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POSTPARTUM USE of PGF_2 and GnRH in DAIRY CATTLE

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Introduction

The fertility of lactating dairy cattle at the time of first insemination (60 DIM) is related positively to the number of previously detected estruses during the postpartum period (1). Cows expressing one or more estruses during the first 30 days post partum required fewer services than cows with no estruses. This observation indicates that the physiological and hormonal events associated with estrus help restore uterine and ovarian function to a state conducive to the establishment of pregnancy.

To help increase the number of estrous cycles prior to first insemination and to reduce the incidence of pyometra and cystic ovaries during the postpartum period, the use of Prostaglandin F_2 alpha (PGF_2) and Gonadotropin Releasing Hormone (GnRH), post partum has been advocated (2,3,4,5,6,7,8,9,10,11,12). However, results from these studies are conflicting.

This paper will review the use of PGF_2 and GnRH in relation to day post partum and its use in cows having an abnormal or normal parturition. In addition, postpartum uterine involution and resumption of ovarian cyclicity will be discussed.

Postpartum Period

Following parturition, and expulsion of the fetal placenta, the uterus begins the process of involution or regression. This process is characterized by a marked reduction in uterine size and histological changes in the endometrium. During the first 4 to 10 days post partum the reduction is slow, although the weight of the uterus is reduced by 70% from calving to day 10 post partum (13). Between days 10 to 14, reduction in uterine size is rapid, the tone is increased and lochial discharge is at its maximum. At this time the diameter of the previously gravid horn is reduced from about 120 mm to 70 mm. Following this time, uterine horn size continues to decrease but it never reaches its pre-pregnancy size.

During involution of the uterus the caruncles which consist mainly of crumpled masses of septae and blood vessels are undergoing necrosis due to vasoconstriction. As a result of this necrosis, PGF_2 in peripheral circulation increases to maximal concentration 1 to 4 days postpartum and declines to basal levels by 17 days post partum (14,15,16,17,18). A longer duration and higher magnitude of postpartum plasma concentration of PGF_2 , have been associated with a faster rate of uterine involution(19,20). Higher concentrations of PGF_2 early in the postpartum period have also been implicated in early resumption of ovarian activity (21,22). At day 10 to 12 post partum, the caruncular surface is denuded of necrotic tissue and regeneration of the epithelium starts from the edge of the caruncles and is completed by 25 to 30 days post partum. The average interval from calving to complete involution of the uterus is about 18 - 22 days in primiparous cows (uterine horn diameter between 20 to 25 mm) to 25 - 35 days in multiparous cows (uterine horn diameter between 30 to 35 mm) (13).

During parturition, the uterus is infected by a variety of bacteria such as E. coli, streptococci, staphylococcus, Clostridium spp., A. pyogenes, Bacteroides and Fusobacterium. The last three species predominate after two weeks post partum as the environment of the uterus changes to an anaerobic one. Most of these bacteria are eliminated during the involution process with an decreasing rate of animals having positive cultures to these bacteria from 93% at day 15 to less than 10% at day 40 to 60. (13).

The cervix is located cranial to the pelvic cavity during the first week post partum. After day 25 post partum, the cervical diameter is greater than that of the previously gravid horn. By 25 days post partum the majority of cows have a uterine tract which is completely retractable into the pelvis with the diameter of the cervix being slightly greater than that of the previously gravid uterine horn. However, the reduction in cervical size occurs much slower in dairy cows with metritis and a poorly involuting uterus and results in a cervical diameter greater than the previously gravid uterine horn (13).

The variability seen in cervical and uterine horn size, type of lochial discharge and continued uterine involution, contributes to spurious diagnosis of metritis during the postpartum period. The designation of postpartum cows to have metritis and to be at risk for impaired fertility, should be based on the occurrence of dystocia, retained fetal membranes (RFM) or both, instead of reproductive tract size and discharge. These conditions have been documented to delay uterine involution, and predisposes cows to metritis post partum (13,23,24). Furthermore, the incidence of dystocia and RFM affected fertility more than did uterine discharge or size.(23)

During this period of uterine involution, the ovaries are changing from a quiescent state to an active one. After parturition, postpartum, hypothalamic-pituitary-ovarian activity is reinitiated by day 10 as evidenced by follicular turnover with development of mature follicles (25,26). Ovulation and resumption of ovarian cyclicity by 30 days post partum has been reported to occur in over 75 % of dairy cows (27,28). However, dairy cows in severe negative energy balance may experience longer postpartum anestrous periods (28)

