂α. Estrus was observed in one cow following PGF₂α.

Experiment 2 consisted of 27 animals assigned to three treatment groups. Group 1 received an injection of 2.5 mg estradiol valerate (EV) plus 1.5 mg SC21009 (a progestin) on day 0. A progestin implant was inserted from day 0 to 9 followed by a 1000 I. U. dose of PMSG at the time of implant removal. Group 2 received only the implant and PMSG while group 3 received no EV-SC21009 injection, implant or PMSG. All groups received a 50 mg dose of PGF₂α on day 23 of the treatment period. Cows were bred by estrous detection for a five day period following PGF₂α and by natural service for the remainder of the 45 day breeding season. Total conception rates for groups 1, 2, and 3 were 66.7, 100.0, and 88.9%, respectively. Average day conception of the breeding season for groups 1, 2, and 3 were 18.2, 9.4, and 23.3, respectively. A significant difference (P<.05) was seen between groups 2 and 3, indicating a positive effect was produced by the progestin implant and PMSG treatment.

Experiment 3 contained 27 cows randomly allotted to three treatment groups. Group 1 received a progestin implant from day 0 to 9 of the treatment period followed by 1000 I. U. of PMSG at the time of implant removal. Group 2 received only the PMSG at day 9 while group 3 received neither the implant or PMSG. All groups were given a 50 mg dose of PGF₂α on day 23. The cows were bred by AI at 72 hours following PGF₂α with Polled Hereford and at 96 hours with Holstein semen. This was followed by natural service to an Angus bull for the remainder of the 45 day

breeding season. Serum progesterone levels at day 23 for groups 1, 2, and 3 were 33.50, 11.17, and 6.22 ng/ml, respectively, indicating an augmented response to PMSG in animals pretreated with the progest implant. Progesterone levels in group 3 tended to increase in a similar pattern as that seen in the PMSG stimulated animals in groups 1 and 2. An inconsistent response to $\text{PGF}_2\alpha$ was observed, indicating incomplete or no regression of luteal tissue in some instances. The average day conception of the breeding season was 8.7, 22.0, and 11.9 for groups 1, 2, and 3, respectively, indicating a negative response may have occurred when using PMSG without prior exposure to the progestin implant.

Cows in experiment 4 were randomly assigned to five treatment groups. Group 1 received a progestin implant from day 0 to 9 followed by 1000 I. U. of PMSG at the time of implant removal. Group 2 received only the 1000 I. U. of PMSG on day 9. Group 3 was similar to group 1 except 750 I. U. of PMSG was administered on day 9. Group 4 received only the 750 I. U. of PMSG on day 9 while group 5 received neither the implant or PMSG. All groups were given 50 mg of $\text{PGF}_2\alpha$ on day 23. The cows were bred by AI at 72 hours following $\text{PGF}_2\alpha$ with Polled Hereford and at 96 hours with Shorthorn semen. This was followed by natural service to an Angus bull for the remainder of the 45 day breeding season. It would appear that 750 I. U. of PMSG is adequate to stimulate an ovarian response in these postpartum cows. Serum progesterone concentrations at day 23 for groups 1 through 5 were 33.03, 8.49, 14.27, 7.90 and

3.51 ng/ml, respectively. An augmented response to PMSG can be seen by comparing groups 1 and 3 to 2 and 4. A significant decrease was seen in groups 1, 2, 3 and 4 following $\text{PGF}_2\alpha$ although individual variation was observed in all groups.

The major conclusions from these studies are:

- 1) The ovaries are responsive to PMSG stimulation, as measured by progesterone concentrations, in anestrous, suckled beef cows at 25 to 42 days postpartum.
- 2) A progestin implant (6 mg) inserted for nine days prior to PMSG stimulation appears to produce an augmented progesterone response to PMSG.
- 3) The varied response to $\text{PGF}_2\alpha$ following induced ovarian stimulation in the anestrous beef cow may be a result of abnormally high physiological progesterone concentrations or an altered ovarian response to $\text{PGF}_2\alpha$. This altered response may be a result of luteinized follicles which respond differently to $\text{PGF}_2\alpha$ than luteinized tissue normally present in corpus lutea.
- 4) Conception rate at 72 and 96 hours following $\text{PGF}_2\alpha$ was quite low while there appeared to be an enhanced fertility and conception rate on the second cycle (about 21 days) following $\text{PGF}_2\alpha$.
- 5) Low doses (1000 or 750 I. U.) of PMSG when used in conjunction with the progestin implant are effective in inducing ovarian activity and affords a treatment that may be effective in decreasing the postpartum interval to conception.