Postpartum Reproductive Management with PGF_2 and GnRH

Secretion of LH after administration of GnRH is restored in dairy cattle by 10 days post partum (6). This is consistent with the early occurrence of postpartum follicular activity. Administration of GnRH by day 14 post partum has been demonstrated to induce ovulation and cyclic activity (26). The induction of ovulation was associated with an increase in the rate of uterine involution following GnRH administration (29). The CL formed after treatment with GnRH at 10 to 14 days post partum will respond to PGF 10 days later, but with a lower a luteolytic rate. Benmrad et al (3), demonstrated a luteolytic rate of 47% after PGF injection around 24 days post partum in cows pretreated with GnRH 10 days earlier. However, this rate was not different from control cows receiving PGF_2 around day 24 post partum, but not pretreated with GnRH. This suggests that the luteolytic response of the CL to PGF injection may be lower during the postpartum period.

A number of studies have been conducted to improve reproductive efficiency by the treatment of postpartum cows with GnRH or GnRH followed later with PGF_2 in cows experiencing a normal or abnormal parturitions. However, results from this studies have been conflicting. A review of several recent studies reinforces this point.

Two studies (3,7) have demonstrated that cows with an abnormal puerperium that were administered GnRH between day 10 and 18 post partum had improved fertility; treated cows ovulated earlier, had a decreased interval from calving to conception, and had fewer services per conception. In contrast, GnRH administered early post partum increased the interval from calving to conception (5). Cows with a normal puerperium treated with PGF_2 early post partum had fewer services per conception than did untreated cows (8). However, services per conception or calving to conception interval, did not improve for cows with an abnormal puerperium treated with PGF_2 from 14 to 16 d post partum (8), 25 to 30 d post partum (2), or 33 and 40 d post partum (12).

To initiate estrous cyclicity in the early post partum period and to help improve fertility, treatment with GnRH, and PGF_2 10 days later has been evaluated. Fertility was not improved in cows with either abnormal (10) or normal (6) puerperium that were treated with GnRH early post partum followed by PGF_2 10 days later. In contrast, the interval from calving to conception was reduced in cows that had a normal puerperium and then were treated with GnRH in combination with PGF_2 post partum (9). In cows with either normal or abnormal puerperium treated with GnRH followed by PGF_2, inseminations per conception were reduced (3).

In a large field trial, 445 Holstein cows that had experienced dystocia, retained fetal membrane, or both at parturition (abnormal calvings) were used to determine the effects of postpartum treatment with

GnRH, PGF_2 , or both, on various reproductive measurements (11). Cows affected with these conditions were assigned to each of four treatment groups. One group of cows was untreated, and three groups received GnRH once at 12 days post partum, or GnRH at 12 and PGF_2 at 26 days post partum, or PGF_2 given at 12 and 26 days post partum. Conception rate after first insemination was higher for cows treated with PGF_2 at 12 and 26 days post partum. Cows affected with these conditions treated early post partum with GnRH alone or followed 10 days later with PGF_2 did not have improved reproductive performance. In these cows treatment with GnRH may have resulted in ovulation or luteinization of follicles in cows affected with dystocia, RFM, or both concurrently with uterine infection. A progesterone-dominated uterus could have exacerbated the uterine infection and reduced fertility. In the report by Etherington et al. (5), cows treated with GnRH and that experienced an increased calving to conception interval also had an increase incidence of pyometra. Furthermore, increase milking frequency (milking three times per day) could negate the effect of GnRH as suggested in two previous studies (4,12).

In the previously cited report (11), the investigators expected that GnRH treatment would hasten ovulation and establish a CL that could be lysed when PGF_2 was administered. Potentially, this induction of a new estrous cycle could aid uterine involution, reestablish cyclicity, and improve fertility, as proposed by Benmrad et al (3), and Thatcher et al (1). However, in some cows, uterine involution may not have been improved despite initiation of an estrous cycle after luteolysis, or an estrous cycle may not have been initiated. In postpartum dairy cows, a significant increase in luteolysis after PGF_2 for cows previously treated with GnRH 10 days earlier was not detected. The CL formed after GnRH treatment 10 to 14 d postpartum exhibited a 47% luteolytic response to an injection of PGF_2 between 20 to 24 d postpartum (3).

In the study by Risco et al (11), progesterone concentration was examined as a covariate and did not have a significant effect on any of the reproductive traits evaluated. The higher first insemination conception rate of the abnormal group treated with PGF_2 at days 12 and 26 post partum was independent of progesterone concentration because a interaction was not found between treatment and progesterone concentration. In a previous report, dairy cows treated with PGF_2 at day 26 post partum had less vaginal discharge, smaller diameter uterine horns, less inflammation and fibrosis in the endometrium, and were less likely to have Actinomyces pyogenes isolated from a biopsy at d 40 than untreated cows (30). These effects were independent of progesterone concentrations at the time of treatment at d 26 postpartum.

Programmed Reproductive Management

A programmed reproductive treatment program (PRT), was evaluated in a large commercial dairy herd, in terms of occurrences of estrous periods and a fertile estrus at days 57 to 62 post partum (31). The PRT utilized sequential treatments of GnRH and PGF_2 .

One hundred ninety nine dairy cows (n=199) were assigned randomly at 14 days post partum to a control (n=100) group or a programmed reproductive treatment group (PRT, n=99). Cows in the PRT group received 8 ug of GnRH (Buserelin, or Receptal® (Hoechst Roussel Agri-Vet Co., Somerville, NJ.) on days 14 and 50 post partum and 25 mg of PGF_2 (Lutalyse® (Upjohn Co., Kalamazoo, Mi.) injected on days 21, 34 and 57 post partum. Cows in the PRT group had a greater frequency of progesterone (P_4) concentrations > 1 ng/ml (50% vs 30%; P<0.01). Frequency of cows having P_4> 1 ng/ml at both days 21 and 34 post partum was greater in the PRT group than in the control group (39% vs 20%; P<0.01). Accumulation of days with a palpable cystic ovary was lower in the PRT than the control group (11.4% vs 6.5%; P<0.05).

Conception rate to all services, services per conception and the interval from calving to conception were not different between the groups. Intensity of estrus detection was low for the study based on a low estrus detection rate (42%) and a low palpation pregnancy rate index (below 68%) throughout the study period. Accuracy of estrus detection was low based on the distribution of normal interestrus intervals for the study (19 to 23 d; 13.5%). It was concluded that programmed reproductive treatments during the postpartum period are functionally effective relative to altered ovarian activity; however, potential advantages to such a system are not evident without good estrus detection practices in a large commercial herd.

An alternative postpartum reproductive management strategy that is less aggressive relative to treatments imposed is implementation of a controlled breeding program at the time of planned insemination. Ferguson and Galligan (32), have implemented a "Prostaglandin Synchronization Program" that is implemented at a time consistent with an established voluntary waiting period (VWP) to first insemination. For example, with a voluntary waiting period of 55 days post partum, the first injection of PGF_2 would be made to all cows > 50 days post partum. All eligible cows could be injected on a Monday with likely occurrence of estrus 3 to 5 days later. Cows detected in estrus will be inseminated to detected estrus. At 2 weeks following the first PGF_2 injection, all cows not detected in estrus can be injected with a second PGF_2 injection and inseminated at detected estrus. With such as system, it is reasonable to expect 90% of the cows to be inseminated following 2 injections of PGF_2 14 days apart. One could use this scheme to evaluate heat detection efficiency in the herd. A goal of inseminating 70% of the cows following the first injection can be established. If heat detection rates fall below 50%, techniques for heat detection or anestrous condition of the cows should be evaluated. Reasonable goals suggested by Ferguson and Galligan (32) are to obtain 80% of cows inseminated by the VWP plus 20 days. The ratio of PGF_2 injections per total inseminations should be less than 1.55.

At the second PGF_2 injection, new cows approaching their VWP could receive their first injection. Cows are rebred if seen in heat 21 days later. Cows are checked for pregnancy around 40 days post breeding, those found open re-enter the pool of cows to be treated with PGF_2. This system is repeated as a routine management program. The program was implemented in 1991 and 1992 and results compared to earlier years of 1988, 1989 and 1990. During the years of a "Prostaglandin Synchrony Program" (1991 and 1992) a decrease in the percentage of open cows during lactation was evident.