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APPENDIX

APPENDIX TABLE I. EXPERIMENT 3 SERUM PROGESTERONE CONCENTRATIONS (ng/ml) OF INDIVIDUAL COWS DURING TREATMENT PERIOD

Treatment	Cow no.	Serum progesterone concentrations (ng/ml)					
		Day 9	Day 14	Day 17	Day 23	Day 27	Day 36
Progestin implant + 1000 I. U. PMSG (group 1)	0079	2.28	.26	.25	4.51	.14	1.86
	422	.13	.91	7.05	9.73	.64	.57
	469	.14	.15	5.30	15.23	.54	----
	525	.33	.25	3.54	24.69	.20	----
	538	8.95	9.92	.93	101.12	1.28	----
	0146	.24	1.67	17.21	16.51	1.27	1.38
	0085	7.74	1.79	5.13	56.64	4.58	1.43
	0201	.57	2.88	11.28	39.71	9.74	2.53
1000 I. U. PMSG (group 2)	9063	.46	2.26	12.73	21.20	.97	5.33
	434	.47	1.56	.53	3.88	.77	----
	421	.31	.49	3.97	10.49	.39	----
	8074	1.48	9.94	13.18	1.24	1.45	4.44
	2004	5.46	.71	2.85	15.24	.78	.52
	0051	2.01	.70	2.59	10.63	1.05	5.30
	0060	2.55	6.33	1.02	14.06	2.41	.67
	8072	.63	3.49	11.61	10.64	.88	2.47
	0168	1.04	5.47	11.54	15.40	.79	4.14
	0112	.83	5.81	9.60	8.95	.59	3.77
Control (group 3)	9026	1.99	5.99	9.35	9.89	1.20	11.08
	0183	.61	2.46	1.07	2.55	.39	5.64
	0165	.50	5.75	10.72	8.02	1.23	.49
	9062	2.15	.51	7.63	11.32	.73	8.31
	9057	4.79	8.06	7.21	.62	2.39	8.21
	0155	.71	7.05	4.10	4.73	.50	4.98
	9051	.95	3.99	5.61	4.02	.81	1.03
	450	.83	3.80	3.19	1.44	1.52	5.43
	216	.42	5.21	8.41	13.43	.43	6.52

APPENDIX TABLE II. EXPERIMENT 4 SERUM PROGESTERONE CONCENTRATIONS OF INDIVIDUAL COWS DURING TREATMENT PERIOD.

Treatment	Cow no.	Serum progesterone concentrations (ng/ml)					
		Day 0	Day 9	Day 18	Day 23	Day 27	Day 36
Progestin implant + 1000 I. U. PMSG (group 1)	631	.30	.31	11.58	2.52	.60	.88
	654	.78	2.13	5.41	12.30	1.39	1.43
	641	.85	.78	8.14	11.35	.48	1.28
	546	.62	4.22	14.94	156.90	3.99	12.67
	606	.39	1.09	4.24	13.41	1.14	.44
	625	.56	1.02	29.73	45.13	2.17	5.73
	715	.94	.97	6.83	14.49	1.14	3.37
	516	.63	2.44	4.97	8.16	.87	3.89
1000 I. U. PMSG (group 2)	528	.21	2.31	.64	.24	.16	2.49
	534	3.58	2.99	17.46	3.59	.98	6.53
	410	.18	.69	.44	.41	.80	2.22
	551	.25	.01	4.90	13.92	.13	5.13
	645	.49	2.83	2.19	5.43	1.47	2.90
	433	.64	1.56	2.70	1.54	.85	3.05
	210	.53	1.40	5.56	29.01	.62	1.43
	503	4.14	.50	4.22	13.77	1.18	4.90
Progestin implant + 750 I. U. PMSG (group 3)	643	1.07	.36	2.60	1.70	1.01	5.90
	646	.42	1.01	5.37	30.57	2.81	5.71
	5540	.67	.16	1.62	6.07	.12	1.72
	627	.56	.14	4.30	21.95	.53	.23
	436	.70	1.96	4.51	4.44	23.36	5.23
	002	.62	.80	9.99	19.06	1.03	7.35
	6646	.48	.45	12.79	21.15	1.12	15.83
	640	.56	.51	6.20	9.20	.80	1.27
750 I. U. PMSG (group 4)	623	.30	.60	6.00	.55	.42	.27
	614	1.06	1.37	.73	2.62	7.66	27.77
	5523	.34	.73	4.58	2.21	.19	.38
	647	.65	.86	4.15	17.30	4.28	3.44
	638	1.00	6.99	35.31	22.80	4.89	9.89
	6621	1.29	.96	5.82	3.62	2.76	6.65
	458	.67	2.06	2.90	4.48	1.16	5.84
	621	1.66	.01	1.13	9.58	.14	.71
Control (group 5)	619	.33	.90	1.07	1.33	.45	.81
	253	.35	1.65	1.63	4.85	3.47	6.03
	439	.67	.54	1.16	.53	5.57	6.67
	5528	4.08	12.85	12.08	6.81	.97	.54
	553	.16	.61	.79	2.37	5.00	12.89
	556	2.25	.82	1.61	2.17	2.28	6.06
	548	.27	1.64	3.64	8.69	14.45	16.10
	228	2.23	.79	.97	1.36	5.66	9.10

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