Conclusion and Recommendations

The variability in results among studies evaluating GnRH and PGF_2 treatment during the postpartum period is difficult to explain. However, different management systems of the study farms as well as unknown factors related to the study are possible explanations. When deciding what hormonal treatment program to use, consideration must be given to the reproductive and nutritional management systems employed in the farm. Simply put, the potential physiological and endocrine effect derived from these treatments, could be masked in herds that have poor nutritional management and inefficient estrus detection practices.

The following suggestions regarding the use of PGF_2 and GnRH during the post partum period in dairy cattle are made with the intent to serve as guidelines and reflect the authors opinion.

1. <u>At what day post partum should PGF2 be given:</u>

Although, some studies (11,33,34) have documented that treatment of dairy cows with PGF_2 around day 26 post partum has a positive effect on reproduction independent of progesterone concentration, the general opinion is that the benefit derived from prostaglandin treatment is by lysing the CL and bringing the cow into estrus.

With this premise in mind, it has been documented (11,14,27) that the majority of dairy cows including those with an abnormal parturition have a CL that should respond to PGF_2 by day 26 post partum. For this reason, it is the authors opinion that injection around day 26 may result in an efficacious use of PGF_2. Further use of PGF2 after day 26 post partum should be used in an attempt to synchronize estrus in relation to the end of the voluntary waiting period.

Another question which is often ask is which prostaglandin product works best? In a Canadian study, Etherington et al (35), demonstrated that a single treatment with any of 3 commercially available prostaglandin products between 24 and 31 days post partum
reduced the calving to conception interval when compared to untreated controls.

2) <u>How many PGF_2 treatments and at what interval?</u> Fertility did not improve for cows with an abnormal puerperium treated once with
PGF_2 at days 14 to 16 post partum (8) or 25 to 30 days post partum (2). Because of these results, Risco et al (11), decided to combine the treatment regime of 25 mg of PGF2 around day 14 and 26 post partum. To the best of our knowledge this is the only study that has evaluated PGF2 treatment around days 14 and 26 post partum. Because we (11) did not evaluate a single PGF_2 treatment either at day 14 or 26, we concluded that treatment with PGF_2 early post partum (around day 12) followed with a second injection 14 days later (day 26) improved first service conception rate in dairy cows having RFM. The question then is, what effect does a PGF_2 treatment have on reproduction when given around day 12 post partum ? It is not known if there is an effect, however the following references suggest a relationship of PGF_2 concentrations early postpartum on ovulation and uterine involution (21,22).

3) <u>Should PGF_2 be used routinely in all cows or used only in cows with an abnormal parturition?</u> Three studies evaluated the use of PGF2 post partum in normal cows; MacClary et al (8), around day 14 post partum and Bonnet et al (30), around day 26 demonstrated a beneficial effect. In contrast, Gay et al (36), did not show any benefit. Perhaps, the lack of response from postpartum PGF_2 treatment in normal cows is related to the fact that the majority of these cows begin to cycle early post partum and have multiple cycles prior to insemination around day 60 (11,14,27).

4) <u>GnRH treatment:</u> Because of its cost ($5.00/dose) and conflicting results in its effectiveness to increase fertility, it is difficult to make general recommendations in the use of this drug. Two postpartum conditions that could affect the efficacy of GnRH treatment are negative energy balance and metritis.

Treatment with GnRH around day 14 post partum results in LH secretion from the pituitary gland and will accomplish one of two things: 1) induce follicular luteinization or 2) ovulation during the first postpartum wave of recruited follicles that would alter the estradiol/progesterone ratio. However, in dairy cows experiencing negative energy balance early post partum the ovaries may be inactive (absence of follicles) and luteinization or ovulation of follicles may not occur. In addition, pituitary sensitivity to GnRH may be refractory in cows experiencing negative energy balance.

During the postpartum period, treatment with GnRH may result in ovulation or luteinization of follicles in cows affected with metritis. The resultant progesterone dominated uterus could prolong the metritis condition and develop into a pyometra. Treatment with PGF_2 10 days after GnRH injection will regress any induced CL, bring the cows into estrus that would help reduce the occurrence of pyometra.

References

1. Thatcher, W.W. and Wilcox C.J. 1973. Postpartum estrus as an indicator of reproductive status in the dairy cow. J. Dairy Sci. 56:608.
2. Archbald, L. F., T. Tran, P.G.A. Thomas, and S. K. Lyle. 1990. Apparent failure of prostaglandin F2 to improve the reproductive efficiency of postpartum dairy cows that had experienced dystocia and/or retained fetal membranes. Theriogenology 34:1025.
3. Benmrad, M., and J. S. Stevenson. 1986. Gonadotropin-releasing hormone and prostaglandin F_2 for postpartum dairy cows: estrous, ovulation, and fertility traits. J. Dairy Sci. 69:800.
4. Cavestany, D., and R. H. Foote. 1985. Reproductive performance of Holstein cows administered GnRH analog Hoe 766 (Buserelin) 26 and 34 days postpartum. J. Anim. Sci. 61:224.

5. Etherington, W. G., W.T.K. Bosu, S. W. Martin, J. F. Cote, P. A. Dorym, and K. E. Leslie. 1984. Reproductive performance in dairy cows following postpartum treatment with gonadotropin - releasing hormone and/or prostaglandin. Can. J. Comp. Med. 48:245.
6. Fernandes, L. C., W. W. Thatcher, E. P. Call, and C. J. Wilcox 1978. Response to PGF2 and GnRH in postpartum dairy cows. J. Anim. Sci. 70(Suppl. 1):359.(Abstr.)
7. Leslie, R.E., P.A. Doig, W.T.K. Bosu, R. A. Curtis, and S. W.Martin. 1984. Effects of gonadotropin-releasing hormone on reproductive performance of dairy cows with retained placenta. Can. J. Comp. Med. 48:354.
8. McClary, D. G., M. R. Putnam, J. C. Wright, and J. L. Sartin, Jr. 1989. Effect of early postpartum treatment with prostaglandin F2 on subsequent fertility in the dairy cow. Theriogenology 31:565.
9. Okuda, K., W.A. Gaona, and K. Sato. 1988. Effects of gonadotropin releasing hormone and prostaglandin F_2 on the reproductive performance in postpartum cows. Theriogenology 29:823.
10. Richardson, G. F., L. F. Archbald, D. M. Galton, and R. A. Godke. 1983. Effect of gonadotropin releasing hormone and prostaglandin F2 on reproduction in postpartum cows. Theriogenology 19:763.
11. Risco, C.A., L.F. Archbald, J. Elliott, T. Tran and P. Chavatte. 1994. Effect of Hormonal Treatment on Fertility in Dairy Cows with Dystocia or Retained Fetal Membranes at Parturition. J Dairy Sci 77:2562-2569.
12. Stevenson, J.S., and E.P. Call.1988. Fertility of postpartum dairy cows after administration of gonadotropin-releasing hormone and prostaglandin F_2 : a field trial. J. Dairy Sci 71:1926.
13. Morrow, D.A., S.J. Roberts, and K. McEntee. 1969. Postpartum ovarian activity and involution of the uterus and cervix in dairy cattle. I. Ovarian activity. Cornell Vet. 59:173.
14. Risco, C.A., M. Drost, W.W. Thatcher, J. Savio and M.J. Thatcher. 1994. Effects of calving related disorders on prostaglandin, calcium, ovarian, activity and uterine involution in postpartum dairy cows. Theriogenology 42:183-203.
15. Edqvist, L.E., H. Kindahl, and G.H. Stabenfeldt. 1978 Release of prostaglandin F2 during the bovine peripartal period. Prostaglandins 63:111-119.
16. Eley, D.S,, W.W. Thatcher, H.H. Head, R.J. Collier, C.J. Wilcox CJ, and E.P. Call. 1981. Periparturient and postpartum endocrine changes of conceptus and maternal units in Jersey cows bred for milk yield. J Dairy Sci 1981; 64:312-320.
17. Guilbault, L.A., W.W. Thatcher, M. Drost, and S.M. Hopkins. 1984. Source of F series prostaglandins during the early postpartum period in cattle. Biol of Reprod 31:879-887.
18. Lewis G.S., W.W. Thatcher, E.L. Bliss, M. Drost and R.J. Collier. 1984. Effects of heat stress during pregnancy on postpartum reproductive changes in Holstein cows. J Anim Sci 58:174-186.
19. Lindell J.O, H. Kindahl, L. Jansson and Edqvist L.E. 1982. Postpartum release of prostaglandin F_2 and uterine involution in the cow. Theriogenology 17:237-245.
20. Madej A., H. Kindahl, W. Woyrro, L.E. Edqvist and Stupnicki R. 1984. Blood levels of 15-keto-13,14-dihydroprostaglandin F2 during the postpartum period in primiparous cows. Theriogenology 21:279-287.
21. Guilbault L.A., W.W. Thatcher, M. Drost and Haibel G.K. 1987. Influence of a physiological infusion of prostaglandin F2 into postpartum cows with partially suppressed endogenous productions of prostaglandins. 1. Uterine and ovarian morphological responses. Theriogenology 27:931-946.
22. Guilbault L.A., W.W. Thatcher and Wilcox C.J. 1987. Influence of physiological infusion of prostaglandin F2 into postpartum cows with partially suppressed endogenous production of prostaglandins. 2. Interrelationships of hormonal, ovarian and uterine responses. Theriogenology 27:947-957.
23. Holt, L.C., W.D. Whittier, F.C. Gwazdauskas, and W. E. Vinson. 1989. Early postpartum reproductive profiles in Holsteins cows with retained placenta and uterine discharge. J. Dairy Sci. 72:533.

24. Oltenacu, P.A., J.H. Britt, R.K. Braun, and R.W. Mellenberger. 1983. Relationship among type of parturition, types of discharge form genital tract, involution of cervix and subsequent reproductive performance in Holsteins cows. J. Dairy Sci. 66:612.
25. Kesler, J.J. Garverick, H.A., Youngquist, R.S., Elmore, R.G. and C.J. Bierschwal. 1977. Effect of days postpartum and endogenous reproductive hormones on GnRH-induced release in daiy cows. J. Anim. Sci., 46:797-803
26. Britt, J. H., R. J. Kittock, and D. S. Harrison. 1974. Ovulation, estrus, and endocrine response after GnRH in early postpartum cows. J. Anim. Sci. 39:915.
27. Stevenson, J.S. Update of reproductive function in the bovine female. Society for Theriogenology Proceedings for Annual Meeting, August 25-27, 1994, Kansas City, Missouri
28. Staples C.R., W.W., Thatcher and J.H. Clark. 1990. Relationship between ovarian activity and energy status during early lactation in high producing dairy cows. J Dairy Sci 73:938-947.
29. Thatcher, W.W., M. Drost, J.D. Savio, K.L. Mcmilan, K.W. Entwistle, E.J. Schmitt, R.L. De la Sota and G.R. Morris. 1993. New clinical uses of GnRH and its analogues in cattle. Animal Reproduction Science 33:27-49.
30. Bonnet, B.N., W.G. Etherington, S. W. Martin, W. H. Johnson. 1990. The effect of prostaglandin administration to holstein cows at day 26 postpartum on clinical findings, and histological and bacteriological results of endometrial biopsies at day 40. Theriogenology 33:1990.
31. Risco, C.A., R.L. De la Sota, G. Morris, J.D. Savio and W.W. Thatcher. 1995. Postpartum reproductive management of dairy cows in a large Florida dairy herd. Theriogenology in press.
32. Ferguson, J.D. and D.T. Galligan. 1993. Prostaglandin synchronization programs in dairy herds. Compendium of Continuing Veterinary Education. April Issue: p648
33. Etherington, W.G., S.W. Martin, B. Bonnett, W.H. Johnson, R.B. Miller, N.C. Savage, J.S. Walton and M.E. Montgomery 1988. Reproductive performance of dairy cows following treatment with cloprostenol 26 and/or 40 days postpartum: A field trial. Theriogenology 29:565-577
34. Young, IM. and D.B. Andersen. 1986. Improved reproductive performance from dairy cows treated with dinoprost tromethamine soon after calving. Theriogenology 26:199-207.
35. Etherington, W.G., D.F. Kelton and J.E. Adams. 1994. Reproductive performance of dairy cows following treatment with fenprostalene, dinoprost, or cloprostenol between 24 and 31 days post partum: A field trial. Theriogenology 42:739-752
36. Gay, J.M. and G.L. Upham. 1994. Effect of exogenous PGF_2 in clinically normal postparturient dairy cows with a palpable corpus luteum. JAVMA 205:870-873.